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P10102-001

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P10100-001

Highly Diastereoselective Synthesis of β²-amino acids Using Novel Fluorinated Oxazolidine (Fox) as Chiral Auxiliary

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Our group recently developed a novel fluorinated oxazolidine (Fox) based chiral auxiliary.(1,2) Alkylation of various amides derived from 2-trifluoromethyl-4-phenyl-oxazolidine occurred with almost complete diastereoselectivity. Moreover, Fox chiral auxiliary allows the use of hindered amides or electrophiles.
In this poster, we report the extension of the Fox methodology towards the synthesis of enantiopure β²-amino acids.(3) *N*-protected amino amide was efficiently alkylated with a very high diastereoselectivity and in good yields using various electrophiles. Reductive removal of the chiral auxiliary and oxidation of the intermediate aldehyde leaded to the enantiopure Boc-protected β²-phenylalanine. The corresponding γ amino-alcohol could also be synthesized in an enantiopure form. In each case the *trans*-Fox chiral auxiliary was recovered in good yields.

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P10100-002

Synthesis of CF_3-Pseudoprolines Units: An approach to conformationally constrained dipeptide

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Conformationally constrained amino acids have recently gained of considerable interest because of their ability to control the conformation of the peptide for investigating structure-activity relationships as well as for the design of peptidomimetics. In particular, incorporation of a proline unit is known to restrict the amino acyl-proline *cis*/*trans* isomerization, to limit the protein folding and consequently to modulate the biological activity of peptides.
Our group is interested in the preparation of enantiopure trifluoromethylated pyrrolidine-type amino acids like CF_3-proline.[1] As a complementary strategy, we have developed the preparation of various CF_3-pseudoprolines starting from L-serine and fluoral or trifluoroacetone. Conformational restrictions as well as unique physical and biological properties imparted by the fluorinated group are expected from the incorporation of such kind of amino acids into a peptide chain.
Convenient conditions were found to perform the acylation of the pseudoproline unit in order to study the impact of the CF_3 group on the *cis*/*trans* isomerization of the amide bond. Preliminary results concerning dipeptides synthesis are also reported.

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P10100-003

Chiral CF_3-allylmorpholinone: a key intermediate for the synthesis of enantiopure α-trifluoromethyl α-amino acids

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Cα,α-disubstituted amino acids is of current interest because of their ability to control the conformation of the peptide backbone. Owing to the unique physical and biological properties induced by the fluorinated group, α-trifluoromethylated aminoacids (α-Tfm AAs) form a special class of Cα,α-disubstituted amino acids and are, therefore, interesting building blocks for peptide synthesis. However, their use in peptide chemistry remains limited due the difficulty to prepare them efficiently, particularly in their enantiopure form.(1)
We will report here a very efficient route for the synthesis of various α-Tfm AAs involving a chiral CF_3-allylmorpholinone as a key intermediate. This building block was conveniently prepared from a chiral trifluoropyruvate-based oxazolidine. Further cyclization reactions gave rise to both enantiomers of α-Tfm proline and derivatives (CF_3-pyroglutamic acid, CF_3-prolinol, ...) in enantiopure form. Moreover, the synthesis of the (*S*)-α-Tfm-α-allylglycine and the novel (*S*)-α-Tfm-α-norvaline were achieved in a few steps from this starting material.(2)

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P10100-004

Amino Acids of Xanthone

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Unnatural amino acids play an important role in design of new bioactive peptides. In this project we intend to synthesize a library of xanthone-based amino acids starting from xanthone.
Our strategy is based on regioselective lithiation of xanthone. Investigation of various strategies of lithiation of xanthone let us to propose a synthetically useful method starting from 9,9-dimethoxyxanthene, which is easily obtainable from xanthone. Under optimal conditions, reaction of 9,9-dimethoxyxanthene with tert-butyllithium, followed by electrophilic quench yields C4/C5 disubstituted xanthone. Introduced functional groups are precursors for side-chains terminated with amino and carboxylic groups of the final aminoacid.

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P10100-005

Enantioselective synthesis of fluorinated *p*-borono-L-phenylalanines enriched with ^{10}B atom

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The boron neutron capture therapy (BNCT) based on the interaction between ^{10}B isotope and thermal neutron has been highly noted in recent years as one of promising techniques for treatment of cancers. *p*-(^{10}B)Borono-L-phenylalanine (L-^{10}Bpa), in which boron atom is enriched with ^{10}B isotope, is now using clinically as an efficient ^{10}B carrier for treatment of patients in particular with malignant brain tumor and melanoma (1). In order to develop a practical method for the synthesis of L-^{10}Bpa, we have recently examined the enantioselective method utilizing the Negishi reaction based on the coupling of 4-(^{10}B)boronoiodobenzene derivative with 3-iodo-L-alanine derivative (2). From the standpoint of diagnosis of cancers, the magnetic resonance imaging (MRI) is noted as one of common techniques. In particular, MRI based on the measurement of ^{19}F atom is becoming a remarkable one. To create practical materials utilizing as not only the 10B carrier but also MRI probe, we had already synthesized the compounds containing both ^{10}B and ^{19}F atoms in a single molecule such as β-[4-(^{10}B)borono-2,6-difluorophenyl]-DL-alanine [DL-^{10}Bpa(2,6F_2)] 3., β-[4-(^{10}B)borono-

2-trifluoromethylphenyl]-DL-alanine [DL-^{10}Bpa(2CF$_3$)] 4..
The present paper describes the enantioselective syntheses of L-^{10}Bpa(2,6F$_2$) and L-^{10}Bpa(2CF$_3$) based on the Negishi reaction.

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P10100-006

Advances in the Stereoselective Synthesis of Homopropargylglycine

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Homopropargylglycine (Hpg) is an unsaturated amino acid which presently has found wide application as methionine surrogate in proteins bioexpressed by procedures of the expanded amino acid repertoire [1,2]. It offers the possibility of further transformations of the protein with the orthogonal click chemistry. Although several syntheses of this alkyne-containing amino acid have been reported, in our hands the reproduction, particularly, in terms of yields proved to be difficult. Thus an improved classic Strecker synthesis was elaborated leading to racemic homopropargylglycine in 60 % overall yield as well as a sulfimine-mediated asymmetric Strecker synthesis (3), which produced the unsaturated amino acid in nearly 90 % yield. Using trimethylsilyl cyanide under optimal reaction conditions an 84 % ee was achieved. When required, transformation into the N-acetyl derivative and its enantiomeric resolution by kidney acylase I results in enantiomerically pure L-homopropargylglycine.

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P10100-007

A substituted proline with a strong preference for the helix conformation: an X-ray diffraction investigation

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L-Proline is conformationally unique among coded amino acids in that its φ torsion angle is blocked (-65 ± 10) by its characteristic five-membered pyrrolidine ring structure and the preceding ω torsion angle (tertiary amide) can undergo cis (0) <-> trans (180) isomerization much easier than the secondary amides of the usual peptide bonds. In addition, its ψ torsion angle is commonly found either in the right-handed 3_{10}-/α-helical region (-30 ÷ -50, or cis' conformation) or in the left-handed, semi-extended, region [-150 ±10, or trans', or poly-(L-Pro)$_n$ conformation]. Methylation at the C$^\alpha$-position of a Pro residue was suggested to block the preceding tertiary amide (ω) torsion angle of the resulting (αMe)Pro to the trans disposition and to restrict the φ,ψ surface to the single region where the 3_{10}/α-helices are found. We have synthesized a large set of Nα-blocked, (αMe)Pro-containing, dipeptide N'-alkylamides having the general formulas P-D-(αMe)Pro-Xxx-NHiPr and P-Xxx-D-(αMe)Pro-NHiPr, where P is Ac or Boc and Xxx is D-Ala, L-Ala, Aib, Gly, D-(αMe)Pro, or L-(αMe)Pro. The results of the present X-ray diffraction analysis clearly show that the region of the conformational map overwhelmingly preferred by (αMe)Pro is indeed that typical of 3_{10}/α-helices, but the semi-extended, type-II poly(Pro)$_n$ helical region can exceptionally be explored by this extremely sterically demanding C$^\alpha$-tetrasubstituted α-amino acid. In addition, the known high propensity for β-turn formation of the Pro residue is even enhanced in peptides based on its C$^\alpha$-methylated derivative. To complete the picture of the preferred conformation of (αMe)Pro, the synthesis and crystal-state investigation of a series of terminally-protected homo-peptides from D-(αMe)Pro are currently in progress in our laboratory

P10100-008

Synthesis of a novel α,α-disubstitute glycine, α-cyclobutylalanine

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The availability of structurally rigid peptides is of value on biochemical and biophysical studies. α,α-Disubstituted glycines are of particular importance in developing small, acyclic, conformationally restricted peptides. In particular, α-methyl-α-amino acids are valuable tools for controlling secondary structures in de novo-designed peptides because they have fairly good reactivity among α,α-disubsutituted glycines. They are known to be α/3_{10}-helices inducers, and also to be often powerful enzyme inhibitors. Previously, we reported that a convenient synthesis of chiral α-methyl-α-amino acids using the Ugi reaction and the excellent chromatographic separation of diastereomers of the resulting Ugi products, as the key steps (1). We have also reported the preparation of a novel amino acid, α-cyclopropylalanine (αCpa) as an application of this method, and the determination of chirality of αCpa by X-ray crystal structural analysis (2). We report here the preparation of another novel amino acid, α-cyclobutylalanine (αCba). A diastereomeric dipeptide containing αCba [Z-(S)-Lys(Z)-(R/S)-(N-Bzl)αCba-NH-cHex] was prepared in good yield by the Ugi reaction using Z-(S)-Lys(Z), benzylamine (Bzl-NH$_2$), cyclobutyl methyl ketone and cyclohexyl isocyanide (cHex-NC). The diastereomers were well separated by chromatography using silica-gel. Thereby, both optically pure enantiomers of α-cyclobutylalanine (αCba) were easily obtained. The configuration of each enantiomer of αCba could be determined by the comparison of CD spectra of N-benzyl-αCba with that of N-benzyl-αCpa.

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P10100-009

Sulfur-containing amino acid amides of phenolic acids

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The antioxidant activity of phenolic derivatives has been widely investigated in relation to their physiological function. Among them a subject of our interest are cinnamoyl amides because of their metabolically stability in comparison to the esters.
In order to broaden the field of our study on the hydroxycinnamoyl amides, we synthesized mono sinapoyl- and mono feruloyl amides of 1,6-diaminohexane and coupled the free amino group to sulfur – containing amino acid. The evaluation of the radical scavenging activity of the newly synthesized compounds, containing unproteinogenic amino acid, against DPPH radical is in progress.

P10100-010

Conjugation of glaucine to hydroxycinnamoyl aminoacid amides

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Recently we found that sinapoyl- and feruloyl amides of phenylalanine possess considerable antioxidant potential against bulk phase lipid peroxidation. On the other hand, data exist about the photoprotective and antioxidant activity of aporphinic alkaloid glaucine. Our study on synthesized hydroxycinnamoyl amides of 3-aminomethylglaucine have shown an increasing of the radical scavenging activity against DPPH* in comparison to the starting aporphinic alkaloid. It could be expected that the coupling of hydroxycinnamoyl phenylalanine amides to glaucine could result in enhancement of the antioxidative activity of the synthesized compounds. The aim of the present study is: - Condensation of feruloyl- and sinapoyl phenylalanine amides to 3-aminomethylglaucine. - Evaluation of the antioxidative activity of the synthesized compounds against DPPH*.

P10100-013

Side-chain hydrazide formation in peptides

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Cleavage of the protected peptide-resins with β-branched amino acids at the C-terminus by hydrazinolysis require particularly harsh conditions. We discovered that under such conditions the side chain Asp and Glu tert-butyl esters [-Asp(OtBu)- and –Glu(OtBu)-] react with hydrazine to form Asp(hydrazide) and Glu(hydrazide) containing peptides. The GLP-1 derivative was synthesized on a SASRIN™ resin using an ABI 433A Peptide Synthesizer and Fmoc/HBTU/DIEA chemistry. The peptide-resin was reacted with 10% hydrazine hydrate/DMF over 2 hours (A) or 20% hydrazine/DMF over 24 hours (B). Protected peptide hydrazides were deblocked using acidolytic cleavage mixture. The main product of A was the expected C-terminal hydrazide of mass M. Product from B was a mixture of two components, M+28 Da and M+42 Da, and after HPLC purification was analyzed by MALDI MS/MS. The +28 Da and +42 Da modifications were determined to be the result of two or three +14 Da additions to acidic residues (Glu^{15}, Asp^{9} and Glu^{3}, but curiously, no modification at Glu^{21}) in the peptide. Automated Edman sequencing of each of the peptides fully corroborated the theoretical sequence. The NMR analysis of purified product from B supported our findings. The model peptides with other than OtBu protecting groups for Asp or Glu were tested. The relative protection against the formation of Asp(hydrazide) containing peptides was decreasing following the O-3-methyl-pent-3-yl (OMpe) > O-tert-butyl (OtBu) > O-2-phenylisopropyl (O-2-PhiPr) esters (NovaBiochem) order. Our results contradict the reported stability of the a-tBu ester group to alkaline hydrolysis, hydrazinolysis, aminolysis, and hydrogenolysis (1).

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P10100-014

Synthesis of peptides containing five-member cyclic *N*-amidino-amino acids

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The interest in guanidine-containing compounds is due to their important role in biological recognition processes. Five-member cyclic *N*-amidino-amino acids represent hybrid structures, combining both proline rigidity and high positive charge of arginine guanidine group. Structurally similar *N*-amidino-proline, *N*-amidino-pyroglutamic acid and cyclocreatine (2-imino-1-imidazolidine acetic acid) were chosen as objects of our study. The problem of their incorporation into peptide structure requires use of substituted derivatives or effective guanidylation technique.
Recently we have presented the synthesis of Mts-protected *N*-amidino-proline. Nevertheless, its application in *N*-termini modification of peptides shows slow condensation rate in classical as well as solid phase synthesis for short peptides (2-5 residues) or completely fails in case of longer ones (10 residues). On the other hand, polymer supported guanidylation of proline by *bis*-Boc-protected carboxamidine-1*H*-benzotriazole seems to be preferred route to *N*-amidino-proline containing peptides. Three methods of *N*-amidino-pyroglutamic acid synthesis have been investigated and model dipeptide has been acquired on Wang polymer by the cyclization of guanidine-glutamic acid. For selective Boc-deprotection on Wang resin the literature technique has been utilized. However, in this case ESI-MS and NMR analyses evidence for side-reaction of triethylamine alkylation by polymer linker fragment. HPLC analysis shows *N*-amidino-pyroglutamyl-phenylalanine stability at acidic and physiological pH but fast ring opening in water solution at pH 9. For cyclocreatine application in peptide synthesis we suggest the use of its *p*-toluenesulfonate having good solubility in DMF and showing effective coupling in *Cl*-HOBt/DIC condensation. The examples of *N*-amidino-amino acids practical application in peptide synthesis will be presented.

P10100-015

Pipecolic Acid Disrupts Collagen Triple Helix Structure in Model Peptides

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Collagen is important protein with ubiquitous occurrence among vertebrates and forms major fraction of their total protein content. It is characterized by triple helical assembly of three left handed helices. Single helix is represented as polymer of repeating unit of tripeptide, Xaa-Yaa-Gly, where Xaa and Yaa positions are frequently occupied by imino acids proline (Pro) and hydroxyproline (Hyp) respectively. Collagen model peptides with different residues have been prepared to understand their effect on their structure and stability. Pipecolic acid (Pip), a higher ring homologue of proline, is widely occurring imino acid and has been substituted in various peptides in position of proline to understand its effect. Here Pip has been incorporated in collagen model peptides to study its effect on triple helix structure. In this study, we designed the three 30-mers collagen model peptides. All Xaa and/or Yaa positions of (Pro-Pro-Gly)10 were replaced with Pip residues to make (Pip-Pip-Gly)10, (Pip-Pro-Gly)10 and (Pro-Pip-Gly)10. These were prepared by Fmoc-solid phase method on Barlos resin with tripeptide coupling. After cleavage of the peptide from the resin, purification was performed by RP-HPLC. These peptides were analyzed by CD spectroscopic measurements. At 4 ‹C in PBS buffer, there was no peak around 225 nm about these peptides indicating triple helical conformation was not formed. Furthermore, we tried to confirm the role of Pip residue in the collagen model peptide. (Pro-Pro-Gly)10 was modified with one Pip residue near the center of the peptide to make (Pro-Pro-Gly)5-(Pro-Pip-Gly)-(Pro-Pro-Gly)4 and (Pro-Pro-Gly)5-(Pip-Pro-Gly)-(Pro-Pro-Gly)4. Only one replacement by Pip induces decrease of the stability of triple helix formation. In spite of being homologue of proline, Pip residues in the collagen model peptides disrupt to form triple helix structure.

P10100-016

Synthesis of novel hydantoin-phosphonic acids and dipeptides consist hydantoin structure with potential biological activity

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Hydantoin derivatives are synthetically valuable, e.g. as precursors to β-amino acid derivatives. Hydantoins substituted at C-5 and aminophosphonic acids are important medicinal compounds. Numerous applications have been found for hydantoin derivatives and aminophosphonic acids: antidepressant, antiviral activities, as inhibited binding of HIV to lymphocytes anti-convulsant, cardiac anti-arrhythmic and anticancer effects. The most familiar derivative, 5,5-diphenylhydantoin (Phenytoin) is extensively used as an anti-convulsant and cardiac antiarrhythmic. Aminophosphonic acids are structurally similar to aminocarboxylic acids. The established antiproliferative effects together with the low mammalian toxicity of these agents have conditioned tremendous interest towards designing novel antineoplastic agents. Taking into account this fact herein we describe the synthesis of novel hydantoin-phosphonic acids and dipeptides consist hydantoin structure with potential biological activity. The novel hydantoin-phosphonic acids were synthesized by reacting phosphorus trichloride/ dimethyl-H-phos¬phonate with formaldehyde and hydantoins/ 3-amino-hydantoins. The novel dipeptides were synthesized by liquid-phase peptide synthesis reacting Boc-/ Z- Amino acids with 3-amino-hydantoins and TBTU/HOBt/DIEA was used as a condensing reagent. Structure-activity relationship will be discussed.

P10100-017

1,3-disubstituted 1,2,3,4-tetrahydro-β≈-carbolines as peptidomimetics of tryptophan: synthesis and conformational analysis

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Tryptophan is often a key pharmacophore which determines the affinity of peptide ligands for their receptors. Cyclic analogues of tryptophan which introduce local constraints and reduce the flexibility of the indol moiety are very valuable tools to probe the bioactive conformation of the peptide ligands. One of the possibilities to freeze indol moiety of tryptophan is the synthesis of the additional 6-member ring by the formation of 1,2,3,4-tetrahydro-β-carbolines. We report the synthesis of 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines via the Pictet-Spengler reaction. Methyl ester of tryptophan or dipeptides with N-terminal Trp (Trp-Ala-OMe, Trp-Leu-OMe) were used as arylethylamine substrates and α-amino aldehydes derived from L and D-amino acids were used as carbonyl components. We determined that there were no differences of the stereoselectivity of the Pictet-Spengler reactions in the case of Trp-OMe or Trp-dipeptides. We also investigated the conformation of the newly created 6-membered ring in cis and trans diastereomers. The ROESY spectra were used to assign the more stable conformation for each isomer. The conformations of cis isomers derived from tryptophan and dipeptides were the same and substituents on C-1 and C-3 were in both cases equatorial. The conformation of trans isomers were depended on the carboxyl part of Trp. For methyl ester of tryptophan the ester group was axial, whereas for dipeptides we observed the opposite conformation with equatorial substituent on C-3. Our results show that the Pictet-Spengler reaction can be successfully performed for amino acids and peptides and the conformation of the newly created 6-membered ring depends on the substrates and the size of C-1 and C-3 substituents.

P10100-018

Helical-screw handedness of peptides composed of diastereoisomeric cyclic amino acids

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Helical-screw handedness in proteins is believed to result from the α-carbon chiral center of L-α-amino acids. Recently we have reported that the helical-screw sense of oligopeptides can be controlled without a chiral center on the peptide-backbone but by chiral centers at the side chain. That is to say, we designed and synthesized an optically active cyclic α,α-disubstituted amino acid (*S*,*S*)-Ac(5)c(dOM), in which the α-carbon atom is not a chiral center but chiral centers exist at the side chain. Conformational analysis of the (*S*,*S*)-Ac(5)c(dOM) peptides revealed that the hexapeptide formed left-handed 3_{10}-helices both in solution and in the solid state, and the octapeptide assumed a left-handed α-helix. Herein we designed new two diastereoisomeric cyclic α,α-disubstituted amino acids; (1*S*,3*S*)- and (1*R*,3*S*)-1-amino-3-(methoxy)cyclopentanecarboxylic acid (Ac(5)c(OM)) having chiral centers both at the α-carbon atom and at the side chain. The amino acids (1*S*,3*S*)- and (1*R*,3*S*)-Ac(5)c(OM) were synthesized starting from L-(-)-malic acid. That is to say, at first, the malic acid was converted to diiodide compound, and bisalkylation of dimethyl malonate with the diiodide gave a cyclic diester. Monohydrolysis of the diester, followed by Curtius rearrangement produced separable mixtures of (1*S*,3*S*)- and (1*R*,3*S*)-Ac(5)c(OM). We prepared both diastereoisomeric (1*S*,3*S*)- and (1*R*,3*S*)-homooligomers by solution-phase methods, respectively, and studied their preferred secondary structures using ^{1}H NMR, FT-IR, CD and X-ray crystallographic analysis.

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P10100-019

Unusual Cleavage of Tripeptides Containing Pipecolic Acid

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Pipecolic acid (Pip), a widespread natural non-proteinogenic amino acid, and its derivatives occur in numerous natural and synthetic products with important biological properties. For example, biologically important natural products such as immunosuppressant FK506, anticancer agent VX710, histone deacetylase inhibitor apicidin, trapoxins etc. contain a Pip. In addition, this imino acid has found widespread utility as Pro mimic and β-turn inducer in many designed peptides and synthetic drug candidates, as a building block in organic synthesis. Replacement of Pro for its higher homologue Pip in peptides is reported to go along with a significant change in bioactivity and leads to interesting model compounds for studies on peptide conformations. Comparative investigations of Pip residues to other cyclic amino acids, especially Pro, incorporated in peptides give detailed insight into local mechanisms of peptide folding processes according to ring size. During the preparation of Fmoc-L-Pip-L-Pip-Gly-OH from Fmoc-L-Pip-L-Pip-Gly-OtBu by the treatment with TFA, unexpectedly Fmoc-L-Pip-D/L-Pip-OH were found as major products. The formation of Fmoc-L-Pip-D/L-Pip-OH indicates the cleavage of Pip-Gly bond and the mechanism involves racemization. Amide bonds are generally known to be stable towards TFA treatment and HCl/dioxane treatment. So, we attempted acid stability of Pip containing peptides. In this study, a series of Fmoc-tri/dipeptide-OBzl

with and without Pip were designed and synthesized. The stability test of these peptides, in 4 M HCl/dioxane at room temperature, was performed by means of HPLC analysis. Results of this study reveal that tripeptides containing Pip at the center are unstable due to the cleavage of Pip-X bond (X= amino acid residue).

P10100-020

Synthesis of a series of cyclic i-to-i+4 side chain-to-side chain 1,4-disubstituted [1,2,3]triazolyl-bridged PTHrP(11-19) derivatives

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Side chain-to-side chain cyclization is used to stabilize a bioactive conformation and to reduce proteolytic degradation. Among the numerous modes of cyclization, bioisosteric modifications and cyclizations that do not require orthogonal protection schemes are of great interest. The recently introduced Cu^{I}-catalyzed azide–alkyne 1,3-dipolar Huisgen's cycloaddition (1-3) known as the click reaction (4) presents a promising opportunity to develop a new paradigm for intramolecular cyclization. In fact, the proteolytic stable 1,4-disubstituted (1,2,3) triazolyl bridge is isosteric with the peptide bond used in the well established lactam-type side chain-to-side chain cyclizations. We reported the preparation of N^{α}-Fmoc-ω-azido-α-amino acids from the corresponding N^{α}-protected α,ω-diamino acids by diazo-transfer reaction or a multistep strategy starting from the ω-hydroxy-α-amino acid. The N^{α}-Fmoc-ω-ynoic-α-amino acids were prepared by alkylation of Ni^{II} complexes of the Schiff bases derived from glycine and a chiral inducer with alk-ω-ynyl bromides. These building blocks were used in solid phase synthesis of series of eight linear nonapeptides derived from the sequence of PTHrP(11-19) where ω-azido- and ω-ynoic-α-amino acids replaced Lys^{13} and Asp^{17}, respectively. Failure to carry out on-resin intramolecular Cu^{I}-catalyzed click reaction led to a solution phase cyclization. Simultaneous deprotection and cleavage from resin generated free peptide that was purified and subjected to intramolecular Cu^{I}-catalyzed cyclization. Optimization of the cyclization reaction yielded a series of i-to-i+4 1,4-disubstituted (1,2,3) triazole-bridged cyclopeptides (5).

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P10100-021

Photoswitchable bisintercalators - synthesis of Triostin A analogues

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Novel highly active and specific DNA binding molecules have a considerable potential particularly with regard to applications in medical science. The major and minor grooves of the DNA serve as recognition areas. It is of high interest for Chemical Biology to control DNA-binding abilities of synthetic molecules. A possible modulator is light in combination with photoswitchable molecules. Triostin A is a natural bisintercalator which belongs to the quinoxaline antibiotics originally isolated from *Streptomyces* S-2-210. It consists of a bicyclic depsipeptide with *N*-methylated amino acids and a Cystine bridge. Its heteroaromatic quinoxaline moieties are able to intercalate GC-specifically into the DNA inducing a change in conformation and therefore inhibit the transcription by blocking spezific enzymes. TANDEM is the *N*-unmethylated derivative of Triostin A which binds AT selectively to the DNA due to the change in the hydrogen bonding pattern. The exchange of the Cystine bridge of TANDEM with substituted azobenzene units leads to photoswitchable Triostin A analogs. The synthesis of the depsipeptidic basic structure which contains the quinoxaline moieties is introduced as well as the coupling with azobenzene amino acids. The double cyclization step is accomplished under pseudo high dilution conditions. In order to differ between *cis*- and *trans*-configurations of the molecule, NMR- and IR-spectroscopy was carried out as well as the photoswitchability of different analogues was tested by irradiation and RP-HPLC analysis.

P10100-022

For synthetic peptide vaccine prototype development: Synthesis and characterization of biodegradable poly(1-azabicyclo[4.2.0]octane) polymers and their conjugates with antigenic peptides

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In this study, the synthesis of a biodegredable polyelctrolyte, poly(1-aza bicyclo[4.2.0]octane), (PC), which has an heteroatom in its structure and their bioconjugates obtained by microwave and carbodiimide methods were explained. For PC obtaining, the synthesis of the monomer, 1-azabicyclo[4.2.0]octane, (C), was carried out by organic methods which then will be used in polymer synthesis [1-2]. Consequently, polymers having different molecular weights and their water soluble bioconjugates were synthesized and the characterization of these polymers and conjugates were done by different methods such as UV, ATR FT-IR, SEC with four detectors and Zeta Sizer. For the chemical modification of PC, bromoacetic acid was used and a water soluble polyampholyte synthesis was achieved with the quaternization of polymer. ATR FT-IR spectra of PC was recorded and molecular weights, polidispersity values of polymers, Mark-Houwink constants and molecular diameters of the polymers were measured with size-exclusion chromatography with four-detectors (light-scattering, refractive index, viscosity and UV). With Zeta Sizer, size-analysis of polymer chains were done and their zeta potentials were measured. It is determined that molecular weights of the polymers were affected by the change in the amount and the type of initiators used and also from the polymerization media. Analysing of having biodegradable characteristics of the synthesized polymers has been being reviewed. After synthesis and modification of PC, its conjugates with antigenic peptides like Avian Influenza Hemagglutinin (HA 98-106) YPYDVPDYA and RGDSGGC cell receptor peptide were obtained. Their characterization was also performed.

P10100-023

Synthesis of biological active cyclopeptides corresponding to 4th loop of nerve growth factor

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cyclo(-Gly-Asp-Glu-Lys-), cyclo(-Gly-Gly-Asp-Gly-Lys-) and cyclo(-Gly-Gly-Gly-Asp-Gly-Lys-) mimicking β-turn residues of the nerve growth factor' 4th loop were synthesized via combination of solid phase and solution methods. Related linear peptides were prepared in 52-83%

yields using Fmoc/t-Bu technique starting from H-Lys(Boc)-OClt resin. BOP, HBTU and DPPA were employed as coupling agents in cyclization experiments which were performed under high dilution conditions (10-3 M). Products of the reactions were identified and their distribution was studied. Cyclyzation promoted by DPPA reagent proceeded most selectively and yielded the desired 5 and 6 membered cyclopeptides exclusively while in case of 4 membered peptide additional dilution to 10-4 M was necessary to suppress unwanted cyclodimer formation. Two other side reactions were encountered in this study, namely, the unexpected premature Boc-group loss during cleavage of protected peptides from Clt-resin by 0.5% TFA in DCM, and substantial racemization of, presumably, Asp residue observed when BOP reagent was used throughout chain assembly. In HT22 cells the synthesized cyclic peptides at the 10-8M concentration significantly decreased the cell survival.

P10100-024

Synthesis of glycopeptides and neoglycopeptides: Fmoc Asp/Glu derived 5-oxazolidinones as key intermediates in the synthesis of several building blocks

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Glycopeptides and neoglycopeptides are important classes of molecules with diverse biological functions. The side chain carboxyl functionalized aspartic acid and glutamic acid represent attractive and easily available starting materials for the synthesis of such molecules and also to carry out several other chemical modifications leading to the synthesise of diverse class of unnatural amino acids and peptide conjugates. Nevertheless in a dicarboxylic substrate, it is synthetically challenging to obtain the suitably protected forms in order to enable chemo-selective modification of only one of the –COOH groups. Traditionally, this was addressed through the protocol involving orthogonal ester protection and deprotection steps. Deviating from this approach, we have developed a concise and practical method to carry out side chain carboxylic acid selective reactions by making use of bidentately protected Fmoc-Asp/Glu in the from of the corresponding 5-oxazolidinones. Fmoc-Asp and Glu derived 5-oxazolidinoes have been obtained by condensing the corresponding acids with paraformaldehyde. The presentation features the synthetic utility of thus obtained oxazolidinones and their corresponding isocyanate derivatives (ù-isocyanates) as key intermediates in the synthesis of the following classes of amino acid derivatives. a) Heterocyclic amino acids; b) glycosylated amino acids; c) orthogonally protected diamino propionic and butanoic acid. Also the synthetic advantages of the present approach and the incorporation of unnatural and glycosylated amino acids into glycopeptides will be dealt with.

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P10100-025

Development of water-soluble click peptides by use of the *O*-acyl isopeptide method: *in situ* production of Alzheimer fs amyloid β peptide

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In Alzheimer fs disease research, sparing water-solubility and uncontrolled self-assembly of amyloid β peptide (Aβ) 1-42 are significant obstacles to establish an experimental system that clarifies a pathological mechanism of Aβ1-42. To solve these problems, we herein disclose water-soluble "click peptides" based on an *O*-acyl isopeptide method. These peptides contain an *O*-acyl instead of *N*-acyl residue at the Gly^{25}-Ser^{26} of Aβ1-42, and are converted to Aβ1-42 via an *O-N* intramolecular acyl migration triggered by pH-change (pH-click) or photo-irradiation (photo-click). These peptides had remarkably higher water-solubility than Aβ1-42. In addition, these peptides clearly adopted monomer state with a random coil structure, which were verified by various physicochemical assays. Importantly, we could establish an *in situ* system predominantly comprised of monomer Aβ1-42 as a result that the monomer click peptide was converted to Aβ1-42 quickly and quantitatively in accordance with pH-change. Both self-assembly and conformational change of the produced Aβ1-42 *in situ* were observed with time. Similarly, the photo-triggered click peptide afforded Aβ1-42 by UV-irradiation. Because the *in situ* production of intact Aβ1-42 from the click peptides could overcome the handling problems of Aβ1-42, this strategy would provide a reliable experimental system for investigating a pathological function of Aβ1-42 in Alzheimer fs disease.

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P10100-026

Hydrolytic action of *Candida Antarctica* lipase B (CALB) and *pig liver* esterase (PLE) upon various carboxyl protecting groups

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The application of biocatalysts in the protecting group chemistry may offer excellent alternatives to chemical methods because enzymes (i) carry out highly chemo-and regioselective transformations, (ii) usually operate at neutral pH values, and (iii) combine a high selectivity for the reaction they catalyze and the structure they recognize with a broad substrate tolerance. Recently we have demonstrated that several carboxyl protecting groups (t-butyl esters, allyl and benzylesters, methyl and chloroethyl esters) can be efficiently removed by *Bacillus subtilis* esterase BS2 and *Candida Antarctica* lipase A (1,2,3). Here, we report that the enzymes *Candida Antarctica* Lipase B (CALB) and *Pig Liver* Esterase (PLE) allow the mild removal of methyl, ethyl and allyl esters from various carboxylic acids, including N-protected amino acids, in high yield. Moreover, the optimum conditions (enzyme-substrate ratio and the reaction time) are investigated for the potential selective removal of a certain type of esters as opposed to the others. Using different esters of N-protected-L- glutamic acid the regio-selective hydrolytic action of the enzymes CALB and PLE on the α- or γ- carboxylate is also studied at the same time.

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P10100-027

Novel semisynthetic full-lenght insulin analogs with modifications in the positions B24-B26

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Insulin is a peptide hormone, which controls the blood glucose and which is one of the central metabolic hormones of the organism. Despite the long term period of extensive research, the interaction of insulin with its receptor still remains poorly understood. The classical binding region has been evolutionary conserved among vertebrates, and thus attention has focused to this surface. The substitution of amino acids in the conserved regions may influence the biological and pharmacokinetic properties of insulin. The C-terminus of the B-chain is considered to belong to the first contact site of insulin with its receptor. We are particularly interested in the substitution of amino acids in the C-terminus of the B-chain of full-length insulin analogues by N-methylated amino acids. N-Methylation is an attractive and subtle modification of peptide bond for several reasons. It enhances hydrophobicity of adjacent peptide part, it leads to the suppression of hydrogen bonding and it changes basicity of the carbonyl group. In this study, we prepared a following series of six new insulin analogs with modifications in the positions B24-B26 of insulin: [PheB26]-insulin, [N-Me-TyrB26]-insulin, [N-Me-PheB26]-insulin, [N-Me-PheB25]-insulin, [N-Me-PheB24]-insulin and [N-Me-PheB25, PheB26]-insulin. We determined the binding potencies of respective analogs and human insulin to the insulin receptor from rat adipocyte membranes. From our results we deduced several clear conclusions about the importance of PheB24-PheB25-TyrB26 amino acids and adjacent peptide bonds in human insulin.

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P10100-029

Hydroxycinnamoylamides of opioide active derivatives

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The antioxidant effect of introducing phenylpropenoyl moiety in either in N-terminal group of analgesic oligopeptides or in C-terminal end of opioide active amino acid, modificated with polyamines have been evaluated. The series of hydroxycinnamoyl peptide and amino acid amides were synthesized by standard method used in peptide chemistry.

P10100-030

Synthesis of natural peptide obestatin

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Obesity is a major public health problem associated with morbidity and mortality and continues to increase worldwide. The gastrointestinal tract and the pancreas release hormones regulating appetite and body weight. Obestatin is a recently described 23 amino-acids peptide derived from preproghrelin. It was identified by bioinformatic prediction (1). Obestatin decreases food intake and body weight gain, decelerates gastric emptying, promotes sleep in rat, inhibits water drinking. It has been described that obestatin effects on feeding behavior and an U-shaped dose-response relationship was found: low doses (0.01-3 nmol/kg) and high doses (1-3 nmol/kg) were ineffective. The 23 amino-acid carboxy-amidated human obestatin nAlaProPheAspValGlyIleLysLeuSerGlyValGlnTyrGlnGlnHisSerGln AlaLeuNH$_2$ and corresponding rat obestatin HPheAsnAlaProPheAsp ValGlyIleLysLeuSerGlyAlaGlnTyrGlnGlnHisGlyArgAlaLeuNH$_2$ were synthesized by different schemes.

Synthesis was developed on the basis of solid phase synthesis of protected peptide fragments followed by their assembly into the final product. Fragments 1 – 8, 9 – 13, 1 – 13, 14 – 20, 14 - 22 were built on the acid-labile 2-chlorotrityl chloride resin using the orthogonal Fmoc/tBu strategy. Fragment 21-23 (HCl•HArgAlaLeuNH$_2$) was obtained in solution. The assembly of the full-length peptide was carried out by fragments coupling in solution or using solid phase method. The free peptide was obtained by treating the protected peptide with mixture of TFA: H_2O:TIS (95: 2.5: 2.5). The peptide was purified with HPLC and isolated by lyophilization with 95%+ purity according to analytical reversed-phase HPLC. The mass of molecular ion determined by MALDI-TOF spectrometry was in fine agreement with calculated value. The inverstigation of biological actions of obestatins is in progress.

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P10100-129

Straightforward Synthesis of Enantiopure Tfm-amino acids from Chiral CF_3-Oxazolidines

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Trifluoromethylated amino acids (Tfm Aas) are current synthetic targets due to their unique properties and their synthesis in enantiopure form remains a challenge. We will report that Chiral 2-trifluoromethyl-1,3-oxazolidines (Fox) are highly versatile synthons for the stereoselective synthesis of various functionalized α-trifluoromethylamino compounds such as α-amino nitriles , α- and β-amino acids, diamines and amino alcohols.[1,2] Moreover, trifluoropyruvate-based oxazolidines proved to be very valuable building blocks for the stereoselective synthesis of both enantiomers of α-trifluoromethyl proline and α-Tfm-pyroglutamic acid, in enantiopure form. [3] Moreover, the synthesis of the (S)-α-Tfm-α-allylglycine and the novel (S)-α-Tfm norvaline were achieved in a few steps from this starting material. We will also report a recent straightforward synthetic route to Tfm-dihydroxyprolines in enantiopure form from trifluoropyruvate-based chiral oxazolidines.

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P10101-028

An efficient method for the synthesis of C-terminal amidated enkephalins

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A central goal in drug discovery is the ability to design small molecules which contain the key structural and functional elements of biologically active peptides. Enkephalins are endogenous peptides with analgesics activities, and also different biological activities. It was shown that the amidation of c-terminus could increase the biological activity and also hydrophobicity of the peptides. Amidated peptides could be

prepared according to the following methods: a) solid-phase synthesis on benzylamine resins; b) ammonolysis of the c-terminal peptide esters; c) using of the recombinant DNA technology by fermentation; d) enzymatic method for the introduction of an β-amido group. All of the reported methods have their merits, such as laborious protection, using of ammonium as gas or another alkylamines in gas form and more reaction steps. We herein present a straightforward route to the synthesis of some Leu- and Met-enkephalin derivatives. The Boc and Fmoc strategy were used for the synthesis of desired enkephalin analogues. The c-terminus was amidated with some ammonium salts. In conclusion, we have developed a novel method for the synthesis of c-terminus amidated enkephalin derivatives. The structures of products were confirmed according to their spectral data and MALDI-Mass spectra. All of the products were purified using prep-HPLC.

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P10102-031

Synthetic Approaches to Cyclic Peptide Natural Products as Chitinase Inhibitors

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Chitinases catalyse the hydrolysis of chitin, the natural homopolymer of β(1,4)-linked N-acetyl-D-glucosamine. Chitin is a key structural component of the cell walls, exoskeletons, and eggshells of pathogenic fungi, insects, and nematodes, respectively, which all rely on the ability to hydrolyse chitin at specific points in their life cycles. Chitinase inhibitors are now attracting considerable interest as novel fungicides and insecticides, as well as chemical tools to study human diseases as diverse as asthma and malaria. In this context, the cyclic pentapeptide natural products, argifin and argadin, are two exciting inhibitors which pose some interesting synthetic challenges. The argifin structure includes two sensitive β-linked Asp residues, as well as an unusual carbamoylated Arg side chain, while argadin contains a unique Asp β-semialdehyde residue, that is cyclised to the peptide backbone to generate a potentially labile hemiaminal. We will describe improved routes to both compounds that allow us to avoid significant side reactions such as aspartimide formation and homoserine-mediated backbone cleavage that are observed in our previously reported syntheses. [1,2] This is achieved by carrying out the assembly and cyclisation of both peptides, including key side chain derivatisation steps, entirely on solid phase. The application of the strategies devised to the automated synthesis of argifin and argadin and related natural products will be described.

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P10102-032

Facile Synthesis of Orthogonally Protected Optically Pure Keto– and Diketopiperazine Building Blocks for SPOC

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During the last two decades combinatorial chemistry has become an important tool for the quick synthesis of large numbers of small molecules used, for example, in the generation of the new lead structures for medicinal applications. Additionally, it allows rapid access to diverse chemical libraries with novel structures and properties. Small molecules libraries are generally prepared on solid support simplifying tedious purification steps of the intermediates and facilitating the entire synthesis. Piperazines and keto-piperazines are amongst the important backbones in today's drug discovery. The piperazine framework has been defined in medicinal chemistry as a "privileged scaffold". It is a molecular backbone with versatile affinity properties representing a frequently-occurring binding motif, and providing potent and selective ligands for a wide range of biological targets. The high number of positive hits revealed in biological screens with the piperazine scaffold urged chemists to develop plenty of different synthetic methods that allow fast and efficient building of these heterocyclic systems on solid support as well as homogenous chemistry. However, the majority of these methodologies is not stereospecific and the complex mixtures of the stereoisomers are generated. In order to preserve important chiral centers in potential hits we propose to use pipearzine backbone, initially prepared in optically pure form, bearing various tethers with orthogonally protected groups applicable via Solid Phase Organic Chemistry (SPOC). We will describe a novel synthesis of keto and diketopiperazine building blocks. These chiral building blocks are applied in "around-the-scaffold" modification strategy by SPOC, introducing valuable physico-chemical properties in 3 independent diversity points.

P10102-033

Solid-phase synthesis of peptides possessing alpha- and beta-amino gamma-lactam constraints for studying relationships between conformation and biological activity

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Solid-phase strategies for constraining peptide structures can serve for elucidating the relationships between conformation and activity. For example, the systematic substitution of natural amino acids by their aza-amino acid counterparts has been accomplished using a Fmoc strategy featuring couplings with aza-amino acid chlorides to provide insight into the biologically active conformers of the melanocortin receptor agonist Ac-His-D-Phe-Arg-Trp-NH2 as well as the calcitonin gene-related peptide antagonist [D31, P34, F35]CGRP27-37 (1-3). Employing cyclic sulfamidates, we have now developed Fmoc strategies for the effective solid-phase introduction of alpha- and beta-amino gamma-lactam constraints into peptides. Since their pioneering use in a somatostatin analog with about 9 fold greater activity (4), lactam restraints have been used to study a variety of relevant targets (5,6). Our strategies now provide effective means for performing lactam scans of biologically active peptides as demonstrated using the growth hormone secretagogue GHRP-6, and an allosteric modulator peptide antagonist of the IL-1 receptor. Our presentation will focus on recent developments in the solid-phase chemistry for synthesizing such constrained peptide analogs and the relationships between peptide conformation and biology, elucidated by these novel methods.

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P10102-034

Solution- and solid-phase chemistry for the synthesis of diazepinones and structural proof for their mimicry of gamma-turn conformations

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Diazepines have been recognized as “privileged structures”, because of their propensity for serving as ligands to various receptors (1-3). The remarkable affinity of diazepine ring systems for membrane bound receptors, such as G-protein coupled receptors, has been suggested to arise from their potential to mimic peptide beta- and gamma-turn secondary structures. Employing X-ray crystallographic analysis of 1,4-diazepin-2-ones, we have demonstrated that the alpha-amino acid moiety encompassed in this heterocycle adopts dihedral angle geometry that mimics the conformation of an ideal gamma-turn (4). Recently, we have developed novel methodology for making diazepinones, which employ solution- (5) and solid-phase approaches. A variety of amino acid building blocks, possessing aromatic, aliphatic and heteroatomic side chains, may now be incorporated into these strategies in order to produce diazepinone libraries for investigation in various biological assays. Our presentation will focus on the novel chemistry for generating these interesting ring systems as well as relevant structural data that demonstrate the ability of diazepinones to mimic peptide turn secondary structures.

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P10103-035

NMe Amide as the best Isostere for Depsipeptides and Thiodepsipeptides

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Peptides natural products are an important source for potential drug candidates. Some of these peptides show a heterodetic structure with disulfide, ester or thioester bonds together with the peptide bond. Depsipeptides, such as Kahalalide F, Aplidine (both at clinical phase II for several cancers) or Romidepsin (orphan drug status) and thiodepsipeptides, such as thiocoraline (advanced preclinical phase), show very often poor pharmokinetic properties. Substitution of the O or S by NH lead very often to an important loss of the activity as it has been shown in Triostin A, another depsipeptide, and very recently in Thiocoraline. Herein, the NMe amide as the best isostere for the ester and thioester bond will be proposed. Thus, several strategies will discussed for the synthesis of oxa-, aza-, and NMeaza-thiocoraline, which involve the use of a myriad of classical protecting groups (Fmoc, pNZ, Alloc, Boc, Trt) as well as the new one by conformationally restricted mobility. Furthermore, optimization of the coupling steps using phosphonium and aminium HOAt-based, or even DIP with the less reactive HOSu will be described. The main conclusion of the present work is that replacement of a thioester bridge by a NMe amide in Thiocoraline results in a compound with nanomolar activity.

P10103-036

Solid-Phase Synthesis of the Rich Cys Containing Peptide Linaclotide

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Linaclotide is a 14-residue peptide currently undergoing phase II clinical trials for the treatment of gastrointestinal diseases such as chronic constipation (CC). Linaclotide, which can be administered orally, is an agonist of the guanylate cyclase type-C receptor found in the intestine. From a structural point of view, this small peptide presents a constrained structure with the presence of three disulfide bridges between Cys1-Cys6, Cys2-Cys10, and Cys5-Cys13.
H-Cys(δ1)-Cys(δ2)-Glu-Tyr-Cys(δ3)-Cys(δ1)-Asn-Pro-Ala-Cys(δ2)-Thr-Gly-Cys(δ3)-Tyr-OH
In order to reach the large amounts required for a marketed peptide, an efficient synthesis needs to be attained. To optimize the synthesis, its fundamental limitations need to be determined and addressed. In the case of Linaclotide, the key points are related to the numerous Cys (some of them consecutive) present in the peptide, for two reasons: the potential risk of racemization upon assembling the linear chain, and the misfolding of the three disulfide bridges. For that, the concourse of different protecting groups and folding conditions as well as the analysis of the disulfide bridges in the final folded peptide has been studied and will be discussed in this presentation.

P10103-037

Synthesis of Novel Gn-RH Analogues Using Ugi 4-MCR

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GnRH analogues have been used for the treatment of steroid-dependent tumors, such as prostate and breast cancers. The development of more potent GnRH analogues depended largely on the important made in the science of peptide chemistry. Ugi-4MCR reaction is known for the synthesis of amide bond. We wish to report herein an efficient method for the synthesis of some GnRH analogues based on ugi reaction using four-component reaction of N and C-terminus peptides, aromatic aldehydes and isocyanides. This ugi-4MCR could describe to build up novel GnRH analogues deriving from triptorelin and gonadorelin. All of the products were purified using preparative HPLC and the structures were assigned according to MALDI mass spectrometry data.

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P10103-038

N→O-Acyl shift in Fmoc-based synthesis of phosphopeptides

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Synthetic phosphopeptides are frequently used as chemical probes to explore protein–protein interactions involved in cellular signal transduction.[(1)] Phosphotyrosine peptides are mainly prepared by two different approaches: a) global phosphorylation, in which the phosphate-group is introduced by a phosphorylating agent (*e.g.* diarylphosphorochloridate or phosphoramidite) after coupling of the amino acid, and b) the use of protected, phosphorylated tyrosine building blocks in the solid phase peptide synthesis (SPPS) known as the synthon method.(2) Most commonly, the solid-phase synthesis of phosphotyrosine-

containing peptides is performed by applying the Fmoc-strategy and N-Fmoc-protected tyrosine derivatives bearing acid-labile phospho-protecting groups.(3)
We observed a side-reaction in the Fmoc-based solid-phase synthesis, the isomerisation at threonine, which furnished a depsipeptide. We show that the rate of *N→O*-acyl migration depends on the sequence context and the deprotection conditions of the phospho-protecting group. Depsipeptides were formed most rapidly when the phosphotyrosine was located in the +2 position. Different phosphotyrosine building blocks were compared and a suitable method that provides phosphopeptides in enhanced purity and yield was developed.(4)

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P10103-039

A Non-Explosive Replacement for Benzotriazole Based Coupling Reagents

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Peptide synthesis is based in a proper combination of protecting groups and in the right choice of the right coupling method. Nowadays, almost all peptide bond formed are carried out in the presence of 1-hydroxybenzotriazole (HOBt) or its derivatives (HOAt, Cl-HOBt). Thus, HOBt derivatives are used in combination with a carbodiimide or another coupling agent or built into a standalone reagent such as immonium (HBTU, HATU, HCTU) or phosphonium (PyBOP, PyAOP, PyClock) salts. Recent reports [Wehrstedt et al., J. Hazard. Mat. A126 (2005) 1] have confirmed the explosive properties of HOBt derivatives. Thus, a replacement of HOBt should be found for preparation of peptides for research purposes and, more important, for the production of peptide based APIs. Herein, several alternatives to HOBt will be discussed taking into account the explosivity properties. Furthermore, a new family of immonium salts, which incorporates an proton acceptor in its carbocation skeleton. The novel proton acceptor coupling reagent has shown superiority to the described previously. An oxygen in the carbocation moiety confers to the reagent more solubility, enhances coupling yields and decreases racemization, allowing the use of just one equivalent of base. Examples on the use of the non-explosive replacement for HOBt together with carbodiimides or built into phosphonium and immonium salts, with the proton acceptor, will be discussed.

P10103-040

Thiocarbamate-linked peptides by chemoselective peptide ligation using phenylthiocarbamate chemistry

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Peptide chemical ligation chemistries, which allow the chemoselective coupling of unprotected peptide fragments, are useful tools for synthesizing native polypeptides or unnatural peptide-based macromolecules. Native chemical ligation (NCL) (1), Staudinger ligation (2) lead to the formation of a native peptide bond at the ligation site. Other methods result in the formation of unnatural covalent bonds such as oxime (3), thioester (4) linkages. These methods are of great interest when native peptide bonds are not absolutely required. In this context, we examined the potential use of the phenylthiocarbamate group in ligation chemistry. The phenylthiocarbonyl group can be easily introduced into peptides on alpha or epsilon amino group using phenylthiochloroformate and standard solid-phase method (5). It reacts chemoselectively with cysteinyl peptides to give an alkylthiocarbamate bond. S,N-shift of the alkylaminocarbonyl group from the Cys side chain to the alpha-amino group did not occur. The method was used for linking two peptides chain through their N-termini, for the synthesis of a cyclic peptide or for the synthesis of di- or tetravalent multiple antigenic peptides.

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P10103-041

Enzymatic synthesis of peptide secondary amides

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Synthetic peptide derivatives which contain secondary amide moiety at C-terminus are inhibitors of serine and cysteine proteinases and thus could be efficiently used as ligands in affinity chromatography in order to isolate these enzymes. There is a number of chemical syntheses of amidous peptide derivatives in the literature, but the reaction yields are usually low and reaction conditions and purification procedures are too complicated. It is also known that the application of enzymatic catalysis allows to obtain high reaction yields with simultaneous simplification of synthetic and purification procedures, and at the same time preserves the optical purity of target compound. Subtilisin 72 sorbed on silochrome could be efficiently used as catalyst of acylation of secondary amides by the esters of acylpeptides. Subtilisin's wide substrate specificity allows to carry out the syntheses with peptides which contain hydrophilic, hydrophobic, dicarbonic- and diamino-acids at C-terminus. Piperidine, morpholine, indole, diethylamine and other secondary amines are used in the syntheses as the amino-components. The reaction yield equals about 60-80% in dependence of the nature of C-terminal amino acid of acylating peptide, pKa of amine and the hydrophobicity of the final compound. The distribution of the reaction product in liquid and solid reaction phases is investigated, the optimal reaction conditions for enzymatic acylation of secondary amines are established. All the compounds obtained are characterized by its NMR and mass spectra. The inhibition constants for some proteolytic enzymes with the compounds of research are defined.

P10103-042

Impact of ionic liquids on peptide acylation reactions

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In comparison to conventional solvents, reactions in ionic liquids (ILs) show a number of altered properties. Besides conversion rates and enantioselectivities, pronounced effects were surprisingly found on the regioselectivity in acylation reactions with peptide reactants. The characteristics of the latter were systematically investigated using comprehensive libraries of ILs, acyl donor esters and peptides containing two amino groups; i.e., the N-terminal N^{α}- as well as the N^{ε}-amino function of the lysine side chain. It was found, that the regioselectivity of the amino group acylations was strongly influenced by the IL used as the reaction solvent. While in reactions 1-ethyl-3-methylimidazolium dimethylphosphate ([EMIM][Me_2PO_4]) show an improved reactivity of the N-terminal amino function, 1-ethyl-3-methylimidazolium diethylphosphate ([EMIM][Et_2PO_4]) mediates the side chain acylation of the lysine's N^{ε} amino group. Based on Eyring kinetics, a significant

effect of the ILs on the activation parameters of the acylation reactions became obvious. Accordingly, N-terminal directing ILs kinetically favor acylations at the N^{α}-amino function while ILs showing a regioselectivity for the lysine side chain kinetically support N^{ε}-acylation reactions. The rational behind is assumed to be based on direct individual IL/peptide interactions. The general existence of such interactions was experimentally proven by studies on the impact of ILs on the cis/trans-equilibrium state of peptidyl-prolyl bonds. The idea of direct IL/peptide interactions as the reason for the observed regioselectivity effect becomes supported by additional ^{1}H-HR-NMR measurements indicating an individual pattern of chemical shifts of aromatic protons of imidazolium cations after peptide addition.

P10103-043

Development of chemical tools for the synthesis of peptides containing methylated arginine and lysine residues

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Post-translational methylation of protein arginine and lysine residues is now recognised to play a very important role in the regulation of protein transcription and other biological processes. Ready access to amino acid building blocks for the synthesis of peptides containing methylation arginine and lysine residues is therefore an important prerequisite for work in this emerging field. In this poster, we outline our synthetic strategies for the production of fully protected amino acid building blocks for the introduction of the naturally occurring methylated arginine and lysine residues and demonstrate their utility in the preparation of peptides containing multiple methylation sites.

P10103-044

Synthesis of C-Terminal Octapeptide B23-30 of B-chain Human Insulin by Classical Peptide Method to be used in semisynthesis of Human Insulin iodinated at Tyrosine B16

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The present work describes the preparation of protected octapeptide derivative (23-30) B-chain Human Insulin Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr and coupling this peptide to B-chain desoctapeptide iodinated at tyrosine B16 of Porcine Insulin. B-chain recombined with natural A-chain giving Human Insulin iodinated at Tyrosine B16. Our approach of synthesis was the conventional method by using stepwise coupling fragment condensation.
The modification at Tyrosine B16 is of great interest for receptor binding, bioassay, and crystallographic studies. Porcine Insulin was split off by oxidative sulphitolysis and A- and B- chain S-sulphonates were separated by ion exchange chromatography. After further purification of B-chain, it was digested with trypsin to give DOP-B (SSO3)2 octa-, and hepta-peptides Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala, Gly-Phe-Phe-Tyr-Thr-Pro-Lys respectively.
Reduction of DOP-B (SSO3)2 with mercaptoethanol led to DOP-B (SH)2. On oxidation of DOP-B (SH)2 by iodine solution in 30% acetic acid we obtained DOP-B (SS) which was separated from polymers formed by Sephadex G.50 chromatography, after which it was iodinated at pH 8.5 to give DOP-B (SS) I-Tyr. Octapeptide B23-B30 of human insulin fragment Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr-OH is synthesized by the conventional method of peptide chemistry using DCCI and hydroxysuccinimide as a coupling agent. Tyrosine and Threonine side chains were protected by convertion of the hydroxyl groups to tertiary butyl ethers, and theω-amino group of B29 lysine by MSc.

P10104-045

Solid-phase synthesis of 5-arylhistidines via a microwave-assisted Suzuki-Miyaura cross-coupling

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Unnatural amino acids are becoming increasingly important substrates in modern drug design, synthesis and discovery research. In particular, arylhistidines naturally occur in the active site of the heme–copper oxidases as well as in cytotoxic and antifungal marine peptides (1).
A method of choice for the preparation of unsymmetrical biaryl systems is the Suzuki–Miyaura cross-coupling of an aryl halide with an arylboronic acid. It has been shown that microwaves significantly enhance this reaction leading to higher overall yields and purities as well as shorter reaction times. Although a great variety of biarylic compounds have been prepared following this approach, so far, it has not been applied to the arylation of the histidine imidazole ring. Initially, we studied a methodology for the synthesis of 5-arylhistidines via a microwave-assisted Suzuki–Miyaura cross-coupling reaction in solution (2).
Taking into account the advantages of the synthesis on solid support, such as the avoidance of tedious work-up which are particularly valuable for palladium-catalyzed reactions, we studied the application of the above methodology on solid-phase. Here, we report the Suzuki–Miyaura reaction between a 5-bromohistidine and an arylboronic acid on solid support. The reaction conditions were optimized by varying several parameters such as the solvent, the reagent concentrations and the reaction time. The optimized conditions were subsequently applied to the synthesis of peptides containing a 5-arylhistidine residue. This work constitutes the first Suzuki–Miyaura coupling involving the imidazole ring of a histidine on solid support.

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P10104-046

Microwave-assisted attachment of Fmoc-amino acids to resins *via* triazine “superactive esters”.

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Functionalization of resins is a key step to get polymer supports suitable for solid phase synthesis. Commercially available resins functionalized with D/L Fmoc-amino acids via the C-terminal carboxyl group, are frequently used for the SPPS following the Fmoc/tBu strategy. Attachment of C-terminal residue to many resins involves the formation of an ester bond usually performed by DCC/DMAP with appropriate symmetrical anhydrides. This procedure can promote side products and require very large excess of Fmoc-amino acids. This is particularly problematic when not commercially available unnatural amino acid have to be coupled and/or in the case of large scale SPPS. It has been proved, that activation of carboxylic acids with our new generation of triazine-based coupling reagents (TBCRs), proceeds via triazine “superactive esters”, highly reactive intermediates useful for peptide synthesis in solution and in solid phase [1,2]. In our experiments herein reported, involving TBCRs high loading of the resin was obtained with a very small excess of Fmoc-amino acids and coupling reagents.

The substitution level of the new functionalized resin was calculated following a standard protocol based on the spectroscopic measurement of the soluble chromophore piperidine-dibenzofulvene. Coupling reactions to the resin proceeds at room temperature in 3 hours. Moreover we carried out the time-consuming attachment of Fmoc-amino acids to the resin by an automatic monomode microwave (MW) instrument (Liberty, CEM), in fact microwave is proposed as a valid alternative to enhance efficiency of coupling reactions and it has been widely applied to SPPS.

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P10105-047

Novel peptide-hetorocycle conjugates: derivatives of 3-(benzimidazol-5-yl)alanine

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In our search for new bioactive peptides we investigate methods for introducing heterocyclic motifs into peptide side chains (1). Considering the biological activity and complexing abilities of benzimidazoles we developed a direct solid-phase synthesis of benzimidazole-peptide conjugates, expecting these new compounds to express novel biological properties (2).

The peptide-heterocycle conjugates are obtained by on-resin reaction between aldehydes and peptides containing a specially designed β-(3,4-diaminophenyl)alanine residue (3). The stoichiometric amount of aldehyde leads to 2'-substituted 3-(1*H*-benzimidazol-5-yl)alanine–containing peptides, the increase in aldehyde content results in 1`,2`-disubstituted derivatives. The reaction with dialdehydes gives novel amino acid residues containing tricyclic systems, in the case of *o*-phthalic aldehyde - the pyrido-[1,2-a] benzimidazole.

The compatibility of our method with the Fmoc solid phase peptide synthesis protocols was proven by the synthesis of analogues of immunosuppressory fragments of ubiquitin and HLA-DQ [4,5]. The biological activity of the modified oligopeptides was compared to that of original fragments 6. as well as to similar quinoxaline-peptide hybrids.

The collision-induced dissociation of substituted benzimidazole-peptide conjugates leads to characteristic fragmentation ions, which may be used as a diagnostic tool in the fragmentation of the benzimidazole-peptide hybrids.

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P10106-048

A novel scalable stereoselective approach for the synthesis of β,β-branched dehydroamino acids

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Dehydroamino acids are an important class of compounds due to their presence in many biologically active natural products including the Antrimycins, Tentoxin, Phomopsin A, or the phosphatase inhibitors like Microcystin and Nodularin. In the last decade an increasing interest in β-branched dehydroamino acids has been developed based on **their importance as commodity chemicals and value as tools in structure relationship studies**. The incorporation into a peptide of such unsaturated amino acids introduces an element of conformational rigidity, as well as changes in reactivity, allowing for **the development of high affinity ligands for receptors**. A variety of methods exist for the synthesis of dehydroamino acids. Some of these approaches rely on thermodynamic control to dictate the alkene geometry. If there is no strong thermodynamic preference, or if the desired product is not the thermodynamically favoured isomer, the existing synthetic methods are often ineffective. We describe here an efficient stereoselective method for the synthesis of β,β-branched dehydroamino acids **(III)** from β-aryldehydroamino acids **(I)**. The β-aryldehydroamino acids **(I)** were prepared from commercially available Z/Boc-phosphonoglycine trimethylester and aldehydes using the Schmidt protocol. The transformation of **(I)** into their β-bromodehydroamino acid derivatives **(II)** is performed by a Hoerrner reaction in a very good yield giving the Trans derivatives. Suzuki cross-coupling on **(II)** with a boronic derivative generated the high value building blocks **(III)**. A variety of side chains can be incorporated using this type of reaction. The stereochemistry of these compounds has been determined using NOE enhancement experiments. We report here a scalable and high yielding synthesis of stereospecific β,β-aryldehydroamino acid derivatives.

P10107-049

Versatile methods for synthesizing acyl-tetramic acids peptide analogs.

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Acyl-tetramic acid derivatives have been identified as potential antiviral and antibacterial compounds. In this work we have improved their synthesis starting from tetramic acid lactams using peptide coupling reagents such as BOP under microwave heating for generating the carbon-carbon bond of the acyl-tetramate. Using this procedure we have synthesized a number of new tetramic acid derivatives in solution and generated a series of acyl-tetramic acid building blocks that were introduced into peptides using conventional SPPS methods. Additionally, we present here a new solid phase microwave assisted synthesis of acyltetramates on pre-synthesized peptides and amino acids. Antibacterial and antiviral activity of the products will be presented.

P10107-050

Synthesis of a New beta Turn Mimetic - the 2,5-oxo-1,4,7-triazacyclodecane system

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Reverse turn mimetics have long attracted attention for their potential to be drug-like analogues of biologically active peptides. The title system was designed on the principle of covalent hydrogen bond replacement. This design approach provides peptide mimetic systems where the peptide side chains are not used in constraining the peptide and where the mimetic framework is close to being isosteric with the peptide backbone. Ideally such mimetics can be incorporated in a

peptide sequence and include all side-chain groups. In practice this is difficult to achieve. The task of making a turn mimetic can be regarded from the perspective of the backbone dihedral angles – to make a beta turn mimetic the phi and psi angles of two consecutive residues have to be controlled. Limiting the dihedral angle space is most effectively accomplished with a cyclic constraint – one or more may be used. In this case a single medium ring cyclisation from the (i) carbonyl to the (i+3) amine – in place of the classical hydrogen bond – forces the four backbone dihedral angles into a turn conformation. Due to the poly-functional nature of peptide systems the introduction of unnatural constraints can be synthetically challenging. In the present case a number of side reactions were encountered and had to be overcome. Some were well known such as diketopiperazine formation and others involving transannular interactions were unexpected and gave rise to interesting byproducts. Ultimately the side reactions were overcome by choice of cyclisation position and synthetic modifications.

P10107-051

Auto-assembling antimicrobial cyclic pseudopeptides including aza-β^3-amino acids

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Antibiotic resistance of pathogens against conventional antibiotics is increasing at a rate that far exceeds the pace of new development of drugs. So, antimicrobial peptides, both synthetic and from natural sources, have raised interest as potential useful drugs in the future. However, due to proteolytic degradation, peptides are not ideal candidates for pharmaceutical development. That is why numerous researches try to develop non natural peptidic analogues for enhancing metabolic stability, bioavailability, and biological absorption. In this class of peptidomimetics, pseudopeptides consisting exclusively or including aza β^3-amino acids have emerged as a promising new class of compounds that favour hydrogen bond formation and can enhance biological activities when compared to natural parent peptides(1). We have designed "mixed" cyclic pseudopeptides composed of α- and aza-β^3-amino acids that target bacterial cell wall and induce the death of the pathogens. Particularly, some of these cyclic pseudopeptides have broad spectrum antibactericidal activities on gram positive and gram negative bacteria with low minimum inhibitory concentrations (MIC). On the other hand, this type of molecules is not haemolytic and cytotoxic at antimicrobial activity levels(2) Now, we try to explain the mechanism of action of our pseudopeptides that act on the microbial membranes. With NMR studies we have demonstrate that in solution cycles auto-associate at high concentrations and we investigate their behaviour in presence of small unilamellar lipidic vesicules (SUV)(3). This phenomenon of auto association seems to be facilitated at the lipid interface.

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P10107-052

Synthesis and Biological Activity of an Oxidatively Stable Analogue of the Antimicrobial Peptide, Lacticin 3147 A2

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Lantibiotics are a class of antimicrobial peptides containing lanthionine (1) and/or β-methyllanthionine (2) residues in cyclic moieties, and are currently used for food preservation. They also have potential as human therapeutics. Lacticin 3147 is a two-peptide lantibiotic produced by L. lactis subsp. Lactis DPC3147, and its components (A1 and A2) are active against a wide range of Gram-positive bacteria by a synergistic mechanism in sub nM concentrations. However, the oxidation of the thioether of lanthionine (1) and β-methyllanthionine (2) is a major source of lantibiotic instability and loss of activity. To investigate structure-activity relationships and determine whether sulfur can be replaced, an analogue of lacticin 3147 A2 in which oxygen atoms replace sulfur was synthesized by solid phase peptide synthesis. Utilizing the stereochemicaly pure oxa-lanthionine (3) and oxa-β-methyllanthionine (4) with orthogonal protecting groups, the conformationally constrained tricyclic moiety in oxa-lacticin 3147 A2 was constructed. The results of biological evaluation of the oxidatively stable analogue will also be described.

P10108-053

Controlling α-helical secondary structure of oligopeptides and its use as a chiral catalyst

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Replacement of α-hydrogen atom of α-amino acids results in α,α-disubstituted amino acids. As an α,α-disubstituted amino acid, achiral α-aminoisobutyric acid (Aib) is well-known, and widely used to construct helical secondary structures of oligopeptides. The helical secondary structures constructed by using Aib usually show 3_{10}-helices, but not α-helices in the case of short oligopeptides. Recently we designed and synthesized a chiral cylic α,α-disubstituted amino acid (*S*,*S*)-Ac_5c^{dOM}, in which the α-carbon atom is not a chiral center but chiral centers existing at the side chain. Homooctapeptide composed of (*S*,*S*)-Ac_5c^{dOM} formed a left-handed α-helix both in solution and in the solid state. Furthermore, in the case that the (*S*,*S*)-Ac_5c^{dOM} was incorporated into L-Leu-hexapeptide, the hexapeptide Cbz-{L-Leu-L-Leu-(*S*,*S*)-Ac_5c^{dOM}}$_2$-OMe preferentially formed a right-handed α-helix in the crystal state, whereas the hexapeptide Cbz-{L-Leu-L-Leu-Aib}$_2$-OMe formed a right-handed 3_{10}-helix. The finding that the propensity of cyclic amino acid Ac_5c^{dOM} is to form α-helix over 3_{10}-helix, stimulated us to use the α-helical oligomer containing the cyclic amino acid as an asymmetric catalyst. We prepared several L-Leu-oligopeptides containing α,α-disubstituted amino acids, analyzed their preferred secondary structures, and studied a enantioselective reaction of prochiral substrate using α-helical oligopeptides as a chiral catalyst.

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P10111-054

FBP28 domains: SPPS using pseudoproline and depsipeptide approaches, stability and structure of glutamine-rich analogs

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WW domains are the smallest naturally occurring three-stranded ß-sheet identified so far, named after the two conserved tryptophan residues, and function as non-catalytical domains of signalling proteins by recognizing proline-containing ligands, serving as model systems for the study of ß-sheet stability and folding. Among them, the FBP28WW domain, GATAV SEWTE YKTAD GKTYY YNNRT LESTW EKPQE LK, was reported to be monomeric and stable, although under certain conditions aggregation and even fibril formation have been observed. The stability of the ß-sheet is dominantly influenced by the hydrophobic core formed by W8, Y20 and P33. Also the ligand-binding face contributes to the stabilization, especially by the side-chains of Y19,21 and W30. In order to study a possible influence of hydrophilic side-chain interactions for the stabilization of the domain, we developed a strategy for the SPPS of FBP28WW and analogs. However, initial attempts to synthesize the FBP28 WW peptide by SPPS failed, not uncommon for the assembly of ß-sheet-forming sequences. Fortunately, the synthesis could be considerably improved by insertion of X-Ser and X-Thr dipeptides in the form of their corresponding pseudoproline derivatives, and alternatively via the intermediate formation of X-Ser/Thr depsipeptide bonds. The depsipeptide strategy was compared with the pseudoproline chemistry and was shown to be equally well suited. Searching for FBP28 WW analogues possessing a reduced aggregation tendency, we found that surprisingly the insertion of Gln residues fulfills the requirements: The peptide (N15,Gln 9, 21, 23, 26, 28)–FBP28 shows the typical fold of FBP28 WW domains (NMR), sufficient stability as well as solubility, and no aggregation in the temperature range 10-90 °C when analysed by FT-IR spectroscopy. NMR analysis shows that Gln–gamma-carboxamide functions are involved at least in part in side-chain-backbone hydrogen bonds, thus stabilizing the monomeric fold of the FBP28 domain.

P10111-055

The [α:*N*-amino]mers: A New Family of Foldamers

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The functional diversity of proteins relies on the unique capacity of these intrinsically flexible polypeptidic chains to fold into well-ordered and compact structures. The formation of protein tertiary and/or quaternary structures relies only on a small set of basic secondary structural elements i) helices, ii) sheets and iii) folds, themselves being induced by the sequence of the aminoacids (primary structure).These α-polypeptidic secondary structures are a great source of inspiration for the elaboration of new oligomeric materials able to undergo a spontaneous self-organisation in a well-defined pattern. In biomimetic chemistry, structural diversity were not restricted to natural linear α-polypeptides but were attainable by a number of aliphatic (β- and γ-peptides) and aromatic ω-oligoamides. These folding oligomers or "foldamers", conceived to adopt regular and predictable conformations, provide good models for studying the main factors governing the formation of 3D-structures in biopolymers. In addition, the predictability of folding in these molecules can be used further to develop molecules with interesting biological functions.

Recently, our group in Nancy has developed an efficient synthesis of *N*-aminodipeptides, in which the hydrogen atom of amide bond was substituted by a nitrogen, by using the Mitsunobu protocol. We demonstrated also that the oligomerization of *N*-aminodipeptides can lead to [α : *N*-amino]mers which can adopt specific secondary structures induced by intramolecular hydrogen bonds. This lecture will present the first results obtained in this field.

P10111-056

Synthesis and conformational studies of octapeptides built from [-Gly-ΔPhe-] blocks

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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 CΔ and CΔβ and two neighboring peptide bonds lead to coupling of ¥ð electrons, which not only influence on side chain but also on all peptide conformation [1-5]. Full knowledge about relation between presence of dehydroamino acid and peptide's conformation is necessary to predict biological properties of new designed peptides.. In this presentation we summarized the results of synthesis and conformational investigations of octapeptides containing four dehydrophenylalanine residues in peptide chain. The general formula of studied compounds is Boc-Gly-ΔX-Gly-ΔX-Gly-ΔX-Gly-ΔX-OMe, where X=ΔPhe with different possible combinations of its isomers. The structural investigations were based on NMR measurements (standard 2D techniques and 1D experiments, typical for detection of hydrogen bonding) and theoretical calculations. Conformational preferences of investigated systems were obtained on base of ROESY and NOESY experiments and calculations by use of X-PLOR.

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P10111-057

Synthesis and NMR investigation of C-terminal exenatide sequence H-Ala-Pro-Pro-Pro-Ser-NH_2

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Exenatide (synthetic Exendin-4), 39-amino acid peptide is a novel drug for treatment of type 2 diabetes mellitus (1). Exendin-4 is a long-acting agonist at the glucagon-like peptide-1 (GLP-1) receptor. It possesses elevated stability in human body and higher biological activity as compared with GLP-1 (2). The present study deals with the development of H-Ala-Pro-Pro-Pro-Ser-NH_2 synthesis. Both solid-phase and classical methods of peptide synthesis have been used. Thereafter, the peptide has been used for Exenatide preparation.Target peptides have been purified by RP-HPLC. The structures of these compounds have been born out by MALDI-TOF spectrometry. The configuration of pentapeptide has been studied by the methods of NMR-spectroscopy.

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P10112-058

Synthesis of hepatitis B virus fusion inhibitors

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Chronic infection with the human hepatitis B virus (HBV) is a major health problem. Looking for new antiviral strategies, we have recently demonstrated that peptides encompassing the 47 terminal amino acids of the N-terminal part of a major envelope protein block HBV infection of primary human hepatocytes in vivo (Nature Biotech., 2008). One peptide will soon be tested in clinical studies. The success depends on efficient synthetic access to large amounts of the peptide. Systematic modifications of the lead structure HBVpreS/2-48 have to be performed resulting in a panel of peptide variants to be studied with respect to their applicability, pharmacokinetics and serum stability. We selected a series of peptides carrying deletions, point mutations, D-amino acid exchanges and sequence permutations. The syntheses of 40 derivates were performed by Fmoc-solid-phase synthesis on a Rink Amide AM resin using HBTU/DIPEA activation on an Applied Biosystems 433A peptide synthesizer. After completion of the peptide sequence, stearic acid was attached to the N-terminus. The products were deprotected and detached from the resin by TFA treatment and subsequently purified by HPLC. The stability of the peptides was determined in human serum. We were able to synthesize all HBVpreS lipopeptides in acceptable yields (7-40%). Preparative HPLC was used to obtain intended compounds in high purity as determined by HPLC and ESI mass spectrometry. The serum stability studies revealed exceptionally long biological half-lives (t1/2 > 24 h for all lipopeptides) mainly due to the fatty acid residue. The peptides belong to a class of intrinsically unfolded peptides and are therefore not prone to aggregations within the synthesis. Consequently solid phase peptide synthesis provides a suitable access to the peptides described. It can be speculated that the peptides are protected against degradation due to an association via their lipophilic end to serum proteins.

P10113-059

Sugar derivatives for self-assembling γ-cyclic peptides

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Last years, numerous inorganic and organic nanotubes have been developed. Self-assembling peptide nanotubes (SPN) made from cyclic peptides have structural and functional properties that may be suitable for various applications in biology and material science. Recently, our group have reported α,γ-cyclic peptide (α,γ-CP) that forms highly stable homo- and/or heterodimers with partial hydrophobic cavities. In the present communication we will describe the design, synthesis and applications of a new class of self-assembling γ-cyclic peptides containing sugar derivatives that modify both the inner and the outer surfaces of the resulting supramolecular entities.

P10113-060

Peptido2.rotaxanes: can a tetramide macrocycle travel to a station by wrapping up around a helical peptide thread?

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Peptide2.rotaxanes based on an achiral tetramide macrocycle locked onto various chiral Gly-L-Xxx dipeptide threads were extensively characterized by Leigh and coworkers (see the recent review-article, E. R. Key and D. A. Leigh, *Pure Appl. Chem.* **2008**, *80*, 1-57). We are currently expanding this field by synthesizing and studying the properties of new sets of peptido2.rotaxanes. The initial set examined includes symmetrical, achiral compounds with a Fmoc stopper at each terminus, threads built up with a central fumaric diamide station and two helical Aib (α-aminoisobutyric acid) homo-peptides of different lengths (from 1 to 5 residues), and an aromatic tetramide macrocycle. These supramolecular systems have been characterized spectroscopically and two of them by X-ray diffraction as well. The fundamental interactions between macrocycle and thread (the same interactions offering the major contribution to the template-directed preparation of this family of molecules) are intercomponent H-bonds comprising the four amide groups in the ring and the two amide bonds in the fumaric derivative. The second, more complex, set of peptido2.rotaxanes examined is represented by non-symmetrical compounds involving a thread based on a central helical -$(Aib)_6$- peptide linker and two stations of opposite chirality (a -D-Leu-Gly-Gly- tripeptide at the N-terminus and a fumaric diamide-L-Leu moiety at the C-terminus). As stoppers and the macrocycle, we selected two diphenylacetyl groups and the usual aromatic tetramide, respectively. As spectroscopically assessed, the macrocycle initially positions on the fumaric diamide-L-Leu station. Subsequently, by using photons as stimuli to induce the fumaric<->maleic equilibrium, we were able to switch partially the relative macrocycle-binding affinity in favor of the -D-Leu-Gly-Gly- tripeptide station. This is the first example of a rotaxane where the ring makes a journey to one of the stations by wrapping up around a helical peptide thread.

P10120-061

Synthetic novel Nα phenyl and bi-phenyl tetra-carboxamides bis peptides. An approach to DNA threading intercalators as expected potential pharmaceutical carriers for catatonic agents.

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Naphthalene diimides were reported to bind to DNA opposite grooves via the threading intercalation mode (1, 2). On the other hand, peptides constitute an excellent class of molecules for rapid drug discovery and lead optimization. The title bis dipeptides may, consequently, offer significant DNA biological probes. Potential anticancer drugs or drug carriers could thus be presumed. Herein, the title bis peptides A and B were suggested and synthesized as new prototype candidates of such class compounds. Pre-assembled and purified peptides via the conventional methods of peptide synthesis are, subsequently, coupled to either 1,2,4,5-benzene tetra-carboxylic dianhydride or 1,4,5,8-naphthalene tetra-carboxylic dianhydride. The expected structures of obtained A and B as preliminary candidates were confirmed via the chemical, chromatographic and spectroscopic methodologies. The chemistry of both A and B will be discussed. Synthesis of other candidates, the corresponding biological, pharmacological and physicochemical investigations are under realization. δ≈≈ X = phenyl or bi-phenyl ring [compound A and B respectivel

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P10120-062

Synthesis and Biological Activity of PTH(1-11) Cyclo-analogues

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In mammals, parathyroid hormone (PTH), an 84 amino acid hormone, plays a vital role in regulating the concentrations of ionized calcium and phosphate in the blood and extracellular fluids. It has been shown that

the fragment PTH(1-34) is sufficient to bind and activate the PTH type I receptor (PTH1R). The molecular mechanisms by which PTH(1-34) binds to and activates the PTH1R have been extensively investigated. Recent investigations focusing on the interaction of N-terminal modified fragments PTH(1-11)NH2 with PTH1R showed that certain modifications can increase signalling potency and that enhancement of the β-helicity in the PTH(1-11) sequence yielded potent analogues of PTH(1-11)NH2. The design of cyclic analogues represents a widely used strategy to increase peptide stability and potency. The structural constraint induced by cyclization reduces conformational flexibility and may enhance potency, selectivity, stability and bioavailability as well as membrane barrier permeability. Initially, the work was concentrated on conformational constrains in N-terminal such as the introduction of Cβ-tetra-substituted amino acids. Global restrictions in the conformation of a peptide are possible by limiting the flexibility of the peptide strand through cyclization. To this purpose, the amino acid side chains that are not involved in receptor recognition are connected together or with the peptide backbone. Previous works on the role of side chains of AA determined through D-scan, analogues which maintained better β-helical structure, contained D-Gln in position 6 and 10. Recently Gardella and co-workers reported that an analogues of PTH(1-11) cyclized between 6 and 10 residues exhibited almost the same activity of linear analogues. So, in this work two new potent cyclic analogues in position 6 and 10 were synthesized directly on SPPS, using side chains of Lys and Glu or Ser. Then they were biologically tested and analyzed by CD, according to our previous work.

P10121-063

Total Synthesis of Neopetrosiamides A and B, Natural Peptides with Antimetastatic Activity

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The neopetrosiamides A and B are two diastereomeric tricyclic peptides that inhibit amoeboid invasion of human tumor cells. They were isolated by Anderson and co-workers from the marine sponge Neopetrosia sp. collected in Papua New Guinea, with their subsequent structure elucidation by the same group in 2005. The peptides are 28 residues in length and contain three disulfide bonds, as well as the unusual amino acid methionine sulfoxide at position 4. The two peptides differ only by being epimeric at this sulfoxide functionality. We report the total synthesis of neopetrosiamides A and B as well as an analog wherein the methionine sulfoxide has been replaced by norleucine, the carbon analog of methionine. The strategy involved solid phase peptide synthesis to generate the linear species, and selective formation of the disulfide bonds from orthogonally protected cysteine residues.

P10121-064

Synthesis of mimetics of the antibody 82D6A3: A new class of antitrombotics.

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In the Lab for Tromboses Research of the KULAK (Belgium) an antitrombotic antibody (Ab) called 82D6A3 has been characterized (1). The Ab inhibits the interaction between the plasma peptide von Willebrand Factor (vWF) and by injury exposed collagen, a prerequisite interaction in thrombusformation in arteries (high blood shear). It has been shown in vivo that the Ab 82D6A3 has an antitrombotic effect without showing the bleeding complications that known antitrombotics exhibit. This makes the Ab an attractive lead for antithrombotic drug design. By X-ray and mutagenesis studies, the paratope (active site) of the antibody has been determined. 6 amino acids, discontinuous in the primary structure of the antibody but a quasi linear unit in space, play an important role in the interaction. Incorporation of the functional groups of the amino acids and fixation of the secondary structure of the paratope will be the aim in the design of the paratope mimetics. With this rational design we will try to develop an orally available, synthetic paratope peptidomimetic with the same antitrombotic effect as the antibody. The development (solid phase and solution peptide synthesis) of the mimetics is a stepwise process. In each step conformational restrictions are introduced. In this we hope to divert away from the peptide and go to a drug like molecule.

1. Synthesis of linear peptides
2. Incorporation of a spacer and cyclisation of the linear peptides
3. Synthesis of small molecules
4. Combination of step 2 and step 3

The poster will present more details of the synthesis and modifications of the small molecules. (2,3,4)

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P10124-065

Backbone cyclization of peptides via N-functionalized phospho tyrosine

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Peptide ligands for SH2- and PTP domains containing phosphotyrosine are of great interest to influence the activity of kinases, phosphatases and other functional proteins. Backbone cyclization can help to stabilize these ligands against proteolytic degradation and to form their bioactive conformation. Till now backbone cyclization was performed with bifunctional and in few cases with trifunctional amino acids but not with phosphotyrosine. The assembly of such peptides requires preformed building units. Because the necessary reductive alkylation of phosphotyrosine derivatives leads only to very low yields we used N-functionalized pseudodipeptides with the unprotected phenolic hydroxyl group. For fragment condensation we synthesized building units of the common structures: Fmoc-AaØ[CO-N(X)-(CH2)n-NH-Alloc)Tyr(OH)-OH and Fmoc-AaØ[CO-N(CH2)m -COOAll)Tyr(OH)-OH. Depending on the steric hindrancy of the N-terminal amino acid these pseudodipeptides were synthesized in solution or at SASRIN-resin. They were purified by flash chromatography and analytically characterized by HPLC, ESI-MS and NMR. We tested our strategy on the synthesis of an octapeptide-ligand for the N-terminal SH2-domain of the phosphatase SHP-1: Glu-Gly-Leu-Asn/Abu-pTyr-Nle-Asp-Leu-NH2. Couplings of the dipeptide units were performed with PyBOP, stepwise coupling to the peptide fragments with unprotected phenolic hydroxyl group with pentafluoro phenylesters. After finishing the assembly the obtained polymer bound octapeptides were consecutively cyclized, phosphorylated, removed from the resin and purified by HPLC. Based on elucidated side reactions the synthetic strategy was optimized. The obtained backbone cyclic and phosphorylated ligands were tested for their influence on the phosphatase activity of SHP-1. The found enzymatic activities are correlated to size, direction, hydrophobicity and conformational flexibility of the lactam bridges.

P10125-066

Evaluation of Tetra “Polyamide Amino Acids” as specific RNA ligands : Thermodynamic Studies by Fluorescence Spectroscopy

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Synthetic molecules that bind with high affinity and specificity to RNA structures involved in biological processes, could potentially disrupt RNA functions, making these ligands attractive tools for molecular

biology and medicine. In this context, we have devised a new family of compounds named « Polyamide Amino Acids » (PAAs), constituted by a PNA (Peptide Nucleic Acids) backbone mimicking RNA sugar-phosphate backbone, onto which aminoacid residues are linked. Therefore, these PAAs could constitute a new type of RNA ligands, liable to specifically interact with an RNA target through an original interaction mode.

To assess the ability of PAAs to be potential RNA binders, we first prepared 8 tetra-PAAs (T1-T8) via solid-phase synthesis, starting from 4 PAA monomers deriving from Alanine, Phenylalanine, Lysine and Arginine residues. Interactions of the 8 tetramers with a HIV-1 TAR RNA fragment, taken as a target model, was investigated by fluorescence spectroscopy and circular dichroism. To give insights about specificity, binding affinities to TAR were also assessed using an excess of a tRNA mixture.

Results showed Kd values varying from 0.08 to 2032 µM, indicating the importance of the aminoacid side chains in the interaction. Thermodynamic analyses revealed that even if electrostatic interactions play a part in the complex formation, the binding is enthalpy-driven, highligthing the importance of non-electrostatic interactions in the recognition process. These results are of special interest since RNA/ligand association specificity is typically assumed to be due to short-range non-electrostatic interactions. Moreover, T1-T4 were shown to be specific to TAR in the presence of an excess of tRNA.

All together, these results are encouraging and they highlight the potential of PAAs as RNA ligands. Indeed, the use of only four PAA monomers as building blocks leads to tetra-PAAs displaying both affinity and specificity for their RNA target.

P10126-067

Studies on the solid phase synthesis and selective detection of peptide derived Amadori products by mass spectrometry

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Mass spectrometric analysis of glycation products of proteins and peptides attracts increasing attention (1). Peptide-based Amadori products could be used as markers of diabetes mellitus, which makes them the subject of interest in clinical chemistry. Recently, several procedures of site-selective synthesis of Amadori-modified peptides has been published [2,3]. In this communication we will present the synthesis of a new, fully protected derivative of glycated lysine which was successfully applied as a building block for incorporation of the glycated lysine moiety into peptide chain according to the standard Fmoc- solid phase synthesis protocol.

We will also present a new and straightforward method of selective detection of peptide-derived Amadori products by ESI-MS basing on characteristic neutral losses in the sugar moiety. The proposed approach in contrast to the neutral loss scanning, which requires triple quadrupol mass spectrometer, can be performed on the instruments with higher resolution and sensitivity, like Q-TOF .

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P10128-068

New APA inhibitors interacting with the S1 subsite bring new insights in the APA substrate specificity mediated by the calcium ion.

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Aminopeptidase (APA, EC 3.4.11.7) is a membrane-bound zinc metallopeptidase involved in the maturation of brain angiotensin III, a peptide which exerts a tonic stimulatory action on blood pressure in hypertensive animals (1). Therefore, developing inhibitors of this enzyme should result in new antihypertensive agents with possible new application in the treatment of certain forms of hypertension (2-3). We and others have previously reported the design of such inhibitors (4-5). Nevertheless, the improvement of the affinities of the inhibitors is relatively impaired since the three dimensional (3D) structure of the enzyme is not available. With the aim of getting insight into inhibitors optimization, a 3D model of APA was constructed in our group as an alternative (6). Furthermore, it is well established that APA substrate specificity and enzymatic activity are calcium dependent. In order to render this model more accurate and so on to identify the amino acids residues involved in calcium binding, we have designed new inhibitors able to explore the APA S1 subsite to better understand its specificity. We will report the synthesis of these new inhibitors, as well as an inversion of the specificity of APA S1 subsite in absence of calcium. These results and the identification of the amino acids implicated in calcium binding will be discussed on the basis of site-directed mutagenesis studies. One of the designed inhibitors, NI 926 (Ki = 70 nM) is to date the more potent inhibitor of APA activity measured without calcium. Altogether, these data should allow the improvement of APA inhibitors by structure aided design. (1) S. Zini et al. PNAS. 1996, 93, 11968-11973. (2) A. Reaux et al. PNAS, 1999, 96,13415-13420. (3) L. Bodineau et al. Hypertension 2008 To be published. (4) C. David-Basei et al. Expert Opin. Ther. Pat., 2001, 11(3), 431-444. (5) N. Inguimbert et al. J Pept Res 2005, 65, 175-188. (6) R. Rozenfeld et al. J. Biol. Chem. 2002, 277 (32), 29242-2925

P10129-011

Synthesis, resolution, and absolute configuration of BpAib, a benzophenone-containing C^{α}-tetrasubstituted α-amino acid

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Photoreactive amino acids with benzophenone side chains, the prototype of which is Bpa (4-benzoyl phenylalanine), have found numerous applications as photo-probes for covalent modification of enzymes and receptors, as well as in intramolecular quenching by a nitroxide free radical in trichogin peptide analogs. However, the remarkable flexibility of the Bpa side chain may question the extrapolation of results of photo cross-linking experiments and photophysical data to protein mapping and intramolecular distances, respectively [M. Saviano et al., *ChemBioChem*, **2004**, *5*, 541-544 and references cited therein]. To overcome this problem, we designed a new “constrained Bpa” amino acid, BpAib, belonging to the sub-class of the $C^{i\alpha}$<->$C^{i\alpha}$ cyclized, C^{α}-tetrasubstituted α-amino acids (strong β-turn and helix inducers in peptides). Racemic Boc-BpAib-OH was prepared by bis(alkylation) of ethyl isocyanoacetate under phase-transfer conditions with 1-benzoyl-3,4-(bis)bromomethyl benzene as alkylating agent, followed by acidic hydrolysis, N^{α}-Boc protection, and saponification of the ester function. Resolution was achieved through

the terminally-blocked dipeptide Bz-BpAib-L-Phe-NHChx with chromatographic separation of the diastereomers and acidic hydrolysis. X-Ray diffraction analysis of a crystal of a Z-BpAib-L-Phe-NHChx diastereomer allowed the assignment of the absolute configuration of the BpAib enantiomers. Photo-crosslinking studies with this residue are currently in progress.

P10129-012

N-Methylation of N$^{\alpha}$-acetylated, C$^{\alpha}$-ethylated, fully-extended homo-peptides: synthetic and conformational aspects

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Peptides characterized by single or multiple N-methylated, C$^{\alpha}$-trisubstituted (protein) α-amino acids are one of the subject of increasing interest in medicinal chemistry. Several naturally occurring peptides, remarkably stable to proteolytic attacks, are based on N-methylated peptides. N Methylation of the -CONH- function is a useful tool for discriminating solvent exposed from intramolecularly H-bonded secondary amide groups in peptides. We are currently extending this reaction to linear peptides based on C$^{\alpha}$-tetrasusbtituted α-amino acids. After having investigated synthesis and conformation of the N-methylated homo-peptides from the C$^{\alpha}$-methylated, helicogenic α-aminoisobutyric acid and C$^{\alpha}$-methylnorvaline residues [A. Moretto et al., Biopolymers (Pept. Sci.) **2006**, *84*, 553-565], in this work we examined the N-methylation reaction on homo-peptides from C$^{\alpha,\alpha}$-diethylglycine (Deg), known to overwhelmingly adopt the fully-extended, multiple C5, conformation. We studied the following peptide series: Ac-(Deg)$_n$-N(Et)$_2$, with n = 1-5. Under the experimental conditions used, only mono-methylation (on the N-terminal, acetylated residue) takes place. Our FT-IR absorption, NMR, and X-ray diffraction analyses support the view that the fully-extended conformation preferred by the original peptides is dramatically perturbed in all of the derivatives mono-methylated at position 1.

P10129-069

Computational Study on Secondary Structure of Oligopeptides Containing Chiral α,α-Disubstituted α-Amino Acids

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We have shown MCMM conformational search method and AMBER* force field using MacroModel is useful to predict secondary helical structures (α-helix, 3_{10}-helix) of oligopeptides prepared from α,α-disubstituted α-amino acids. Moreover, we have studied conformational analysis of oligopeptides containing chiral α,α-disubstituted α-amino acids to predict the helical screw sense of helical structures. We calculated α,α-disubstituted peptide using MCMM conformational search with various force fields (AMBER*, MMFF, OPLS) . In the case of using AMBER* force field the results were in agreement with those of x-ray and were most stable conformation evaluated by 3-21G level molecular orbital calculation. These results indicated that computational simulation using conformational search calculations with AMBER* force field is most useful for conformational analysis of oligopeptides containing α,α-disubstituted α-amino acids.

P10200-070

A New Colorimetric Test for Solid-Phase Amines and Thiols.

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Simple colorimetric tests still remain the most convenient tests for providing information on the course of solid-phase reactions. A colour test, which indicates the presence or absence of a functional group by visual detection, is a simple, practical tool to monitor the completeness of the reaction. Although the variety of colour tests for amines, there is still a need for a reliable and sensitive test in some synthetic approaches. Here, we wish to report a new simple, fast and sensitive colorimetric assay for the visual detection of solid-phase bound primary, secondary amines and solid-phase bound thiol groups using 1-alkyl-2-aryl-imidazo[1,2-a]pyrimidinium salts. In the search for a new synthetic approach to polysubstituted 2-aminoimidazoles (1), we have reported a new procedure, employing substituted 2-aminopyrimidines and β-bromocarbonyl compounds. The intermediate imidazo[1,2-a]pyrimidinium salts in the synthesis of substituted 2-aminoimidazoles appeared to give a strong colour when reacted with primary and secondary amines. Due to the strong colour change of amino functionalised resins after reaction with the "DESC" reagent (2), a protocol for the detection of resin bound primary and secondary amines has been developed. The "DESC" test is a new, practically, simple, quick and reliable test for the visual detection of resin bound primary and secondary amines in a sensitive way. The "DESC" reagent is accessible from the cheap starting materials and can be prepared in two steps.

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P10203-071

Application of traceless enantioselective coupling reagents in SPPS

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Tetrafluoroborates of chiral N-triazinylammonium salts were found stable and useful in enantioselective (ee up to 98%) peptide synthesis from racemic amino acids in solution. Several chiral N-triazinylammonium tetrafluoroborates were obtained as stable L and/or D selective coupling reagents (1). Broad range of common amines (strychnine, brucine, sparteine etc.) were found useful as a chiral auxiliary, opening access to amino acids of both required configurations. We have attempted to expand the scope of application of this family of reagents expecting their efficiency in enantiodifferentiating reactions in solid phase peptide synthesis (SPPS). Tetrafluoroborates of chiral N-triazinylammonium salts were obtained by treatment 2-chloro-4,6-dimethoxy-1,3,5-triazine with tetrafluoroborates of appropriate tertiary amines in the presence of sodium bicarbonate (2). Enantiodifferentiating reagents were used in synthesis on Wang resin under classic condition for triazine based coupling reagents (3). The most important advantage of application of chiral N-triazinylammonium tetrafluoroborates is the predictability of configuration and repeatability of enantiomeric purity of incorporated amino acid. Even if only threefold excess of racemic carboxylic acid, enantiomeric purity of coupling products in SPPS reached ee 98-99.

Acknowledgement: This work was supported by Ministry of Science and Higher Education, Grant PBZ-KBN-126/T09/15.

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P10203-072

Traceless, chiral triazine coupling reagents for synthesis of peptides from racemic amino acids with fully predictable optical purity and configuration of product

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The demand for diversity of non-proteinogenic amino acids, willingly used as new building blocks, is severely restricted by laborious procedures leading to enantiomerically homogeneous individuals. Typical sources such as isolation from natural products, biotechnological methodology, even the asymmetric syntheses posses a limited value because complex or tedious synthetic procedures or limited access to the pool of chiral auxiliaries. Herein we present the novel, general approach allowing enantioselective incorporation of any enantiomer of amino acids directly from racemic substrate. According to our procedure, the chiral auxiliary is used only at the stage of enantioselective activation. The departure of chiral auxiliary yields in the traceless way, the activated derivative of N-protected amino acid identical with those which is obtained with the well known, classic, achiral triazine coupling reagent (1). Therefore, the traceless chiral coupling reagents can be successfully applied directly in the coupling stage without additional studies of their reactivity. Several chiral N-triazinylammonium tetrafluoroborates were obtained as stable L and/or D selective coupling reagents. Broad range of common amines (strychnine, brucine, sparteine etc.) were found useful as a chiral auxiliary, opening access to amino acids of both required configurations. The most important advantage of application of chiral N-triazinylammonium tetrafluoroborates is the repeatability and predictability of enantiomeric purity and configuration of incorporated amino acid. Even if only twofold excess of racemic carboxylic acid enantiomeric purity of coupling products determined by GC on ChirasilVal reached ee 98-99 with Kagan's coefficient s = 90-100.

Acknowledgement: This work was supported by Grant PBZ-KBN-126/T09/15.

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P10300-073

Solid Phase Synthesis of Azapeptides using activated Nβ€™-Substituted Ddz Protected Hydrazines

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Azapeptides are peptide analogues in which one or more of the α-carbons, bearing the side chain residues, has been replaced by a nitrogen atom. Azaamino acid residues conserve the pharmacophores necessary for biological activity while inducing conformational changes and increased resistance to proteolitic degradation. These properties make azapeptides an attractive tool for structure-activity relationship studies and drug design.

A general approach for solid phase synthesis of azapeptides has been developed based on the in-situ activation of N-2-(3,5-dimethoxyphenyl)propan-2-yloxycarbonyl (Ddz), Nβ€™-substituted hydrazines, with phosgene, followed by introduction to N-terminus of resin-bound peptide. The Ddz-aza-amino building units include aliphatic, aromatic and functionalized side chains, protected for synthesis by the Fmoc strategy. Solid-phase azapeptide synthesis is demonstrated including selective mild deprotection of Ddz with $Mg(ClO_4)_2$ and coupling of the next amino acid with triphosgene. The mild Ddz deprotection is also orthogonal with Boc chemistry. We describe the synthesis of Nβ€™-substituted Ddz protected hydrazines which have wide applications in the synthesis of azapeptides as well as in general synthesis of substituted hydrazines and aza containing peptidomimetics. Ddz protected hydrazines offer the possibility to construct novel and unique drug-candidate structures such as branched and cyclic azapeptides.

P10300-074

Fast Conventional Synthesis of Parathyroid Hormone (1-84) on the Symphony®

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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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P10301-075

Improved synthesis of insulin-like peptides

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The A- and B-chains of insulin-like peptides were synthesized by various methods. The best results were achieved by dividing the sequences of the A-chain into three protected fragments and that of the B-chain into two fragments. The fragments were prepared on 2-chlorotrityl resin and/or 4-methoxybenzhydryl resin using Fmoc-amino acids. Condensation of the fragments was carried out either by solution phase or solid phase techniques. The joining of the A and B chains was also studied using either biomimetic or chemoselective oxidative folding methods.

P10301-076

A new approach for amides and peptides chemical synthesis by means of phosphonic acid/alkylene oxide chemistry

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Few different derivatives of L-Phe were synthesized, using originally discovered method of phosphonic acid/alkylene oxide chemistry. This method provides the successful synthesis of variety amides and dipeptides without preliminary protection of alfa-NH2 group of L-amino acids. The supposed mechanism displays the formation of N-phospholane carboxyanhydride (1-oxy-3-aza-2-phospholane-5-one), or an O-hydroxypropyl H-phosphonate salt of an amide of L-Phe. The N-phospholane carboxyanhydride is protected at the N-terminus, as well as is activated at the C-terminus. This pathway favors the desired reaction with a variety of nucleophiles from the N- to C-terminus. This method also allows the synthesis of different esters of amino acids employing alcohols as nucleophiles previously described by us (1).

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P10301-077

CLEAR-OX™: Synthesis of Disulfide-Bridged Peptides Under Mild Conditions

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Formation of disulfide bonds in synthetic peptides is one of the more challenging transformations to achieve in peptide chemistry, in view of possible formation of oligomeric by-products and other side reactions, as well as occasional solubility problems in aqueous oxidizing media. The recently introduced polymer-supported oxidant, CLEAR-OX™, has proven to be a very valuable tool in the preparation of disulfide-bridged peptides. [1,2] Oxidations using CLEAR-OX were carried out at pH ranging from 4 to 7 in water/acetonitrile solutions, with concentrations 10-15 times higher than comparable solution oxidations. The latest progress in the preparation of various monocyclic and bicyclic peptides will be presented.

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P10301-078

Mild and Selective Boc Deprotection on Acid Cleavable Rink-Amide MBHA Resin

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Although a number of reagents have been reported for t-butoxycarbonyl (Boc) deprotection on acid-sensitive resins[1-3], to the best of our knowledge, all attempts to remove Boc on acid-labile resin have resulted in some cleavage from the resin. Tin tetrachloride has been shown to be an excellent mild and selective reagent for deprorection of Boc from amino acid and guanidines in solution[4,5].

We examined the removal of the Boc group with tin(IV) chloride during solid phase peptide synthesis on acid sensitive Rink-amide MBHA resin. The presented method enables Boc deprotection with no threat to other carbmate-based amino protecting groups and no detectable cleavage from the resin.

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P10301-079

Covalently linked Aβ dimers

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The amyloid β-peptide (Aβ) is believed to play a causal role in Alzheimer's disease (AD). One of the hypotheses of Aβ neurotoxicity is that it induces the generation of reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) formation by binding to metals such as copper and iron. It is hypothesized that the sole tyrosine residue plays an important role in Aβ-mediated toxicity, due to its ability to form a dityrosine cross-link from tyrosyl radicals generated in the highly oxidative environment in the brain. Hence, studies of the dityrosine cross-linked Aβ peptide dimers would increase our understanding of the oxidative alteration and dimerisation of Aβ in amyloid formation and AD related neurodegeneration.

We are investigating the synthesis of the Aβ peptide dimers containing the dityrosine cross-link. Dityrosine, suitably protected for solid-phase peptide synthesis (SPPS), has been prepared from iodotyrosine using a Miyaura borylation-Suzuki coupling method. Studies on the synthesis of dityrosine-linked peptide dimers through incorporation of dityrosine in SPPS are underway. Initial model studies employing 2,6-diaminopimelic acid (DAP) have validated this approach. Preparation of the model DAP-linked Aβ peptide dimers will be discussed, as well as progress towards the dityrosine-linked Aβ peptide dimers.

P10301-080

Switch-Peptide via Staudinger Reaction

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The Staudinger reaction, discovered nearly a century ago, occurs between a phosphine and an azide to form an aza-ylide. This transformation was further explored and developed, leading to several reactions of highly synthetic importance. Traceless Staudinger ligation is one example of such modifications, in which an ester moiety is placed within the phosphine structure to capture the nucleophilic aza-ylide, by intramolecular cyclization, leading to a stable amide bond. While the aza-ylide intermediate is known to be stable in organic solvents, it tends to hydrolyze rapidly under aqueous media to furnish the primary amine product. In the traceless Staudinger ligation, however, the reduction of the azide to amine is a competing side reaction, as it would reverse the capture step leading to two peptide fragments. We have found that such reduction step would be beneficial if an electrophile and an azide (rather than the phosphine) are placed within the switch peptide system. Investigating various reducing reagents led us to the discovery that tris(2-carboxyethyl) phosphine hydrochloride (TCEP) is excellent azide reducing reagent. Both the reduction and the acyl transfer steps are rapid and occur in a few minutes. Applying the TCEP-mediated triggering in switch peptides, derived from the Aβ to understand the currently unclear processes of pathological folding, self-assembly, and aggregation of amyloid β peptide will also be reported.

P10301-081

Fast Fmoc-deprotection reagent for peptide synthesis

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The standard reagent for Fmoc-deprotection in solid phase peptide synthesis is 20% piperidine in DMF or NMP (1). Piperidine is a drug precursor, and therefore there is a strong legal control of its distribution, and very much time-consuming paperwork to fill-in. Although there are studies on stronger deprotection reagents, used for difficult cleavage, and smoother reagents, described to avoid side reactions for base sensitive sequences and suppression of aspartimide formation. Only a few literature is found on an actual subtitute for piperidine (2). Recently we developed a new Fmoc-deprotection reagent , which is reacting faster than piperidine as shown by kinetic studies in solution . The new reagent was compared to piperidine by synthesis' on a model peptide (IIKKSTALLG). The ultimate quality of the peptides was assessed by using mass spec techniques. With shortened deprotection times the new reagent show less Fmoc-deletion peptides in comparison to piperidine.

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P10301-082

The Nmec group: A new carbamoyl protective group that facilitates purification of hydrophobic peptides

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Difficult sequences have long been known in solid phase peptide synthesis, theses sequences can experience sever aggregation both during synthesis and under purification. The aggregation is believed to depend mainly on β-sheet formation or/ and hydrophobic properties of the peptide. Now a days there are several strategies to circumvent theses problems, one of the most successful is to incorporate a backbone protective group as the Hmb group, develop by Johnson and co-worker(1), and thereby prevent aggregation due to β-sheet formation. An additional charge will increase the solubility of peptides towards aqueous solutions and thereby facilitate the purification by HPLC (using acetonitrile and water system) and analysis (by MALDI-TOF mass spectrometer) of the peptide. Here we present the Nmec (N-methyl- N-[2-(methylamino)ethyl] carbamoyl) group(2) that is a orthogonal protective group for tyrosine side chain and the hydroxyl moiety of the Hmb group. The Nmec group is Fmoc compatible and is protected during the synthesis with a Boc group. The final cleavage from the resin with TFA renders the amine of the Nmec group protonated and will increase the solubility of the peptide during purification and analysis. The Nmec group can be easily removed with a mild alkaline treatment by a cyclization elimination reaction. The Nmec group has been employed in the synthesis of difficult and hydrophobic peptides as the membrane spanning sequence of the calciton receptor, β amyloid (25-35) and β amyloid (1-42) with success.

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P10301-083

Isopeptide method: an efficient preparation of difficult peptides on solid support

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In 2003, we discovered that the presence of an *O*-acyl instead of *N*-acyl residue within a peptide backbone significantly changed the secondary structure of a native peptide. A target peptide was subsequently generated by an *O*-*N* intramolecular acyl migration reaction. These findings led to the development of a novel method, called e*O*-acyl isopeptide method f, for the synthesis of peptides containing difficult sequence [1, 2].

Recently, the *O*-acyl isopeptide method has further evolved as a general method for peptides synthesis with our developments of e*O*-acyl isodipeptide units f [3-5] and eracemization-free segment condensation methodology f 6.. Isodipeptide units have enabled routine use of the *O*-acyl isopeptide method by avoiding the often difficult esterification reaction on the resin [3, 4]. For example, Influenza A virus-related peptide (H-GILGFVFTL-H) with a difficult sequence was synthesized using *O*-acyl isodipeptide unit. Analysis of the crude peptide revealed high purity of the product with no by-product derived from the difficult sequence or epimerization. Using CH_2Cl_2 as solvent in coupling the isodipeptide unit, Aβ1-42 was also synthesized with almost no major side reaction 5..

Racemization-free segment condensation method was developed by employing *N*-segments possessing a C-terminal urethane-protected *O*-acyl Ser/Thr residues 6.. The synthesis of long peptides/proteins by racemization-free segment condensation has thus become possible at Ser/Thr residues and not only C-terminal Gly/Pro residues.

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P10305-084

Different proteases immobilized inside chitosan film can catalyze synthesis and hydrolysis of peptide substrates

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Growing interest to peptide substrates, inhibitors and other peptidomimetics required new highly effective synthetic techniques. The important goal of enzymatic peptide bond formation is the optical purity of the peptide, which facilitates the product isolation. Thus using enzymatic condensation as a last step in peptidomimetics production is preferable. Fixing of enzymes on/in suitable insoluble supports has many advantages: high operational stability, ease of separation, possibility of recycling and improved activity in low water media. Chitosan, natural hydrophilic polysaccharide, was used as a matrix as it has distinct advantages over the other supports due to (a) its renewable nature - it is available in large quantities as a waste product from fishing industry and (b) its excellent film-forming ability, allowing attachment to reactor walls for advanced processing. Serine proteases subtilisin and chymotrypsin, and cysteine protease papain were immobilized onto chitosan. The films of biocatalysts were prepared by drying the mixed solution of chitosan and enzyme in acetate buffer pH 5.6. Treatment with glutaraldehyde was found to give material with high stability and good mechanical properties. The obtained biocomposites showed high amidase activity against specific chromogenic peptide substrates and protein substrate azacasein. Immobilized subtilisin and chymotrypsin also possess esterase activity against p-nitrophenyl acetate. The long-term storage in aqueous buffer and acetonitrile had a little effect on the hydrolytic activity of subtilisin-based biocomposite. The dependence of subtilisin/chitosan hydrolytic activity on temperature and pH was studied. Obtained samples possessed high synthetic activity and were capable to catalyze peptide bond formation in DMFA/MeCN mixture in reaction ZAALOMe+FpNA→ZAALFpNA for subtilisin, ZAAOMe+LpNA→ ZAALpNA for papain. The product yield reached 60-100% in 24 h.

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P10305-085

Studies on the synthesis of the kazal-type inhibitor LEKTI domain 6

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LEKTI (Lympho-Epithilial Kazal-Type Inhibitor) is a novel multidomain proteinase inhibitor consisting of 15 potential serine proteinase inhibitory domains. Defects within the gene encoding LEKTI have been associated with several skin diseases and atopic disorders, including Netherton syndrome and atopic dermatitis. Therefore, LEKTI represents a potential drug candidate for treating these disorders. Here we report our studies towards the synthesis of the domain 6 of LEKTI, consisting of 68 amino acid-residues and two disulfide bonds. For the assembly of the linear sequence two main strategies were investigated: a) the fragment condensation of large protected fragments, either in solution

or on solid phase and b) the chemical ligation of the [Cys12(Acm)]-(1-25)-thioester with the [Cys48(Acm)]-(26-68) segment, both deprotected and HPLC purified. The required intermediate peptides were prepared by optimized Fmoc-based methods either stepwise or by convergent synthesis. For the formation of the two disulfide bridges a two step procedure was investigated, involving a DMSO oxidation step to form the Cys26-Cys45 linkage, followed by iodine oxidation to form the Cys12-Cys48 bond.

P10305-086

Native chemical ligation at valine

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Native chemical ligation is perhaps the most useful technique for peptide segment coupling in water.(1) A *C*-terminal peptide thioester reacts with an *N*-terminal cysteine. The requirement for this rare amino acid represents the major bottle neck to the synthesis of native proteins. Several strategies have been developed to overcome this limitation. In the extended ligation, N-terminally attached auxiliaries allow access to other ligation junctions by mimicking the cysteine-thiol moiety. The ligation-desulfurization approach employs β-thiol amino acids for fragment coupling followed by sulphur removal. Herein, cysteine acts as precursor of the abundant alanine(2) and phenylalanine can be obtained by using its β-mercapto derivative.(3).
We demonstrate the application of penicillamine in the generation of valine junctions employing the ligation-desulfurization approach. The β,β-dimethylcysteine building block is commercially available with various protecting group patterns suitable for routine solid phase synthesis of peptides. The ligation at penicillamine proceeded surprisingly fast despite the steric demand at the thiol group. Even Leu-Val ligation sites, which appear in hydrophobic peptide segments, are accessible. We also present an improved method for achieving metal-free desulfurization and show applications in the synthesis of valine-containing peptides.(4)

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P10305-087

The application of N-Alkyl cysteine (NAC)-assisted thioesterification reaction to the synthesis of polypeptides by the thioester method

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Peptide thioester is a key compound in the synthesis of proteins by the thioester method as well as the native chemical ligation. The peptide thioester has been mainly synthesized by the Boc method using an established protocol. Due to the increasing interest in the post-translational modifications, such as glycosylation, peptide thioester synthesis by the Fmoc method, which do not use harsh acidic conditions, is desired. Recently, we have developed a novel SPPS thioesterification, in which N-alkyl cysteine residue at the C-terminus of a peptide is used as a N to S acyl transfer device (1). In the presence of appropriate thiols, such as 3-mercaptopropionic acid, the peptide with N-alkyl cysteine at its C-terminus is easily converted to peptide thioester at room temperature. Thus, this method is fully compatible with conventional Fmoc method. In this presentation, the usefulness of the method was demonstrated by the application to the synthesis of protein (2), glycoprotein, glycopeptide dendrimer.

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P10305-088

Azide as a protecting group for lysine side chains on the solid phase peptide synthesis oriented toward the peptide condensation by the thioester method

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The solid phase peptide synthesis (SPPS) is usually limited to approximately less than 50 residues long. For the synthesis of longer peptide sequences, condensation methods of two or more peptide segments are used. Thioester method is one of the condensation methods of peptide segments. In this method, any residue at the ligation point can be used, although the protecting groups for amino and thiol groups are required. For protection of thiols, acetoamidomethyl group can be used because of its stability in both basic and acidic conditions. Inconveniently, Boc groups should be introduced to the amino groups in the peptide segments used for this method after the cleavage from the resins and the purification. To overcome this problem, a new hydrophilic protecting group for amines stable for both acids and bases was desired. Azide moiety was a good candidate for such purpose. However, the methods for introducing to the peptide side chains and deprotection (reduction) method have not yet been developed. In this study, we have synthesized two pigment dispersing hormones (PDHs) of the prawn, *Marsupenaeus japonicus*, by the thioester method using the azido peptides as building blocks. Fmoc-Lys(N_3)-OH was synthesized from Fmoc-Lys-OH by the copper(II)-catalyzed diazo transfer method, and introduced to the C-terminal segments by the ordinary Fmoc-based SPPS. The N-terminal thioester segment was prepared by N-alkyl cysteine (NAC)-assisted thioesterification method developed in our previous study. The N- and C- terminal segments were condensed in DMSO containing HOObt/DIEA. After condensation, N-terminal Fmoc group was removed by addition of piperidine, and the azido group was converted to amine by Zn/AcOH without any significant side reactions. The synthetic PDHs were obtained by reverse-phase HPLC in good yield.

P10305-089

N to S Acyl Shift Reaction for Preparation of Peptide Thioester and for Peptide Ligation

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Peptide ligation chemistry has been developed based on the use of peptide thioester as a building block in the thioester method (1) and native chemical ligation (2). We found that a cysteine-containing peptide is transformed into the corresponding S-peptide (peptide thioester) by the N to S acyl shift reaction (3). On the other hand, in 1985, Zanotti et al. reported that a diketopiperazine thioester, cyclo(-Cys(COCH2Ph)-Pro-) (**1**) was formed when a dipeptide p-nitrophenyl ester, PhCH2CO-Cys(S-t-Bu)-Pro-ONp (**2**), was treated under reductive aqueous conditions (4). Thioester **1** would be formed via intramolecular N-S acyl shift reaction followed by diketopiperazine formation. Based on these observations, we designed a cysteinyl prolyl ester (CPE) autoactivating unit for preparation of the peptide thioester and for the peptide ligation [5,6]. A peptide containing the CPE unit, peptide-CPE **3**, was transformed into a peptide thioester of diketopiperazine, cyclo(-Cys(peptide)-Pro-) **4**, then thiol-thioester exchange reaction with an external thiol produced the peptide thioester **5** and a diketopiperazine, cyclo(-Cys-Pro-) (**6**). The

peptide-CPE **3** was also able to ligate with a cysteinyl peptide **7**, via the thioester in one-pot, to give a polypeptide **8**. The peptide-CPE can be prepared by standard Fmoc solid phase peptide synthesis, because it contains no thioester moiety.

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P10305-090

Synthesis of a Homogeneously Glycosylated Enzyme: Ribonuclease C

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Christian Piontek, Daniel Varon, Christian Heinlein , Stefano Mezzato, Claudia Pöhner, Carlo Unverzagt Bioorganische Chemie, Universität Bayreuth, Gebäude NWI, 95440 Bayreuth, Germany Native chemical ligation (1) has enabled the synthesis of entire proteins including those carrying posttranslational modifications. One of the most frequently encountered modification of eukaryotic secretory and cell surface proteins is the attachment of oligosaccharides to asparagine residues (N-glycosylation). Despite many efforts in this field the function of N-glycosyation is poorly understood, which is mainly caused by the lack of pure glycoproteins. Since purification of natural glycoproteins is quite tedious due to heterogeneity in the sugar part, the total synthesis of homogeneous glycoproteins has become an attractive target (2). We have addressed this topic by choosing RNase C as a model glycoprotein. Instead of the oligomannosidic type RNase C is containing a complex type N-glycan. For synthetic reasons ligations had to be conducted in a sequential manner (3) by first reacting the recombinant 40-124 Cys-segment (4) with an N-terminally protected and glycosylated thioester 26-39 5.. Selective deprotection of the ligation product 26-124 and ligation with synthetic thioester 1-25 in a one pot manner was accompanied by unexpected side reactions. These drawbacks were finally overcome leading to full-length glycosylated RNase C displaying enzymatic activity.

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P10305-091

A new pseudo-native ligation: multiple, successive azide-alkyne cycloadditions.

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Copper-catalyzed Cycloaddition of Azides on terminal Alkynes (CuAAC) affords 1,4-disubstituted 1,2,3-triazoles under mild conditions. Since discovered in 2002,[1] the reaction has become a classics. As triazoles are stable to acid and basic hydrolysis, and reductive and oxidative conditions, the reaction has been widely used in chemistry and biochemistry, proving to be an useful tool in combinatorial, bioconjugate or medicinal chemistry. The reaction has been applied in the peptide field as an easy way to synthesize cyclic, labelled and side chain modified peptides including efficient synthesis of pseudo-glycopeptides. Conformational and structural studies prove that the triazole ring is an excellent trans-amide surrogate and thus, can be considered as a new pseudo-native linkage.[2] In order to explore the potential of the reaction as a way to successively assemble several peptide chains to generate mimics of proteins, we have elaborated a new strategy for consecutive triazole formation based on a semi-orthogonal alkyne protection scheme.[3] So far, we have improved our first one-pot successive cycloaddition approach performing the first three successive CuAAC in mild conditions thanks to a highly selective deprotection of two different alkyne groups.

The application of this strategy represents a new promising method for the chemoselective pseudo-native ligation dedicated to the synthesis of protein chimera or for the decoration of molecular templates.

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P10306-092

New Economical, Scalable Amphipathic Resins for Peptide Synthesis

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Solid phase synthesis using lightly crosslinked polystyrene supports has proved to be a successful route to the manufacture of peptides. The methodology introduced by Merrifield nearly fifty years ago has remained largely unchanged. During the last twenty years or so, it has been argued that incorporating a hydrophilic polymer within the conventional hydrophobic polystyrene matrix is beneficial. Others have suggested that polyamide or polyether-based resins may prove to be superior to polystyrene. Despite this, large-scale peptide manufacture has generally relied on traditional polystyrene supports. This is due in part to the difficulty in producing composite supports economically in large volumes, but also due to the handling difficulties that are often associated with very high swelling polar polymers. Other problems arise from the fact that many of these types of resin are relatively low loading and so yields are greatly diminished. In this poster we offer a solution to these problems: a polystyrene derived support which is modified to create economical, amphipathic resins suitable for large scale peptide synthesis. Attachment of appropriate handles or linkers enables both peptide acids and peptide amides to be produced. The synthesis of a variety of peptides is demonstrated.

P10306-093

Peptide Synthesis in Water Using Boc-Amino Acids Nanoparticles

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There has been increasing interest in environmental-friendly synthesis and technology to achieve reduced use of organic solvents and utilization of low-toxic reagents. Peptide synthesis methods using Boc or Fmoc chemistry are well-established technique. However, these procedures consume large quantities of organic solvents. Recently we succeeded in development of an organic solvent-free, environment-conscious, solid phase synthesis method using gwater h as an eco-friendly reaction solvent, and reported the solid-phase synthesis of Leu-enkephalinamide using water-dispersible Fmoc-amino acid nanoparticles (1). This novel technology is based on coupling reaction of suspended nanoparticle

reactants. Here, we studied in-water solution-phase method using water-dispersible nanoparticulate Boc-amino acids, which are the most common building blocks but are difficult to use for peptide synthesis in water. Water-dispersible Boc-amino acid nanoparticles were prepared by pulverization using a planetary ball mill in the presence of PEG, and the size of the resulting water-dispersible nanoparticles was determined by dynamic light scattering analysis. The scanning electron microscopy image of water-dispersible Boc-Phe-OH nanoparticles also revealed nanosize particles. We studied in-water coupling reaction using water-dispersible Boc-amino acid nanoparticles, and Leu-enkephalinamide was successfully synthesized in water according to the Boc chemistry.

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P10306-094

Synbeads rigid, hydrophilic, macroporous, methacrylate functionalized polymers for efficient SPPS

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Peptides are key to modern drug discovery. They are currently produced in mg-scale for research purpose, in order to better understand the function of biological systems. Many peptide drugs are now produced in multi-tons scale on solid support.(1) Here we demonstrate that the use of rigid and highly porous Synbeads, allows a significant reduction of solvents, reagents and time consuming in the synthesis of peptides of pharmaceutical interest. Synbeads are methacrylate functionalised hydrophilic supports with very high physico-chemical stability in several harsh conditions. (2) Moreover it has already been evidenced that these supports have a perfectly calibrated functional group density and thanks to their characteristic macroporous structure optimal reagent diffusion is guaranteed. Peptide synthesis on Synbeads can be easily performed from gram to ton scale allowing very high yields and product purity, even in comparison with other commercial swelling supports, such as ChemMATRIX, PS, Tentagel. Synbeads have been tested in the synthesis of Somatostatine, Terlipressin and also in the most common Fuzeon. Traditional coupling reagents as HOBt and DIC can be successfully employed on Synbeads instead of expensive HCTU.

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P10307-095

An alternative way for conopeptide formation

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Cysteine-rich conopeptides are important synthetic targets taking into account their diverse bioactivities and thus broad range of application.(1) Conotoxins are classified according to their three-dimensional conformations which are caused by distinct patterns of disulfide bonds. Indeed, the chemical synthesis and characterization of conotoxins is challenging, mainly because of oxidative folding problem. A variety of strategies for the solid phase synthesis of the linear precursors as well as the oxidative folding protocol were already published in the literature.(2) However, a major drawback of all these reports is the low yield of the desired products.(3) We investigated an alternative method of conopeptide formation with the aim to obtain higher peptide yields and in this way to improve accessibility to different classes of conotoxins. Members of different conotoxin families have been selected for this study, among them μ-SIIIA, μ-PIIIA, δ-EVIA and δ-SVIE. The synthesis of the linear conopeptides has been accomplished using Tenta Gel Ram resin and TFFH as coupling reagent. The oxidative folding was performed with air in a biocompatible ionic liquid (1-ethyl-3-methylimidazolium acetate ([C2mim](OAc)). This procedure enables the efficient formation of both hydrophilic as well as poorly water soluble conotoxins without the use of buffer solutions and redox-active agents.

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P10309-096

Synthesis of cysteine-rich peptides

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The chemistry used to oxidize the free thiol bonds to the corresponding disulfide bond in a controlled fashion remains a significant challenge in spite of many advances in peptide chemistry. The primary reason of this lies in the difficulties involved in the formation of multiple regioselective disulfide bonds. In this work we focused on the elucidation of synthetic strategies for the preparation of multiple disulfide containing peptide venoms regulating the ion channels of immune cells- Anuroctoxin and Tc32-, and a neuropeptide with regulatory functions- Orexin A. For the synthesis of these naturally occuring Cys-rich peptides we had chosen the oxidative folding being the most simple of the methods available. In the case of anuroctoxin we isolated the native form of the peptide toxin with high selectivity and we optimized the folding conditions, so within an hour the majority of the linear peptide folds in the cyclic form with all four disulfide bonds in the correct form. The regioselectivity of the isolated isomer was verified with biological measurements. For the synthesis of Orexin A we optimized the folding conditions and, using a polymer-supported oxidant- the Clear-Ox resin-, within two hours we could isolate the correctly folded isomer as the major reaction product. The correct structure was proven by coelution of the isolated isomer and the commercially available Orexin A. But oxidative folding does not always give the correctly folded isomer. The best proof for this is the Tc32 scorpion toxin which albeit our tryings always gave the misfolded isomers if we used the linear, unprotected peptide. Therefore a new chemical synthesis using different side-chain protection needs to be taken into consideration. The synthesis of orthogonally protected Tc32 and its use for the preparation of the correctly folded peptide to be underway.

P10309-097

Glycopeptides – a synthetic challenge

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The number of possible combinations of the twenty proteinogenic amino acids is enormous even for smaller oligopeptides. However, Nature was not satisfied with this variability. Great amount of new compounds, hardly similar to the mother compound, can be formed by partly post-translational modifications following the ribosomal protein synthesis, partly modifications of amino acids by mostly plants, microorganisms and fungi. Beside glycosylated, phosphorylated, etc. peptides and proteins, such compounds are amino acid- and peptide-based heterocyclic compounds (alkaloids and some antibiotics, etc.) widespread in the plant kingdom and used as medicines. These post-translational modifications

have fundamental importance in biological recognition processes. One of the most challenging task among of them the rational preparation of the glycosylated peptides especially having oligosaccharide moieties. There are two main strategies for the synthesis of glycopeptides: the synthon and global (convergent) method. Both of them can be implemented in liquid or solid-phase. Since the glycosylation could appear on O and N atoms of the amino acid side-chain, due to the different reactivity of the glycosidic linkage different chemical strategies will necessitate. In this presentation we compare several chemical strategies for the preparation of two model peptides (Leu-Lys-Asn*-Gly-Gly-Pro, Gly-Val-Glu-Asp-Ile-Ser*-Gly-Leu-Pro-Ser-Gly,*site of glycosylation). As glyco-part several mono, di and trisaccharide including chitobiose, galactosil-xilose, mannosil-N-acetyl-glycosil-N-acetyl-glucosamine were used and several of the used strategies led to successful preparation of these glycoconjugates.

P10309-098

Site-specific PEGylation of human IgG1-Fab using a rationally designed trypsin variant

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In the present contribution we report on a novel, highly selective biocatalytic method enabling C-terminal modifications of proteins with artificial functionalities under native state conditions. The approach is based on the fourfold trypsin variant K60E/N143H/E151H/D189K which was generated by site directed mutagenesis and initially specified in terms of selectivity and activity using a peptide library of the general structure Bz AAX$_{aa}$X$_{bb}$X$_{cc}$AAG-OH. The trypsin variant was found to bear a restricted proteolytic activity towards the rare recognition sequence YRH with an occurance of less than 0.5% in native proteins. The specificity is zinc ion dependent due to an artificial metal binding site in the S2´-subsite (1). Placing of the recognition sequence at the C-terminal region of respective proteins via standard mutagenesis protocols provides suitable precursor targets for site-specific modification via a transamidation reaction. Due to the great demand for polymer-modified antibody fragments for pharmaceutical purposes we evaluated the function of the approach on example of the C-terminal PEGylation (PEG...polyethylene glycol) of the human IgG1 Fab fragment. The fragment itself was expressed with the recognition sequence and an additional Strep-fusion enabling efficient purification. The derivatization of the antibody fragment with 40 kDa PEG via the trypsin variant (MW ~ 24 kDa) proceeds efficiently with a total yield of isolated modified protein of 40%. Interestingly, no undesired cleavage reactions could be detected leading to fully active modified antibody fragment.

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P10317-099

2-Chloro-4,6-bis-(2,2,2-trifluoroethoxy)-1,3,5-triazine and N-4,6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl)ammonium tetrafluoroborates as highly efficient coupling reagents

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We expected that modification of substituents in the triazine ring improve activity, stability and solubility of new triazine based coupling reagents. In order to increase activity we prepared 2-chloro-4,6-(2,2,2-trifluoroethoxy)-1,3,5-triazine by treatment of cyanuric chloride with 2,2,2-trifluoroethanol. Taking advantage of modular structure of triazine coupling reagents the entire family of N-(4,6-(2,2,2-trifluoroethoxy-1,3,5-triazinyl-1)ammonium tetrafluoroborates have been obtained. Efficient coupling reagents should be useful for amide bond formation between broad range of substrates, work in stoichiometric quantities, be soluble and stable in most of the solvents. It should function efficiently in solution as well as in SPPS, to get high purity crude products and minimize racemization of the products. We found N-(4,6-(2,2,2-trifluoroethoxy-1,3,5-triazinyl-1)ammonium tetrafluoroborates useful for activation of carboxylic components. The participation of triazine “superactive ester” as intermediate in the condensation has been proved in the model experiments. Utility of reagents N-(4,6-(2,2,2-trifluoroethoxy-1,3,5-triazinyl-1)ammonium tetrafluoroborates were confirmed by peptide synthesis in solution in high yield. In our study we focused our attention on the performance (in terms of purity of the crude and extent of racemization) testing the solid phase synthesis of ACP(65-74) and model peptides with AibAib fragment, which are a good example of difficult peptide sequence.

P10317-100

Purification of chemically synthesised proteins by use of cleavable IMAC- based Nβ-terminal protein protecting groups (tags)

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Despite the progress in protein purification over the past forty five years since the achievement of solid phase peptide synthesis (SPPS) by R.B. Merrifield (1), there remains a challenge in terms of time and cost, for the separation of impurities from the chemically synthesised product in SPPS. This is particularly acute in the case of protein synthesis where the crude product mixture contains capped (acetylated) truncates of comparable size and structure to the protein product. Such a common situation results in extensive, and expensive, purification techniques such as large scale HPLC. To this end we designed a hydrophobic tag, Tbfmoc (2), which incorporated the base-labile characteristics of the Fmoc (3) group. The Tbfmoc group causes retention on hydrophobic columns and hence separation from untagged impurities. Although this was effective, we were of the opinion that a complementary tag was required which would have the characteristics of metal-complexation and cleavage by β-elimination for subsequent removal of the tag from the protein product after IMAC. Considerations implicit in the design of such an Nβ-terminal protecting group, or tag, will be discussed , and applications to the chemical synthesis of chemokines will be dealt with in the presentation. 1. R.B.Merrifield, J.Am.Chem.Soc., 1963, 85, 2149 2. R. Ramage and G. Raphy, Tetrahedron Lett., 1992, 33, 385 3. L.A.Carpino and G. Han, J.Org. Chem., 1973, 37, 3404

P10320-101

Solid phase synthesis of 4,5,8-trihydroxy-9,10-anthraquinone- 1-yl-(tuftsin or retro-tuftsin) derivatives

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Anthracycline antibiotics are an increasingly numerous group of compounds which have found a clinical application in treatment of leukemia or solid tumor. However, they can show a lot of negative effects what limited their application. Both high cardiotoxic activity and multi-drug resistant led scientists to research rather novel analogues avoid such disadvantages and possess better pharmaceutical properties [1,2]. Continuing our search for potential anticancer agents, we suggested the solid phase synthesis of anthraquinone linked to tuftsin analogues. We hope the covalent bond between tuftsin analogues and 1,4,5,8-tetrahydroxyathraquinone will improve anticancer activity of presented compounds.

Tuftsin-anthraquinone conjugates were synthesized using a classical Fmoc solid phase technique. Entering protected amino acids were

activated with DIC as the coupling reagent and the additive of HOBt in the presence of 1% Triton in DMF, DCM and NMP mixture. The modification of tuftsin chain was achived by introducing lysine residue, protected at ε-amino group with Mtt. The orthogonal group assured the obtainment of tuftsin analogues containing isopeptide bond. The condensation between leuco-1,4,5,8-tetrahydroxyanthraquinone and *N*-termini of peptide-resin was achieved during reaction in DMF under nitrogen and heated to reflux for 24h. Then peptidyl-anthraquinone-resin was oxidized in air at room temperature (3). Simultaneous deprotection of peptide side chain and the liberation from resin was achieved using standard TFA cocktail. The final products were purified by SPE and characterized by MS and ^{1}H-NMR.

Acknowledgement: This work was supported by the Polish State Committee for Scientific Research (Grant No. NN 405064134) and University of Gdansk (Grant BW No. 8000-5-0126-8).

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P10321-102

Solid phase synthesis of conjugates of tuftsin analogues with 1-nitro-acridine derivatives

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In last few decades, acridines which are known as anticancer, antimicrobal and antiviral agents have been in the center of interest of scientists. The heterocyclic molecules demonstrate a noteworthy group of compounds which interact with biological targets e.g. topoisomerase I, II [1,2]. We wish to pay our attention to a new group of tuftsin-acridine conjugates. Tuftsin offers a wide range of biological activities such as supporting of immune system, bactericidal, tumoricidal activity. However tuftsin is unstable in plasma which reduces its efficacy. That is the reason for search of new analogues that were more resistance to proteolysis degradation (3). Tuftsin-acridine conjugates were synthesized using a Fmoc solid phase strategy. The elongation of peptide chain was based on two-step procedure: deprotection and coupling reaction with DIC and the additive of HOBt in DMF/DCM/NMP mixture. Tuftsin analogues were modified at ε-amino group of lysine via introduction of the simple amino acids to obtain isopeptide bond. Tuftsin analogues were conjugated to the acridine molecule via flexible linkers. The carboxylic group of linker was connected to *N*-terminal group of peptide-resin using standard method for amide bond formation. The coupling reaction was performed with peptide-resin and the 4-fold excess of 1-nitro-acridine derivative using TBTU, HOBt in the presence of DIEA in DMF for 24h. Simultaneous deprotection of peptide side chain and cleavage from resin was done with the TFA cocktail. Final products were purified by SPE and characterized by elemental analysis, MS and ^{1}H-NMR spectroscopy.

Acknowledgement: This work was supported by the Polish State Committee for Scientific Research (Grant No. NN 405064134).

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P10400-103

Solid-Phase Peptide Synthesis at Elevated Temperatures - A Comparison of Conventional and Microwave Heating Technology

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The effect of microwave irradiation under controlled temperature conditions on the solid-phase synthesis of peptides was investigated. For optimization studies a model peptide (H-Gly-Ile-Leu-Thr-Val-Ser-Val-Ala-Val-OH) was selected which suffers from poor synthetic efficiency under standard SPPS conditions. Synthesis of the nonapeptide was performed using various combinations of solid supports (polysterene, Tentagel, ChemMatrix) and solvents employing Fmoc/But orthogonal protection strategy. Applying controlled microwave heating, the reaction times were significantly reduced while maintaining a high purity of crude product with no racemization being observed. The optimized microwave synthesis method was succesfully applied for longer, aggregated peptides. Microwave coupling and cleveage were accomplished in a dedicated reactor setup that allowed accurate internal reaction temperature measurment using a fiber-optic probe system. Comparison studies between microwave- and conventionally heated reactions will be presented.

P10401-104

Comparison of Microwave Mediated Peptide Synthesis in Comparison to Conventional Peptide Synthesis

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During the last few years microwave assisted peptide synthesis has became popular and it was reported to be useful in some cases. There is also an ongoing discussion about an additional “microwave effect”. Based on this background we have synthesized several model peptides on a microwave synthesizer and compared with the synthesis on conventional batch and continuous flow synthesizers with and without microwave irradiation. During this investigation we have also compared reaction rates and purity of the peptides synthesized under the influence of microwave irradiation or conventional heating on this different synthesizer systems by various conditions. In no case we can find any additional postulated “microwave effect”. The results of this investigation will be presented and discussed.

P10403-105

Enhanced Microwave Assisted On-Bead Disulfide Bond Formation Method. Synthesis of α–Conotoxin MII

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Conotoxins form a large family of peptide toxins from cone snail venoms that act on a broad spectrum of ion channels and receptors. The subgroup β-conotoxins specifically and selectively bind to subtypes of nicotinic acetylcholine receptors (nAchRs), which are targets for treatment of several neurological disorders. The aim of this work is to develop an improved method able to generate conotoxins in high yield and purity. This will overcome a key barrier currently preventing the efficient synthesis of small focused libraries in order to investigate the structure-activity relationship (SARs) of those peptides. The development of general synthetic strategies for the preparation of conotoxins and analogues are essential to efficiently approach important questions within the area of neurobiology and for the development of novel drugs for treatment of various neurological diseases. A new and highly efficient synthetic strategy for the synthesis of alpha-Conotoxin-MII has been developed. This strategy combine solid phase synthesis with microwave assisted heating to produce the two disulfide bonds peptide in high yield and purity. We first report here the use of microwave assistance in order

to form a disulfide loop. This technique demonstrates the advantage of preparing the first disulfide bridge while the peptides are resin bound. This step is critical to provide the dicyclic native peptide, that was performed by a followed classical in solution oxidation by iodine strategy. An enhanced total procedure for the synthesis of βCtxMII is recommended, which can be efficient applied at several small disulfide rich peptides of biological importance.

P10403-106

Solid phase peptide synthesis in aqueous environment using microwave assistance heating

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Since the first reports on the use of microwave heating more than 20 years ago, microwave-assisted organic synthesis (MAOS) has become an important tool for rapid and efficient synthesis of organic molecules. Microwave assisted heating has been further applied to peptide synthesis in order to accelerate the rate of synthesis and to improve the yields for the synthesis of difficult sequences. As far as water as solvent is concerned, numerous recent publications report the combination of water as an environmentally benign solvent for chemical transformations with the use of microwave irradiation as an efficient heating method. We report herein, the combination of the microwave-assisted heating and the use of water as solvent for solid phase peptide synthesis. A variety of common amino acids derivatives and coupling reagents have been studied in order to optimize coupling reactions in water by microwave-assisted heating. We also describe the total synthesis of a small peptide using water as solvent. The solid-phase synthesis using the environmental friendly aqueous medium dramatically reduces the cost of the synthesis and could be broadly applied in research or in industrial production of peptides.

P10403-107

New Developments in Microwave Assisted Solid Phase Peptide Synthesis

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The application of microwave energy for solid phase peptide synthesis (SPPS) represents a major breakthrough for overcoming incomplete and slow reactions typical of conventional SPPS. Microwave energy has been applied successfully in a manual and automated approach for enhancing synthesis of peptides and peptidomimetics. Common side reactions such as racemization and aspartamide formation have been studied and shown to be easily controllable with optimized methods that can be applied routinely. We will present the latest research on improving the synthesis of difficult peptides with microwave energy.

P10404-108

Synthetic antifreeze glycopeptides and analogues: synthesis, structural analysis, and functional studies

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Glycosylated peptides are involved in various biological processes such cell adhesion and differentiation. In contrast to mucin-type *O*-glycan peptides which are present on the membrane of mammalian cells antifreeze glycopeptides (AFGPs) are a little investigated example of glycosylated peptides containing similar components. AFGPs usually consist of a varying number of repeating units of (Ala-Ala-Thr) with minor sequence variations and the threonine hydroxyl oxygen glycosylated with the disaccharide β-D-galactosyl-(1-3)-α-*N*-acetyl-D-galactosamine. Antifreeze activity has been proven by different experimental observations like suppression of recrystallisation and ice nucleation, thermal hysteresis and change of the crystal habitus. Although it is known that the *N*-acetyl group at the C2 position of the galactosamine, the α-configured glycosidic bond to the threonine hydroxyl group and the γ-methyl group are essential, the adsorption mechanism is not yet understood. (1).
In contrast to fragment condensation, solid phase peptide synthesis gives the possibility to prepare one defined product and to introduce structure inducing amino acids such as proline. The disadvantage of SPPS with the bulky glycosylated amino acids is the low coupling efficiency. This problem was overcome by using more active coupling reagents and microwave-enhanced methods during coupling leading to sufficient coupling efficiency without having to apply great excess of the glycosylated amino acid.
After purification the peptide structure was examined by CD and NMR in water and DMSO at different temperatures. Additionally, the substances were microphysically analysed according to their recrystalisation inhibition activity and their influence on the crystal habitus.

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P10405-109

Peptide and Polyelectrolyte-Peptide Bioconjugate Synthesis by Microwave Assisted Method

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Microwave technology applied to SPPS has been recently proposed as valid support to the enhancement of coupling rates. We also used microwave energy in conjugation process between polyelectrolyte-peptide bioconjugation with EDC, HBTU. The use of peptide epitops of viruses particles in the composition of polymeric conjugates as vaccine has several potential advantages over whole viral or bacterial preparation. Recently, our group report a novel approach to a totally synthetic vaccine which consists of FMDV (Foot and Mouth Disease Virus) VP1 peptides, prepared by covalent conjugation of peptide biomolecules with membrane active carbochain polyelectrolytes In the present study, peptide epitops of VP1 protein both 135-161(P1) amino acid residues (Ser-Lys-Tyr-Ser-Thr-Thr-Gly-Glu-Arg-Thr-Arg-Thr-Arg-Gly-Asp-Leu-Gly-Ala-Leu-Ala-Ala-Arg-Val-Ala-Thr-Gln-Leu-Pro-Ala) and triptophan (Trp) containing on the N terminus 135-161 amino acid residues (Trp-135-161) (P2) were synthesized by using the microwave assisted solid-phase methods. Synthesis of peptides were performed by microwave assisted SPPS. Peptides characterized by LC-MS and purified by RP-HPLC. Bioconjugation between polyelectrolytes-peptide were synthesized by two different microwave assisted method. The first one is classical carbodiimid activating method. The second one is HBTU activating method. The second method is a novel and effective method for bioconjugation process. Peptides and polyelectrolytes-peptide bioconjugates analysed and comparised by GPC system with four dedector (UV. Refractive Index, Light Scattering, Viscosity).

P10417-110

Microwave-assisted solid-phase synthesis of peptide probes to detect specific biomarkers: shifting off limitations affecting conventional synthetic strategies

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Biomarkers play a key role in development of diagnostic/prognostic tools. In fact, since their involvement in several diseases such as autoimmune diseases (i.e., multiple sclerosis, rheumatoid arthritis) and neurodegenerative diseases as Alzheimer's disease, biomarkers represent a great promise for the development and set up of tuned therapies. For autoimmune diseases, we proposed the development of synthetic post-translational modified peptides as antigenic probes for characterization of specific and high affinity autoantibodies as biomarkers. By an innovative "Chemical Reverse Approach" we propose optimisation of antigenic probes by a statistically significant screening of sera guided by autoantibodies circulating in patients' biological fluids (1). SPPS *via* the building block approach is the principal strategy leading to modified peptides. High purity is a *condicio sine qua non* for efficient biomarker detection. These syntheses can present several difficulties as result of internal aggregation of resin-bound peptides during elongation steps, reducing reagents penetration, and significantly decreasing reaction rates in both acylation and deprotection steps. Such events strongly affect purity of the crude peptides and therefore, final yield. We demonstrated that application of microwave (MW) energy in SPPS of modified complex peptide probes exhibits several advantages improving coupling rates, possibly because of the decrease of chain aggregation during the synthesis (2). We report the MW-assisted synthetic conditions of difficult peptides (i.e. glycosylated, citrullinated, multiple antigenic, amyloidogenic peptides, etc.) by which we strongly improved preparation of diagnostic/prognostic probes in terms of crude purity, final yield, and time-consuming compared to conventional protocols.

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P10500-111

From Coiled-Coil Interactions to Chemical Biology

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The alpha-helical Coiled-Coil structure is a versatile protein-interaction-domain, in which the Coiled-Coil is composed of at least two right-handed amphipathic alpha-helices, which are coiled up around each other into a left handed supercoil. This widespread structural motif is involved in many biological procedures like transcription, scaffolding or signalling. In addition, the most characteristic and important identifying feature of a Coiled-Coil is the recurrence of a periodic heptad repeat sequence. Coiled-Coils are able to form complexes up to heptamers of different orientation. Based on this significant occurrence and also their structure Coiled-Coil peptides have the ability to function as molecular recognition molecules. Our goal is to use self-associating Coiled-Coil sequences as molecular building blocks (Lego brick). The ligation of desired functionalities on Coiled-coil sequences opens up the opportunity to add different functionalities on artificial complex peptide molecules that stick together via Coiled-Coil moieties. This makes Coiled-Coil sequences to useful tools during complex oligopeptide assembly. We want to present novel chimeric oligopeptides which are build up by two segments, one responsible for association the other for functionality. As a model for the association segment several GCN4-leucine zipper mutants (1) and as functionality segments amongst others WW domains were used. Both segments were linked by the native chemical ligation approach. We will present in detail the synthesis of a GCN4-leucine zipper mutant and also the following C-terminal thioester formation for the subsequent ligation step. Furthermore, we will present the synthesis of the functional device, a WW-domain with a N-terminal cystein residue and also the native chemical ligation of both synthesized peptide fragments supported by HPLC and Mass-chromatography.

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P10501-112

New insights into β2-microglobulin amyloidogenesis using site-directed-isotope labelling, Fourier transformed infrared spectroscopy and native chemical ligation

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Dialysis-related amyloidosis, a disease arising from long-term dialysis is characterised by gradual accumulation of β2-microglobulin (β2-m) amyloid fibrils in bones and ligaments. Although α2-m is known to form amyloid fibrils in vitro under acidic pH conditions, seeding of preformed amyloid fibrils or as an effect of ionic strength, the mechanism underlying aggregation of soluble β2-m into insoluble fibrils under physiological conditions is largely unknown. Interestingly, Eakin and co. recently showed that the chemical basis of the amyloidogenesis process is a backbone isomerisation of the conserved Pro 32. Our aim is to understand the molecular mechanism associated with β2-m amyloidogenesis using Fourier transformed infrared (FTIR) spectroscopy and site-directed-isotope labelling, a method in which the vibration of the labelled residue is shifted from its original position in the spectrum. We already demonstrated that FTIR spectroscopy along with site directed-isotope-labelling is a promising approach to obtain information on the microenvironment of tyrosine side chain residues.2 To use this approach in the case of β2-m amyloidogenesis, we decided to synthesise an isotopically labelled β2-m at crucial residues such as Pro 32 that should undergo drastic variations in their microenvironments during the misfolding. With its 99 residues, β2-m is over the limits of a reasonable synthesis using SPPS but the two suitably positioned cysteines in its sequence offer the possibility of a chemical synthesis using native chemical ligation. Thus, we were able to realise the chemical synthesis of an isotopically labelled β2-m in a good yield using a three segments strategy with disconnections at the two cysteines and thiazolidine as a masked cysteine for the middle segment. The synthetic β2-m obtained was in full agreement with the characteristic β2-m FTIR spectra.

P10503-113

A Novel Cyanophycin Synthetase from *Thermosynechococcus elongatus* BP-1 Catalyzes Non-Primer-Dependent Cyanophycin Synthesis.

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Cyanophycin (multi-L-arginyl-poly[L-aspartic acid]) is synthesized by cyanophycin synthetase (CphA). It was believed that cyanophycin synthetase requires Asp, Arg, ATP, Mg^{2+} and primer (low-molecular mass cyanophycin) for cyanophycin synthesis and catalyzes the elongation of low-molecular mass cyanophycin. Despite extensive studies of cyanophycin, the mechanism of primer supply is still unclear. In the present study, we searched for a cyanophycin synthetase that synthesizes cyanophycin from Asp and Arg without added primer *in vitro*. Cyanophycin synthetase from *Thermosynechococcus elongatus* BP-1 (Tlr2170 protein) was produced by an *Escherichia coli* gene-expression system as a C-terminal His-tagged protein. We found that Tlr2170 protein synthesized cyanophycin without added primer. The Tlr2170 protein had strict substrate specificity and used only Asp and Arg as substrates. The optimal pH was 9.0, and Mg^{2+} or Mn^{2+} was essential for cyanophycin synthesis. ATP could not be substituted by GTP, CTP, or TTP. The molecular mass of the Tlr2170 protein as estimated by gel-filtration chromatography was 400 } 9 kDa. Thus, the Tlr2170 protein appeared to be a homo-tetramer of 100-kDa subunits including the His-tag sequence. The Tlr2170 protein had thermal stability and fully retained its activity after a 15-min incubation at 60 ‹C. Additionally, we

examined cyanophycin synthesis at 30 ‹C, 40 ‹C, 50 ‹C, and 60 ‹C. SDS-polyacrylamide gel electrophoresis showed that the molecular mass of cyanophycin increased with increased reaction temperatures.

P10503-114

Synthesis of peptide thioester by Fmoc chemistry through hydroxyl side chain anchoring

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Since developed by Kent and co-workers in 1994 (1), native chemical ligation has been used to synthesize various natural polypeptides and proteins. Up to now the preparation of the C-terminal peptide thioester using Fmoc chemistry remains the limiting stage of this methodology. Several available approaches have been developed with the aim of circumventing the instability of thioester under standard Fmoc-SPPS conditions. Among them, the strategy which involves the side chain anchoring of trifunctional amino acids (2) seems the most attractive for the synthesis of long peptide thioester. In order to prepare a 53-mer peptide thioester with a serine at the antepenultimate C-terminal position, we set out to extend the side chain anchoring strategy to the hydroxyl side chain of serine. The 53-mer peptide thioester corresponds to the N terminal fragment of mitogaligin, a cytotoxic 97-residue protein. Our strategy, inspired by Hannesian (3) and Mayer (4) works involves the hydroxyl reaction of Ser or Thr to the trichloroacetimidate derivative of Wang resin, a stepwise elongation using Fmoc chemistry, the selective allyl ester deprotection, the solid phase carboxyl activation to couple an amino thioester, and a final TFA treatment to release the deprotected peptide from the resin. The feasibility of this strategy has been demonstrated using a short model peptide Ac-Ser-Arg-Ser-Thr-SR.(5) Special care was taken to minimize racemization during the amino acid thioester coupling. Then, the model peptide was ligated to the pentapeptide H-Cys-Thr-Trp-Ser-Leu-OH.

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P10503-115

Synthesis of Azapeptides by Chemical Ligation

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The substitution of amino acids by aza amino acids in a peptide has been shown to improve its stability towards proteases, to modulate its conformation or biological activity. (Melendez and Lubell, 2004).

For example, azapeptides have been recently described as hormone analogues (Boeglin and al., 2006), or protease inhibitors (Bailey and al., 2004)(Kato and al., 2005). Azapeptides are usually synthesized on solid phase. A method, permitting the convergent synthesis of azapeptides starting from unprotected fragments, would offer the possibility to study aza amino acids effect in complex polypeptides or proteins. We have focussed our study on ligation reactions leading to the formation of an azaGly residue at the ligation point, as Gly is a frequent amino acid in peptides or proteins.

The first synthetic strategy relies on the reaction between a peptide thioester and a N-terminal azaglycine peptide. Experimental conditions were found which permitted the chemoselective formation of azapeptide but with racemisation of the C-terminal amino acid of the thioester fragment.

Alternately, a synthetic strategy based on the chemistry of the phenylthiocarbonyl group, permitted the successful synthesis of azapeptide without racemisation.

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P10504-116

Recombinant Fragments for the Synthesis of Homogeneously Glycosylated Human Interleukin 6 by Native Chemical Ligation

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The investigation of the function of the glycosylation pattern of glycoproteins is still hampered by the absence of efficient methods for the separation and isolation of single glycoforms. Thus, the synthesis of homogeneously glycosylated proteins becomes increasingly important (1). Using native chemical ligation we have designed a semisynthesis of glycosylated Human Interleukin 6, a cytokine with pleiotropic functions in cell proliferation, immune response or thrombopoiesis. Due to the short half-life of recombinant, non-glycosylated IL-6 in the use as a thrombopoietic reagent (2), native glycoforms are of therapeutic interest. Our retrosynthetic strategy implies sequential native chemical ligation (3) and the split of IL-6 into three fragments. An N-terminal fragment IL-6 (1-42) thioester and the cysteine fragment IL-6 (49-183) were expressed in *E. coli*. The carbohydrate carrying fragment IL-6 (43-48) was synthesized via SPPS. To obtain the recombinant fragments specific termini were required. The N-terminal IL-6 (1-42) was fused between two inteins and after expression and refolding from inclusion bodies the target peptide was released as a C-terminal thioester after dual intein cleavage. The cysteine fragment IL-6 (49-183) was expressed as a single intein fusion also leading to inclusion bodies. This enabled the release of IL-6 (49-183) with an N-terminal cysteine after intein cleavage of the refolded fusion protein. The central segment IL-6 (43-48) contains a sugar residue attached to the *N*-glycosylation-site at Asn44. This fragment features both a C-terminal thioester and an N-terminal cysteine protected as a thiazolidine. Sequential ligations leading to the full length IL-6 glycoprotein will be presented.

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P10521-117

Novel synthetic approach to the prion protein: Kinetic study optimization of a native chemical ligation

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Prion proteins are suspected of causing several neurodegenerative diseases such as CJD, BSE etc. (1) Despite of extensive research in this area, there are still a lot of questions. Synthetic modified prion protein could solve many of those. Although recombinant prion protein is available, it is not easy to modify it. Moreover synthetic prion may solve viral (2) and protein-only hypothesis (3). Prions possess more than 200 AAs which disfavor straightforward peptide synthesis. It requires

syntheses of shorter fragments which are finally linked together (4). This "small building block approach" should also simplify synthesis of modified prion protein.

In our plan of mouse prion (MoPrP) synthesis, we have employed consecutive chemical ligations. We have started with MoPrP(203-231) in which it is possible to study native chemical ligation between cysteine in the position of 213 and methionine in the position of 21(2)

Several MoPrP(203-212) peptide thioesters with aryl and alkyl thiols were prepared for further study of the native chemical ligation of MoPrP(213-231) and MoPrP(203-212). The optimal ligation conditions were found by a kinetic study which was carried out in a range of pH and with various thiols. Influence of electron withdrawing and electron donating groups was studied in both aromatic and aliphatic thiols and it was found that it is possible to affect the ligation by choosing an appropriate thiol. Ligation optimal conditions, kinetic study results, and suitability of various thiols for the ligation are discussed.

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P10603-118

Low-cost industrial chemo-enzymatic synthesis of pharmaceutical, nutraceutical and diagnostic oligopeptides

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The production costs for oligopeptides and derivatives thereof by chemical synthesis are extremely high. Therefore DSM Pharmaceutical Products embarked on a research programme on chemo-enzymatic peptide synthesis. Important advantages of this technology are that no expensive stoichiometric coupling reagents nor side-chain protection are required and that no racemisation occurs. Focus is on elongation in the N ¨C terminal direction using novel enzymatic C-protecting group interconversion methods. One of the preferred building blocks are amino acid amides, but selective deprotection of peptidic C-terminal amides is a challenge. We discovered[1] that using a Peptide Amidase[2] C-terminal peptide amides can be directly converted to methyl esters in almost pure methanol. Thus, separate enzymatic hydrolysis and reactivation steps are no longer required. Other versatile building blocks are amino acid t-butyl esters. We discovered that peptide C-terminal t-butyl esters can be transformed, using commercially available proteases, to activated alkyl esters such as methyl- and benzylesters which can be directly used in another protease-mediated coupling reaction.[3] Furthermore, we found that using commercial enzymes side chain unprotected peptides with a free C-carboxyl terminus can be directly transformed to C-terminal thioesters[4] and C-terminal aryl amides[5] in the presence of the corresponding thiol and aniline, respectively. Finally, a real-life example of large-scale industrial chemo-enzymatic peptide production will be shown.

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P10700-119

Synthesis of antimicrobial peptide parasine I

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The advent of bacterial strains, resistent to currently used antibiotics, is the permanent necessity for searching new effective antimicrobial drugs. Antimicrobial peptides are very promising candidates since they kill target cells rapidly and specifically, and possess unusually broad activity spectra. In 1998 Park et al. (1) described parasin I - novel cationic peptide isolated from catfish (Parasilurus asotus) epithelia. It demonstrated strong antimicrobial activity against a wide spectrum of microorganisms without any hemolytic activity. Parasin I has a molecular mass of 2000.4 Da and consists of 19 amino acids, including three arginines and five lysines,which contribute to the net charge of +8. HLys-Gly-Arg-Gly-Lys-Gln-Gly-Gly-Lys-Val-Arg-Ala-Lys-Ala-Lys-Thr-Arg-Ser-SerOH

The current study deals with the synthesis of Parasine I for intensive biological study. We used the convergent synthetic strategy. The protected peptide fragments were synthesized with high purity by SPPS using Fmoc-chemistry on 2-chlorotritylchloride resin. The final couplings were performed both in solution and on resin. After all, target peptide was deprotected and purified by RP-HPLC. The mass of molecular ion determined by MALDI-TOF spectrometry was in good agreement with calculated value.

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P10801-120

Exogenous delivery and molecular evolution: peptides based on C^{α}-methylated α-amino acid as asymmetric catalysts in the syntheses of simple sugars

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It is known that chiral amino acids, as well as their dipeptides, may catalyze the asymmetric condensation of glycolaldehyde in water. On the basis of the particularly large erythrose enantiomeric excesses (*ee*) obtained when utilizing the chiral L-Val-L-Val catalyst and given the possibility of an abundant delivery of other types of amino acids to the early Earth, we have studied the catalytic effect on this synthesis of the peptides based on C^{α}-methylated α-amino acids, such as Iva (isovaline or C^{α}-methyl, C^{α}-aminobutyric acid) and C^{α}-methylvaline, (αMe)Val, that are abundant in meteorites.

Results of the catalysis experiments showed the all C^{α}-methylated peptides to the tetramer level exhibit significant chiral influence on the synthesis of tetroses and mimic the effect of the L-Val-L-Val catalyst in having a larger erythrose ee than threose ee, as well as in their configuration relationship with the sugars (the product erythrose acquires *ee* of configuration opposite to that of the catalyst in case of peptides, while it is the same for amino acids). Interestingly, the largest *ee* (45% for erythrose) was obtained with the Iva homo-tetrapeptide under mild conditions. The homo-dipeptides of both Iva and (αMe)Val also produced a significant *ee* (41% for erythrose) that appears to increase with time.

Because C^{α}-methylated amino acids are non-racemic in meteorites, do

not racemize in aqueous environments, and are known to be (3_{10})-helix formers in peptides with as few as four residues, these results suggest that meteoritic, C^{α}-methylated, α-amino acids may have contributed to molecular evolution upon delivery to the early Earth by catalytically transferring their asymmetry to other prebiotic molecules.

P11300-121

Synthesis' and application of peptide adhesives for wound healing

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Adhesives for medical application have special requirements in physical and chemical properties, for instance in respect to biological resorption. Degradation characteristics are important for application in surgery. General requirements for the application are biocompatibility, functionality and the avoidance of carcinogenic, mutagenic, toxic, and allergic reactions. Mussel adhesive proteins (MAP) are potential new adhesives. They are able to form permanent and strong but also flexible bonds with special amino acids. The products are biodegradable and nontoxic to the human body and do not exhibit immunogenicity. Mimicking MAPs oligopeptides with the structure [Tyr-Lys]n are synthesized. A new adhesive technique for fast occlusion of wounds basing on the polymerisation of such peptides by the help of polyphenoloxidases is described in patent (1). In earlier experiments, a composition of peptides with the structure [Tyr-Lys]n ; n = 4–25 was successfully used as adhesive. To test the required lengths of the oligopeptide, we synthesized peptides with several defined lengths, [Tyr-Lys]10 and [Tyr-Lys]15, by solid phase peptide synthesis on a 433A peptide synthesizer. Adhesive experiments with the 20 and 30 mer peptides show the same quality as the oligopeptide mixture. The new type of adhesives shows better cohesiveness than the adhesives available on the market. Possible applications are: - Wound healing - Fixation of small bone pieces after comminuted fracture - Augmentation of bone screws

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P11403-122

Synthesis, characterization and enzymatic degradation of peptide-triazole based polymers prepared by microwave-assisted click chemistry

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Natural polymers with repeating peptide sequences, like spider silk and mussel glue have interesting properties that can be used for biomedical applications. However, the isolation from their natural sources is rather difficult and the desire to introduce modifications necessitates the development of novel methods to synthesize such polymers with repeating peptide sequences. Recently, we have shown that the microwave-assisted copper(I)-catalyzed 1,3-dipolar cycloaddition can be used in the polymerization of the model dipeptide azido-Phe-Ala-propargyl amide. By varying the reaction conditions we could either obtain small cyclic oligomers (4-20 AAs) or linear polymers which consisted up to 300 AAs residues (1). To broaden the scope of this polymerization reaction, two novel biodegradable monomers, azido-Phe-Ala-Lys-propargyl amide and azido-Phe-Ala-Glyc-Lys-propargyl amide were polymerized. Both monomers are water-soluble and contain recognition sites for the proteases trypsin and chymotrypsin; moreover, the tetrapeptide can be chemically hydrolyzed depending on the pH of the solution. The molecular weight of the polymers could be tailored between 4 – 14 kDa (33 to 100 AAs residues). The enzymatic degradability of the polypeptides was monitored by a ninhydrin-based colorimetric assay and by MALDI-TOF. The results showed that the peptidic polytriazoles were smoothly degraded by trypsin and chymotrypsin. From these data we can conclude that the microwave-assisted copper(I)-catalyzed 1,3-dipolar cycloaddition reaction is an effective tool to synthesize biodegradable functional polymers which provides new opportunities to design (novel) biomedical materials. Details of monomer synthesis, polymerization reactions and degradation studies will be presented.

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P11502-123

Synthetic Peptides-What Kind of Peptide for Which Purpose?

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Peptide synthesis on cellulose membranes, known as SPOT synthesis, is an ideal tool to determine biological interactions and/or key positions in proteins. In general we can differ in two kinds of synthetic peptides with different possibilities:

1. Soluble peptides

Over the past 15 years synthetic peptide library techniques have emerged as powerful approaches to determine T-cell epitopes and to specify peptide binding to MHC-I and/or MHC-II molecules. The sequence-based approach is a straightforward method to directly identify T-cell antigens belonging to a specific target (e.g. a virus of interest) without the need of any further deconvolution steps. The entire protein sequence is presented as a set of overlapping peptides (peptide scan), which are subsequently screened for T-cell stimulation. Several CD-8 T-cell epitopes in selected CMV proteins have been identified using standard SPPS techniques. However, the peptide sequences synthesized by standard SPOT synthesis can only be cleaved without authentic C-termini (ß-alanine or glycine as C-terminal amino acid). Here, we summarized three different established methods to generate cleavable peptides with authentic C-termini in adequate amounts, with sufficient purity and within a justifiable time-scale.

2. Membrane bound peptides

The standard SPOT synthesis to generate membrane bound peptides use glycine or ß-alanine membranes to map epitopes of B-cells, allergenic proteins, antibodies etc. Furthermore, we have also established the "method of inverted peptides" to screen protein domains requiring peptidic ligands with free C-termini such as PDZ- or 14.3.3 domains. Another highlight of peptide libraries is the screen of posttranslational modification in proteins by phosphorylation at serine, threonine, and tyrosine residues which plays a role in eukaryotic cell cycle regulation, cell differentiation, apoptosis, or cytoskeletal regulation.

P11602-124

Construction of O-glycoside peptide libraries by Fmoc-SPPS using a building block strategy

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Labeled peptide libraries, consisting of α-helices, β-loops and β-sheets peptides, have been successfully prepared for use in peptide arrays that act as a protein-detection system and have been applied for protein-recognition studies [1-3]. It is known that more than half the proteins in a mammalian cell are post-translationally modified by such processes as phosphorylation, glycosylation, and acetylation. Such modifications play an important role in various bio-recognition, for instance glyco-

proteins are involved in cell-adhesion, infection, and biological protection. Hence the designed peptide library used for arrays has been expanded to include fatty acid attached peptides and glycopeptides. Fatty acids were easily introduced by conventional Fmoc-SPPS, while the synthesis of glyco-peptides was not easy. In the present paper we describe the efficient construction of O-glycoside structured and labeled peptides by Fmoc-SPPS using a building block strategy. Glycosylated threonine has been used as a building block and was prepared in large amounts. Glucose, mannose, galactose and lactose were acetylated and coupled to the hydroxyl group of Fmoc-Thr in the presence of BF3OEt2. The resulting protected sugar-amino acids were manually assembled on to the peptidyl resin containing a fluorescent dye using a Peti-Syzer® (HiPep Laboratories). After construction of the glycol-peptides the acetyl groups on the sugar were removed by hydrazine hydrate, and the peptides were then cleaved and purified. The resulting glycopeptides were characterized by LCMS. The present protocol has been used to prepare ca. one hundred glycopeptides that have been tested against various carbohydrate-binding proteins.

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P11716-125

Parallel small scale peptide synthesis meets a fast, low-cost purification method for the production of high quality peptide microarrays to analyze DNA/protein interactions

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Synthetic peptides are versatile tools for all kind of biological applications, e.g. for the analysis of protein-protein interactions, epitope binding, enzyme activity and so on.
Herein, a parallel synthesis in a small scale (0,1 µmol) which takes place in 384-micro well plates is demonstrated. With regard to a fast but yet efficient, low-cost purification in a parallel manner a ''Fmoc-on'' purification method, which is integrated into the synthesis process, was developed. Peptides were cleaved from the resin with their Fmoc-group still on. The crude products were transferred by extraction into another 384-micro well plate filled with purification material. Purification takes place due to the high affinity of the terminal Fmoc-group to this material. HPLC measurement shows a high recovery (98%) and purity (90%). Cleavage of the Fmoc-group during the purification process is also possible. Here, HPLC measurement demonstrated a recovery of 93% and a purity of 92%. A fully automated synthesis of more than 300 peptides in a 384-micro well plate combined with a parallel ''Fmoc-on'' purification is shown. The peptides were used for the production of microarrays to analyze DNA/protein interactions. The "Fmoc-on" purification allows easy and cost-efficient purification of peptides for all kind of biological applications.

P12001-126

Synthesis of desmicosin analogs containing peptides at 4'-position

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Desmycosin (Des) is an antibiotic-macrolide related to a group of tylosin (Tyl) which structure is based on 16-member lactone with carbohydrate substitutes attached. Macrolides are well known translation inhibitors, they bind to ribosomal tunnel (RT) in a way that their lactone ring is located orthogonally to the long axis of the RT, covering most of its cleft; hence, the mechanism of protein synthesis inhibition by macrolides relies on the mechanical obstruction they provide to the passage of nascent polypeptide chain through the RT. Recently we have designed and synthesized a number of peptide derivatives of macrolides where the peptide part modeled the growing chain, while the antibiotic served as an "anchor" for positioning the peptide at the specific site of RT. These derivatives are of interest both as antibacterial agents and as potential probes for investigation of the interactions of nascent peptide chain with the specific sites of the RT and their influence on the translation.
Now we report a new type of peptide - macrolide conjugates in which peptide binds by its α-amino function through a spacer to the 4'-hydroxyl group of the mycaminose residue of Des. Two proline-containing peptides are chosen: GP and GGP. Proline residues of these peptides are supposed to interact with the peptidyl transferase center region when the complexes of these 4'-peptidyldesmycosins with bacterial ribosomes are formed. The first step of the synthesis was acetylation of 2'-, 2''-, 4''- and 4'''-OH-groups of Tyl by Ac2O in pyridine following by hydrolysis in 1N sulphuric acid resulted in Des with all protected OH-groups except 4'-OH-group of mycaminose. This free hydroxyl group was used for reaction with succinic anhydride leading to formation of reactive carboxyl group. The next step was a reaction between this carboxyl function with α-amino groups of peptide methyl esters. The structures of new 4'-peptide derivatives of Des were proved by MS MALDI and NMR-spectroscopy.

P12104-127

Synthesis and Characterization of Hemagglutinin 98-106 (YPYDVPDYA) and Biodegradable Polymer Conjugates As a Peptide Based Vaccine Prototype for Influenza Virus

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In this study, the synthesis of a biodegredable polyelctrolyte, poly(1-aza bicyclo[4.2.0]octane), (PC), which has an heteroatom in its structure and their bioconjugates obtained by microwave and carbodiimide methods were explained. For PC obtaining, the synthesis of the monomer, 1-azabicyclo[4.2.0]octane, (C), was carried out by organic methods which then will be used in polymer synthesis [1-2]. Consequently, polymers having different molecular weights and their water soluble bioconjugates were synthesized and the characterization of these polymers and conjugates were done by different methods such as ESI LC-MS, UV and ATR FT-IR spectroscopy. For the chemical modification of PC, bromoacetic acid was used and a water soluble polyampholyte synthesis was achieved with the quaternization of polymer. It is determined that molecular weights of the polymers were affected by the change in the amount and the type of initiators used and also from the polymerization media. Analysing of having biodegradable characteristics of the synthesized polymers has been being reviewed. ATR FT-IR spectra of PC and PC-hemagglutinine peptide was recorded. For peptide synthesis we have used microwave assisted solid phase peptide synthesis method. LC-MS was used to determinate of molecular weight of hemagglutinin. After we have obtained m/z values of peptide we used Preparative HPLC for purification of hemagglutinin. After synthesis and modification of PC, its covalent conjugates with Hemagglutinin (HA 98-106) were obtained by carbodiimide activation. Its characterization was also performed.

P12900-128

Reactivity of the phosphonium salts in front of the aminuim salts in peptide synthesis

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The peptide coupling reagent field has clearly evolved in the last decade from carbodiimides to onium (phosphonium and uronium) salts. The era of industrial coupling reagents began in 1955 with the introduction of dicyclohexylcarbodiimide (DCC), which at that time was already known and well studied, as a reagent for the formation of amide bond. Unfortunately, carbodiimides did not comply with the concept of ultimate coupling reagents because its high reactivity provokes racemization and side reactions during the coupling reaction. At the beginning of the 70's, 1-hydroxybenzotriazole (HOBt) was proposed as an additive to DCC to reduce racemization and from then on other benzotriazole derivatives such as 1-hydroxy-6-chlorobenzotriazole (Cl-HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt) have also been used. The OBt active esters are less reactive than the O-acylisourea, but are more stable and less prone to racemize. The use of the most reactive aminium salt, HATU , is inconvenient because of the price, which makes its use detrimental for industry. HCTU/TCTU, based on Cl-HOBt, are a good alternative to HBTU/TBTU, because of the presence of the chlorine atom that stabilizes the structure, hence, making these reagents less hazardous. Herein, PyClocK, the phosphonium salt of the Cl-HOBt is introduced

P20100-001

N-Methylation of cyclic Enkephalin analogues

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N-Methylation of biologically active peptides is of interest because it results in increased conformational integrity and stability against enzymatic degradation. Furthermore, *N*-methylated peptides have a decreased ability to form H-bonds to water molecules and, consequently, a better ability to cross biological barriers. This is exemplified by the naturally occurring peptide cyclosporine which is orally active. In an effort to improve the blood-brain barrier permeability of the cyclic enkephalin analogues H-Dmt-c[D-Cys-Gly-Phe-D(or L)-Cys]NH_2 (Dmt = 2',6'-dimethyltyrosine), we prepared analogues that were *N*-methylated at Phe^4 and/or Cys^5. *N*-Methylated Cys derivatives were prepared either by direct methylation (CH_3I)/NaH) or by using oxazolidinone chemistry. Single *N*-methylation of the two cyclic peptides at Phe^4 or D(or L)-Cys^5 produced four analogues that all showed very high mu and delta opioid agonist potencies in the guinea pig ileum and mouse vas deferens assays. The two analogues N-methylated at both Phe^4 and D(or L)-Cys^5 also retained high agonist activity at both receptors. Results from molecular mechanics and molecular dynamics studies on the conformational behavior of these peptides will be presented.

P20100-002

New amide analogues of isoform 2 and 3 of antistasin

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During the last years the number of deaths due to hemostatic impairments such as coronary angioplasts, coronary thromboembolisms, myocard heart attack, pulmonary embolism etc. has become equal to those caused by cancer formations. Haemostasis is a key process whose correct functioning is an important defence mechanism of the human organism. It is a blood coagulation process activated in case of injury of the blood system. If it is functioning correctly vascular-motor and cell reactions are triggered and the blood coagulation cascade is activated. One of the most important enzymes in the blood coagulation cascade is Factor Xa. That's why its inhibitors are promising alternative against thrombotic disorders. In our previous work, we reported the synthesis of hybrid structure between isoform 2 and 3 of antistasin and the active sequences D-Phe-Pro-Arg; D-Arg-Gly-Arg; Phe-Ile-Arg and Tyr-Ile-Arg (1). Beside the analogues with C-terminal COOH group, the peptide D-Phe-Pro-Arg-Pro-Lys-Arg-NH2 was synthesized. The biological activity of the last one was 60 times bigger than natural isoform 3 of ATS and some times more active than all other synthesized analogues. In the current work we described synthesis and biological activity of C-terminal amide analogues of all early synthesized peptides in order to deduce the structure-activity relationship. The anticoagulant activity according to the APTT and IC50 was determined.

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P20100-003

Synthesis of the S129-I229 fragment of the extracellular domain of the VEGF receptor 1 embedding the ligand binding site.

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The neo-angiogenesis is the formation of new blood vessels from a pre-existing vasculature which enables the supply of the nutrients and oxygen necessary for tumor growth. Consequently, inhibiting the blood vessels sprouting in order to starve the tumour constitutes an attractive anti-tumoral therapy. The vascular endothelial growth factor or their receptors (VEGFR-1 and 2) are key mediators of neo-angiogenesis and constitute nowadays validated targets for anti-angiogenic therapies (1). Despite this fact, strategies aiming to develop pure receptors antagonists and not inhibitors of the tyrosine kinase activity remain scarce. We have previously designed antagonists that interact with the extra-cellular domain of VEGF receptor 1 and thus prevent VEGF binding (2). These antagonists were proved to be potent and able to interfere with the VEGF signalling pathway. Because these compounds were rationally designed by using the co-crystallized structure of VEGF with the immunoglobulin like fragment 2 of VEGFR-1, we raise the following question: do these antagonists really target the immunoglobulin like domain 2 of VEGFR-1? In order to respond to this question but also with the future aim of optimizing our antagonist in a rational way we need to co-crystallize at least one of our antagonists with the d2 fragment of the VEGFR-1. Furthermore, since human VEGFR-1 d2 is a potential target in the development of angiogenesis modulators, the availability of this protein domain, and mutants, should be useful in the screening and development of novel specific ligands. Up to now, the 101-amino acid polypeptide chain of VEGFR-1d2 has been only produced by gene expression (3). Here, we report the first solid phase peptide synthesis of the VEGF-binding domain of the VEGF receptor 1 and the in vitro experiments which permitted to verify its biological activity.

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P20100-004

Short Substrate-Based Peptides as Inhibitors of Human Sirtuins 1 and 2

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Sirtuins are potential targets for drug discovery. They are a conserved protein family and are found in all domains of life. There are seven human sirtuins (SIRT1-7), which belong to the class III of histone deacetylases. Apart from histones, sirtuins deacetylate also other proteins and regulate diverse cellular functions. For example, SIRT1 deacetylates histone H4 and several important transcription factors like tumor suppressor p53, FoxO3, PGC-1α and NF-κB. SIRT1 activation has been linked to the extension of life-span while SIRT1 inhibition was recently shown to decrease tumour growth.1 SIRT2 deacetylates α-tubulin and knockdown of SIRT2 results in α-tubulin hyperacetylation. A variety of drug treatments modulate the level of tubulin acetylation, including the anticancer drug paclitaxel. SIRT2 inhibitors have potential as anticancer drugs.1 Recently, it was observed that a SIRT2 inhibitor protected against α-synuclein-mediated toxicity in a cellular model of Parkinson disease.2

The deacetylation reaction catalyzed by sirtuins is a NAD+ dependent reaction where an acetylated lysine residue of a substrate protein and a cofactor NAD+ are converted to a deacetylated substrate protein, nicotinamide and 2'-O-acetyl-ADP-ribose. Thioacetylated substrate-based peptides consisting of 18 or 11 aminoacids have been shown to inhibit human sirtuins.3,4 We have synthesized a series of substrate-based short peptides which possess a thioacetylated lysine residue. The most potent peptides inhibit SIRT1 at submicromolar and SIRT2 at low micromolar level. The interactions of the peptides with sirtuins are studied by molecular modeling. These peptides serve as a promising starting point for the development of peptidomimetic SIRT inhibitors.

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P20100-005

A Useful Scaffold For β-Turn Scan In Peptides. Enkephalin And Morphiceptin Analogues Containing a 4-Aminopyroglutamic Acid Residue

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Different types of turns are important elements of secondary structure in peptides and proteins. The most common ones are different kinds of β-turns involving four consecutive amino acid residues. The dipeptide unit containing the second and the third residues of such turns exists in the *cis*-conformation. Our *cis*peptide bond motif 4-aminopyroglutamic acid promotes the β-turn type VI/VI', and can be treated as a hybrid of glycine and alanine. This feature prohibits its utilization as a replacement for any possible dipeptide unit. In order to convert our compound into a more valuable tool for probing the existence of a particular peptide bond in the *cis*-conformation, we have elaborated the synthesis of N-monoalkylated derivatives of 4-aminopyroglutamic residue through a reductive alkylation reaction performed on solid support. Following this idea, we have obtained analogues with N-benzylated and N-(p-hydroxy)benzylated 4-aminopyroglutamic residues as scaffolds for the Phe-Ala and Tyr-Ala dipeptides units, respectively. Only one of 12 enkephalin analogues, [N(Bzl)-(R,R)-apy4-5]- enkephalin amide, possesses similar μ agonist potency as Leu-enkephalin in the GPI assay, indicating that the C-terminal part of this peptide may assume the *cis*-conformation upon binding to the receptor. This is the first active opiod analogue containing a 4-aminopyroglutamic acid residue. Supported by KBN (Poland) grant 2 P05F 00129 and NIH (U.S.) grant DA-04443

P20100-006

Non-protein amino acid analogues of melanocyte inhibiting factor (MIF-1): Synthesis and effects of nociception

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MIF-1 (L-Prolyl-L-Leucyl-Glycine amide, PLG) is a brain peptide, presented in hypothalamic tissue, as well as the C-terminal tripeptide of the neurohypophiseal hormone oxytocin and inhibits the release of melanocyte-stimulating hormone (MSH) in some systems. Recently, some chemical modifications of MIF-1 to enhance opiate agonist/antagonist actions as well as binding activity of analogues have been reported. On the other hand, the concept of structural modification in peptide fragments to confer them specific properties is of current interest in the study and design of new bioactive targets. A well known example is the incorporation of unnatural amino acids into the molecule of natural biologically active peptides leading to analogues with significant theoretical and practical importance. With this idea in mind we studied possibilities of introducing unnatural amino acids canaline (Can), nor-canaline (NCan), canavanine (Cav), nor-canavanine (NCav) and sLys into MIF-moiety in order to achieve a better analgesic effect. To obtain the peptide mimetics, both Fmoc- and Boc-based SPPS approach were used. Analgesic activity was determined by the paw-pressure (PP) and HP tests. The experiments were carried on male Wistar rats. The changes in the mechanical nociceptive threshold of the rats were measured by the Randall-Selito paw pressure test using analgesimeter (Ugo Basile). It was found that substitution of Leu in position 2 of MIF-molecule by unnatural amino acids increased the pain threshold. The analgesic effect with Cav-substitution was highest, whereas parent MIF-1 showed only a minute increase of pain-threshold.

P20100-007

In vivo and *in vitro* activities of new L-Valine derivatives: Structure-activity relationships

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Four compounds, derivatives of L-Valine, were studied as potential drugs. The compound structures differ by the position of L-Valine connected by amide bonds to pyridine residue in m-(M) or p-(P) positions as well as the length of the spacer (3 and 6 methylene groups) between the L-Valine terminal groups. In experiments in vivo (in Albino mice) and in vitro (on cell cultures) the compounds showed low toxicity. At the same time they demonstrated neuropharmacological activity on learning and memory, orientation and nociception in doses 125 and 250 mg/kg b. wt. The compound M6 had the strongest effect on learning and memory and the compound P6 showed the strongest and fastest analgesic effect. The compound M6 and P6 were also able to modify the effect of the model CNS-drugs (hexobarbital and pentylenetetrazole). Theoretically and experimentally determined octanol/water logP values of the compounds correlate with their CNS-effects. M6 and P6 had higher logP than M3 and P3 and showed better antinociceptive and anticonvulsant activity in vivo. Compounds possessed also chelating activity towards Fe ions. The stronger chelating activity of the compounds with the 6-spacer clearly correlated with their better neuropharmacological activity in vivo (in comparison to the compounds with the 3- spacer). The position isomery may also contribute for the variations in their pharmacological activity. The limited number of the compounds does not allow derivation of well-defined structure-activity relationships, however, their 3D models show possibility for many low energy conformers with different atoms involved in formation of Fe chelating complexes.

P20100-008

Synthesis of β^2-amino acids and their application in endomorphin analogues

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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 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 β^2-amino acids. The procedure involves Knoevenagel condensation of the methyl cyanoacetate and aromatic aldehydes (for aromatic path) or alkylation of the methyl cyanoacetate with various alkyl halides (for aliphatic path) at first then reduction and Boc-protection (performed in one pot) of the resulting first step-products.

We focused our attention on applying above method to the synthesis of different β²-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 in every position with exception of Pro were substituted by their respective β²-analogues. As templates, endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2), endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) and its D-Ala2-analogue (TAPP) have been used. In this communication the pharmacological consequences of such modification in endomorphins will be discussed.

P20102-009

Solid-phase synthesis and effects of amino acid and peptide analogues of non-protein amino acid Canavanine on nociception

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Non-protein amino acids have been widely used as components of peptides to enhance biological activity, proteolytic stability, and bioavailability. It is well known that unnatural amino acids with guanidine functionality exhibit diverse pharmacological effects when introduced in biologically active systems. Our previous efforts were focused on the preparation and the characterization of unnatural amino acids, particularly those containing a basic functionality in the side chain. We have synthesized numerous unnatural amino acids, structural analogues of arginine and lysine, which demonstrated certain biological effects. Recently, as part of our ongoing research focused on the search of novel arginine mimetics, we developed an efficient approach for solid-phase synthesis of unnatural amino acids nor-canaline (NCan) and nor-canavanine (NCav). Next we studied the possibilities of introducing NCan and NCav into the molecule of biologically active peptides. We also studied their antinociceptive effects using the paw pressure (PP) and HP tests.

P20102-010

Development of a synthetic receptor for trace determination of endocrine disruptor chemicals

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In the last few decades, considerable attention has been devoted on the potential role and activity of certain environmental pollutants (EDCs) in increasing anomalies that involve the endocrine system of wild species and Man. (1)The development of a fast high-throughput detection system for the quantitative analysis of EDCs requires the development of a novel sensitive solid phase EDCs extraction method. Because of the high affinity of EDCs with the estrogen receptor, this has been greatly investigated. This study has leaded the recognition of the amino acids located in the ligand binding domain of the receptor which interact with EDCs. Use of a dipodal scaffold molecule and different combination of the crucial amino acids, will allow the generation of a library of small to medium size biomimetic receptors.
In this communication, we will disclose our first results in the preparation of a small library of biomimetic receptors, with and without the dipodal scaffold, to evaluate their affinity towards EDCs and the role played by the scaffold structure.

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P20102-011

Synthesis of a peptidomimetic library mimicking the hormone binding domain of the extrogen receptor

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Endocrine disrupting chemicals (EDC's) are an important class of pollutants, which have in common that they show affinity for the hormone binding domain of the estrogen receptor, thus disturbing the endocrinal system. Detection in waste water has not been succesful due to their low concentration. Therefore, new sensitive screening methods are highly demanded. A possible solution is the use of estrogen receptor mimics for affinity chromatography. To this end, scaffold 1 will be synthesized and used for building a tetrapodal peptidomimetic library. Amino acids known to be important for the estrogen-receptor interaction will be preferably incorporated. First of all, we set out to prepare one dipodal peptide library member 6. The orthogonally protected scaffold 2 was synthesized in one single step starting from the commercially available 3-amino-5-nitrobenzoic acid.2 Using Fmoc solid phase chemistry on Wang resin as the solid support, this dipodal scaffold allowed the attachment of two different oligopeptide chains.Employing this methodology the synthesis of a small library will be performed, and the affinity of the different peptidomimetics for EDC's will be investigated.

P20104-012

On–resin microwaves–assisted Ring Closing Metathesis for the synthesis of Octreotide dicarba–analogues

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We synthesised via RCM (Ring Closing Metathesis) several Octreotide dicarba–analogues lacking the disulfide bridge, of the general formula H–D–Phe^2–c[Hag^4–Phe^7–D–Trp^8–Lys^9–X^{10}–Hag^{14}]–$Thr(ol)^{15}$–OH (X= **1**: Thr; **2**: Phe; **3**: Tyr(Bzl)) (SRIF numbering) [1;2]. Octreotide is an antitumoral agent used mainly as a carrier of radionuclides for cancer diagnosis and therapy. It shows the same disulphide bridge of the parent SRIF that is prone to be opened by oxidizing and reducing agents. This prompted us to search a more stable tether bridging the active motif of the cognate molecule. The premier reaction of RCM was performed in an oil bath under severe experimental conditions i. e. anhydrous argon atmosphere and long reaction times [3;4]. The microwaves assisted version was, instead, efficient for the cyclopeptides yield and required very short reaction times. We also evaluated the efficacy of different Grubbs catalysts in the microwaves assisted RCM, operating in different conditions of temperature and time.

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P20107-013

Potential inhibitors of dapE and argE enzymes as the new antimicrobial agents: Synthesis and characterization

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In the search for potential antimicrobial drugs, we have been dealing with two microbial enzymatic systems: N^{α}-succinyl-L-diaminopimelic acid desuccinylase (dapE [1,2]) and N^{α}-acetyl-L-ornithine deacetylase (argE [3,4]), which might be promising targets for potent and selective enzyme inhibitors based on the modification of N^{α}-succinyl-diaminopimelic acid (DAP) and N^{α}-acetyl-ornithine (Orn). The inhibition of both the enzymes would possibly interrupt the pathways leading to development of bacteria due to a role of *meso*-DAP as essential component of peptidoglycan based bacterial cell walls and Orn, as well, being a component of biosynthetic pathway for arginine that can serve as a source of both the carbon and nitrogen in microorganisms. Our effort was focused on the synthesis and characterization of two series of N^{α}- substituted derivatives of DAP and Orn, potentially interfering with the hydrolytic action of dapE and argE in the processes of bacterial growth. In this introductory study, the compounds prepared were also assayed against bacterial strains *Escherichia coli* and *Bacillus subtilis* and inhibitory activity of Orn derivatives was found with regard to differences in the structure of N^{α}-substituent.

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P20107-014

New bradykinin antagonists – influence of the C-terminal modifications on their pharmacological properties

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The first report on practically effective bradykinin (BK) antagonists for B_2 receptors was published in 1984. The key to conversion of bradykinin into an antagonist was replacement of Pro^7 with an aromatic D-amino acid; D-Phe was first used. However, our studies demonstrated that the D-amino acid residue at position 7 of the bradykinin antagonist, until recently considered to be necessary for B_2 antagonism, can be replaced by suitable L-amino acid or achiral residue or, together with the amino acid occupying position 8, by a sterically restricted dipeptide unit. Having all this in mind we synthesized and bioassayed two new analogues of bradykinin. The peptides were designed by substitution of position 7 of bradykinin B_2 receptor antagonist ([D-Arg^0,Hyp^3,$Thi^{5,8}$,D-Phe^7]BK), previously described by Stewart's group, with structural isomer of proline: β^2-iso-Pro or its homologue: β^3-homo-Pro. It's worth emphasizing that position 7 in bradykinin molecule is occupied by proline residue.

Our previous results demonstrated the importance of the position in the peptide chain into which the sterically restricted 1-aminocyclohexane-1-carboxylic acid residue (Acc) was inserted. These findings prompted us to investigate how introduction of L-pipecolic acid residue (L-Pip) in position 7 or 8 of Stewart's antagonist will affect pharmacological properties of resulting compounds. In comparison to the Acc residue, the ring of L-pipecolic acid also consists of six atoms, but includes the nitrogen atom.

Bearing in mind that acylation of the N-terminus of several known B_2 blockers with a variety of bulky groups has consistently improved their antagonistic potency in the rat blood pressure assay, the aforementioned four analogues were also synthesized in the N-acylated form with 1-adamantaneacetic acid (Aaa).

The activity of eight new analogues was assessed in isolates rat uterus and in rat blood pressure test

P20109-015

Modifications of the Myelin Basic Protein epitope MBP87-99 divert Th2 to Th1: Immune responses in peripheral blood mononuclear cells (PBMC) from Multiple Sclerosis patients.

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Postranslational modifications (citrullination, phosphorylation, deamidation, methylation, glycosylation) are common biological processes that alter specific parts of a protein after synthesis. Nearly all known proteins undergo some form of postranslational modification and almost all amino acids can be altered by one or more of these processes. The modified protein thus, contains new or rare amino acids or new specific side groups that can have critical influence on the structure and function of the protein molecule. Conversion of arginine to citrulline, an important postranslational modification, was first described by Fearon. Arginine residues in proteins can undergo this modification and the resulting citrulline remains part of the protein in the position of arginine. Citrulline is not a natural amino acid in proteins, and may induce immune responses. Such responses have been recently implicated in the pathogenesis of autoimmune/inflammatory diseases such as MS and rheumatoid arthritis 1. Herein we investigated cytokine secretion in peripheral blood mononuclear cells (PBMC) of 7 MS patients and 7 controls, and attempted to correlate cytokine polarization with the nature of the antigenic stimulus. We synthesized peptide analogs that map to the myelin basic protein (MBP) wild type epitope 87-99 (P1): a linear [Arg91, Ala96] MBP87-99 analog (P2), a cyclo(87-99)[Arg91, Ala96] MBP87-992 analog (P3), a linear [Cit91, Ala96, Cit97] MBP87-99 analog (P4) and a cyclo(87-99) [Cit91, Ala96, Cit97] MBP87-99 analog (P5). Analogs P4 and P5 resulted from the citrullination of the 91 and 97 arginine residues in epitopes P2 and P3. We then tested MS and control PBMC with various concentrations of the peptides and investigated cell proliferation by the BrdU proliferation assay and cytokine secretion by ELISA. We suggest that citrullination of self-antigens maybe an important step in triggering disease in susceptible individuals.

P20111-016

Incorporation of aza-β3-amino acid into 26RFa(20-26), the endogenous ligand of GPR103 : Structural Analysis.

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Aza-β3-peptides, mixing α- and aza-β3-amino acids (the aza analogs of β3-amino acids), represent a novel and exciting type of peptidomimetics.1 In particular, we have shown that aza-β3-amino acid induces a N-N or hydrazino turn, stabilized by an eight-membered-ring intramolecular hydrogen bond between the carbonyl acceptor group of the residue i-1 and the amide proton of the residue i+1. Interestingly, this N-N turn promotes a well-defined γ-turn formation (hydrogen bond between the CO of the residue i-2 and the hydrazidic proton of the aza-β3-moeity) when an α-amino acid is foregoing.2 26RFa, a novel neuropeptide of the RFamide superfamily, exhibits high affinity for GPR103 and induces a potent orexigenic effect in mice.3 In biomimetic environment, 26RFa encompasses an α-helix between Pro4 and Arg17 residues and a canonical γ-turn centered on Ser23. 26RFa(20-26), whose sequence is strictly

conserved across species, is about 100 times less potent than 26RFa. This heptapeptide shows important distortions of the γ-turn that may be responsible for its weak potency. The aim of this study was to restore the γ-turn formation in 26RFa(20-26) (GGFSFRF-NH2) by the presence of an aza-β3-amino acid. For this purpose, we have (i) synthesized the aza-β3-counterpart of each residue of 26RFa(20-26), (ii) individually incorporate the surrogate into the heptapeptide and (iii) investigated the 3D structure under NMR restraints of the hybrid peptides. The results will be presented with a particular attention on the serine position.

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P20111-017

The analysis of the structure-antithrombotic activity correlation for peptide receptors of adhesive glycoproteins with the general formula Arg-Xaa-Asp

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Structural analogues of the Arg-Gly-Asp sequence with the general formula Arg-Xaa-Asp, where Xaa – Ala, D-Ala, β-Ala, γ-Abu, ε-Ak, Pro, D-Pro, Asn-Trp, were synthesized using classical methods of peptide chemistry in solution, the levels of their antithrombotic activity were determined and stable conformations were calculated using a pairwise–additive approximation method. Interatomic distances in the obtained conformers were calculated between various atoms in the functional groups, including carbons in the carboxylic groups, nitrogens in the α-aminogroups, amino and imino nitrogens in the guanidine group, 16 distances in total. The analysis of correlation of the calculated interatomic distances and measured values of antithrombotic activity was carried out using statistical methods. We show that the distance between the amino nitrogen of the guanidine group of arginine and the β-carboxyl carbon of aspartic acid determines the antithrombotic activity. It has the optimum value in the Arg-β-Ala-Asp peptide, which was the most active among the synthesized analogues of the Arg-Gly-Asp sequence (Ic50 – 10.6 mkM, ADP, 1.5 mkM).

P20113-018

Microbial proteases with narrow specificity as an instrument of peptide chemistry

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The problem of selective removal of short N-terminal peptides from the gene-engineered proteins is actual by many reasons. At first it is connected with gene-engineering method of protein preparation in form of its precursors. The problem of N-terminal formyl-Met removal from gene-engineered proteins, which are produced by bacillacae may be solved by introduction of specifically-cleaved insert after formyl-Met. Subsequent specific removal of such short N-terminal peptide results in mature protein. Moreover, the analysis of cleaved short peptides could characterize the process of mature protein formation. We suggest microbial enzymes with narrow specificity to solve the mentioned problem. For this aim we have isolated the trypsin-like enzyme and postproline-specific endopeptidase from Aspergillus sp. and glutamyl-specific protease from B. intermedius. These enzymes have high specificity approved by hydrolysis of short synthetic peptides and long polypeptides. For example, specificity of postproline protease was established by hydrolysis of mellitin. The final peptide mixture was analyzed by RP-HPLC and mass-spectra. The use of enzymes with narrow specificity is demonstrated in the analytic method of detection of formyl-Met presence at the N-terminus of gene-engineered α-interferon. Trypsine hydrolysis of α-interferon leads to more than 14 peptides are formed including the 12-membered N-terminal peptide. Being hydrolyzed with glutamyl-specific enzyme α-interferon is converted to 6 peptides, including 41-membered N-terminal peptide. Application of postproline-specific enzyme for α-interferon hydrolysis leads to formation of 1 short 4-membered peptide and 4 long fragments. Experimental data show that in case of analytic control of α-interferon maturity the utilization of postproline-specific protease is preferable. In other cases the enzyme selection is ruled by the features of protein primary structure.

P20114-019

Bimodal action of cystatin related epididymal spermatogenic (CRES) protein and its reactive site loop derived S-S bridge bicyclic peptides on proprotein convertase-4 (PC4) activity

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Proprotein Convertase4 (PC4) is a member of Ca2+-dependent mammalian subtilase Proprotein Convertases (PCs) or Proprotein Convertase Subtilisin Kexins (PCSKs). PC4 plays a key role in mammalian fertilization, sperm maturation and sperm-egg fusion. Several precursor proteins found on sperm surface and reproductive tissues were proposed or confirmed as substrates of PC4. These include ADAM proteins, growth factors proIGF1 and 2 and hormonal protein PACAP. Lack of PC4 leads to impaired fertility in mice, suggesting its important role in reproduction. PC4 is thus considered an important target for development of nonhormonal contraceptive agents. PC4-inhibitors are expected to find therapeutic, clinical and biochemical applications. A lot of interest has grown to develop specific PC4 inhibitors. Recently natural inhibitor of serpin family have been described for PC1, 2, and furin, but not for PC4. However, a new serpin, CRES, has been reported from epididymis fluid, where PC4 may be found. So far, CRES has only been shown to inhibit PC2, which is not present in reproductive tissues. We propose that CRES may represent a natural regulator of PC4, based on localization and other studies. In this study we generated recombinant human CRES protein (139 aa), and its reactive-site loop (RSL) derived acyclic and cyclic 43-mer peptides with various S-S linkage combinations. CRES inhibited PC4 with IC50 ~50uM, while RSL-peptides were found to be more potent with IC50 50-250nM, depending on the S-S linkage locations. Furthermore, we noted that at lower concentrations, CRES and its peptides first enhanced PC4 activity before any inhibition occurred. This bimodal behavior may be due to cleavage. Molecular modeling showed strong interactions between CRES and PC4 via some of their key residues. Overall, we noted that "RSL" is the most crucial element required for PC4 inhibition by CRES.

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P20114-020

Design, Characterization and Applications of Conformationally Restricted Peptides

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The de novo design of peptides and proteins has assumed considerable interest didehydroresidues, in particular alpha,–in the recent years. alpha, beta- beta-didehydrophenylalanine (deltaPhe) are being considered important conformational constraints inducing tools in de novo peptide design. deltaPhe is a noncoded, achiral residue, an analog of the naturally occurring phenylalanine amino acid with a double bond between Calpha and Cbeta atoms. Introduction of deltaPhe in peptide sequences is known to induce conformational constraint, both in the peptide backbone as well as the side chain, and to provide the peptide with increased resistance to enzymatic degradation. In small peptides containing eta turn structure, and in peptides containing–a single deltaPhe, a type II b more than

one deltaPhe residues, helical structures are stabilized. A number of deltaPhe peptides varying in length, content and position of deltaPhe residues have been found to contain 310 helices of both screw senses. Following these design principles, we were able to design, synthesize and characterize super secondary structural motifs like helix-turn-helix, helical bundle and glycine zipper. We have extended this work to the de novo design of peptides with antibiotic and anti-fibrillization activity. A series of cationic peptides containing deltaPhe residues have shown remarkable antibiotic activity and are being developed further. deltaPhe containing peptides may have longer in vivo half life owing to their ability to resist enzymatic degradation. More recently, we have observed that small peptides containing deltaPhe self-assemble in nanotubular and nanovesicular structures. These nanostructures have also been used to entrap small drug like molecules. We have fully characterized these systems and are exploring their potential as delivery agents in biological systems.

P20121-021

Building β-peptide foldamers via stereochemical LEGO approach

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The synthesis and design of peptidomimetic oligomers that adopt designed, compact conformations (foldamers) have become a challenging task in recent years. Among them, β-peptide foldamers exhibit rich diversity of secondary structures.[1,2] A relationship has been established between the backbone chirality pattern and the prevailing secondary structure, which underlines the role of stereochemical control in the β-peptide foldamer design.(3) An important challenge is to introduce proteinogenic side-chains to generate diverse anchor points on the molecular surface promoting their application in drug discovery.
It has been proved that stable helices can be obtained when sequences were coupled with β-amino acid enantiomeric pair motifs in a LEGO approach.(3) In this work, α-amino acids and open chain functionalized β-amino acids were inserted between the enantiomeric pair design element for **1** and **2**, respectively.
Configurations in the backbone were designed to promote helix formation. NMR and ECD measurements augmented with *ab initio* calculations revealed that **1** forms a H9-12 helix and **2** forms a H14/16 helix. These helices are unprecedented in the literature. To prove that the stereochemical patterning is crucial in the folding, we perturbed the configuration motifs of the backbones by swapping the sequence of the central β-amino acids. The peptides with exchanged residue order could not fold into helices. These novel structures can be useful scaffolds for biomedical applications.

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P20121-022

Synthesis and Antiproliferative Activity in Breast and Colon Cancer Cells of Peptidomimetics Based on Substance P C-Terminal Region Hexapeptide

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Introduction Small length synthetic peptides, based on SP C-terminal fragment, increase the secretion of TNF-α and prevent the proliferation of several cancer cell lines. We have already shown the antiproliferative activity of tri- and tetra-peptoids in breast and prostate cancer cells. The aim of this study was the synthesis of hexa-peptoids, analogs of SP C-terminal region, containing the residues D-Trp and Tic and the peptoid ones NHN(R)CH2CO, NPhe and NAla in their sequence and their evaluation against cancer cells proliferation. Methods and Results All the syntheses were carried out stepwise using the Fmoc/But methodology on the solid support 2-CLTR resin and DIC/HOBt as coupling reagent. All analogs were purified (HPLC) and identified (ESI-MS). The highly invasive breast cancer epithelial MDA-MB-231 and HT-29 colon cancer cells of high tumorigenity were cultured in absence or presence of hydrazino-peptoids for 48h, in presence and abscence of serum and cell proliferation was documented by WST-1 method. 1. Glp1-NPhe2-Gly3-[NH-N(Bzl)-CH2-CO]4-D-Trp5-Leu6-OH 2. Glp1-NAla2-Gly3-[NH-N(Bzl)-CH2-CO]4-D-Trp5-Leu6-OH 3. Glp1-Tic2-Gly3-[NH-N(Bzl)-CH2-CO]4-D-Trp5-Leu6-OH 4. Glp1-NPhe2-Gly3-D-Trp4-[NH-N(Bzl)-CH2-CO]5-D-Trp6-OH 5. Glp1-NAla2-Gly3-D-Trp4-[NH-N(Bzl)-CH2-CO]5-D-Trp6-OH 6. Glp1-Tic2-Gly3-D-Trp4-[NH-N(Bzl)-CH2-CO]5-D-Trp6-OH It was found that all peptoides significantly inhibited the proliferation of MDA and HT-29 cells in a dose-dependent manner in absence of serum, even at low concentrations. However, in presence of serum only the peptoids 2 and 3 inhibited the cell proliferation of MDA, whereas none inhibited HT-29 cells. Conclusions Hydrazino-peptoids incorporating D-Trp act as potent antiproliferative agents in breast and colon cancer cells and further studies are warranted to evaluate their inhibitory effects in the view of the new analogs.

P20123-023

Design and Synthesis of Cyclotheonamide Analogs with a Basic P3 Residue as Inhibitors of Human beta-Tryptase

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Cyclotheonamides constitute a family of structurally related cyclic pentapeptides of marine origin that inhibit trypsin-like serine proteases. Their binding mode has been elucidated by the X-ray structure of cyclotheonamide A in complex with trypsin. An extended peptide conformation which is stabilized by macrolactamization allows to address in a substrate-like manner beside the S1 pocket also the S1' and S2 pocket. The S1 ligand, (S)-3-amino-6-guanidino-2-oxo-hexanoic acid, interacts via its guanido function with Asp 189 at the bottom of the S1 pocket. In addition, the ketone covalently modifies the gamma-oxygen of Ser 195 by hemiketal formation (1). Recently, two novel cyclotheonamides have been isolated from a marine sponge of the genus Ircinia. One of them, cyclotheonamide E4, is a potent inhibitor of human beta-tryptase (2). In this study, cyclotheonamide E4 was modified at two positions: (i) the S1 ligand was replaced by beta-homolysine or as beta-homoarginine to obtain reversible acting tryptase inhibitors, and (ii) the alpha amino function of (S)-2,3-diamino propionic acid, which is not part of the cyclic backbone, was used as anchoring point for basic P3 residues to exploit interactions with the negatively charged Glu 217 of tryptase. These analogs were synthesized by a combination of solid phase and solution phase chemistry 3.. Synthetic details as well as the inhibitory profile of these novel cyclotheonamide E4 analogs will be discussed.

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P20126-024

Metal ion interactions within the 1-16 N-terminal region of β-Amyloid

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Aggregation of the amyloid β-peptide (Aβ) into insoluble fibrils is a key pathological event in Alzheimer's disease (AD).(1) Several in vitro and in vivo studies have shown an important role of metal ions, such as zinc(II) and copper(II), both in Aβ's aggregation and cytotoxicity.[2-4] It is now well-established that the metal-binding site is located at the N-terminal hydrophilic region encompassing the amino acid residues 1-16 (Aβ1-16). However, different coordination modes, stability constant values and metal-assisted polypeptide conformation changes have been proposed for metal-Aβ1-16 complexes. Here we report a detailed study on the zinc(II) and copper(II) complexes with a new polyethylene glycol peptide conjugate (Aβ1-16-PEG). Complex formation was studied using different metal ion to peptide ratios, ranging from 1:1 to 4:1. We employed a combined potentiometric and spectroscopic approach to determine the binding modes of both zinc(II) and copper(II) with the title peptide. The results were also validated by limited proteolysis experiments carried out in the same metal-peptide ratios.

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P20126-025

Oostatic peptides containing D-amino acids: Activity and degradation in the flesh fly *Neobellieria bullata*

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Deteriorating effect of C-terminus truncated 4P and 5P analogues [1,2] of decapeptide H-Tyr-Asp-Pro-Ala-Pro_6-OH (3) on ovarian development (i.e. oostatic effect) of insect species Diptera, Orthoptera and Hemiptera has stimulated an analysis of metabolic degradation of corresponding peptides after application to the insect body [4-7]. Radiolabeling in different positions of the peptide chain allowed determination of the degradation decisive steps - the fast splitting off the C-terminal Pro from the 5P followed by successive cleavage of Tyr and Asp from the N-terminus. After introduction of corresponding D-amino acids into peptide chain of 5P, we could see the same or even stronger oostatic effect of the analogues in comparison with parent peptide after application on the flesh fly *Neobellieria bullata*. In the degradation assay, an elimination of enzymatic cleavage of peptide bonds pointing from the central labeled Pro residue to either D-Ala or D-Asp residues in the neighbourhood was observed, resulting in total increase of the analogs stability.

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P20200-026

Structure-activity relationships(SAR) studies of CamK II inhibitors.

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Ca2+/calmodulin-dependent protein kinase II (CaM-KII) is an oligomeric Ser/ Thr protein kinase that regulates numerous physiological functions. In fact, in vitro it can phosphorylate up to 40 proteins, including enzymes, ion channels, transcription factors and a number of these proteins appear to be physiological substrates. For example, CaM-KII is highly concentrated in the postsynaptic density of glutamatergic synapses where it phosphorylates and potentiates current through the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor ion channel (AMPA-Rs). In fact, this family is encoded by four genes (α, β, γ and δ), whereas the γ and δ isoforms are expressed in diverse tissues and α, β isoforms are most prominent in neural tissues. The identification of CamK II inhibitors is important to better define its physiological. In literature is reported a 79-aa brain-specific protein that and potently inhibited kinaseβ and αbound the catalytic domain of CaM-KII activity with an IC50 of 50 nM. The inhibitory protein (CaM-KIIN), and a 27-residue peptide derived from it (CaMKIINtide, KRPPKLGQIGRAKRVVIEDDRIDDVLK), was highly selective for inhibition of CaM-KII with little effect on CaM-KI, CaM-KIV, CaM-KK, protein kinase A, or protein kinase C. CaM-KIIN interacted only with activated CaM-KII (i.e., in the presence of Ca2+/CaM or after autophosphorylation). Here we report a structure- activity relationships study using as template the CAMKIINtide. We synthesised a peptide contains all 28 amino acids present in CaMKIIN-tide, however, in random sequence (CaMKIIN-tide Scramble). Then we operated a progressive deletion of amino acids from C-terminal and dall'N-terminal to detect minimum active sequence. Moreover CamKIINtide and CanKIINtide scrumble was made cell-permeable by N-terminal addiotion of an antennapedia (RQIKIWFQNRRMKWK). Here we report the biological results of our study. αβα

P20200-027

PTPRJ: a receptor-type protein tyrosine phosphatase as a target of new peptides with antitumoral activity

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Protein tyrosine phosphatase, receptor type, J, also known as Ptprj, is a human gene. The protein encoded by this gene, which is composed of an extracellular domain containing eight fibronectin type III repeats, a transmembrane domain, and a single cytoplasmic catalytic domain, is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known

to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. It has been demonstrated that PTPRJ (also known as HPTPeta, DEP-1, CD148) is able to inhibit cell growth promoted by specific PTK some of which are over-expressed in certain types of cancer. Moreover a role for PTPRJ was also assessed in vasculogenesis; infact its reduced activity in enhanced VEGF-induced VEGFR2 activity leads to increased cellular responses, supporting a PTPRJ-VEGFR2 interaction. On these basis it is important the identification of molecules with agonist activity which are able to stimulate the function of residual PTPRJ in malignant cells and stopping the proliferation and possibly trigger programmed cell death. Using as a template a peptide, already identified, with agonist activity against PTPRJ(H-[Cys-His-His-Asn-Leu-Thr-His-Ala-Cys]-OH), here we report a structure-activity study carried out through endocyclic modifications (Ala-scan, D-substitutions, single residue deletions, substitutions of the disulfide bridge) and the preliminary biological results of this set of compounds.

P20201-028

Selection of peptomeric inhibitors of bovine α-chymotrypsin and cathepsin G based on trypsin inhibitor SFTI-1 using combinatorial chemistry approach

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Isolated in 1999 from the sunflower seeds trypsin inhibitor SFTI-1 is up to date the smallest naturally occurring peptidic proteinase inhibitor and therefore is an excellent starting structure to design peptidomimetic serine proteinase inhibitors. Peptomeric library consisting of 360 monocyclic analogues of SFTI-1 was designed and synthesized by the solid phase method with the intension of select chymotrypsin and cathepsin G inhibitors. All peptomers contained in positions 5 and 12 N-benzylglycine (Nphe) that mimics proteinogenic Phe. In the synthesized library different peptoid monomers were introduced in the segment 7-10. This is a turn region that makes an important contribution to structural integrity and rigidity of SFTI-1. Deconvolution of the library against both proteinases by the iterative method in solution revealed that the highest chymotrypsin inhibitory activity displayed analogue with N-[4-(2-aminoethyl)morpholyl]-glycine (Naem) in position 8. This analogue was even more active than the one with Pro in this position which is absolutely conserved in Bownan-Birk inhibitors. While, deconvolution carried out against cathepsin G indicated that analogue with N-piperonylglycine (Npip) in position 8 and 9 and with N-butylglycine (Norleu) in position 10 presents the highest inhibitory activity.
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P20207-029

Antimicrobial peptides tailored for plant protection

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A platform for the design and optimization of new antimicrobial peptides effective against specific pathogens has been set up. The main features expected for these peptides are low environmental impact, broad spectrum of activity, reasonable bacterial selectivity, and low eukaryotic cytotoxicity. Our approach also includes the use of a design of experiments protocol in order to find peptide sequences that fit these features. The obtained peptides would represent an alternative to currently used antibiotics or pesticides. This work focused on finding new control agents against economically important plant pathogenic bacteria such as Erwinia amylovora, Pseudomonas syringae and Xanthomonas vesicatoria for which the available methods are not sufficiently effective. Nowadays, their control is mainly based on copper compounds and antibiotics. Although antibiotics are highly efficient, they are not authorized in several countries and resistance has been developed on plant pathogens. We have synthesized combinatorial libraries of cyclic decapeptides and linear undecapeptides. These libraries have been screened for antibacterial activity and eukaryotic cytotoxicity, and have led to the identification of peptides with MIC values of 1.6-12.5 microM. Notably, cyclic peptides active against E. amylovora have been found, constituting the first report of this type of peptides with activity towards this bacteria. The best peptides are bactericidal, display a low eukaryotic cytotoxicity at concentrations 30-120 times higher than the MICs, and show a low susceptibility towards protease degradation. Best peptides have been tested in vivo by evaluating their preventive effect of inhibition of P. syringae, X. vesicatoria and E. amylovora infections. The most active peptide is slightly less effective than streptomycin, currently used in field. Therefore, the best analogues can be considered as good candidates for the development of antibacterial agents for use in plant protection.

P20215-030

Selection of chromogenic and fluorogenic substrates of neutrophil serine proteases using combinatorial chemistry approach

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Human serine neutrophil and mastocytes proteases such as cathepsin G, neutrophil elastase, proteinase 3 and β-tryptase are involved in several physiological processes. Unwanted activity of those enzymes yields to severe pathological states like inflammation, Wegener granulomatosis or various types of cancer. Therefore monitoring of the activity of these proteases is crucial for the proper therapeutical treatment. The simplest method for determination of protease activity is the use of synthetic substrates. They are also useful for characterization of the enzyme specificity. In this work we report selection of chromogenic and fluorogenic substrates of human serine neutrophil and mastocytes proteases applying combinatorial chemistry approach. Peptide libraries were synthesized by the portioning-mixing method. Deconvolution of synthesized libraries was performed using iterative approach in solution. 5-Amino-2-nitro benzoic acid attached to the C-termini of synthesized peptides served as a chromophore released upon the interaction of peptide with enzyme. Additional introduction of 7-methoxy-4-coumaryl acetic acid or 2-amino benzoic acid on á-amino groups of the chromogenic substrates converted them into FRET displaying compounds. The most active substrates were subjected to further modification applying non-proteinogenic amino acids. As a result, one of the most selective substrates of these proteases was obtained. We also attempted to construct a simple tools to detect activity of human serine neutrophil and mastocytes proteases by immobilization, through amide and peptoid based linkers, of selected substrates on solid phase.

P20223-031

Angiogenesis modulation: identification of tetrameric tripeptide as inhibitor of VEGFR-1 by the screening of peptide combinatiorial libraries

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Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1, Flt-1) and their ligands are involved in complex biological processes associated to severe pathological conditions, like angiogenesis, inflammation and metastasis formation (1). Thus, the search for antagonists of Flt-1 has recently gained a growing therapeutic interest. In order to identify new molecules able to selectively bind Flt-1 and neutralize its activity, a screening of a combinatorial tetrameric tripeptide library built with non-natural amino acids has been carried out by a competitive ELISA-based assay. The library has been designed on a branched tetrameric structure in order to obtain molecules with a high recognition surface and, using 30 building blocks, a complexity of 27.000 different peptides has been achieved. Peptide mixtures composing the library have been utilized as competitors of the Flt-1/PlGF (placental growth factor) interaction and the most active components have been isolated following an iterative deconvolution procedure. The selected most active hit shows a selective binding to Flt-1 over KDR and inhibits in vitro its interaction with both PlGF and VEGF-A. The peptide is fully stable in biological environments, prevents Flt-1 phosphorylation and blocks HUVEC capillary-like tube formation stimulated by PlGF or VEGF-A. In vivo the peptide inhibits the VEGF-induced neoangiogenesis in chicken embryo chorioallantoic membrane assays and also stimulates cornea neovascularization (CNV) by displacing VEGF from a complex with sFlt-1. All data suggest that the compound has potential applications in diseases characterized by pathological angiogenesis, such as tumor growth and ischemic retinopathy.

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P20300-032

Racemization-free Synthesis of Glycopeptides using Fragment Condensation with C-terminal Pseudoprolines

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Since the purification of homogeneously glycosylated glycoproteins remains a difficult task the synthesis of entire glycoproteins is a field of emerging interest (1). Especially the synthesis of the required protein fragments in high purity and good yields demands for efficient methods. Fragment condensation of protected peptides can reduce deletions in the crude product and thus facilitates purification. Condensation of peptide fragments in CSPPS (2) is however subject to epimerization upon C-terminal carboxyl activation. To overcome this problem the use of C-terminal Gly or Pro is usually performed. Peptides containing C-terminal pseudoprolines can also be coupled without stereomutation because the serine or threonine residue has been reversibly protected as a proline-like oxazolidine (3). This concept facilitates the synthesis of long peptides by epimerization-free fragment condensation at C-terminal Ser/Thr residues in addition to Gly/Pro residues thus increasing the number of safe condensation sites. After solving several problems associated with the generation of peptide acids with C-terminal pseudoprolines the synthesis of the glycosylated RNase fragment [1-39] was carried out as a racemization-free fragment condensation.

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P20307-033

Solid phase synthesis and biological activity of linear tuftsin and retro-tuftsin derivatives

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Tuftsin is liberated from Fc-domain of the heavy chain of IgG by two specific enzymes. Being the tetrapeptide of biological origin is extremely important product because it can activate a few elements of immune system such as granulocyte and macrophage. Tuftsin indicates not only immunological stimulating factor but also antibacterial, antivirial and antitumor properties. In spite of its wide range of activity, the peptide is unstable in plasma and it has become the aim of the formation of novel analogues more resistant to proteolysis degradation. The introduction of the additional residue at ε-amino group of lysine caused that new bond became stronger than peptide bond in central chain [1-3]. We synthesized linear tuftsin derivatives that were prepared on the solid phase using a Fmoc/*t*Bu procedure. The method of elongation of peptide chain was based on two-step procedure: deprotection and coupling step. Segment coupling reaction was carried out with TBTU, HOBt and in the presence of DIEA in DMF/DCM/NMP mixture. The introduction of the simple amino acid (Ala, β-Ala, Val, Ile or Gly) at ε-amino group of lysine let us obtain the isopeptide bond. The modification was achived by introducing lysine residue, protected at ε-amino group with Mtt. The selective removal of Mtt group was caused by 2% TFA treatment. Peptides were cleavage from resin and purified. Tuftsin derivatives were confirmed by MS, amino acid analysis, elemental analysis and RP-HPLC analysis. Peptides were sent to assay their microbiological properties and the results will be described as a structure-activity relationship.

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P20311-034

The synthesis and structural study of iso-Aß(1-42)

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Aß(1-42) is prone to aggregate, if it is dissolved in aqueous solution. Because of that, the solubility of the peptide is poor and it cannot be stored for prolonged time in solution. Sohma and Kiso(1) attached Gly^{25} to the hydroxyl side chain of the Ser^{26} via an ester bond in their synthesis using Fmoc-chemistry. The solubility of the '*O*-acyl isopeptide' is greater than that of the Aß(1-42), because it will not form fibrils as Aß(1-42) does. It also can be stored in acidic solution for days. This isopeptide will be rearranged at pH 7.4 to Aß(1-42) through an *O*→*N* acyl shift. Thus this peptide can be used as a precursor of Aß(1-42). The Aß(1-42) peptide is difficult to synthesize, because of its high tendency for aggregation during the synthesis. Both the couplings and the Fmoc-removal can be troublesome, due to the steric hindrance. The incorporation of the ester-bond disturbs the structure, thus ease the synthesis after the 25^{th} residue. If the peptide would be synthesized using Boc-chemistry the removal of the α-amino protecting group would be less problematic, because the 50% TFA/DCM mixture solubilizes well the peptide chain. Thus a Boc-strategy was devised for the synthesis of iso-Aß(1-42).

The purified peptide was studied with CD-spectroscopy and dynamic light scattering. These structural studies revealed that the isopeptide has some ß-sheet content even in a pH 2 solution. It was realized also that small aggregates are present in the precursor peptide. Both techniques mentioned above showed, that after altering the pH to 7.4, the ß-sheet content of the peptide increases and aggregation takes place. With the use of the isopeptide, Aß oligomers and – with prolonged incubation – fibrillar structures can be formed for biological studies.

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P20320-035

Synthesis of Conjugates of MDP and nor-MDP linked to Tuftsin Derivatives as Potential Immunomodulators

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Continuing our program of syntheses of muramyl dipeptide (MDP) and nor-muramyl dipeptide (nor-MDP) conjugates as potential immunomodulators, we designed novel conjugates of MDP or nor-MDP with tuftsin derivatives containing isopeptide bond between ε-amino group of lysine and carboxylic group of simple amino acids such as alanine, glycine and valine. The synthesis of a greater number of conjugates will enable structure-activity relationship studies. Tuftsin analogues containing isopeptide bond showed increased chemical resistance and activity in relation to tuftsin. The introduction of the additional residue at ε-amino group of lysine by NHCO- formation caused that isopeptide bond became stronger than peptide bond in central chain. The protected pentapeptides (H-Thr-Lys(Y)-Pro-Arg(NO_2)-OBn, Y= Ala,Gly,Val) were synthesized by the conventional chemical procedure using mixed anhydride method. Acylation of the Thr amino group of partially protected pentapeptides by 1-benzyl-MDP or 1-benzyl-nor-MDP was performed using the mixed anhydride method with isobutyl chloroformate and N-methyl-morpholine (NMM) in dry DMF. The protected conjugates were isolated and purified with a preparative TLC. The identities of the protected products were confirmed by high resolution ^{1}H-NMR (500 MHz, COSY, TOCSY, ROESY, gHSQC, gHMBC) spectroscopy. The final products were hydrogenated with H_2/Pd/C in 50% methanol-acetic acid and purified with preparative TLC. The identities of the conjugates were confirmed by TLC qualitative amino acid analysis, and elemental analyses. Finally, the combined use of muramyl peptides with other immunomodulators, e.g. such as tuftsin, retro-tuftsin other chemotherapeutics is promising in the therapy of different infections, autoimmunological diseases and anticancer therapy.

P20400-036

Efficient microwave-assisted synthesis of myelin epitopes MOG35-55 and MOG97-108 using CLTR-CL resin

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Multiple Sclerosis (MS) is a slowly progressive, immunologically mediated disease of the Central Nervous System (CNS), characterized by inflammation and demyelination of white matter in the brain and spinal cord. The myelin oligodendrocyte glycoprotein (MOG) is a minor CNS myelin-specific protein that is an important candidate autoantigen in MS. Two immunodominant epitopes, MOG35-55 (MEVGWYRPPFSRVVHLYRNGK) and MOG97-108 (TCFFRDHSYQEE), were synthesized with Microwave Enhanced Solid Phase Peptide Synthesis (SPPS) utilizing 2-chlorotrityl chloride resin (CLTR-Cl). Microwave energy represents a fast and efficient way to enhance both the Fmoc deprotection and coupling reactions using Fmoc/tBu methodology. Unlike conventional heating, microwave energy directly activates any molecule with a dipole moment and allows for rapid heating at the molecular level. The protected peptides were synthesized using the CEM Liberty automated microwave peptide synthesizer in 18 and 13.5 hours respectively, with a 3-minute Fmoc deprotection (25% piperidine solution in DMF) and 5-minute coupling reactions using DIC/HOBt in DMF solution. The maximum temperature reached during both the deprotection and coupling reactions was 80 °C except for the coupling of FmocHis(Trt)OH (50 °C). The final crude products were of high purity as identified by analytical RP-HPLC.

P20405-037

Siah up-regulates the Hif-1alpha hypoxic response pathway: Siah binding peptides coupled to CPPs inhibit Siah activity and demonstrate proof of concept that Siah is a viable anti-tumor drug target

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Growth and metastasis of solid tumors is, in part, dependent on activation of the transcription factor Hif-1, a central component of the hypoxic response pathway. Components of this pathway, such as Hif-1*f*Ñ and VEGF, have been targeted for the development of cancer therapeutics. VEGF, a secreted downstream component crucial for angiogenesis, has been successfully targeted clinically using antibody therapy (Avastin). The hypoxic response, however, activates other pathways that stimulate tumor growth, suggesting that inhibitors of upstream components in the pathway may be useful to give a broader spectrum of inhibition. We have focused on the Siah proteins that regulate Hif-1*f*Ñ levels by ubiquitylation and degradation of the prolyl hydroxylases (PHDs) immediately upstream of Hif-1*f*Ñ in the hypoxic response pathway. In a proof-of-principle study, we have shown that Siah inhibition by expressed protein fragments (from a Drosophila high affinity interacting protein, Phyllopod) can inhibit the stabilization of mammalian Hif-1*f*Ñ during hypoxia and limit tumor growth in a mouse tumor model. A 23 amino acid peptide sequence (Phyl) from the Phyllopod protein has been identified as the interaction site with Siah. The aim of this work was to show that a small peptide could mirror the activity of the transfected recombinant Phyllopod protein. To achieve this, Phyl was covalently attached to cell penetrating peptides (CPPs) and tested for its ability to inhibit Hif-1*f*Ñ stabilization and hypoxic response in human U2OS cells. Both the TAT sequence and Penetratin were utilized as CPPs and attachment was via disulfide, maleimide or through a Pro10 spacer sequence. The CPP¡VPhyl constructs were found to have varying activities but a Penetratin-Pro10-Phyl was found to be inhibitory in the U2OS cell-line Hif-1*f*Ñ stabilization assay. This result demonstrated proof of concept that Siah inhibition could be attained by targeting a restricted and specific protein-protein interaction site.

P20411-038

Investigation of the Structure of the N-terminal Region of Prion Protein (PrP) via the Microwave Synthesis of Peptide Fragments up to 111 Amino Acids in Length

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Prion diseases such as CJD (Creutzfeldt-Jakob disease) in humans or scrapie in sheep and BSE (bovine spongiform encephalitis) in cattle are

diseases caused by changes in protein conformation (1). The principal event in the development of prion disease is the transformation of the normal cellular prion protein (PrPc), which is highly helical in nature, into the pathogenic isoform PrPsc which is insoluble and has an extensive *fÒ* sheet structure. The events surrounding this transformation are very poorly understood, but the mechanism of the detailed steps involved in this process is fundamental to the understanding of prion disease pathogenesis. In an endeavour to study the structure of polypeptide component of the N-terminal section of PrPc, synthesis of a range of PrP polypeptides were assembled on a CEM Liberty microwave synthesiser. These fragments ranged in length from 20 to 111 amino acids and span the protein sequence from position 1 to 144. Standard CEM synthetic coupling cycles were used, except when peptides were greater than 30 amino acids in length, whereby longer coupling cycles were employed. A number of purification strategies were employed, such as C4 and C18 RP-HPLC at either 25C or 60C and size exclusion chromatography. The purified peptides were characterised by RP-HPLC and ESI-MS. In addition, they were analysed for secondary structure by CD spectrometry, and evaluated for cell toxicity and fibril forming ability with ThT.

P20423-039

The synthesis of a 60mer peptide for investigating the mechanism of action of the HIV fusion inhibitor T20

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T20 (Fuzeon), derived from the HIV-1 envelope glycoprotein transmembrane- subunit gp41 C-terminal heptad repeat (CHR) region, has been approved by FDA as the first member of HIV fusion inhibitors. However, the mechanism of action of T20 is still in debate. It is believed that peptides derived from gp41 CHR region may share a common mechanism, by binding to gp41 NHR coiled coil and preventing formation of the fusogenic gp41 core-six helix bundle (6HB), thereby inhibiting fusion between the virus and target cell membrane. We have synthesized a 60-mer NHR peptide- N60- covering all the binding sites for any length of the CHR-peptide. Synthesis of the polypeptide was carried out using conventional as well as microwave assisted solid phase synthetic methods. Details, including comparison of the synthetic approaches will be presented. Using C34, another anti-HIV CHR peptide containing the pocket-binding domain, as a control, we analyzed the activity of T20 to interact with N-60 to form 6HB and to inhibit the 6-HB formation between N-60 and C34 by CD spectroscopy and ELISA. We found that T-20, unlike C34 could neither form a stable 6HB with N60, nor inhibit the 6-HB formation of the fusogenic 6HB core. Our results thus suggest that T-20 and C-34 peptides inhibit HIV fusion by different mechanisms of action.

P20517-040

FRET-based assay for sensing a cancer key protein functional state and inhibition by oligopeptides: *Site-specific Protein Chemical Modifications*

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Thymidylate Synthase is a key enzyme required for DNA synthesis; cessation of this pathway halts cell replication and leads to apoptosis of rapidly dividing cells just like tumoural ones(Houghton et al., Antifolate drugs in cancer chemotherapy, 1999, Humana Press).
The oligomerisation equilibrium of TS is modulated by a fine tuning; shifting this equilibrium towards the monomeric form would cause TS inactivity and low translation, overcoming resistance mechanisms encountered for (co)substrate-like inhibitors as the inactive monomer regulates its own expression.
Our group designed some small ligands to interfere with Thymidylate Synthase dimerisation, including short peptides taken from the interface sequence that represent the natural ligand for this region, mimicking the other subunit without forming a functional dimer. Interesting biological data are arising from the activity tests but a rapid screening assay is necessary to prove they are really interfering with TS oligomerisation.
FRET is a spectroscopic phenomenon whose intensity depends on the distance between two fluorophores; when this value varies due to conformational changes of the protein the probes are linked to, consequent FRET variation can be used to sense the state of the protein(s) (Yan et al., Curr. Opin. Chem. Biol., 2003, 7,635). This strategy was exploited to study the effects of potential inhibitors on the oligomerisation state of TS, by derivatising each monomer with a different probe.
A site-specific conjugation protocol was set up in order to tag the protein on a selected Cys residue without affecting the catalytic pocket; in this manner, the same screening assay can test both the oligomerisation state and residual activity. The assay is meant for being scaled-up on 96-wells plates.
Acknowledgements: This work is a part of LIGHTS project (LIGands to interfere with Human Thymidylate Synthase, 6FP STREP funded, LSHCCT-2006-037852,www.lights-eu.org) and of MIUR-PRIN 2006-2006030430_004 CSTMPL.

P20520-041

Photoactivatable SDF-1α Analogues as Useful Prodrugs in Regenerative Medicine

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Stromal Derived Factor-1α (CXCL12) is a 68 amino acid CXC-chemokine with critical role in homing, migration and guiding of different cell types including hematopoietic progenitor cells (HPC), stem cells, tumour cells and neuronal cells during embryogenesis and in adults (1). These various functions in physiological as well as pathophysiological processes make this small protein interesting for regenerative medicine. To apply this chemoattractant in medicine, it is needed to form spatially and temporally controllable concentration gradients of active SDF-1 in response to a non-tissue damaging trigger like visible or near UV-light. After irradiation of an inactive prodrug dramatic change in conformation or lack of sterical hindrance should then lead to fully biological activity under physiological conditions within a few minutes.
To prove the principle and assess its potential in photodynamic therapy of neuronal injuries a water-soluble, photosensitive SDF-1 analogue has been developed. For this the expressed protein ligation (EPL) approach has been used, in which one segment has been recombinantly expressed and purified using the IMPACT®-System to yield the corresponding peptide thioester (2). The modified peptide fragment has been chemically synthesized on solid phase using Fmoc-strategy. The photocleavable moiety, the 6-nitroveratryloxycarbonyl (Nvoc) protecting group, has been introduced at a side chain amino group. Furthermore, the analogue has been characterised by physico-chemical methods and has also been tested *in vitro* on transfected COS-7 cells in order to determine its biological activity after activation.
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P20520-042

Modification of the Regenerative Chemokine SDF1 α to Allow Fluorescence Imaging

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SDF-1 is a chemokine that plays a major role in trafficking of hematopoietic stem cells (HSC). Thus it enables the formation of bone marrow during embriogenesis and later in adult life it supports retention and homing of these cells in the bone marrow. Furthermore it is involved in organogenesis and regeneration, respectively.[1] Due to these promising features the subform SDF-1α could serve as a therapeutic target. For studies on the small protein concerning its molecular properties as well as its therapeutic potentials, it needs to be modified chemically. In order to reach these goals, the N-terminus SDF-1α_{1-49} has been cloned and expressed recombinantly in E. coli ER 2566 as a thioester, while the C-terminus SDF-1α_{50-68} has been synthesized via solid phase peptide synthesis. Modifications are thereby introduced at the C-terminus at Lys56. Up to now carboxyfluorescein has been coupled to the ε-amino group of the lysine residue. The two fragments then have been ligated via Expressed Protein Ligation (EPL), a subform of the Native Chemical Ligation (NCL).[2] Activity studies and fluorescence microscopy on HEK293 cells transfected with the SDF1-specific G-protein coupled receptor CXCR4 have been conducted.

P20621-043

Development of Peptide Based Vaccines for the Treatment of Renal Cell Cancer (IMA901)

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IMA901 is a multiple peptide vaccine for the treatment of renal cancer (RCC). The tumor-associated peptides (TUMAPs) contained in IMA901 were identified by immatics directly from primary renal cells (= primary RCC tumor tissue samples), selected regarding their over-expression in RCC and proven to be immunogenic using in vitro T-cell assays. IMA901 consists of 10 individual peptides (10 TUMAPs) and non-active ingredients which are used as excipients of the pharmaceutical presentation of IMA901. All 10 peptides are synthesized by conventional Fmoc chemistry. The sequences of the peptides will be presented and technical issues will be discussed. In the final formulation of IMA901 578 µg of each peptide plus excipients are filled into glass vials and lyophilized. The challenges of the production of multi peptide drugs will be discussed. Such challenges comprise the synthesis, the production of the formulation as well as the analyses of the final presentation of IMA901. Results of the phase 1 trial in 28 vaccinated RCC patients showed that (1) IMA901 was safe, (2) multiple T-cell responses to vaccinated peptides correlated with favourable clinical outcome and (3) patients with a lower percentage of regulatory T cells (Tregs) were more likely to develop a vaccine-induced multiple T-cell response.

P20700-044

Novel peptides α-MSH analogs with high candidacidal activity

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α-Melanocyte stimulating hormone (α-MSH) is an endogenous linear tridecapeptide with potent anti-inflammatory effects. It was demonstrated that α-MSH and its C-terminal sequence Lys-Pro-Val (α-MSH [11-13]) have antimicrobial effects against two major and representative pathogens: Staphylococcus aureus and Candida albicans. In an attempt to improve the candidacidal activity of α-MSH and to better understand the peptide structure-antifungal activity relations, we designed and synthesized novel peptide analogs. Because previous data suggested that the peptide [DNal-7, Phe-12]-α-MSH(6-13) has greater candidacidal activity than α-MSH and is the most potent of the analogs tested in the past, this compound has became our lead (1,2). From this lead compound we have synthesized a new library of peptides where we have replaced the glycine in position 10 with unconventional amino acids. Here, we report new analogs with a strong antimicrobial and candidacidal activity. The obtained results are very encouraging in that they show the great potential of these peptides as a truly novel class of candidacidal compounds.

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P20700-045

Novel antimicrobial peptides from the venom of solitary bees

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A novel antimicrobial peptide named melectin was isolated and characterized from the venom of the cleptoparasitic bee *Melecta albifrons*. By Edman degradation and ESI-QTOF mass spectrometry, the primary sequence of melectin was established as H-Gly-Phe-Leu-Ser-Ile-Leu-Lys-Lys-Val-Leu-Pro-Lys-Val-Met-Ala-His-Met-Lys-NH2. In the same way three structurally related peptides were identified in the venom of parasitic bee *Lasioglossum laticeps* and were named lasioglossin I, II, and III. Their sequences are: H-Val-Asn-Trp-Lys-Lys-Val-Leu-Gly-Lys-Ile-Ile-Lys-Val-Ala-Lys-NH2, H-Val-Asn-Trp-Lys-Lys-Ile-Leu-Gly-Lys-Ile-Ile-Lys-Val-Ala-Lys-NH2 and H-Val-Asn-Trp-Lys-Lys-Ile-Leu-Gly-Lys-Ile-Ile-Lys-Val-Val-Lys-NH2 respectively. Synthetic melectin and lasioglossins exhibit potent antimicrobial activity against both Gram-positive and Gram-negative bacteria and low hemolytic activity against rat erythrocytes. Unlike the lasioglossins melectin degranulates the rat peritoneal mast cells. Lasioglossin III has a strong potency to kill different cancer cells. The CD spectra of melectin and lasioglossins measured in the presence of trifluoroethanol and sodium dodecyl sulfate showed a high content of α-helical conformation which indicates that these peptides can adopt an amphipathic α-helical secondary structure in the anisotropic environment such as bacterial cell membrane.

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P20700-046

Influence of the adjacent amino acid on antimicrobial activity of 3-(2-benzoxazol-5-yl)alanine derivatives

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3-(2-benzoxazol-5-yl)alanine derivatives are a group of unnatural amino acids which can be used as fluorescent probes (1, 2). Moreover, some of them are active against *Bacillus subtilis*, *Pichia pastoris*, *Candida albicans*, *Aspergillus niger* (3) as well as cytotoxic to the tumour cell lines (4). To modify the biological activity of 3-(2-benzoxazol-5-yl)alanines dipeptides with their selected derivatives were synthesized. Both, amino and carboxyl groups of each benzoxazolylalanine

derivative were modified by the same amino acid to establish influence of the adjacent amino acid on antimicrobial activity of the compounds studied. 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.

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

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P20700-047

A Combined Chemistry and Biology Approach to the Development of Novel Agents for Protection Against Livestock Pests

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The cyclotides are a family of naturally occurring macrocyclic peptides that combine the unique features of a head-to-tail cyclic backbone and a cystine knot motif, which impart extraordinary stability to this peptide family. A recent study demonstrated that the prototypic cyclotide kalata B1 possesses significant activity against two economically important sheep nematodes Haemonchus contortus and Trichostrongylus colubriformis. An alanine scan of the molecule highlighted the residues critical for activity. In this work, we explore the relative importance of positively charged residues in different regions of the molecule to aid the understanding of the structural and biological basis of its nematocidal activity. A lysine scan has been conducted, in which each of the non-Cys residues in this 29 amino acid peptide has been successively replaced with lysine and the suite of peptides have been assayed against the two sheep nematodes. Substitution of residues in loop1, V10, G12, N15, T16, W23, V25, L2, P3, and V4 decreased or completely abolished the activity, suggesting that these residues are critical to the nematocidal activity of kalata B1. On the other hand, incorporation of a positive charge in positions G18, T20, T27, N29, and G1 significantly enhanced the anthelmintic activity of the grafted peptides, up to four-fold, compared to native kalata B1. These increases in activity after lysine incorporation into the kB1 scaffold raise the possibility of being able to engineer greater anthelmintic activity into the peptides, further highlighting their potential as anthelmintic agents.

P20700-048

Collagen Peptide Fragment Regulates Spreading of Murine Fibroblasts

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Interaction of cells with extracellular matrix (ECM) affects many aspects of cell behavior including growth, morphology, migration and differentiation. Integrins are known to mediate cell adhesion to proteins of ECM. Ligand-binding properties of integrins depend not only on composition of ECM ligands and level of integrin expression in cell, but also on spectrum and activity of soluble factors indirectly influencing cell-matrix contacts. Interaction of cells with substratum may be divided into cell adhesion and spreading. Following an initial cell attachment event, cell may or not spread depending on cell type and the nature of the molecular signals they receive. Oligopeptides released from different proteins during their proteolysis in or out of cells may act as the such short-time existing signals. In the present study we investigated the effect of multiply repeated peptide fragment in different collagen types on cell spreading. Murine embryonic fibroblasts (200000/ml) were allowed to adhere for 45 min at 37° C with or without peptide (in various concentrations) to plastic surface pre-coated or not with gelatin. It was shown that the synthetic peptide increased the number of spread cells and caused shape changes in cells spread on different substrata. A 30-min pretreatment of cells with the peptide (before conducting of cell spreading assay) resulted in inhibiting of cell spreading on gelatin. Our results suggest that the peptide regulation of cell spreading could be related to its effect on re-distribution of integrin receptors in sites of cell contacts with substratum.

P20700-049

Bioactive peptides from cyanobacteria

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Cyanobacteria are versatile source of small peptides from which vast majority are cyclic. Cyanobacterial culture collection in University of Helsinki contains over 1000 strains and this collection is used for screening of new bioactive compounds. Cyclic heptapeptides, microcystins are the best known cyanobacterial peptide family. Over 80 structural variants have been described from which most are strong hepatotoxins. Our research group have participated to the determination of the structure of many novel microcystins and other cyclic and linear cyanobacterial peptides. In recent years we have found highly toxic and novel microcystins from lichen associated cyanobacteria (1) and from Anabaena strains of Baltic Sea (2). In the structural analysis of new peptides from the known peptide families we have used liquid chromatography ion trap mass spectrometry which have proven to be very effective method and is in many cases the only method needed in the verification of a new structure. With this LC-ITMS method we have found many new structural variants from Anabaenopeptin, Anabaenopeptilide and Spumigin peptide families. In collaboration with many research groups we have found cyanobacterial compounds/extracts which inhibit protein kinase C activity, inhibit/activate boar sperm motility, disintegrate cell membranes or are antidotes for microcystin toxicity. Structures of the compounds are under study except the microcystin antidote which structural analysis showed that it belongs to a rare peptide family containing imino bond in cyclic skeleton.

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P20700-050

The results of N-terminal modification of Arginine Vasopressin with cis-1-amino-4-phenyl-cyclohexane carboxylic acid. The highly potent oxytocin receptor antagonists

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Arginine vasopressin (AVP) is a cyclic nonapeptide with multiple functions. The main peripheral physiological roles of AVP are the regulation of water balance, the control of blood pressure, and the release of adrenocorticotropin hormone (ACTH). Moreover, AVP also exhibits to some extent typical oxytocin (OT, a closely related neurohypophyseal peptide) activities such as the galactogogic and the uterotonic effects.
Many of vasopressin agonists and antagonists have been designed and synthesized in the course of extensive investigation of structure – activity relationship. A great deal of evidence showing that the conformation of the N-terminal part of arginine vasopressin analogues is crucial for their pharmacological activity. We decided to check how substitution of position 2 with bulky cis-1-amino-4-phenyl-cyclohexane carboxylic acid (cis-Apc[2]) would reflect in the values of biological potency of the analogues. The analogues have the following structure: [cis-Apc[2]]AVP (I), [Mpa[1],cis-Apc[2]]AVP (II), [cis-Apc[2],Val[4]]AVP (III), [Mpa[1],cis-Apc[2],Val[4]AVP (IV).
All peptides were tested for the pressor, antidiuretic and uterotonic in vitro activities in the rat.
cis-Apc[2] modification at position 2 of AVP is sufficient to change the pharmacological profile of the peptides. Analogues I – IV were moderately potent antidiuretic agonists with prolonged action. In regard to the uterotonic activity, all peptides with cis-Apc were highly potent antagonists, except compound I. It supports our earlier hypothesis that an amino acid residue in position 2 has significant impact on pharmacological activities.

P20700-051

Analogues of arginine vasopressin modified in the N-terminal part of the molecule with α-2-indanylglycine. Highly active and selective antiuterotonic agents.

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The incorporation of unnatural non-proteinogenic α-amino acids into peptides has emerged as a novel and promising approach in peptide modification. The conformationally restricted amino acid derivatives are of particular interest.
This thesis are also supported by our already 12-years research focused on the effects of steric restriction and the presence bulky substituent in the N-terminal part of AVP molecule on biological properties of the resulting analogues.
We decided to check how substitution of position 2 with bulky α-2-indanylglycine (Igl) would reflect in the values of biological potency of the analogues. We designed, synthesized and determined some pharmacological properties of four new analogues of AVP where the above mentioned modification was combined with Mpa[1] and/or Val[4] substitutions. These peptides have the following structure: [Igl[2]]AVP (I),[Mpa[1],Igl[2]]AVP (II), [Igl[2],Val[4]]AVP (III), [Mpa[1],Igl[2],Val[4]]AVP (IV).
All peptides were tested for the pressor, antidiuretic and uterotonic in vitro activities in the rat.
All the analogues were devoid of the pressor potency and exhibited only negligible antidiuretic activity. Interestingly, in regard to the uterotonic activity, the new compounds exhibited moderate (I) or high (II – IV) antioxytocic potency. It should be point out that single substitution e.g. replacement of Tyr in AVP molecule with Igl results in moderately potent and highly selective antagonists of oxytocin (I).
Our new Igl[2] substituted peptides proved that the presence of the sterically restricted amino acid residue may result in high active and selective antiuterotonic agents. These in our opinion interesting finding demonstrates the usefulness of our approach in the design of new highly active and selective analogues with desired pharmacological properties.

P20700-052

Influence of (2S,4R)-4-(2-naphthylmethyl)-pyrrolidine-2-carboxylic acid replacing position 2 of Arginine Vasopressin (AVP) and its analogues on their pharmacological properties.

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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. As a hormone AVP exerts its biological effects upon binding to three receptor subtypes termed: V_{1A}, V_{1B} (V_3), and V_2. Furthermore, AVP to some extent can interact with the oxytocin receptor (OT).
Biological activity of peptides is determined by their structure and conformation. Conformational restriction of bioactive peptides is therefore a well-established strategy to change their pharmacological profile. Peptide flexibility can be restricted by a local constraint imposed, e.g. by introducing amino acids with limited conformational freedom, that has an impact on specific orientations of the peptide backbone and the side chains. In this work, we decide to check the influence of the bulky (2S,4R)-4-(2-naphthylmethyl)-pyrrolidine-2-carboxylic acid (Nmp) at position 2 of the AVP and some of its analogues on the pharmacological properties. The synthesized analogues have the following structures: [Nmp[2]]AVP (I), [Mpa[1],Nmp[2]]AVP (II), [Nmp[2],D-Arg[8]]AVP (III), [Mpa[1],Nmp[2],D-Arg[8]]AVP (IV).
All peptides were tested for the pressor, antidiuretic and uterotonic in vitro activities in the rat.
Analogues I – IV exhibited moderate antioxytocic potency and were week pressor antagonists with pA_2 values ranging from 5.70- 6.31. All the analogues exhibited only negligible antidiuretic activity. It is worth to point out that only Nmp[2] modification at position 2 of AVP is sufficient to change the pharmacological profile of the peptide.
The results offer new information about structure – activity relationship of AVP analogues and may be profitably used for the design of new analogues with better pharmacologica

P20700-053

Design, Synthesis and Biological Activities of Temporin A and Temporin L Analogues.

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Temporins A (TA) and L (TL) are antimicrobial peptides isolated from the skin of Red European frog "Rana temporaria". Temporins are active against a broad spectrum of microrganism: TA (FLPLIGRVLSGIL-NH2) is preferentially active against Gram-positive bacterial strains; TL (FVQWFSKFLGRIL-NH2) has the highest activity against fungi, and bacteria, including resistent Gram-negative strains, but it shows haemolytic activity too. TA exerts its antimicrobial activity by its ability to form a transmembrane pore via a 'barrel-stave' mechanism or to form a 'carpet' on the membrane surface via the 'carpet-like' model. Recently we investigated the preferential conformation of TL and TA in SDS and DPC solutions which mimic bacterial and mammalian membranes, respectively. In SDS, the peptides prefer a location at the micelle-water interface; in DPC, they prefer a perpendicular location to the micelle surface, with the N-terminus imbedded in the hydrophobic core. TL shows higher propensity, with respect to TA, in forming α-helical structures in both membrane mimetic systems and the highest

propensity to penetrate the micelles (1). On these results we designed and synthesized new TA and TL analogues and found interesting differences in their efficacy against microbial species, and finding a new potent antimicrobial agent without haemolytic activity. 1. Carotenuto, A.; Malfi, S.; Saviello, M.R.; Campiglia, P.; Gomez-Monterrey, M.I.; Mangoni, M.L.; Marcellini Hercolani Gaddi, M.; Novellino, E.; Grieco, P. Journal Medicinal Chemistry, in press.

P20700-054

Biological Evaluation of New Arginine Vasopressin (AVP) Analogues Containing Non Natural Amino Acids

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Arginine vasopressin (AVP), a neurohypophyseal nonapeptide hormone [cycle1-6 (H-Cys[1]-Tyr[2]-Phe[3]-Gln[4]-Asn[5]-Cys[6]-Pro[7]-Arg[8]-Gly[9]-NH_2)], elicits a variety of responses both centrally and peripherally by acting on three distict G-protein coupled receptors: V_{1a} (vascular), V_{1b} (pituitary) and V_2 (renal). It also binds to the oxytocin (OT) receptor. In addition to its well-known antidiuretic activity, AVP has also complex cardiovascular actions and adrenocorticotropic hormone (ACTH) releasing activity. Binding of AVP to the V_{1a} receptor subtype also stimulates glycogenolysis in the liver and promotes platelet aggregation. It is generally accepted that the conformation of the N-terminal part of neurohypophyseal hormones analogues is important for their pharmacological activity. In continuing our work aimed at the design of selective AVP analogues, we synthesized twelve new analogues of AVP containing mercapto propionic acid (Mpa) or S-salicylic acid (Sal) in position 1, D-Tyrosine(Ethyl) [D-Tyr(Et)] or D-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid [D-Tic] in position 2, L-Arginine (Arg) in position 3 and L-Î±-*t*-butyglycine [Gly(But)] or L-*Î²*-(2-thienyl)-alanine [Thi] in positions 4 or 9. We also studied the effect of modified C-terminal amide on biological potency of the new AVP analogues. The analogues were synthesized by Fmoc/Bu^t solid phase methodology and were tested for their rat uterotonic in vitro activity, rat pressor activity and antidiuretic activity using conscious rats. The modifications performed had a significant impact on pharmacological activities of the analogues.

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P20700-055

Three-dimensional structure and mechanism of action of an antifungal peptide generated from hemocyanin cleavage in a penaeid shrimp

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An antifungal peptide, PvHCt, which corresponds to the 23 amino acid C-terminal sequence of the shrimp respiratory protein hemocyanin, has been previously identified in the plasma of the penaeid shrimp *Litopenaeus vannamei* (1). It is generated by proteolytic cleavage in response to a microbial challenge. Similarly, a C-terminal fragment of hemocyanin displaying antimicrobial activity has been isolated from crayfish plasma (2). These peptides are believed to contribute to the crustacean defence. The phenomenon of in vitro antimicrobial peptide generation from a respiratory pigment already observed with hemoglobin thus appears not to be restricted to mammals (3). PvHCt displays a broad spectrum of antifungal activity with minimum inhibitory concentrations (MICs) in the range 3-50 µM (12.5 µM against the shrimp pathogen *Fusarium oxysporum*). Its activity would be based on the inhibition of spore germination (1). To contribute to the elucidation of the mechanism of PvHCt antifungal activity, we determined its three-dimensional structure by circular dichroism (CD), NMR and molecular modelling and examined its effects on the *F. oxysporum* spore ultrastructure by transmission electron microscopy (TEM). CD and NMR data indicate that PvHCt is unfolded in an aqueous environment but adopts a similar helical structure in methanol solution and in dodecylphosphocholine (DPC) micelles used to mimic biological membranes. The structure consists of an amphipathic α-helix spanning residues 8 to 18. TEM shows that PvHCt induces structural changes of the plasma membrane accompanied by a disorganization of the cytoplasm and a significant decrease of lipid bodies.

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P20700-056

A single amino acid substitution changes the pharmacology of a peptide hormone, Oxyntomodulin, from a co-agonist at GLP-1/Glucagon receptors to GLP-1R-selective agonist

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Glucagon-like peptide-1 (GLP-1), glucagon (Gcg), and oxyntomodulin (Oxm) are highly homologous peptide hormones derived from post-translational processing of the preproglucagon gene. The sequence of Oxm in particular, is identical to the sequence of Gcg, with an 8-amino acid extension at the C-terminus. Pharmacological doses of Oxm activate both the GLP-1 receptor (GLP1R) and the glucagon receptor (GcgR) albeit with lower affinity compared to GLP-1 and glucagon, respectively. In order to understand the origin of this dual specificity, we synthesized a number of Oxm analogs in which one or more of the native amino acids were replaced. First, a chimera was produced in which all the residues differing between GLP-1 and Gcg were grafted into the Oxm native sequence, yielding an analog with the same pharmacologic profile as GLP-1. Further studies showed that surprisingly, the switch from GLP1R/GcgR co-agonism to GLP1R-selective agonism could be obtained by a single amino acid substitutions at position 3 of Oxm. In particular, the analog with Gln3 substituted by Glu (Oxm-Q3E) showed the same activity as native Oxm on GLP1R, but complete loss of activity on GcgR. Oxm and Oxm-Q3E were compared in a hyperglycemic clamp study performed in diet-induced obese (DIO) mice. Due to the short half-life of the peptides, both were infused intravenously at ~16 µg/kg/min in chronically catheterized mice. The amount of exogenous glucose required to maintain the hyperglycemic level was 2-fold greater with the selective GLP1R agonist Oxm-Q3E than with the GLP1R/GcgR co-agonist Oxm, showing that abolishment of GcgR activity significantly improves the glucose-lowering effect. We believe that these findings could be useful for the development of a peptide therapeutic for the treatment of type 2 diabetes.

P20700-057

Structural elucidation of thuricin, a two-component antimicrobial peptide

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Hospitals across Europe and North America have lately been plagued with infections caused by *Clostridium difficile*, a difficult to treat bacterium due to its resistance to many commercially available antibiotics. Recently, we isolated a two-component bacteriocin, thuricin, produced by *Bacillus thuringiensis* that exhibits activity against *C. difficile*. We found that these peptides, called Trnα and Trnβ, operate together in a synergistic fashion to inhibit bacterial growth. The peptide combination was found to be highly active against a wide range of *C. difficile* strains, including the virulent epidemic strain of the O27 ribotype, as well as most other clostridia and some bacilli and *Listeria* species. MALDI mass spectrometry revealed that both peptides have molecular weights that are lower than those predicted from their genetic sequences, indicating that they are post-translationally modified. Sequencing of the mature peptides through tandem mass spectrometry showed that each peptide has three modified amino acid residues near its C-terminus. These residues were found to be two units lighter than their expected natural amino acid masses, suggesting a loss of two hydrogen atoms through dehydrogenation or oxidation. In order to confirm these proposed modifications, we are investigating the production of ^{13}C- and ^{15}N-labeled Trnα and Trnβ for structure elucidation by NMR. Isolation of labeled peptides will be achieved by growing *B. thuringiensis* on defined media containing [U-^{13}C] glucose and $(^{15}NH_4)_2SO_4$. Subsequent NMR experiments will enable us to determine the three-dimensional solution structures of Trnα and Trnβ, individually and bound together. By elucidating thuricin's structure, we aim to better understand its mechanism of action against *C. difficile*.

P20700-058

Analogues of bradykinin B_2 receptor antagonist

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The most potent peptidic human bradykinin (BK) B_2 receptor antagonist is HOE-140 (Icatibant). In this study we present the synthesis of nine new analogues of HOE-140 substituted at position 7, 8 or 9 with chosen D-amino acid residues and/or nonproteinogenic amino acid residues, e.g. N-cyclohexylglycine (Nchg), 1-aminocyclohexane-1-carboxylic acid (Acc), octahydroindole-2-carboxylic acid (Oic), piperidine-3-carboxylic acid (Nip) and 4-phenylpiperidine-4-carboxylic acid (Ppc). In the next nine peptides we combined the above mentioned modifications with the placement of 1-adamantane acetic acid (Aaa) at position 0. All new analogues were tested on human umbilical vein for their antagonistic potency. Only three compounds containing Nchg^8, Nchg^9 or Ppc^9 exhibited noticeable antagonistic activities on human bradykinin receptors thus still less potent then original HOE-140 sequence. Each of them with Aaa^0 showed lower activity then parent analogues. Peptides: D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Nip-Arg and Aaa-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Nip-Arg, although strongly potent antagonists against BK-induced blood pressure lowering responses in rats, did not show noticeable antagonistic activity on BK-induced contraction of the isolated human umbilical vein, a well-established B_2R bioassay system, suggesting a species dependent activity of the compounds.

P20700-059

A Scalable Fmoc-SPPS Method for PT-141 and MT-II as Potential Drugs

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Due to advances made in the peptide field during the last few years, peptides as therapeutics have continued to gain more interest. The interest in peptide therapeutics generally comes from their high specificity to targeted sites, their diversity and their usually low toxicity. PT-141, also known as Bremelanotide, and MT-II are potential future drugs and both are heptapeptides with a 23-membered cyclic monomeric lactam bridge. They are melanocortin receptor agonist and an analog of alpha- melanocyte stimulating hormone (a-MSH). The PT-141 molecule has a c-terminal acid group while MT-II has a c-terminal amide function. Both neuropeptides have been tested in treating male sexual and erectile dysfunction as well as female sexual arousal disorder. PT-141, patented by Palatin Technologies, New Jersey, USA, is the only known synthetic aphrodisiac and unlike other erectile enhancers like Viagra, it does not act upon the vascular system. Instead, it directly increases sexual desire and is used nasally as a spray. A scalable method for the synthesis of both peptides using Fmoc-chemistry and the problems involved during the synthesis process will be discussed in details.

P20700-060

Creation of novel spider toxin analogs to adopt as probes for visualization of glutamate receptors

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L-Glutamate (Glu) is known to be major excitatory neurotransmitter not only in the mammalian central nervous system but also in the ganglia of arthropods. Binding of spider toxins to glutamate receptors (GluRs) results in the inhibition of Glu-mediated neurotransmission. In order to elucidate the mode of binding between Glu and GluRs, we focused on the visualization of GluRs by complex formation with fluorescent-labeled analogs of NPTX-594 (**1**), a spider toxin with the structure of N^1-(2,4-dihydroxyphenylacetyl-L-asparaginyl)-N^{12}-L-lysyl-4,8-diaza-1,12-dodecanediamine [Dhpa-Asn-Dada(12Lys)]. In the present study, the modified NPTX-594, i.e., Dhpa-Asn-Dada(12Abg) (**2**) in which the Lys residue of **1** was replaced with the *N*-(4-aminobutyl)glycine (Abg) residue, was employed as a template compound to create the fluorescent-labeled analogs of NPTX-594, since the biological activity of **2** is three times higher than that of **1**. We thus carried out the modification of Dhpa in the analog **2**, i.e., 1) Dhpa was replaced with the coumarin-type acyl residues (**Type-1**); 2) the phenylacetyl residues having alkyne side chains that can be converted into suitable fluorophores based on the click chemistry (**Type-2**); and 3) the coumarin-type acyl residues having the mercapto group to form disulfide bond with the Cys residue in GluRs (**Type-3**). This paper presents the synthesis and biological activity of various NPTX-594 analogs to adopt as probes for visualization of glutamate receptors.

P20701-061

An investigation of the functional requirements of Apidaecin Ib C-terminal fragment by means of peptoid-peptide hybrids

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Pro-Arg rich peptides constitute an important class of antimicrobial peptides isolated from mammalian and insect sources. They kill bacteria

by acting on one or more intracellular targets, without damaging the cytoplasmatic membrane. Their particular killing mechanism and their low toxicity against mammalian cells make them attractive for the development of new antibiotics. Structure-activity relationships studies have been carried out on short Pro-Arg rich antimicrobial peptides isolated from insects and the characterization of various natural isoforms of the 18-residues peptide apidaecin Ib allowed to identify an evolutionary conserved region in the C-terminal part of the molecule. Even a single point mutation in this region results in reduction or loss of antimicrobial activity. We recently described the synthesis of some apidaecin Ib peptoid-peptide hybrids in which each arginine was replaced by the corresponding N-alkyl glycine residue (1). The afforded modification made the resulting peptoid-peptide hybrids more resistant to proteolysis but moving the [Narg]residue from the N- to the C-terminal end of the molecule progressively reduced the antibacterial activity. Here we report the synthesis of a series of novel analogues containing a N-*homo*arginine or N-*nor*arginine residue in position 4, 12 or 17. The effect of the size of the side-chain of the peptoid residue on the antimicrobial activity is also reported. In order to strengthen the peptide resistance to proteolysis and by considering that enzymic cleavage of the Arg^{17} - Leu^{18} peptide bond yields a fully inactive compound, we also prepared the [$Nleu^{18}$]-apidaecin analogue. The conformational properties of the resulting peptoid-peptide hybrid, which is devoid of any antimicrobial activity, will be compared to those of apidaecin Ib and the other [Narg]peptoid-peptide hybrids.
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P20701-062

Enhancement of Antimicrobial Activity of a Natural Antimicrobial Neuropeptide of *Sepia officinalis*

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A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. However, antimicrobial activity is not the main function of some of these peptides. In the Cuttlefish *Sepia officinalis* we observed an antibacterial activity for the neuropeptide : H-ALSGDAFLRF-NH_2(1). This decapeptide belonging to the FMRFamide family involved in regulation of reproduction and chromatophore function and is able to inhibit the growth of marine bacteria. Circular dichroism studies have revealed for this amphiphilic peptide a helical structuration in SDS micelles and in 50% TFE. To improve this antimicrobial activity, we first introduce a Lysine residue instead of Aspartic residue, the antimicrobial activity of this new peptide has been improved by augmentation of the positive net charge (+1 to +3). Moreover preliminary results have shown that the incorporation of aza-β^3-amino acids analogues could create original hybrid pseudopeptide with superior activity . The natural peptide is not efficient against *Staphylococcus aureus* whereas the pseudopeptidic analogue revealed a Minimum Inhibiting Concentration (MIC) between 8-16µM. Therefore some α-amino acids were replaced by aza-β^3-amino acids. We will show in this communication that depending on the substituted residue and on the structuration, these modifications could lead either to no activity or to a drastic enhancement of the antimicrobial activity, demonstrating that aza-β^3-amino acids can facilitate the burying in lipidic bilayer of antimicrobial peptides that acts on bacterial membranes(2)

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P20709-063

Isolation and structural characterization of capistruin, a lasso peptide predicted from the genome sequence of *Burkholderia thailandensis* E264

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Lasso peptides are a structurally unique class of gene-encoded, bioactive peptides characterized by a knotted arrangement where the C-terminus threads through an N-terminal macrolactam ring. The rigid lasso structure confers a particular stability against temperature and proteolytic degradation. Most of these lasso peptides were isolated from Actinobacteria and act as enzyme inhibitors or receptor antagonists. A typical lasso peptide is microcin J25 (MccJ25), which is secreted by *Escherichia coli* and displays antibacterial activity by inhibiting the RNA polymerase. The *mcjABCD* gene cluster is required for MccJ25 biosynthesis: *mcjA* encodes the linear precursor, *mcjD* is necessary for MccJ25 export and self-immunity, and *mcjB* and *mcjC* code for the two processing enzymes that are sufficient for the conversion of McjA into bioactive MccJ25. Similarity studies revealed homologues of McjB, McjC and McjD in *Burkholderia thailandensis* E264. Additionally we identified a 144 bp ORF coding for the putative 47 amino acid precursor protein CapA and we proposed the C-terminal 19 aa to form the mature lasso peptide. LCMS analysis of the culture supernatant of *B. thailandensis* E264 revealed a compound of the exact mass expected. The poor overall fragmentation behavior in MS^n studies suggested a branched cyclic peptide with a rigid lasso structure. Optimization of the fermentation conditions increased the production by 200-fold and subsequent NMR structural studies proved the lasso structure of the peptide that was named capistruin. Heterologous production of the lasso peptide in *E. coli* showed that the identified genes are sufficient for the biosynthesis of capistruin, which exhibits antimicrobial activity against closely related *Burkholderia* and *Pseudomonas* strains. To our knowledge, this is the first rational based identification of a novel lasso peptide and the presented approach should be advantageous for the isolation of further lasso peptides in the future.

P20710-064

Novel antimicrobial peptides from marine invertebrate animals

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Endogenous antimicrobial peptides (AMPs) are the earliest molecular factors in the evolution of innate immunity. Marine invertebrate animals have no acquired immunity with a system of antibodies diversification. They are presumed to use an AMPs-based system as principal defense against potential pathogens. We have discovered a new family of small (21-residue) AMPs, termed arenicins, in coelomocytes of marine polychaeta lugworm Arenicola marina. These AMPs exhibited activity against Gram-positive, Gram-negative bacteria and fungi. Complete amino acid sequences were determined for each isoform. Arenicins have one disulfide bond (Cys3-Cys20). Arenicins have no structure similarity to any previously identified antimicrobial peptides. A novel 40-residue antimicrobial peptide, aurelin, exhibiting activity against Gram-positive and Gram-negative bacteria, was purified from mesoglea of a scyphoid jellyfish Aurelia aurita. Complete amino acid sequence of aurelin was determined. Aurelin has 6 cysteines forming three disulfide bonds. The total RNA was isolated from the lugworm coelomocytes and from the jellyfish mesoglea, RT-PCR and cloning were performed, and cDNAs were sequenced. A 202-residue preproarenicin contains a

putative signal peptide (25 amino acids) and a long prodomain. A 84-residue preproaurelin contains a putative signal peptide (22 amino acids) and a propiece of the same size (22 amino acids). Aurelin reveals partial similarity both with defensins and K+ channel blocking toxins of sea anemones and belongs to ShKT domain family. Overlapping of biological properties of marine animal AMPs and toxins along with their sequence homology might be a consequence of divergent evolution from a common ancestor. Antimicrobial peptides from marine organisms could afford design of new antibiotics manifesting broad-spectrum antibacterial activity.

P20711-065

Cyclic enkephalin analogs containing two alkylurea units

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We shall present some structure-activity results for 8 enkephalin analogs, derived from the exhaustive combinations of D-Lys and D-Orn in position 2 with Lys, Orn, Dab and Dap in position 5, both positions coupled ¦Ø-¦Ø¡¯ by means of the urea bridge. Accordingly, they all are restrained by 14-18-membered rings. In addition, we introduced the -NH-ethylurea unit instead of -NH*2* of amide group in previously published analogs [1-3]. Their in vitro activities were determined in the GPI and MVD assays. The peptides are more active than enkephalin in the GPI while have similar activities to the latter in the MVD assay. The effect of the introduction of ethylurea unit at the C-terminus on the activities is also discussed. Chemical shifts of the peptides in water were fully assigned and their sequences confirmed. Several cross-peaks between the protons of amidoalkylurea unit and preceding residues have been observed in each case, suggesting that the unit may be involved in specific interactions between residues 4 and 5.

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P20711-066

Understanding the structure/activity relationships of hepcidin

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Iron is an essential element for nearly all living organisms and plays a key role in a range of processes including oxygen transport and storage, catalysis of redox reactions, production of metabolic intermediates and host-defence (1). Until recently, little was known about the regulatory elements involved in the control of iron uptake and distribution within the body. The recently discovered peptide hepcidin has been shown to be a key regulator of iron metabolism within the body in response to a range of conditions, including inflammation, hypoxia and anaemia (2). This talk will focus on work towards elucidating structure/activity data for hepcidin with the aim of gaining a better undertstanding of the interaction between hepcidin and its receptor, ferroportin. We hope that this information will facilitate the design of synthetic agonists or antagonists of ferroportin to be used as potential drug leads.

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P20711-067

Influence of bulky 3,3-diphenylalanine isomers replacing position 2 of AVP analogues on their conformations

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It is believed that a tyrosine residue at position 2 of AVP plays a part initiating the pressor response of AVP. Moreover, it is known that inhibitors of the uterotonic activity have been traditionally produced by introduction of β-carbon substituents into position 1 and/or by substitution of L-tyrosine at position 2 of OT and AVP with aromatic D-amino acid. In this study, we present the results of the conformational analysis of four vasopressin (AVP) analogues modified at position 2 with 3,3-diphenylalanine (Dpa) isomers, [Mpa^1,Dpa^2,Val^4,D-Arg^8]VP (I), [Mpa^1,D-Dpa^2,Val^4,D-Arg^8]VP (II), [D-Dpa^2,D-Arg^8]VP (III) and [Mpa^1,D-Dpa^2]AVP (IV). All the peptides are very potent antidiuretic agonists with significantly prolonged action. Moreover, the analogues II, III and IV have high antioxytocic potency. Additionally, all the peptides are devoid of pressor or antipressor activities with the exception of analogue II which display very weak antagonistic properties.

Three-dimensional structures of investigated analogues were determined using two-dimensional NMR spectroscopy and molecular dynamics simulations with time-averaged restraints. The analysis of structural differences exhibited by different modifications provides the basis for understanding conformation – activity relationships and thereby the mechanism of interactions of the analogues with receptors.

P20711-068

NMR structure of the micelle-bound 26RFa and 43RFa, two peptide ligands of the GPR103 receptor

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A novel RFamide peptide, named 26RFa and with no meaningful similarity with other members of this family, has been recently characterized. Its precursor encompasses several potential cleavage sites and thus may generate various mature peptides including an N-terminally extended form of 26RFa, termed 43RFa. Both peptides act as endogenous ligands of the G-protein coupled receptor GPR103. This receptor has been recently implicated in the bone metabolism regulation

The determination of the 3D structure of such peptides is essential for the elucidation of their structure/function relationships and for the design of potent agonists or antagonists. Although structure elucidation of a ligand in the absence of the target receptor can deliver limited insight into the bioactive conformation, there is emerging evidence that interactions with the cell membrane is a key step required for receptor recognition. In this context, we have investigated the solution conformation of 26RFa and 43RFa by CD, NMR and Molecular Modelling in different media, in particular in one miming the “free” form of the molecule (methanol or mixture TFE/water) and in a cell membrane mimetic medium (DPC micelles). In an organic solvent, both peptides adopt the same conformation, i.e. an amphipathic alpha-helical structure, flanked by two

N- and C-terminal disordered regions. When bound to DPC micelles, the N-terminus remains flexible and an helix is present at the same position as in the "free" form for both molecules. In contrast, the C-terminal extremity becomes structured adopting an inverse gamma-turn conformation. These data represent the first step for the rational design of new molecules that might be used in the treatment of osteoporosis.

Acknowledgements: Supports were obtained from INSERM and IFRMP 23. The NMR spectrometers are supported by grants of the Conseil Régional de Haute-Normandie. NMR and Molecular Modelling facilities are provided by the Centre de Ressources Informatiques de Haute-Normandie.

P20711-069

Antimicrobial activity of analogues of a peptide isolated from venom glands of social wasps Polistes major major inhabiting the Dominican Republic

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Recently we have described isolation and biological activities of several new peptides from the venom glands of social wasps Polistes major major found in Dominican Republic. We have also reported the synthesis of their analogues in order to investigate structure-activity relationship with respect to the antimicrobial and hemolytic activities (1). Here we report the activities of a few further analogues of one of the peptides called PMM (H-Ile-Asn-Trp-Lys-Lys-Ile-Ala-Ser-Ile-Gly-Lys-Glu-Val-Leu-Lys-Ala-Leu-NH2). The parent sequence or its truncated analogues were modified on the N-terminus with 6-aminocaproic acid, glycolic acid, palmitoic acid, and 9-acridinyl and 9-(1,2,3,4-tetrahydro)acridinyl groups. The new analogues were tested for their antimicrobial activity (determination of minimal inhibitory concentration values – MIC - using broth dilution method) and hemolytic activity (determination of the IC50 value using suspension of rat erythrocytes). The palmitoylation unfortunately did not enhance antimicrobial activity against the tested microorganisms (Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa). Substitution of amino acids Ser8 or Glu12 subsequently for alanine, serine or lysine did not influence the activity of the peptides significantly.

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P20712-070

New analogues of the antimicrobial Gramicidin S

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Gramicidin S, GS, c-(Val-Orn-Leu-D-Phe-Pro)2, was isolated from Bacillus brevis. It forms a two-stranded antiparallel β-sheet flanked by two II'β-turns. It was found that the distribution of hydrophobic and hydrophilic residues on the opposite sides of the sheet is a structural feature required for GS antimicrobial (AM) activity. Despite its wide Gram+ and Gram- antimicrobial activity GS is useless in therapy because of its high hemotoxicity in humans. It was found, however, that the analogues of GS-14 (GS with Lys-Leu inserted into each strand) got more AM selective, when their amphipatic moments were perturbed by swapping adjacent Lys↔Leu/Val or configuration reversal at Lys (1). Here, we report on effects of similar perturbations put on GS original c-decapeptide, using the following examples: c-(Val-Lys-Leu-D-His-Pro)2, **1**, 1. as the mother compound, and its three analogs, viz. c-(Val-D-Lys-Leu-D-His-Pro-Val-Lys-Leu-D-His-Pro) – Lys2 converted to D, c-(Lys-Val-Leu-D-His-Pro-Val-Lys-Leu-D-His-Pro) – Val1-Lys2 swapped, and c-(Val-Leu-Lys-D-His-Pro-Val-Lys-Leu-D-His-Pro) – Lys2-Leu3 swapped, **2-4**; all having reduced ring-sequence symmetry. The peptides were synthesized by solid-phase methods using 9-fluorenylmetoxycarbonyl (Fmoc) methodology. Having solved their structures by 2D-NMR and having tested their activities/selectivities, we confirmed that only **1** had relatively favorable bio-profile, as already published (1), while the other – did not.

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P20712-071

Cereulide, a foodborne peptide highly toxic towards the insulin producing beta-cells of the pancreas

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Cereulide is a heat stable cyclic, lipophilic (log Kow 5.96) peptide (1152 g mol/1) produced by certain strains of *Bacillus cereus*, a bacterium connected to emetic food poisonings. It is insoluble in water, soluble in ethanol, methanol, DMSO, food oils. Cereulide exposure caused collapse of mitochondrial membrane potential in all tested human cells at low (ng/ml) exposure concentration (NK cells, T lymphocytes, Caco2, Calu3, neural Paju, Hela). Toxicity is caused by its action as ion carrier with a selectivity of K^+: Na^+ = >1000 : 1. In various foods connected to human illness, concentrations of 0.01 to 3 µg/g of cereulide were measured. In a case where the remains of a meal that had caused acute serious illness of two adult persons, were obtained for analysis, 1.3 µg of cereulide was found /g of food. We undertook to explore the effects of purified cereulide, and cereulide containing bacterial extracts, on porcine pancreatic islet cells in culture. Foetal porcine islet cells were exposed to heat killed extracts from food-borne *B. cereus* strains producing or not producing cereulide and to purified cereulide. Effects were assayed using viability staining with fluorochromes and cellular contents of DNA and insulin. Exposure to 1 ng/ml of purified cereulide caused necrotic cell death of the islet cells impairing their insulin content within 2 days. Cell extracts of cereulide positive *B. cereus* strains connected to food poisoning or isolated from food items were toxic, corresponding to their measured cereulide content. Extracts of *B. cereus* strains producing or not producing the *B. cereus* diarrhoeal toxin, but not cereulide, were tolerated by the porcine islet cultures up to concentrations 1000 fold higher compared to extracts from strains containing cereulide, produced substance toxic towards porcine fetal Langerhans islets and beta cells.

P20712-072

Application of non-sequential pharmacophore concept for design of antimicrobial peptide dendrimers

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Unique structure of dendrimeric compounds consisting of a central core and several generations of branches provides opportunity of multiple practical solutions in the area of medicine. Location of a high number of functional groups at the surface, allows to present multiple pharmacophoric units to the receptors, with immediate application in the design of a new generation drugs or vaccines, tools for studying autoimmune diseases or understanding gene delivery mechanism.
Here we present another possible application of dendrimeric compounds – preparation of drug molecules, which mimic active conformations of various macromolecular ligands. We focused on low molecular weight basic dendrimeric peptides, which mimic active conformations of recently discovered natural antimicrobial peptides. Structurally, natural compounds are linear cationic peptides consisting of 10-50 amino acids that kill a broad spectrum of microbes destabilizing ordered structure of their cell membrane. It is generally accepted that positive charge and an induced amphipathic conformation are necessary for their antimicrobial activity. The project is related to multi-drug resistance of numerous bacteria against conventional antibiotics and involves de novo design of 1-2 generation dendrimeric peptides, which mimic sequence-related active conformations of natural compounds (non-sequential pharmacophore concept). Apparently, several groups of small peptide dendrimers were synthesized and structurally characterized (NMR, CD). They are potent antimicrobials, active against broad spectrum of species including MRSA and ESBL strains. Interactions between model membranes and peptide dendrimers of various structure will be discussed.
Acknowledgements: Financial support from the Ministry of Science and Higher Education of Poland, Grant 3T09B 115 28 is acknowledged.
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P20713-073

Identification of Antimicrobial Peptides Capable of Reducing Biofilm Growth in Seawater Desalination Processes

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Seawater desalination is most commonly done today by reverse osmosis (RO) using thin-film composite membranes. A major problem in RO desalination is biofouling, caused by adhesion and growth of bacteria to form biofilm on the membrane surface. Recently we proposed a new approach to reduce biofilm formation on RO membranes that is based on immobilization of antimicrobial peptides (AMPs) onto the membrane surface. In this study we screen AMPs capable of reducing biofilm growth under conditions simulating seawater desalination, and search for mode of binding to the membrane without affecting the peptides bioactivity. A specific bioassay was developed for screening peptides activity in high salinity conditions in order to evaluate the inhibition of biofilm growth, based on growing biofilm-forming bacteria in a 96-wells microtiter plate. We prepared various AMPs known from the literature by solid phase peptide synthesis (SPPS) using Fmoc-chemistry. The bactericidal activity of AMPs was examined in fresh water and compared to high salinity water. Most AMPs lost their activity in high salinity conditions; yet, few peptides possessed their bactericidal activity and were used in subsequent experiments. Searching for mode of linkage was performed by evaluating the bactericide activity of AMPs modified with numerous types of linker molecules. Based on literature studies that showed no decrease in bactericidal activity of AMPs upon N-terminal modification, we prepared the corresponding peptides with modified spacers on their amino-terminal and evaluated their antimicrobial activity in solution. Indeed, we obtained Gly_3-spacers that retained activity, which were used subsequently as linkers to RO membranes. The mode of binding, as well as bactericide activity of the peptides and of the membranes will be presented and discussed. This study will lay the bases for a novel approach to decrease biofilm formation on the surface of RO membranes during RO desalination.

P20713-074

Bioactive tripeptides Ile-Pro-Pro and Val-Pro-Pro protect endothelial function in vitro in normotensive and hypertensive rats

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Milk drink containing casein-derived bioactive tripeptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) has been shown to decrease blood pressure both in animal models and clinical studies. This effect can be attributed to the tripeptides. It has been suggested that one possible blood pressure lowering mechanism of the tripeptides could be angiotensin-converting enzyme (ACE) inhibition. However, not all studies support this finding.
The effect of tripeptides IPP and VPP on vascular function was investigated in vitro using rat mesenteric arteries. Superior mesenteric arteries isolated from male Wistar-Kyoto and spontaneously hypertensive (SH) rats were incubated in Krebs solution containing 1 mM of the peptide (either IPP or VPP) in +4 ºC for 48, 24, 12 or 1 h. After incubation mesenteric artery rings were mounted in an organ bath chamber and extensive vascular reactivity measurements were performed. Acetylcholine-induced endothelium-dependent relaxation was better preserved ($P < 0.05$) in mesenteric arteries of both strains incubated with IPP or VPP compared to the control. Clear differences were not observed in sodium nitroprusside-induced endothelium-independent relaxation.
The ACE-inhibitory activity of IPP and VPP was studied by measuring the response to a single administration of angiotensin I and II in organ chambers. Proportioned to KCl-induced contraction, no clear reduction in angiotensin I -contraction was seen. Thus, ACE-inhibition may not be the main mechanism for the long-term effects of IPP and VPP in the protection of endothelial function.
We suggest that the tripeptides do not affect smooth muscle but they protect endothelium during incubation indicated as preserved acetylcholine-induced endothelium-dependent relaxation.
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P20716-075

Short host defense peptides – determination of sequence requirements for killing Pseudomonas aeruginosa

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Host defense peptides are a part of the innate immunity and can often kill both Gram negative and Gram positive microorganisms rapidly

and directly and modulate other parts of host innate immunity. Today, more than 800 cationic peptides have been identified. They all have certain conserved physical features including a net positive charge, contain approximately 50% hydrophobic amino acids and have sizes ranging from 12 to 50 amino acids. However, virtually any -sheet, loop includingβ-helix, αtype of secondary structure can arise including -turn and extended. The multitude of cationic peptide sources, structures andβa spectra of activity is matched by a number of complex and controversial models attempting to describe and explain their modes of action. Little is known about the sequence requirements of short host defense peptides like bactenecin (12mer). With help of our novel technique using a artificially created luminescence producing gram negative bacteria and peptide synthesis on cellulose we can investigate the sequence requirements of such peptides. Hundreds of peptides are tested for their ability to kill Pseudomonas aeruginosa. Complete substitutional analyses of different bactenecin variants as well as a semirandom peptide library with about 2000 members were measured. The complete substitutional analysis will give us information about the importance of each single position whereas the peptide library will give us broader information which composition of amino acids results in an active antimicrobial peptide. The data will be analyzed using the quantitative structure-activity relationship approach (QSAR) to identify sequence patterns that discriminate between superior activity cf. equivalently active and inactive. This will give us mechanistic cues for a better understanding of the mode of action of the short antimicrobial peptides. The results of these measurements and analyses will be discussed in detail.

P20719-076

A flow cytometric method to detect internalization of antimicrobial fluorescently-labeled peptides in bacterial cells

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Antimicrobial peptides inactivate bacteria *via* different mechanisms of action, some of which are mediated by internalization of the peptides without any apparent membrane damage. In this case, immunoelectron transmission microscopy and confocal microscopy are the techniques used to confirm peptides' uptake. However, these techniques are quite complex to perform and time consuming, and the results are sometimes ambiguous. Here we propose a simple and rapid flow cytometric method to assess internalization of the peptides in bacteria. The method is based on the use of fluorescently-labeled peptides and of the extracellular quencher Trypan Blue to discriminate between a cell surface and cytoplasmic localization of the tested molecules. To his aim, we used BODIPY-labeled peptides showing different modes of action. These included some fragments of Bac7, a proline-rich peptide known to penetrate bacterial and eukaryotic cells without membrane damage[1,2], and polymyxin B, a peptide antibiotic that binds to LPS and to the cell membranes. By using this approach coupled to flow cytometric analysis, we showed that the fluorescence intensity of *E. coli* and *S. typhimurium* cells treated with sub-inhibitory BODIPY-Bac7 concentrations did not decrease despite extensive washing and addition of the quencher Trypan blue. In contrast, the fluorescence of cells treated with BODIPY-polymyxin B, as well as that of bacteria treated with a fluorescein-labeled anti LPS antibody, were promptly and almost totally quenched by addition of Trypan Blue, indicating their accessibility on the bacterial surface. These results confirm the suitability of this method to rapidly infer the localization of labelled molecules in an accessible or inaccessible compartment of the treated bacterial cells.

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P20720-077

Effect of Synthetic Peptides against Multi-Drug Resistant Bacteria from Otitis Media

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HP (2-20) is an antimicrobial peptide derived from the N-terminus of Helicobacter pylori Ribosomal Protein L1 (RPL1). In our previous study, several analogues of HP (2-20), with amino acid substitutions that increased or decreased net hydrophobicity, were designed and showed that an analogue, A3 designed by substituting Gln and Asp with Trp at positions 17 and 19, respectively, caused increased antibacterial activity in minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) without having hemolytic activity. The peptide A3 acted also synergistically with known antibiotics including chloramphenicol against bacterial cells. Fluorescence activated flow cytometry showed that A3-treated cells had higher fluorescence intensity than untreated cells, similar to that of melittin-treated cells. The peptide A3 showed a strong antimicrobial activity against antibiotic-resistant Pseudomonas aeruginosa from otitis media, clinically isolated MRSA and VRSA including biofilm-forming bacteria in vitro and in vivo. The ototoxicity of A3 was studied in vivo by topical application to the middle ear in guinea pig model. Twenty guinea pigs (5 groups, each group n=4) were each injected by transtympanic approach with 20ul of 8ug/ml,16ug/ml,32ug/ml, 128ug/ml of A3, and 0.4% gentamicin sulfate was instillated as a control. Auditory brainstem responses (ABR) to click were measured between 1st and 7th days after injection. Histologic investigation of cochlea was performed by scanning electron microscope and light microscope. The results showed that topical application of A3 to the middle ear is well tolerated without cochlear damage. The present study, therefore, demonstrates that the usefulness of antimicrobial peptides for multi-drug resistant bacteria including as a new ototopical agent for CRPA otitis media.

P20720-078

Structure-activity relationship study of KiSS-10: identification of an antagonist of GPR54

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Metastin, also known as kisspeptin-54 (KiSS-54), a fragment of the protein encoded by the KiSS-1 gene, is the endogenous ligand of GPR54. This peptide, which has 54 residues and a C-terminal amide, inhibits tumor metastasis. Two other biologically active peptides derived from the KiSS-1 gene, KiSS-14 and KiSS-13, have been isolated from human placenta. KiSS-10, a synthetic decapeptide amide from the C-terminus of KiSS-54, has a 10-fold greater affinity for GPR54 than KiSS-54, identifying KiSS-10 as the pharmacophore for receptor binding. All these peptides are collectively named kisspeptins. In addition to described roles in cancer metastasis and placentation, the kisspeptin/GPR54 pair has received growing attention following the discovery that it acts as a molecular switch for puberty. The aim of this work was to study the Ca^{2+}-mobilizing activity of Ala-substituted KiSS-10 analogs in GPR54-transfected cells. Administration of KiSS-10 induced a dose-dependent increase in $[Ca^{2+}]i$ with an EC_{50} of 15 nM. Sequential Ala-substitution of the native residues 1-5 and 7, led to analogs which exhibited similar potency to KiSS-10. Conversely, [Ala^6], [Ala^8] and [Ala^9]KiSS-10 were 450, 6 and 11 folds less potent than KiSS-10, respectively. Whatever the

dose, [Ala^{10}]KiSS-10 did not modify the basal [Ca^{2+}]i. On the other hand, [Ala^{6}]KiSS-10 (but not [Ala^{10}]KiSS-10) dose-dependently inhibited the KiSS-10-evoked [Ca^{2+}]i increase. Altogether, these data show that (1) the biological activity of KiSS-10 is located in the C-terminus, (2) the native Phe^{6} residue is involved in GPR54 activation and the Phe^{10} residue in affinity, and (3) the introduction of an aliphatic residue in position 6 may lead to potent antagonists. This SAR study constitutes the first step towards the development of ligands that will be used for the rational design of drug candidate useful for the treatment of reproductive disorders.

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P20720-079

Peptides from CcdB protein as novel inhibitors of DNA gyrase

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The bacterial protein CcdB (11.7 kDa) is encoded by the F plasmid as a component of the toxin-antitoxin module (TA systems). In this module CcdB acts as a toxin and CcdA, an 8.7 kDa protein, as the antitoxin. In the absence of CcdA, CcdB inhibit the cell division and can kill bacteria by a mechanism that involves the DNA gyrase. Bacterial DNA gyrase is unique among the type II topoisomerase with ability to negatively supercoil DNA. The enzyme consists of two subunits, A (GyrA) and B (GyrB) and operates as an A_2B_2 heterotetramer. The mechanism of the inhibition of the gyrase activity by CcdB is still an object of many debates, but is clear that R462 residue of GyrA and the C-terminus of CcdB (W99-I101) play a crucial role in the Gyrase-CcdB interactions. As an approach for a better understanding of this mechanism as well as for development of new gyrase inhibitors, we have synthesized peptide analogues of the CcdB protein and studied its activity by supercoiling assays and bacterial growth. Five fragments ($CcdB_{ET1}$, $CcdB_{ET2}$, $CcdB_{ET3}$, $CcdB_{ET4}$ and $CcdB_{SS1}$) of the natural CcdB were designed and synthesized by SPPS. For the design, we considered the 13 residue C-terminal α-helix (residues E87 to W99), the loop that connects two strands of the wing sheet (residues R40 to L50) and an N-terminal region that includes the first of the five-stranded antiparallel β-sheet. All peptides, except $CcdB_{ET4}$, showed inhibition of the supercoiling activity of the DNA gyrase, especially $CcdB_{ET2}$ with a MIC = 15 μM. Free Peptides not showed antimicrobial activity, but when encapsulated in Liposome (SUV) were able to inhibit the bacterial growth in liquid culture medium. The growth inhibition in vitro for $CcdB_{ET2}$ was about 70% for Gram negative bacteria. Our findings revealed a novel synthetic inhibitor of DNA gyrase and $CcdB_{ET2}$ analogue is a good starting point for the development of a new and specific class of antibacterial agents based in the DNA gyrase inhibition.

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P20720-080

Synthesis and neuroprotective properties of short peptides consisting solely from glycine and proline residues

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Now appears more and more data on biological activity of short peptides consisting solely from glycine and proline residues. It is suppose, that these peptides, named as "glyprolines" (GPs), can be formed from collagen, ÅÑÌ and related proteins. However an effect of these peptides on CNS is not yet understood. The aims of this work were to improve a methodology of GPs synthesis and to study cytoprotective properties of some new GPs in culture of neuronal cells. GPs with a common structure (GP)n, (PG)n, and (PGP)n (n=1-3) were synthesised by consecutive growing of peptide chain and fragment's condensation. Synthesised peptides were characterised by HPLC, mass-spectrometry, element analysis etc. Cytoprotective activity of GPs was assessed on an increase of survival of cultivated ÐÑ12 cells after $Í_2Î_2$-induced oxidative stress. It was shown, that from all tested peptides only (GP)n and PGP reveal cytoprotective activity. At the concentration 100μM these peptides reduced an amount of damaged cells 1.7-2.0 times in comparison to the control. Thus preliminary data received show that some GPs demonstrate a strong cytoprotective activity and therefore it is advisably to put them on further study on in vivo models.

P20721-081

Antiviral (HSV) activity of selected insect peptides

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Natural products isolated from arthropods are an important source of bioactive compounds. However, relatively little data are available on molecules from insects with antiviral activities. The subject of our work was a search for new biological properties of a series of insect peptides, such as alloferon (H-His-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH) (I) isolated from the blow fly *Calliphora vicina*, its four analogues modified at position 1 ([des-His^{1}]-(II), [Arg^{1}]-(III), [Lys^{1}]-(IV), [Ala^{1}]-alloferon (V)), the pentapeptide Any-GS (H-Asp-Ile-Leu-Arg-Gly-NH_2) (VI) isolated from the wild silkmoth *Antheraea yamamai*, and its eight analogues ([2-5]-(VII), [3-5]-(VIII), [1-4]-(IX), [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 ($HSV\text{-}1_{MC}$) in Vero celles line, infected with $HSV\text{-}1_{MC}$ $1TCID_{50}$/cell and 2/ their cytotoxic activity was evaluated in Vero cell line by the MTT (3-[4,5 dimethylthiazol-2-yl]-2,5 diphenyl tetrazoliumbromide) method. In preliminary investigations we found that all the peptides inhibited to a high degree the replication of HSV-1 in Vero cells. Moreover, these compounds did not show any cytotoxic activity against the Vero cells.

P20721-082

Sexual dimorphism of HLDF-6 peptide neuroprotective action in Alzheimer's models in *vivo* and *vitro*

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We established that HLDF-6 peptide, biologically active fragment of Human Leukemia Differentiation Factor (HLDF), restores ability for learning, and also prevents loss of long-term memory and decrease in the exploratory behavior of Wistar rat-males with experimental Alzheimer's disease induced by intrahippocampal injection of beta-amyloid peptide Aβ (25–35) and ibotenic acid. The HLDF-6 peptide was shown to render protective influence directly on primary culture of hippocampal and cerebellar neurones, isolated from newborn male brain, under conditions of the beta-amyloid toxicity. Protective action of HLDF-6 peptide on newborn rat-male neurons is connected with

its ability to reduce 5-alpha reductase mRNA expression in more than 30 times, blocking testosterone metabolism into dihydrotestosterone (DHT) and thus interfering hyper activation of NMDA receptors in CA1 areas of hippocampus. The protective action of HLDF-6 peptide was investigated also and on female-rats with experimental Alzheimer's disease. It was shown, that in contrast to males at which both forms of memory are broken: and long-term and working ones, in the case of females, injections of Aβ (25-35) + ibotenic acid result in infringement of only working memory, at safety of long-term memory. Introduction of HLDF-6 peptide restored the broken working memory at females as effectively, as at males. However the mechanism of protective action of peptide on primary culture of hippocampal and cerebellar neurones, isolated from newborns female-rat brain, is connected not with the decrease in hyper activation of NMDA receptors, but with ABAD (17 beta-hydroxysteroiddehydrohenase of the 10th type) mRNA expression enhancement which is a target of beta-amyloid peptide action and blocking of progesterone conversion into 5 alpha-dihydroprogesteron due to 5-alpha-reductase mRNA expression inhibition.

P20721-083

In vitro and *in vivo* studies of P-19, an antimicrobial peptide active against multidrug resistant Gram positive cocci

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Gram positive cocci (GPC) are common cause of hospital associated infections resulting in increased mortality, morbidity and cost of treatment. In last few decades, development of resistance has been noted in major human pathogens. This highlights the need for the novel therapeutics for effective treatment/control of these resistant pathogens. A large number of peptides that exhibits antimicrobial activity have been isolated from animal and plant. Among them, cationic peptides are the new therapeutic antibiotics.
We have designed octapeptide based on the sequence of Sepecin B, an antibacterial protein of *Sacrophaga peregrina*, effective against Gram + bacteria. We systematically incorporated internal hydrophobic residues for a cationic, α helical amphipathic structure effective for its high antimicrobial activity. It is also modified C-terminally by a dehydro Leucine residue effective for its structural stability. The synthesis of dehydro amino acid was done by solution phase and other amino acids were coupled by solid phase peptide synthesis method. Anti-bacterial activity of the peptide was done by standard micro broth dilution technique against clinical isolates of GPC including methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus* (MSSA), HLAR, Group A and Group B Streptococci cultured from pus, wound and throat swab. All these isolates were known to be responsible for nosocomially acquired infections. *S. aureus* ATCC 25023 was used as quality control strain. *Invivo* efficacy of this peptide was also tested in a mouse model with epidermal lesions caused by MRSA. The MIC obtained for the peptide was 10μg/ml (average) for the tested strains. The peptide showed no hemolytic activity against human red blood cell. In the *invivo* studies the healing was induced early (after 72 hrs total healing was seen) in the experimental animals. This novel peptide has a potential to evolve as a therapeutic option in infections caused by resistant GPC.

P20721-084

Synthesis and Anti-Aggregatory Activity of $\beta_{\acute{E}\acute{E}b}$ 313-320 Hybrid Analogues Incorporating the (S,S) -CDC- Motif

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Fibrinogen (Fg), an adhesive protein, is recognized by the activated platelet integrin $\acute{a}_{\acute{E}\acute{E}b}\beta_3$ through the Arg-Gly-Asp (RGD) sequence resulting to platelet aggregation and thrombus formation. Inhibition of this process can be achieved by RGD peptide analogues that bind to $\acute{a}_{\acute{E}\acute{E}b}\beta_3$ receptor. However, this class of inhibitors upon binding to receptor cause an outside-in signaling which induces a further activation of the platelet. In previous studies we presented cyclic (S,S)–CDC– containing compounds (IC_{50}~2ìM) and $\acute{a}_{\acute{E}\acute{E}b}$ derived sequences (Y^{313}MESRADR^{320}, $\acute{a}_{\acute{E}\acute{E}b}$ 313-320) (IC_{50}~250ìM) that exhibit a non-RGD-like inhibitory activity[1,2]. This interesting aspect could be the basis for the design and development of a new class of anti-platelet agents that could overcame the drawback of platelet activation through the outside-in signaling. To this aim we designed, synthesized and tested for their inhibitory potency various $\acute{a}_{\acute{E}\acute{E}b}$ 313-320 hybrid analogues incorporating the (S,S)–CDC– motif. Cyclization reduces the allowed conformations, of both the backbone and the side chains, and possibly induces a favourable for the biological activity orientation of the charged side chains. The inhibition assays on the ADP induced platelet aggregation revealed that incorporation of the (S,S)–CDC– motif considerably increases the inhibitory activity of the $\acute{a}_{\acute{E}\acute{E}b}$ 313-320 analogue.

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P20723-085

The unique toxic effect of acrebol, a novel peptide from *Acremonium exuviarum*

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A novel peptaibol, named acrebol, was isolated and purified from the fungal strain BMB4 found in water-damaged wood-based indoor building material. The strain BMB4 was identified as *Acremonium exuviarum* based on morphology, ITS sequence, and cycloheximide resistance. MS/MS analysis showed that acrebol is a mixture of two almost identical peptaibols composed of 16–17 amino acid residues with masses 1726 and 1740 Da, AcePhe-Iva/Val-Gln-Aib-Ile-Thr-Leu-Aib-Pro-Aib-Gln-Pro-Aib and AcePhe-Iva/Val-Gln-Aib-Ile-Thr-Leu-Val-Pro-Aib-Gln-Pro-Aib, respectively. The C-termini of the peptaibols was SerOH however the sequence (mass of 216 Da) between b13 and SerOH could not be interpreted. Both isoforms of acrebol had a strong toxic effect on boar spermatozoa, feline fetus lung cells, murine neuroblastoma, and mouse insulinoma MIN 62 cells. We found that, unlike other peptaibols, acrebol in toxic concentrations did not increase the ionic and solute permeability of membranes of isolated rat liver mitochondria. Acrebol did not disturb the ionic homeostasis and osmotic balance of mitochondria and induced no release of apoptogenic proteins (cytochrome *c*) from the intermembrane space of mitochondria. Acrebol strongly inhibited complex III of the respiratory chain (IC_{50} ~ 110 ng/ml), presumably, the outer quinone-binding center and, similarly to myxothiazol but in contrast to antimycin A, decreased the production of superoxide anion in the outer compartments of mitochondria. In the boar spermatozoa, acrebol, blocking the respiratory chain, caused the ATP depletion due to the oligomycin-sensitive reversion of the reaction of ATP synthesis, which resulted in the inhibition of progressive movement. Acrebol induced necrosis-like death of mouse insulinoma

MIN 62 cells whose energetic metabolism is strongly dependent on oxidative phosphorylation in mitochondria. Thus, acrebol is a unique peptaibol with a specific pattern of the toxic effect.

P20724-086

Delta Sleep Inducing Peptide (DSIP), its analogues and Deltaran®: biological activity and mode of action

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For the last decade we have been engaged in studies on the endogenous neuromodulator DSIP (WAGGDASGE) and a large group of its derivatives in respect of both their physiological activity and mechanism of action. A wide range of evidences confirmed beneficial effects of DSIP and some active analogues under experimental stress models. DSIP has emerged as promising and potentially effective therapeutic agent due to strong and unique adaptive and stress protective activity revealed during the study. DSIP related drug Deltaran® registered in Russia has also showed the significant efficiency in animal test models and clinic. The peptides of DSIP family often do not demonstrate any effects under normal and comfortable conditions or even cause slight prooxidative and stress promoting effects. These properties and established wide profile of biological activities of DSIP complicate the work on DSIP mode of action. Cellular and subcellular effects of this peptide still remain poorly studied. Previously we investigated some biochemical events underlying the stress protective efficiency of DSIP and its derivatives. In continuation of these studies we have attempted to evaluate the putative DSIP influence on classical cellular processes utilizing stress-protective heat shock proteins (HSPs) and apoptosis. Effect of DSIP on the level of HSP70 expression in human erythroleukemia cell line K562 was detected.. We have found that DSIP down regulates the increase of intracellular HSP70 level during incubation of cells in high density cell culture. According to our preliminary data DSIP increased HSP70 level in murine T-cell line CTLL-2 similar to α- and ß-adrenergic receptor agonists. In murine thymocytes DSIP increased both apoptosis and HSP70. We propose that registered effects of DSIP are mediated through adrenergic receptors. Cellular mechanisms of DSIP and related peptides action are under way.

P20726-087

Identification, chemical synthesis, and antimicrobial activity of TBD-1 – the first β-defensin isolated from reptiles

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Antimicrobial peptides (AMPs) and proteins have been discovered in single and multicellular organisms indicating the importance of this peptide class for the innate immune system. AMPs are active against bacteria, fungi and viruses by affecting either the microbial cytoplasmic membrane, thus increasing its permeability or interacting with specific targets. Defensins form one of the major subfamilies of AMPs, among which α- and β-defensins play a significant role in bridging innate and adaptive immunity in mammals. The cationic β-defensins are cysteine-rich and vary in length from 36 to 44 residues. The β-stranded structure of β-defensins is stabilized by a characteristic arrangement of three conserved disulfide bonds between cysteines 1-5, 2-4, and 3-6. Although β-defensins lyse the bacterial membrane at higher concentrations it has been shown that they modulate also the immune system, e.g. being chemotactic for T-cells. We have isolated a novel 40mer β-defensin called TBD-1 from leukocytes of the European pond turtle and deduced its complete sequence *de-novo* by combining different tandem mass spectrometry techniques (MALDI, ESI; CID and ETD) and Edman degradation. It was also possible to identify the disulfide pattern in a tryptic digest by MALDI-mass spectrometry. The deduced peptide sequence was afterwards confirmed by solid phase peptide synthesis. Thus two fragments were synthesized by standard Fmoc/^{t}Bu chemistry using three orthogonal cysteine protecting groups (Trt, Acm, tBu) to selectively form the right disulfide bridges. After native chemical ligation of the two peptide fragments the first two cysteines were oxidized on air followed by iodide oxidation for the second and DMSO oxidation for the third disulfide bridge. In antimicrobial activity assays, TBD-1 synthesized in β-conformation was active against Gram-positve, Gram-negative bacteria, and fungi similar to the native peptide.

P20728-088

Trichogin GA IV binds Ca(II) and lanthanide ions

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Trichogin GA IV (TrGA), an antimicrobial peptide of the lipopeptaibol family, continues to reveal peculiar properties since the time of its former identification by Rebuffat and coworkers. X-Ray diffraction studies showed that TrGA is folded in a mixed 310/a-helix conformation, while NMR, CD and IR absorption experiments proved that this structure is predominantly populated in solution, although more disordered structures contribute to the conformational landscape of TrGA. We have recently shown by time-resolved experiments, that an equilibrium between helical conformers and more compact, folded conformers takes place in solution, with interesting transition dynamics in the microsecond time scale. In our conformational studies on TrGA we realized that the 3D geometry of the peptide chain reproduces the structural environment of the ion coordination site of Calcium-binding proteins. This finding urged us to investigate the binding properties of TrGA with respect to Ca(II), Gd(III) and Tb(III), because lanthanide ions have been widely employed in biochemical studies as best substitutes of Ca(II). The binding of Ca(II), Gd(III) and Tb(III) to TrGA gives rise to a conformational transition, monitored by CD spectra at different ion-peptide molar concentration ratios. At high r values, the CD curves of all the ion-peptide complexes show a positive maximum at ~214nm, typical of type II β turns, suggesting the population of bent structures. The quasi-isodichroic point found for all systems between 198 and 202 nm, indicates that an equilibrium between extended helical and bent conformations actually takes place. Fluorescence experiments on the Tb(III)/TrGA adduct have shown that, upon ion binding, the fluorescence quantum yield of Tb(III) markedly increases, due to the release of water molecules from the ion coordination inner shell. Molecular dynamics calculations on the peptide/Ca(II) adduct were also performed to obtain structural and dynamical information.

P20729-089

Biological Studies of the Peptide *Hy-a1* and Analogs

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Antibiotic resistant bacterial strains represent a global health problem with a strong social and economic impact. Thus, there is an urgent need for the development of antibiotics with novel mechanisms of action. Castros group isolated and determined the sequence of the peptide *Hy-a1* (IFGAILPLALGALKNLIK) of skin secretion from the frog *Hypsiboas albopunctatus* which showed antimicrobial activity. The aim of the present work was evaluated 4 analogues to supply information

about the relationship structure-biological activity. The peptides were synthesized by SPPS using the Fmoc chemical approach. The biological activities were assayed by measuring growth inhibition of two types of Gram-positive bacteria and others two types of Gram-negative. The synthesis and purification of peptides by HPLC was efficient and a high purity level (96%) was obtained. The peptide containing Trp in position 6 (for fluorescent studies) replacing Leu presented MIC values comparable to wild type sequence: 32 uM, 32 uM, 8 uM and 2 uM for *E. coli, P. aeruginosa, S. aureus* and *B. subtilis*, respectively. Two peptides with this modification but containing at the N-terminal region one group acetyl or a residue of Asp showed MIC values of 128 uM for *E. coli* and *P. aeruginosa*, although 4 uM for Gram-positive bacteria. Different results were observed when the residue added was Lys. In this case, the activity against whole bacteria was sustained or increased. Conformational properties were investigated by CD techniques in water, TFE and in zwitterionic micelles (LPC). The CD experiments demonstrated that in water, the peptides have a random structure, but in TFE and LPC solutions they acquired an ordered structure, composed mainly by α-helix. However, these data there is no relationship between the structure and activity against bacteria Gram-positive. These results showed that the N-terminal region of the peptide *Hy-a1* develop key roles in its antibacterial action different types of bacteria.

P20729-090

Novel Chitosan-Pexiganan Conjugate for the Treatment of Infected Diabetic Foot Ulcers: Synthesis and Characterization by FT-IR and Amino Acid Analysis

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Pexiganan is an antimicrobial peptide remarkably effective against bacteria causing skin infections, and has been commercially developed as a topical cream to treat infected diabetic foot ulcers.[1-3] As peptide drug-based therapy often suffers with low drug stability in vivo, suitable delivery systems must be developed. With this purpose in mind, we have designed a pexiganan-chitosan conjugate to combine the exceptional bioadhesion and tissue regenerating abilities of chitosan [4-6] with the excellent antibiotic properties of pexiganan. We herein wish to report our first results on the successful synthesis, FT-IR and amino acid analysis of a pexiganan-chitosan conjugate prepared by covalent attachment of a Cys-containing pexiganan analogue to the chitosan's amino groups, by means of the heterobifunctional cross-linker Sulfo-EMCS.7.

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P20729-091

Overexpression of *E. coli* oligopeptidase B confers resistance to the proline-rich antibacterial peptides

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The Proline-Rich AntiMicrobial Peptides (PRAMPs) are a large group of cationic peptides isolated from mammals and insects, which show a spectrum of antibacterial activity limited to Gram-negative bacteria and a remarkably low cytotoxicity towards eukaryotic cells. PRAMPs are thought to act in a permeabilization-independent manner, *via* energy-dependent internalization into bacteria followed by recognition and inactivation of internal molecular targets. With the aim of investigating their mode of action, we have isolated a number of *E. coli* clones showing increased resistance to the bovine proline-rich peptide Bac7 after transformation with a DNA library from Bac7-resistant mutants. Among the recombinant plasmids responsible for resistance, some of them harboured the gene coding for the oligopeptidase B (OpdB), a serine peptidase belonging to the prolyl oligopeptidase family (POP) broadly distributed among unicellular eukaryotes and Gram-negative bacteria, which has emerged as an important virulence factor. OpdB was cloned and expressed under the control of an inducible promoter and the transformants tested for susceptibility to a panel of antimicrobial peptides, including PRAMPs. Olipopeptidase activity of purified OpdB was then tested in vitro against the same peptides. The results indicate that the clones overexpressing OpdB are more resistant to the PRAMPs and that the degree of resistance correlates with the expression level of OpdB. In addition, *in vitro* incubation of PRAMPs with the purified peptidase, followed by mass spectrometry analysis, showed that it promptly hydrolyzes all the peptides assayed to short, inactive fragments. These results suggest that OpdB may contribute to cleavage and inactivation of antimicrobial peptides that are internalized into the target cells and support the notion that OpdB is a novel virulence factor.

P20820-092

Peroxidase mimetics DhHP-6 elevate antioxidant enzyme activity in Caenorhabditis elegans

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Great progresses have been made in the research on identifying molecules of therapeutical potential for delaying aging. Using Caenorhabditis elegans, we had tested the effects on stress resistance and life span of treatment with DhHP-6 (Deuterohaemin-AlaHisThrValGluLys), synthetic mimetics of the antioxidant peroxidases, which neutralizes peroxide. To further test the mechanisms of DhHP-6 on life span extension, exogenous protein SOD and catalase levels were measured. We show that DhHP-6 is able to elevate in vivo SOD and catalase activity levels after two days administration. Treatment with exogenous DhHP-6 affected endogenous protein SOD and catalase levels and elevated the expression of sod-3::GFP in the head and vulva. On the other hand, DhHP-6 can extend life span of C. elegans lacking sod-3 or ctl-2 gene expression by RNAi. This suggested that the antioxidant enzyme in C. elegans may not the only target affected by DhHP-6.

P20820-093

DhHP-6, a Synthetic Microperoxidase, Perfectively Prevents Hydrogen Peroxide-Induced Cell Damage in H9c2 and neonatal rat ventricular myocytes

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High concentration of hydrogen peroxide (H2O2) induces nuclear DNA fragmentation, lipid peroxidation and has been implicated in many diseases including heart failure, Parkinson's disease, and cancer. In a systematic attempt to develop an effective scavenger of H2O2, we have successfully synthesized an artificial microperoxidase, Deuterohaemin -AlaHisThrValGluLys (DhHP-6) as core catalytic center to which 6 amino acid peptide was covalently attached. DhHP-6 exhibited potent peroxidase activity, favorable membrane permeability and thermal stability.Two experimental models of oxidative injury were established in order to investigate the anti-oxidative effect of DhHP-6: one was induced by hypoxia-reoxygenation in cultured heart-derived H9c2 cells

and another was caused by H2O2 in cultured neonatal rat ventricular myocytes. DhHP-6 could protect cells against oxidative injury by determining MTT cell proliferation assay, LDH leakage and Ca2+-ATPase activity. Furthermore, DhHP-6 repressed the apoptosis gene expression, such as p21, Hsp70 and heme oxygenase¢ñ, that proved DhHP-6 had protective effect in cultured neonatal rat ventricular myocytes . Taken together, this small microperoxidase exhibited excellent ¡°druggable¡± properties, and could be a promising agent for ROS-associated diseases with unmet needs.

P20900-094

Antibacterial activity and resistance to proteolytic degradation of trichogin GA IV and selected analogues

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Trichogin GA IV is the most extensively investigated member of the class of lipopeptaibols that are linear peptide antibiotics of fungal origin, characterized by the presence of a variable, but remarkable, number of Aib residues, a fatty acyl group at the N-terminus, and a 1,2-amino alcohol at the C-terminus. Several analogues of trichogin GA IV with amino acid substitutions or deletions were designed which allowed determination of the minimal inhibition concentration against Gram-positive and Gram-negative bacteria and various pathogenic fungal cells. The natural peptide exhibits a specific activity against *S. aureus* and only a marginal hemolytic effect. Interestingly, trichogin GA IV is active also against several methicillin-resistant *S. aureus* strains. Studies on synthetic analogues demonstrated that substitution of the C-terminal leucinol by Leu-OMe, or substitution of one Aib residues by the EPR label TOAC do not perturb significantly the biological activity of the peptide. On the other hand, removal of 3 or 7 N-terminal residues eliminated any antibacterial activity. Finally, studies of proteolytic degradation on trichogin GA IV and analogues where the 3 Aib residues are replaced by Leu demonstrated that the presence of several non-coded Aib residues endows the natural peptaibol with remarkable resistance to proteolysis. The present results indicate that trichogin GA IV is a promising lead compound for the development of new, selective and protease-resistant, antibacterial drugs.

P20907-095

The siderophore microcin family: from the genetic systems to the antimicrobial peptides

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Microcins are low molecular weight antimicrobial peptides secreted by enterobacteria and involved in microbial competitions within the intestinal tract. They are synthesized by the ribosomal pathway. We have isolated the first siderophore peptide (1), a post-translationally modified form of the chromosomally encoded microcin E492 (MccE492) from *Klebsiella pneumoniae*, having a potent bactericidal activity mainly directed against *Escherichia coli*. The post-translational modification consists of a glycosylated catechol-type siderophore linked to the C-terminus. We have recently identified the genes responsible for the acquisition of this modification and proposed a model for its biosynthesis (2). In order to identify novel siderophore peptides, we analyzed the genetic systems of several microcinogenic strains. Based on the genetic organization of the microcin gene clusters, three microcins which had never been isolated until now, MccM, MccH47 and MccI47, were hypothesized to carry a post-translational modification similar to MccE492, putatively forming the siderophore microcin family. The gene clusters of these microcins were found in the genomic DNA of four *E. coli* strains (Nissle 1917 [the probiotic agent Mutaflor® used against intestinal diseases] H47, CA46, CA58). They are closely interwoven, but genes *mchA* and *mchS1*, putatively responsible for the post-translational modification are lacking in strain Nissle 1917. Complementation experiments showed that MccM and MccH47 carry the same post-translational modification as MccE49(2). MccM and MccH47 were isolated for the first time under their unmodified and modified forms. They were identified by MALDI-TOF MS. These two microcins become new members of the siderophore microcin family.

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P20923-096

Preparation of a close mimic of the N-terminal part of the C5A receptor (C5AR) by selective introduction of sulfated tyrosine residues

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Introduction of an O-sulfated tyrosine residue in a peptide is still considered a difficult task, mainly because of the acid lability of the sulfate group during the acidic deprotection steps. In this contribution we will present a new strategy for selectively incorporating several O-sulfated tyrosine residues in peptides by Fmoc solid phase synthesis. We have applied this strategy to synthesize several peptide fragments representing the N-terminal portion of C5a Receptor (C5aR). The N-terminus of the C5aR contains two O-sulfated tyrosine residues at positions 11 and 14. Using isothermal titration calorimetry (ITC) measurements and cell based calcium mobilization experiments we have demonstrated the importance of the O-sulfated tyrosine residues of the C5aR mimic upon binding of a natural inhibitor, the Chemotaxis Inhibitory Protein of *Staphylococcus aureus* (CHIPS)(1). Furthermore, we have shown that the sulfated C5aR7-28 peptide can be used as a close model of the C5aR for studying the inhibition by CHIPS. We have elucidated the structures of free CHIPS(2) and CHIPS in complex with our sulfated C5aR-model by multi-dimensional NMR techniques. Based on these interaction data and the solution structure of the complex, CHIPS-based C5aR inhibitors for use in anti-inflammatory therapy might be designed.

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P21004-097

Synthesis, confirmation, and receptor binding and activity of human insulin-like peptide 5 (INSL5)

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Insulin-like peptide 5 (INSL5) was first identified through a search of the expressed sequence tags (EST) databases. Primary sequence analysis showed it to be a prepropeptide that is predicted to be processed in vivo to yield a two-chain sequence (A and B) containing the insulin-like disulfide crosslinks. The high affinity interaction between INSL5 and the receptor RXFP4 (GPCR142) coupled with their apparent co-evolution and partially overlapping tissue expression patterns strongly suggest that INSL5 is an endogenous ligand for RXFP4. Given that the primary function of INSL5/RXFP4 pair remains unknown, an effective means of producing sufficient quantities of this peptide and its analogues is needed in order to systematically investigate its structural and biological properties. A combination of solid phase peptide synthesis methods together with regioselective disulfide bond formation were used to obtain INSL5. Both chains were identified as being ¡§difficult sequences¡¨ and were unusually resistant to standard synthesis protocols including those mediated by microwaves. The A-chain was also prone to significant aspartimide formation. The B-chain, in particular, required the use of the strong tertiary amidine, DBU, for more effective N*f*Ñ-deprotection during its assembly. Following chain combination and sequential disulfide bond formation, the resulting synthetic INSL5 was obtained in good overall yield and shown to possess a similar secondary structure to human relaxin-3 (H3 relaxin). The peptide was able to inhibit cAMP activity in SK-N-MC cells expressing the human RXFP4 receptor with a similar activity to H3 relaxin. In contrast, it had no activity on the human RXFR3 receptor.

P21007-098

Novel cyclic bacteriocin-like peptides from strains of *Anabaena* (cyanobacteria)

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Most of the biosyntheses of bioactive secondary metabolites described in cyanobacteria are non-ribosomal. The bacteriocin-like ribosomal peptide biosynthesis has recently been described in three cyanobacteria; *Prochloron* spp.(1,2), *Trichodesmium erythraeum*(3) and *Microcystis aeruginosa*(4). The genome sequence of filamentous and diazotrophic cyanobacterium *Anabaena* 90 revealed a putative gene cluster (*acy*) encoding a bacteriocin-like peptide. One of the *acy* genes encoded a prepeptide, which showed N-terminal homology to the known cyanobacterial prepeptides but the mature peptide product in *Anabaena* 90 could not be predicted. We sequenced prepeptide genes from several *Anabaena* strains to find a prepeptide containing either cysteine or methionine, since sulphur containing amino acids enable the detection of the mature peptide product through 34S-labelling and LC-MS. The nucleotide sequence of the prepeptide genes revealed enormous variety in closely related *Anabaena* strains. One strain, *Anabaena* 844B contained a methionine in the prepeptide, and a cyclic heptapeptide product corresponding to the precursor was discovered in this strain. Since the cleavage sites were found to be conserved in all *Anabaena* strains the products could be predicted from the amino acid sequence and identified by LC-MS. In addition, the biosynthesis of the decapeptide anacyclin in *Anabaena* 90 was verified by heterologous expression of the *acy* genes in *E. coli* and the structure of the peptide was confirmed with a synthetic reference peptide. Altogether, new cyclic peptides were found in 19 strains of *Anabaena* with very little sequence conservation. Cyanobacteria seem to be versatile producers of peptides using both ribosomal and non-ribosomal biosynthetic pathways.

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P21020-099

CRF fragment Pro-Pro-Ile has CRF-like central effects

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It is well established that N-terminal flank of corticotropin-releasing factor molecule is crucial for its biological activity. Little, however, is known about in vivo effects of CRF-derived peptides. This study was aimed to investigate possible actions of a tripeptide CRF fragment 4-6 Pro-Pro-Ile (PPI). Tripeptide PPI was found to exert effects similar to full-size CRF molecule after central administration. The tripeptide induces behavioral activation in home cage, but inhibits behavioral activity under stressful conditions. PPI increases blood pressure and heart rate, blood glucose level and body temperature, decreases pain sensitivity, increases EEG amplitude and large doses of PPI induce seizures. PPI inhibits sexual motivation and performance in mating tests in males. CRF antagonist α-helical CRF_{9-41} abolishes PPI influence on circulatory system, glucose metabolism, thermoregulation and pain sensitivity. Adrenalectomy does not interfere with hyperglycemic and hyperthermic actions of PPI, while pancreatectomy prevents hyperglycemic effect. Nonselective beta-adrenergic blocker obsidan prevents hyperglycemic and decreases hyperthermic effects of the tripeptide and ganglionic blocker hexamethonium abolishes both effects. Taken together effects of PPI correspond to stress-reactions, involving corticotropin-releasing factor and evidence that PPI is either 1) a part of a CRF molecule active site, or 2) physiologically active CRF derivative, realizing its effects through activation of CRF receptors, or 3) an independent regulator, affecting CRF-ergic neurons.

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P21100-100

Hairpin Peptide Inhibitors of Amyloid Fibril Formation

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Amyloid fibril formation is associated with at least 17 human diseases; among these, the human-amylin(hAM)-derived deposits in type II diabetes were the discovery system and hAM aggregation is one of the more thoroughly studied systems. To date, inhibitors of beta-aggregate and fibril formation have been polyphenols, mutants of hAM, or short peptide related to the hAM(22-29) sequence, NFGAILSS. We now report that stable beta-hairpin scaffolds displaying Trp and Tyr residues are effective inhibitors, delaying the onset of both the CD changes associated with beta structure formation and the nucleation time and net enhancement of the fluorescence observed with added Thioflavin-T (ThT). Under our test conditions (8 microM hAM, 2% HFIP in 5mM phosphate buffer, pH 7), hAM begins to display an increase in beta structure by CD at 40 min with a constant maximal value from 85 – 220 min. ThT fluorescence also indicates a circa 50 min onset time with a rapid (<20 min) rise to the full response. Inhibition (delayed onset and reduction in the maximal fluorescence enhancement) has been observed with a number of hairpins; those with two Trp residues on a single face of the hairpin are more potent. Of these, our best inhibitor to date, KKLTVWIpGKWITVSA (p = D-Pro), increases the onset time more than 2-fold at equimolar concentrations. At 4 molar equiv., the onset time is greater than 320 min and ThT fluorescence levels out at < 40% of the control value. In analogy to the report by Prof. Ghosh (JACS 2006, p. 14456) that a beta-sheet protein with added Tyr and Trp residues inhibits fibril formation by the Alzheimer-related Abeta peptide; we expect that designed beta-hairpin scaffolds will be more generally applicable and will afford new insights into the recognition phenomena of amyloidogenesis.

P21100-101

Artificial Repeat Protein Receptors

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The main advantage in utilizing proteins for the development of sensors and receptors, over the more often used approach that exploits small molecules, is the ability to manipulate large recognition surfaces, which expose toward the bulk solution number of different functional groups. We search for stable artificial proteins that can be utilized as 'universal' recognition entities. To that end, modular chemical syntheses are exploited for the preparation of repeats-proteins. Leucine Rich Repeat proteins (LRRs) are 20-29 residue sequence motifs present in several proteins with diverse functions. Internalin B (InlB), a surface LRR protein of the human pathogen Listeria monocytogenes, promotes invasion into various host cell types by inducing phagocytosis of the entire bacterium. Consensus design uses statistical analysis of sequence alignments of families of homologous proteins for protein engineering. We show here that using consensus design, series of protein mutants that differ in the recognition surfaces are synthesized and will be probed for their ability to bind different natural and non-natural ligands.

P21100-102

Comparative structural studies of potent neuroprotective peptides of the Humanin family

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The discovery of Humanin (HN, MAPRGFSCLLLLTSEIDLPVKRRA) in 2001 with rescue activity against insults related to Alzheimer's disease, opened new horizons on the development of new therapeutic strategies targeting neuroprotection. Continuous research in the field resulted in new potent HN derivatives: (a) HNG, a 1000-fold more active peptide having a Gly in place of Ser14 of HN, (b) desLeu-AGA(C8R)HNG17, 1000-fold more potent than HNG, based on the 17-amino acid core of HN, and finally, (c) Colivelin (CL), a new hybrid peptide composed of the Activity-Dependent Neurotrophic Factor fused to the AGA(C8R)HNG17 sequence, displaying neuroprotection at fM concentrations. Although the rescue activity of HN peptides has been linked to a number of signaling pathways and receptors, their mechanism of action remains unknown.
In this work CD and NMR data on the above peptides are presented and compared in an effort to define structural characteristics related to their function. Evaluation of our findings in combination with existing structure-function relationship data for this class of peptides, brings forth flexibility as an important structural feature that may facilitate interactions with functional counterparts of the neuroprotection pathway. The ability to adopt a partial helical conformation in the presence of low concentrations of TFE is another common structural feature that may be defining the interactions of these peptides in the environment of cell membranes. DesLeu-AGA(C8R)HNG17 and CL display a more complex behavior shifting from α-helical to β-sheet conformations depending on pH, peptide concentration, and % of TFE present in solution. This fact may be related to their high potency since in HN literature evidence for self-association ability has been linked with neuroprotection. Our CD and NMR experimental data combined with theoretical modeling will hopefully provide important clues for the elucidation of the mechanism of action of the HN family of peptide

P21100-103

Recycling in Aβ(1-42) amyloid fibrils

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The process of molecular recycling describes a dynamic mechanism by which individual amyloid fibrils are continuously dissolving and reforming[(1)]. The present work aims at the study of the dynamic properties of the β-amyloid, Aβ(1-42), amyloid fibrils. Since the formation of Aβ aggregates has been suggested as a key process in the pathology of Alzheimer's disease, the study of the dynamic nature of Aβ(1-42) amyloid fibrils can be of great significance for the design of therapeutic strategies. The methology we have used to prove the dynamic nature of Aβ(1-42) amyloid fibrils is based on hydrogen/deuterium (H/D) exchange experiments. Aβ(1-42) amyloid fibrils have been exposed to deuterated buffer for different periods of time, then solubilized into monomers by transfer to a DMSO solution, and their deuterium content subsequently analyzed by electrospray ionization mass spectrometry (ESI-MS). The mass spectra show two well-resolved peaks indicating that two distinct, isotopically labelled population of Aβ(1-42) are present within the fibrils which confirms that molecular recycling occurs.To further learn about the specific factors that determine the recycling nature of Aβ(1-42) amyloid fibrils, we have produced fibrils of different morphologies and studied their recycling times.

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P21100-104

Controlling and Monitoring the Structure and Function of Coiled Coil Proteins using Light

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The coiled coil structure is widely distributed in natural proteins, such as transcription factors, receptor proteins and enzymes. This motif has been recently used by chemists for the design of functional synthetic proteins. Specific coiled coil sequences can be utilized as templates for self replication processes(1,2). The stability and the activity of these replicators depend on the characteristics of the amino acid residues in the recognition interface. It has been suggested that it is possible to control protein structure and the replication process by external triggers such as light, and that the light can also be used to monitor the conformational changes and/or to follow the protein functionality(3). We show here our ability to control the folding stability of coiled coil peptides and their reversible or irreversible self replication efficiency by light, and we demonstrate the possibility to exploit FRET couples to facilitate in-situ monitoring of the folding and reactivity. We use 'caged' mutants with a photo-switchable molecule in the recognition interface of the peptide- which disrupts its coiled coil structure - as inactive species. Deprotection of the caged proteins is used as a mechanism to restore the self replication process. The ligation is followed by monitoring the changes in fluorescence of either the Donor or Acceptor of the FRET couple. We will describe synthesis and structural characterization of caged and cage-free peptides and measurements that show, as expected, that the cage free peptides are more stable as coiled coils and better catalyst for their own formation than the caged analogs. Moreover, we discuss the reversible replication process and how we can shift and control its equilibrium staes.

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P21107-105

Structural studies on human neuropeptide FF

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Neuropeptide FF (NPFF; FLFQPQRF-NH_2), a member of the RFamide peptide family, has been reported to have pronociceptive and analgesic activities as well as pro- and anti-opioid effects. These contradictory effects seem to result from NPFF capacity to bind two different G-protein coupled receptors (NPFF1 and NPFF2). Although the exact role of NPFF1 and NPFF2 receptor is still unclear, this complex system appears to play an important role in pain regulation. Structure-activity relationship (SAR) studies using analogs of NPFF and derived peptides have shown that the C-terminal part of the molecule (i.e. PQRF-NH_2) is crucial for affinity and activity. However, some of the essential features for ligand recognition by NPFF receptors are still missing to design highly selective molecules.

In order to provide further insight into ligand recognition, we have investigated the solution conformation of NPFF in different media using circular dichroism and NMR spectroscopy. Our results showed that (1) in the presence of methanol or trifluoroethanol, turn-like elements are present in NPFF structure, and (2) a secondary structure is stabilized in dodecylphosphocholine (DPC) micelles, a membrane-interface mimetic environment. The 3D structure of NPFF bound to DPC micelles was thus solved by molecular modelling under NMR restraints. NPFF membrane-induced conformation consists of two successive β-turns encompassing residues Q4 to F8. These structural features provide an amphipathic character to the C-terminal part of the molecule, which corresponds to the crucial region for biological activity. Such a correlation suggests that NPFF structure in DPC micelles constitutes a valuable starting point for the rational design of new analogs.

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P21107-106

Controlling Amyloid β-Peptides Toxicity by Biocompatible Polymers

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Mechanisms causing Alzheimer's Disease (AD) are not yet established, although genetic and animal models have shown a causal role of Amyloid β-Peptide (Aβ) in AD. However, recent debate has focused on whether amyloid fibrils or soluble oligomers of Aβ are the main neurotoxic species contributing to neurodegeneration and dementia. One approach for preventing aggregation would be the conversion of the peptide conformation. Prior investigations indicate that polymeric nanoparticles offer strong advantages in modulating the secondary structure of the peptide (1, 2). These results have encouraged us to extend our work on polymeric nanostructures for conformational transformations contributing to the development of new therapies for these diseases.

Complexes of polyampholytes and dodecanoic or perfluorododecanoic acid were prepared (2) resulting in nanoparticles with hydrodynamic diameters ranging from 3 to 5 nm. The fluorinated nanoparticles induced α-helix rich structures in Aβ peptide, whereas their hydrogenated analogues were less efficient leading in most cases to aggregation or β-sheet formation, as determined by circular dichroism spectroscopy. The degree of fluorination, the hydrophilic balance and the charge density of the fluoropolymers, as well as the size of the nanoparticles in aqueous solution, are decisive for the interactions.

The impact of these structures on the Aβ-induced toxicity in cultured neurons was studied. We report that the fluorinated nanoparticles increased the Aβ-mediated MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) reduction, which indicates a higher cell viability. The anti-apoptotic effect of the complexes was evaluated by determining the activation of caspase-3. This assay also confirms the decrease of Aβ-mediated cytotoxicity in the presence of fluorinated biocompatible complexes.

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P21108-107

Alternative strategy to investigate enzymatic activity using peptides containing TOAC spin probe

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Following recent report (1), the present work extended the investigation of the specificity of angiotensin I-converting enzyme (ACE, EC 3.4.15.1), a dipeptidyl carboxypeptidase which cleaves the C-terminal dipeptide from angiotensin I (AngI, DRVYIHPFHL) to produce the potent vasoconstrictor angiotensin II peptide (AngII). The use of paramagnetically labeled AI analogues attaching the TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid) probe (2) advantageous allows the monitoring of their conformation and its enzymatic hydrolysis specificity through the EPR and fluorescent methods, the latter due to the quenching effect induced by the stable free radical TOAC probe upon the Tyr4 residue of AngI. The study of TOAC-attaching AI analogues at positions 0, 1, 3, 5, 8, 9 and 10 indicated that the first four analogues are substrates for ACE in the decreasing order 0 ~ 1 > 3 > 5, thus confirming that greater the proximity of the unnatural probe to the cleavage site (8-9) of the sequence, the smaller are the substrate specificity of analogues. Otherwise the quenching effect of Tyr4 fluorescence by TOAC decreased with increasing distance between both residues, thus suggesting overall flexible structures for most of analogues. These findings were also corroborated in a combined CD and EPR studies although some differences were detected among the derivatives either in the variation of pH or amount of the structuring TFE studies. Finally, differences between EPR spectral lineshapes of some labeled analogues and their corresponding cleavage products seems to allow a real time monitoring of the enzymatic reaction.

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P21114-108

The effect of the so called "β-sheet breakers" (BSBs) on Aβ 1-42 aggregates

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Inhibition of aggregation of amyloid β-peptide seems to be a critical step in the therapeutic approach to prevent amyloidosis in Alzheimer's disease. The therm "β-sheet breaker" (BSB) had been introduced by Soto C. et. al. as a 5-residue peptide in 1998, that inhibits amyloid β-protein fibrillogenesis. We synthetized several derivatives of the "Soto peptide" as well as a big number of peptidomimetics. Their mechanism of action has been studied by NMR spectroscopy, CD and transmission electron microscopy (TEM). All of these methods showed that the Soto peptide LPFFD and the similar peptides can not prevent Aβ aggregation. These compounds bind to the surface of Aβ aggregates and decrease

the specific surface area of Aβ which accessible for the cell-membrane-bound receptors, can lead to a decreased toxicity. The β-sheet breaking effect does not work up to an Aβ peptide–small peptide ratio of 1:10. As a consequence, these compounds are not β-sheet breakers, only they modify the surface of Aβ fibrils and rather speed up the formation of β-sheet structure.

P21117-109

Structure and dynamic characterization of βA aggregates using H/D exchange experiments by mass spectrometry

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The objectives of this proposal are to define the structure and to probe the dynamic character of the different aggregates assembled during β-amyloid (Aβ) aggregation. To this end, we are developing new methodologies based on hydrogen/deuterium (H/D) exchange experiments that can provide both structural and dynamic information. Structural information is based on solvent accessibilities: amide protons that normally undergo rapid exchange with solvent deuterons experience much slower exchange when involved in H-bonded structures and/or when sterically inaccessible to the solvent. Dynamic information is related to the continuous dissolving and reforming of amyloid fibrils: when fibrils produced in a H_2O based buffer are placed in a D_2O based buffer, dissociated molecules fully exchange with the solvent on a much shorter timescale than that required for their reassociation to another fibril, when protein molecules reincorporate into the fibrils they are fully deuterated. A possible H/D exchange experiment consists on exposing Aβ ïŀ fibrils to deuterated solvent for different periods of time, solubilize them into monomers by transfer to a DMSO solution, and subsequently analyze the deuterium content by electrospray ionization mass spectrometry (ESI-MS).

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P21126-110

Designing trehalose-conjugated peptides for the inhibition of Alzheimer's Aβ oligomerization and neurotoxicity.

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by loss of memory and language skills, damaged cognitive function and altered behaviour.(1). Besides these clinical symptoms, the central histopathological feature of AD is the presence of extracellular senile plaques, found in the hippocampus and neocortex, associated with synaptic loss and cell death.(2) The principal protein component of these plaques is the β-amyloid peptide (Aβ), a 39-42 residues peptide fragment generated by the proteolysis of cellular amyloid precursor protein (APP).(2) It is now believed that early stages of aggregation of Aβ in the brain, initiate a cascade of events that result in neuronal cell death and leads to cognitive decline.(3) Inhibiting Aβ self-oligomerization might, therefore, provide a useful approach to treating and controlling the pathogenic pathways underlying AD. Several small molecules capable of binding to Aβ have been identified, among these trehalose and the pentapeptide LPFFD have been reported to have effects on the aggregation as well as on Aβ neurotoxicity.[4,5] We hypothesized that the conjugation of trehalose with the pentapeptide LPFFD would result in new compounds with higher affinity for Aβ thereby acting as new effective inhibitors of Aβ's cellular toxicity. In this communication we report the synthesis and the spectroscopic characterization of three new trehalose conjugates with the LPFFD peptide. All the synthesized compounds were tested as inhibitors of both Aβ's fibrillogenesis and toxicity toward pure cultures of rat cortical neurons. In addition, the effects of these glycopeptides on the morphology of Aβ aggregates were analyzed by AFM microscopy.

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P21126-111

Oligopeptide-Porphyrin Interaction Studied by Chiroptical Spectroscopies: Axial Ligand Effect of Metalloporphyrins on Peptide Matrix Conformation

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Interactions between cationic or anionic porphyrins and polypeptide templates with charges that are opposite to those of porphyrins have been extensively investigated for their possible applications in biomedicine and photodynamic therapy (PDT). The influence of porphyrin on the conformation of the peptide part of the complexes is studied in this work. Non-covalent interactions of cationic tripeptide L-lysyl-L-alanyl-L-alanine (KAA) with anionic *meso*-tetrakis(4-sulfonatophenyl)porph yrin (TPPS) and its copper(II) (without axial ligand), iron(III) (one axial ligand), and manganese(III) (two axial ligands) derivatives were investigated in aqueous solutions by vibrational (VCD) and electronic circular dichroism (ECD) spectroscopies. Although both the CD spectroscopies are sensitive to conformation, particularly VCD is extremely sensitive to subtle conformational changes. The VCD spectra of pure KAA in the amide I' (C=O stretch vibration) region showed the spectral patterns typical for left-handed Polyproline II helical conformation (PPII). Interaction of KAA with non-metallated TPPS was accompanied by change of the amide I' VCD patterns - loss of VCD intensity and arising of a new negative band at ~1630 cm^{-1} - that was interpreted as a partial change of PPII into less compact conformation as extended helix or β-sheet segment. In case of Cu(II)- and Fe(III)-TPPS, the loss of the amide I' VCD intensity of KAA was observed only. For Mn(III)-TPPS having two axial ligands, the VCD pattern was unchanged compared to the pure tripeptide indicating that this derivative is not able to change the conformation of KAA in peptide-porphyrin complex.

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P21126-112

Conformational and Thermal Stability Studies of Sequential Oligopeptides $(Lys\text{-}Ala\text{-}Ala)_n$ in Aqueous Solution

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Sequential cationic oligopeptides containing positively charged aminoacids as lysine (Lys), arginine (Arg) or ornithine (Orn) are

suitable models of biologically important small-sized proteins – histons – located in the chromosomes of eucariotic cells. They are able to interact with negatively charged functional groups of many biologically important molecules as DNA, polyuronic acids, and porphyrins and thus influence a broad range of biological functions. In this work, the solution conformation of synthetic oligotripeptides (L-lysyl-L-alanyl-L-alanine)$_n$ [n = 1, 2, 3] was investigated at the different temperatures and pH using combination of vibrational (VCD) and electronic circular dichroism (ECD) spectroscopies. VCD spectra of all the oligopeptides measured at room temperature show a negative couplet (positive to lower frequency) in amide I' region. This spectral pattern is indicative of left-handed Polyproline II helical conformation (PPII), which is stable at wide range of pH. The temperature dependence of the VCD spectra indicates that PPII conformation of all the oligopeptides remains stable even at 90°C, independently on length of oligopeptide chain. These results were confirmed by temperature dependent ECD experiments, where the characteristic negative and positive bands at about 195 and 220 nm, respectively, were observed. Taken together, VCD and ECD results suggest that PPII conformation of (Lys-Ala-Ala)$_n$ sequence is the dominant conformation within the range of temperature from 20 to 90°C. All the results including spectral data analysis are discussed in detail.

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P21128-113

Structural characterization of H-[(*cis* or *trans*)-ACPC-2*S*-aza-ACPC]$_3$-NH$_2$ peptides

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β-peptides are probably the most thoroughly investigated peptidomimetic oligomers. To extend the field of β-peptides towards the construction of possible new secondary structures, the replacement of the C$^{\alpha}$ and C$^{\beta}$ atoms of the β-amino acid with heteroatoms could be an attractive modification, for example C$^{\beta}$-atom of β-peptides by an NR moiety, leading to hydrazine peptides. In the literature, there are only a few studies about hydrazine peptides [1-3], and hydrazine peptides with cyclic side-chain have not been studied yet.

In order to determine the secondary structure preference of H-[(*cis* or *trans*)-ACPC-2*S*-aza-ACPC]$_3$-NH$_2$ peptides (Figure 1), their potential energy hypersurface were probed at the *ab initio* B3LYP/6-311G** level. The *cis* (1*R*,2*S*) formed 10/12-helices, while the opposite ACPC enantiomer resulted 6 strand. The *trans* (1*S*,2*S*) formed 8-strand, while the opposite ACPC enantiomer resulted 12-helix.

The hybrid-peptides in question were synthetized on solid support, and their high-resolution 3D assignments were made by using NMR, which was supported by ECD, VCD, QLS and TEM methods. The hydrazino modification resulted in better water solubility than that of the ACPC homooligomers. This result is very important concerning the further biological applicability.

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P21128-114

Conformational Studies of Cyclolinopeptide A Analogues Modified by β-Prolines

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Cyclolinopeptide A, (CLA, 1), a cyclic nonapeptide cyclo(–Pro^1-Pro^2-Phe^3-Phe^4-Leu^5-Ile^6-Leu^8-Val^9–), possesses strong immunossupresive and antimalarial activity as well as the ability to inhibit cholate uptake into hepatocytes [1,2]. The mechanism of cyclolinopeptide A activity is similar to that of cyclosporine A.

The object of our investigations are six CLA analogues with Pro^1 and Pro^2 residues replaced by β^2-isoproline or β^3-homoproline.

The immunosuppressive activity of these new CLA analogues was evaluated on the basis of the mouse splenocyte proliferation assay 3.. NMR spectra analysis (chemical shifts assignment and structural constraints) was based on 1D and 2D NMR experiments at 700 MHz. Long (300 ns) molecular dynamics calculations were carried out using GROMACS program (GROMOS G53A6 force field) for isomers of at least 30% content.

In comparison with 1, the modified CLA molecules are less conformationally flexible or conformational exchange is much slower in NMR time scale, and moreover all of them exist as mixtures of four isomers due to *cis/trans* isomerization of the Xxx-Pro bond (Xxx=Val or Pro). The replacement of one or both prolines by β-prolines changes the backbone and side chain conformations, in particular mutual orientation of the Phe^3 and Phe^4 aromatic rings.

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P21315-115

Design and Synthesis of Photo-switchable Peptides and Proteins

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Thiol-reactive azobenzene-based cross-linkers provide a straightforward means for introducing a photo-isomerizable unit into a peptide or protein structure. We have shown that the conformational response of the peptide in such systems is critically dependent on the manner of attachment of the cross-linker as well as the cross-linker structure. Cross-linkers can be introduced in which trans-to-cis photoisomerization leads of formation of helical structure, or conversely to loss of helical structure. These effects can be understood in a semi-quantitative manner by calculating the degree of mismatch between the steric requirements of the linker and the conformational ensemble of the peptide. Kinetic studies reveal that in general photoisomerization of the linker is fast (several ps) whereas subsequent peptide folding or unfolding occurs over hundreds of microseconds. Such cross-linkers are thus potentially useful photo-triggers for studying protein folding processes, as well as for controlling the equilibrium stability of different conformational states. Applications to photo-control of bioactive peptides and proteins will be discussed.

P21420-116

Short corticotropin-like peptides with stress-protective activity

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It was synthesized 87 linear è 9 cyclic corticotropin-like peptides, which contained from 13 to 2 amino acid residues. The ability of each of the synthesized peptides to inhibit the specific binding of tritium-labelled corticotropin (11-24) to the adrenal cortex membranes of rat in vitro was investigated. On the base of the obtained results 12 peptides with the highest inhibitory activity were selected for in vivo tests. The

influence of the selected peptides on the level of 11-oxicorticosretoids and catecholamines in the adrenals and blood of rats in the experiments on acute hemorrhage and hypobaric hypoxia, cold and heat shock and low doses of g-radiation were studied. It was established that intravenous injection of 5 short peptides at the dose of 1 µg/kg could correct disturbance of 11-oxycorticosretoids-catecholamines system in the adrenals and plasma of rats that were subjected to hemorrhagic shock and hypoxia. The rest of investigated peptides possessed lower stress-protective activity. It was also shown that under cold or heat shock, thrice-repeated intranasal injection of these peptides into rats at the dose of 10-20 µg/animal abolished temperature induced changes in the level of 11-oxycorticosretoids and catecholamines in the adrenals as well as the content of free histamine and the activity of diaminoxydase in the myocardium. It has been shown that the stress-protective activity of corticotropin-like peptides is mediated by the corticotropin receptor in cortex of the adrenals.

P21500-117

A study of immunobiological activity of the WRNWDYYK octapeptide

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Synthetic octapeptide WRNWDYYK is a fragment of the α-chain of a high affinity receptor for immunoglobulin E (FcεR1A), fragment 110-117, and is an active domain for binding IgE. The programme of investigation of the biological properties of the octapeptide included the investigation of its properties using standard sets of reagents for immunoferment determination of concentration of the general class E immunoglobulin in the blood serum produced by closed joint-stock company ”Vector-best”, Russia, catalogue number A-8660. The investigations showed that introducing only the WRNWDYYK-peptide labelled by horse-radish peroxidase in the holes of the plotting board containing the test system A-8660 with primary monoclonal antibodies against IgE leads to a dose-dependent increase of the optical density of the solution. The concentration of horse-radish peroxidase in the conjugate of the WRNWDYYK-peptide introduced in the holes was 0.01 mg/ml. 20.0 to 180.0 µl of the conjugate was added to the holes. The minimum optical density of the solution at the working wavelength of 450 nm and background wavelength of 620 nm was found after adding 20.0 µl of the conjugate and was equal to 0.33±0.057 units of optical density, while the maximum, achieved after adding 160.0 µl of the conjugate, was 1.694±0.092 units of optical density. A strong correlation was found between the amount of added WRNWDYYK-peptide, conjugated with horse-radish peroxidase, and the optical density of the solution after adding the substrate chromogenic mixture (r=0.884). The experiments demonstrated a high binding activity of the synthetic WRNWDYYK-peptide with primary monoclonal antibodies against IgE, which allows us to conclude that antibodies against a certain epitope of IgE are able to bind to FcεR1A and can contribute to the development and course of a number of allergic illnesses.

P21507-118

The Evaluation of the Effect of Japanese Herbal (Kampo) Medicines using Bioavtive Peptides as Biomarkers.

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In many cases, Japanese herbal (Kampo) medicines have been used in the empirical treatment of chronic hypofunction. However, the Kampo medicines consist of several herbs, whose pharmacological mechanism is not clear. In western medical science, medical doctors usually treat patients according to disease diagnosis. In eastern medical science, treatment is based on diagnostics called gsho h, which is a unique concept in Kampo medicines and quite different from diagnosis. The concept of gsho h is difficult for non-professionals to understand, furthermore, there are responders and non-responders when non-professionals prescribe Kampo medicines. The concept of gsho h focuses on individuals, not diseases, therefore, it is difficult to gain a given effect for everyone by general clinical trials. In this study, we investigated the effects of prokinetic Kampo medicines on plasma levels of gut-regulatory peptides (somatostatin, motilin and gastrin) and compared with that of dopamine receptor antagonists, the western prokinetics. The five Kampo medicines, including *Pinelliae Tuber* and *Zingiberis Rhizoma*, three dopamine receptor antagonists or placebo was orally administered. Venous blood samples were taken before and till 240 min after administration. Plasma peptide levels were measured using a sensitive enzyme immunoassay. The dopamine receptor antagonists and Kampo medicines caused significant increase of plasma gut-regulatory peptide levels compared with placebo group. In recent years, chronic hypofunction without mechanical problem, such as non-erosive reflux disease and functional dyspepsia, is difficult to cure using western medicines. The preliminary study indicated the plasma somatostatin levels of patients with any symptoms are high and plasma motilin levels of them are low compared with those of healthy subjects. To evaluate Kampo medicines using bioactive peptides as biomarkers, it may be possible to cure diseases that is difficult to treat by western medicines.

P21507-119

Receptor-Mediated Targeting of Metastatic Melanoma with Radiolabeled DOTA-α-MSH Analogs

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Radiolabeled α-MSH analogs are potential candidates for melanocortin-1 receptor (MC1-R)-mediated melanoma targeting. Several short α-MSH peptides carrying a DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) metal chelator were designed and evaluated as potential diagnostic (e.g. with 111In, 67,68Ga) or therapeutic (e.g. 90Y, 67Cu) radiopharmaceuticals. The analogs tested to date showed high affinity for the MC1-R in vitro, excellent internalization into the tumor cells, as well as a good incorporation in tumor xenografts and a low uptake in normal tissues in vivo, except the kidneys where considerable uptake is observed. Our current studies attempt to influence the pharmacokinetic parameters in order to address specific uptake (i.e. by melanoma) versus non-specific uptake (i.e. by the kidneys). As glycosylation had been shown to improve tumor-to-kidney ratios in the case of somatostatin and to reduce peptide re-uptake by the tubular system of the kidneys in general, we investigated glycosylated analogs of [Nle4, Asp5, D-Phe7]-α-MSH4-11 (NAPamide). Carbohydrate moieties such as glucose, galactose and maltotriose were introduced at various positions on the MSH peptide carrying the metal chelator DOTA for labeling with 111In. The peptides were evaluated in vitro in both murine and human cell lines for MC1-R binding and cellular localization, and in vivo in B16F1 tumor-bearing mice for tissue distribution. The tumor-to-kidney ratio for Gal-NAPamide (4-48 h AUCs) was superior to any of the previously published MSH peptides. Other glycopeptides showed very good binding affinities but lower selectivity in vivo. In additional, a series of non-glycosylated dimeric derivatives, bearing one or two moieties of the chelator complex, were developed which displayed excellent receptor affinity but tended to result in higher kidney accumulation. By contrast, at least one negatively charged DOTA-NAPamide showed excellent tumor-to-kidney ratios.

P21509-120

Discovery and Validation of Peptide Biomarkers with Novel Mass Spectrometry Based Workflows

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There is a critical lack of validated early biomarkers for most conditions and diseases. Early diagnosis does enable treatment of less severe disease states, the use of less invasive techniques and could potentially reduce the costs of healthcare systems. However, it is most likely that peptides in tissue or blood – or their modifications – can be specifically associated with different disease states. The discovery and verification/validation of such disease markers are two distinct workflows. During the discovery phase, a relatively small number of samples with a high number of potential biomarker candidates are screened. The high-throughput provided by the iTRAQ™ reagent strategy allows for simultaneous analysis of such samples. Once biomarker candidates have been identified with initial statistical significance, these have to be validated. This validation workflow involves analyzing a large number of samples with a relatively small number of candidates to establish the biological significance of the biomarker candidates. Rather than switching to immunological techniques for this validation step, we suggest a mass spectrometry based approach. This orthogonal strategy is a novel targeted, high throughput quantitative multiplexed multiple reaction monitoring (MRM) approach. The approach relies on assay development using a combination of MRMs to target specific peptides identified in discovery, followed by MS/MS to confirm that the quantitative MRM signal results from the target peptide. In addition to being a quantitative method, this validation approach is extremely specific and sensitive. Several published examples of the application of this MRM-based approach utilizing the actual or hypothetical physical properties of peptides in complex mixtures will be presented.

P21518-121

Influence of the charge on the *in vivo* behavior of radiolabeled bombesin analogues

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Prostate and breast cancers are the second leading cause of cancer death in men and women, respectively. The side effects related to the treatments that are available today have a great influence on the patients' quality of life. Therefore, the development of new diagnostic and therapeutic strategies may have an important impact in the outcome of these cancers. Gastrin-releasing peptide (GRP) receptors are present in high quantities in a variety of cancers, prostate and breast tumors among them. Targeting of over-expressed GRP receptors with radiolabeled bombesin (BBS) analogues would offer an interesting tool for tumor imaging and therapy, depending on the radionuclide used. Some analogues of BBS, based on the fragment 7-14, were functionalized with the (N^{α}His)-chelator for labeling with the ^{99m}Tc- and ^{188}Re-tricarbonyl-core. Despite an increased metabolic stability, these analogues showed very low tumor uptake. Additional insertion of a βAla-βAla linker led to increased tumor uptake but still unfavorable *in vivo* properties. In order to further improve the biodistribution, novel polar linkers with different charge were introduced in the molecule. A positive charge resulted in increased kidney uptake, whereas one single negative charge led to a significant increase in the tumor uptake and also significantly higher tumor-to-tissue ratios. Co-injection with natural BBS importantly inhibited the uptake in the tumors and receptor-expressing tissues, which confirmed the specificity of the *in vivo* uptake. Moreover, imaging of the tumor xenografts by SPECT/CT was also much clearer with the analogues bearing one negative charge. Additional negative charges, however, resulted in a loss of binding affinity and internalization, and unfavorable biodistribution. In conclusion, BBS analogues with one single charge in the linker hold a greater potential for imaging and therapy of GRP receptor-overexpressing tumors

P21525-122

Peptide-macrolide conjugates as novel instruments for protein biosynthesis study

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Bacterial ribosomes are targets of numerous therapeutic agents. Macrolide (ML) antibiotics prevent nascent polypeptide growth by blocking the ribosomal exit tunnel (RT). Availability of high-resolution structures of complexes of ribosomal 50S subunits with ML enabled developing novel ideas concerning their inhibitory activity of translation. Formerly we obtained peptide derivatives of ML in which the peptide part modelled a growing chain, while an antibiotic moiety served as an "anchor" for positioning the peptide in the RT. The goal of this study was to synthesise tryptophan-containing peptide derivatives of 5-*O*-mycaminosyltylonolide (OMT) to monitor location of the specific Trp binding site that modulate the activity of the peptidyl transferase centre of the ribosome. The peculiarity of compounds designed in this study lies in that of a Trp containing peptide fragment, connected to the primary hydroxyl group in position C23 of OMT, is oriented in the RT towards the hypothetical Trp binding site. The following peptides were used: Boc-*L*-Trp-Gly-OH, Boc-*L*-Trp-*β*Ala-OH, Boc-*L*-Trp-*γ*Abu-OH, Boc-*L*-Trp-*δ*Ape-OH. Variable distance between Trp residue and macrolide ring was achieved by introduction of glycine, *β*-alanine, *γ*-aminobutyric acid and *δ*-aminovaleric acid as C-terminal amino acids of the dipeptides. The peptides were obtained from Boc-*L*-Trp-OH and ethyl esters of the corresponding amino acids by condensation with BOP followed by saponification of the peptide esters. The reactions between peptides and OMT were produced using DCC and DMAP as condensation agents. As a result peptide-macrolide derivatives were obtained. All compounds were purified by column chromatography on silica gel and characterized by HPLC, mass-spectrometry and NMR. It was shown by means of chemical probing that Trp containing OMT derivatives specifically bound to RT of the ribosome. Moreover these compounds displayed an antibiotic activity in testes with several *Staphylococcus* strains.

P21529-123

Novel Chelators for Metal Conjugation of Neuropeptide Y (NPY) Y1-Receptor Selective Peptides for Breast Cancer Targeting

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NPY is a 36 amino acid peptide amide that belongs to the pancreatic polypeptide-hormone family (1). It is the most abundant neuropeptide in the brain and triggers a number of central activities, such as regulation of food intake as well as stress induced reactions [2-4]. NPY forms a selective interaction with at least three receptors, which are called Y1, Y2 and Y5. In the past it was shown that Y1-receptors are overexpressed in >90% of all breast tumors as well as in 100% of the derived metastases. Normal breast tissue expresses Y2-receptors, while the neoplastic tissue expresses Y1-receptors 5.. As a result, the NPY-Y1-receptor system can be used for tumor targeting and therapy. In the first step we synthesized the truncated and modified NPY analogue [Pro30,Nle31,Bpa32,Leu34]NPY(28-36) (Nle–norleucine; Bpa–benzoyl-phenylalanine) by solid

phase peptide synthesis using Fmoc/tBu strategy. The NPY analogue was characterized with respect to in vitro binding affinity and selectivity at Y1-receptor expressing human breast cancer MCF-7 cells and the metabolic stability in human blood plasma, respectively. Then we coupled different potential chelators to [Pro30,Nle31,Bpa32,Leu34]NPY(28-36), which was N-terminally modified by two ß-alanines as spacer between the peptide sequence and the chelator. Next, we determined their ability of metal conjugation of diverse metals, as Cu2+ and Re3+. Metal conjugated peptides were characterized by Maldi-ToF-MS (matrix assisted laser desorption/ionization mass spectrometry), RP-HPLC (reversed phase high performance liquid chromatography) and IR-Spectroscopy (infrared). After optimization of the metal conjugation first binding affinity studies were performed.

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P21600-124

Identification of biologically active peptides from the N-terminal domain of laminin alpha 2 chain

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Laminins, a major component of the basement membrane, consist of α, β, and γ chains and have various biological activities including promotion of cell adhesion, growth, migration, differentiation, and angiogenesis. So far, five α (α1-α5), three β (β1-β3), and three γ (γ1-γ3) chains have been identified and at least 15 isoforms of laminin have been discovered by various combinations of each subunit. These laminin isoforms are tissue- and/or developmental stage-specifically expressed. The laminin α2 chain is mainly expressed in skeletal muscles and peripheral nerves, and interacts with cell surface receptors such as integrin, dystroglycan, and heparan sulfate proteoglycans. Biological functions of the laminin α2 chain N-terminus including integrin binding and heparin/heparan sulfate binding were found previously. Here, we focused on the N-terminal region of the mouse laminin α2 chain (position 1-1566) and screened the biologically active sequences using 142 peptides. The synthetic peptides were generally 12 amino acids in length and overlapped with neighboring peptides by 4 amino acids. Cell attachment activity of the peptides was evaluated using a peptide-coated plastic plate assay and a peptide-conjugated Sepharose bead assay using HT-1080 human fibrosarcoma cells. Eleven peptides showed cell attachment activity on plate assay and five peptides showed cell attachment activity on beads assay. Previously we screened active sites on the laminin α1 chain and identified several active sequences. When we compared with active sequences of the α1 and α2 chains, A2-31 (YYDETVASRNLSLN) and A2-112 (GGKLKYAIYFEA) are unique active peptides only within laminin α2 chain. These results suggest that the biological activity of A2-31 and A2-112 are α2 chain specific and are useful for investigating the α2 chain specific functions of laminin α2 chain.

P21613-125

Novel peptide biopharmaceuticals by using phage display technology

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Libraries of random peptides displayed on the surface of bacteria, mammal cells or bacteriophages are an essential tool that enables systematic study of target molecule interactions. The identification of ligands from large biological libraries by phage display has now been used for almost 15 years. In a last few years several improvements have led to numerous high affinity peptide ligands that express various biological activities. Phage-displayed peptide libraries have been used successfully to isolate peptide ligands directed to a functional site for which the natural ligand is or is not a protein or peptide. By using a modified, in-house developed selection proctocol, we successfully selected several phage clones with high affinity to pancreatic lipase, ghrelin and cysteine protease cathepsin K as target proteins. Based on their deduced animoacid sequences, twenty heptapeptides with the highest affinity to target proteins were synthesized and characterized for their capacity to inhibit enzyme or acceptor function. The most succesful peptide candidates inhibited pancreatic liapase with the apparent inhibition constant of 16 uM, and cathepsin K with the apparent inhibition constant of 0,10 uM. A set of 22 peptidomymetic compounds were sinthesyzed based on the aminoacid sequence of selected peptides and their inhibitory activity was determined. The most potent candidates were selected for further development of peptide biopharmaceuticals aganist obesity (inhibitors of pancreatic lipase and ghrelin) and in the prevention of osteoporosis (cathepsin K inhibitors).

P21617-126

Novel approach to non-specific elution in phage display using ultrasound

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Phage display is used to select and optimize peptides or protein domains binding to virtually any protein and sometimes even non-protein targets. Several rounds of screening are performed, until the increase in phage output, or binding assays performed with phage pools, indicate that the population of binding phages has been adequately enriched. In cases where ligands of particular target are not known or available, target-bound virions are released by non-specific elution for example with acidic buffer, or competitively with free target molecule, or by addition of bacterial host directly to the target-bound phages.

We used streptavidin, immobilized by adsorption, as a model target protein for affinity selection of peptides from phage display library. The efficiencies of a number of well-known typically used non-specific elution strategies in selecting and retrieving a phage clone displaying the tripeptide biotin mimetic (HPQ) from a streptavidin coated surface were compared.

All the commonly used elution strategies have failed to elute and select high affinity HPQ-bearing phage clones bound to streptavidin. The failure was shown to be due to the inability of eluants to break the interaction of high affinity clones with the target, which is thus likely to be the cause for failed selection with other targets also. To surmount this, we have introduced a new elution strategy, combining low pH elution buffer with sonication which, in addition to loosening the peptide-target interaction, also serves to detach the target molecule from the immobilization surface. This ultrasound-based method enabled single step selection of a high affinity peptide from a library of diversity greater than 10^9, and thus represents a dramatic improvement in searching for novel, specific peptide ligands.

P21620-127

Screening of a novel octamer peptide, CNSCWSKD, that induces apoptotic cell death

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Therapeutic peptides and small molecules, rationally designed to trigger cell death have attracted strong attention. Cell death inducible peptides were screened from the sequences of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) that is known to induce

apoptosis to various tumor cells but not in normal cells. Using Fmoc solid phase synthesis, cellulose membrane-bound octameric peptide library of TRAIL scan was prepared and cell viability assay was directly performed on peptide disk with Jurkat cells. Six peptide sequences that could induce cell death were found, and particularly RNSCWSKD (TRAIL227-234) peptide was shown the strongest effects. Then, peptides of stronger effects were found through amino acid substitution, and the CNSCWSKD peptide induced >90% cell death in treated cells. Features of apoptosis, such as DNA fragmentation, activation of caspase, phosphatidylserine externalization, chromatin condensation, and competition with TRAIL for binding to the death receptor (DR) 4 or DR5 were observed, suggesting that this peptide is a TRAIL mimic. Caspase-3 activation was observed in various tumor cells treated with this peptide as well as with TRAIL, while no activation was observed in human normal fibroblasts. The CNSCWSKD peptide is a potential candidate for use in cancer therapy.

P21620-128

The Study on the Exendin-4 Mimetics for the Treatment of Type 2 Diabetes

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Being an incretin mimetic, exendin-4 exerts the same action as GLP-1 including the glucoregulatory and the insulinotropic effects through GLP-1 receptor. Exendin-4 may be preferable to GLP-1 on the aspect of stability. However it was comprised with 39 amino acids and was not suitable for developing as an oral drug. Recently, there was an upsurge in the development of exendin-4 mimetic as potential therapy for type 2 diabetes. In this study, a receptor-binding region on the surface of RINm5F cell was used as the target to screen peptide ligands for the receptor in a phage 12-mer peptide library. DNA sequencing revealed a group of closely related peptides from the fourth round of selection. Through the activity of decreasing blood glucose assay in vivo, the cell proliferation assay and capability against DPPIV in vitro, one highest bioactivity peptide (QPSVGMKPSPRH, Ex-12PA) was acquired. The bioactivity of Ex-12PA was almost identical with exendin-4 on the promoting cell proliferation in a dose-dependent manner. The decreasing blood glucose effect of Ex-12PA was up to 45 min after administration. The stability against DPPIV of Ex-12PA maintained good performance that 35% parent peptide remained after 48h. In summary, Ex-12PA as a shorter GLP-1 receptor agonist mimicked the action of exendin-4 and built a good foundation for designing the oral diabetes drug. Key words: exendin-4, mimetic, phage display peptide library, screen

P21623-129

Peptide inhibitors of MurD and MurE, essential enzymes of bacterial cell wall biosynthesis

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Over the last decades we have witnessed the emergence of bacterial resistance to virtually all clinically important antibiotic types. Therefore, continuous development of antibacterial agents with completely novel modes of action accompanied by rationalization of chemotherapeutic prescription is the key strategy to adopt in a long-run struggle against the growing problem of pathogen resistance.
Numerous indispensable antibiotics interfere with peptidoglycan cell wall biosynthesis making this unique metabolic pathway a well validated target for antimicrobials. While nearly all of these antibiotics inhibit late stages of murein synthesis occurring on the extracellular side of plasma membrane, initial cytoplasmic steps have not been extensively exploited as drug targets. We have performed affinity selections from random linear and conformationally constrained (disulphide cyclized) peptide libraries displayed on bacteriophage particles against two essential bacterial enzymes MurD and MurE, involved in the cytoplasmic synthesis of peptidoglycan monomer precursor. Selected peptides were found to inhibit respective targets in an *in vitro* assay with IC_{50} values of 140 μM to 1.5 mM. Reported inhibitory peptides should be regarded as templates for design of low-molecular-weight peptidomimetics. Resulting MurD and MurE inhibitors with improved potency and/or physicochemical characteristics (especially membrane permeability) have the potential to act as broad spectrum chemotherapeutics.

P21623-130

Analyses of coiled-coil associations by SPOT technology and biophysics

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Coiled coil (CC) sequence motifs are common structural motifs and versatile protein interaction modules. The CC is composed of at least two right-handed amphipathic a-helices that wrap around each other into a left-handed supercoil such that their hydrophobic surfaces are in contact. CC can associate up to heptamers, form homomeric and heteromeric complexes at different stoichiometries, and be aligned parallel and antiparallel. A characteristic of all coiled coils is the presence of heptad repeat sequences [abcdefg]i, where i denotes the heptad number. Although CC motifs can be predicted with a high degree of confidence, predicting the association states and topologies is still a great challenge. We will report on synthetic peptide arrays useful to study CC associations at the amino acid level. In contrast to rational design, which mostly depends on short model peptides, our approach relies on the full-length homodimeric GCN4 – and the heteromeric cJun/cFos coiled-coil domain formation. The influence of amino acid substitutions on association is tested without restrictions and presumptions and the stoichiometry is examined by biophysical methods. Furthermore, we generate arrays comprising hundreds of (putative) CC sequences and subsequently probe them for association to a set of several native CC sequences. An interactome can be drawn for each of the investigated CC sequences, enabling one to interpret the biological functions. We will present: (i) association analyses of all single substitution variants of the GCN4-, cFos and cJun leucine zipper probed with the associated native leucine zipper; (ii) the exposure of a heteromeric CC-network deduced from GCN4 variants and the determination of the network-stoichiometry; (iii) the CC-interactom of several native coiled coils like the CC sequences of AKAP 18δ, PQBP1 18, Orai1 and others.

P21723-131

New Results in the RGD Field

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Almost two decades ago we used the spatial screening technology to optimize activity and receptor subtype selectivity among RGD recognizing integrins. This culminated in the avb3 selective superactive pentapeptide c(-RGDfV-) which gave rise to a number of peptidomimetic modifications. Among them we studied all retro-, inverso- and retro-inverso peptides of this structure. Only one retro-inverso peptide exhibits full activity: the peptide c(-dGRvf-) strongly inhibits avb3/vitronectin binding (4 nM) but not aIIbb3/fibrinogen binding (>10 000 nM). All investigated retro-sequences show very low affinity for avb3. Recently it was discovered that the NGR sequence in the fibronectin domain FI5 after rearrangement into iso-Asp-G-R exhibits high binding affinity for integrin avb3. This surprising result stimulated us to investigate cyclic peptides containing the iso-Asp-Gly-Arg sequence3.. After optimisation we obtained peptides which bind with high activity to integrin a5b1 and lower, but significant activity for avb3. In the ongoing work we investigate if receptor modelling can help to understand these results. Selectivity and activities for these two integrins have been obtained recently in our group using two different peptidomimetic scaffolds

(tyrosine based and diacylhydracine based) using a homology model of the integrin a5b1 for which no X-ray structure is known. Binding of integrin ligands involves binding of a carboxyl group to the metal ion (Ca or Mn) in the so-called MIDAS region of the integrin. So far all integrin ligands contain a carboxyl group and any attempt to substitute this group by an isoster failed. We recently found that hydroxamic acids (-CONHOH) allow for the first time a substitution of this carboxyl group. The activities and selectivities for integrin subtypes avb3, a5b1 and aIIbb3 have been explored and give interesting results.

P21819-132

Development of GnRH-III—antracycline conjugates as multifunctional drug delivery systems for targeted chemotherapy

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Targeted chemotherapy based on the cell specific or overexpressed receptors on tumors might be an efficient therapeutic approach for the treatment of cancer. Expression of gonadotropin-releasing hormone (GnRH) receptor was identified on different types of tumors.1 It has been shown that GnRH-III (Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH2) isolated from see lamprey has antiproliferative activity on numerous tumor cells, and significantly less potency on releasing gonadotropin hormones (LH, FSH); therefore, it is a more selective antitumor agent than the human GnRH derivatives.2 In our work, GnRH-III was used as targeting moiety for the preparation of multifunctional drug delivery systems for targeted cancer chemotherapy. Daunomycin (Dau) and doxorubicin (Dox) as antineoplastic agents were attached to the side chain of 8Lys of GnRH-III through amide, oxime, hydrazone or ester bonds, either directly or by insertion of an enzyme cleavable tetrapeptide spacer. Stability studies of the conjugates were performed in human serum, as well as in the presence of chymotrypsin. The effect of chemical structure of the conjugates on in vitro antitumor activity was studied using different cancer cell lines (MCF-7 human breast, HT-29 human colon and C26 murine colon cancer cells). Oxime bond linked Dau-GnRH-III conjugate was selected for in vivo experiments using C26 colon tumor bearing mice. The conjugate showed similar antitumor activity as the free drug, but less toxic side effect and longer survival of the animals was determined in the case of the application of the conjugate.

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P21819-133

Fluorescent Agonists for the Human Vasopressin V1b Receptor

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We recently reported that d[Leu4,Lys8]VP is the first selective agonist for the rat vasopressin (VP) V1b receptor (1). It was also shown to be highly selective for the human V1b receptor (1). We now report the synthesis and some pharmacological properties of three fluorescent hV1bR ligands (A,B,C), based on modifications of the Lys8 residue in d[Leu4,Lys8]VP, with Alexa 488 (A), Alexa 647 (B) and Antraniloyl (Atn) (C). The fluorescent peptides A, B and C exhibit the following affinities for the hV1bR: (A) Ki = 1.2 nM, (B) Ki =186 nM and (C) Ki = 0.65 nM. Peptides A and C exhibit moderate affinities for the hOTR and very weak affinities for the hV1aR and for the hV2R. On V1b-transfected AtT20 cells, they activate PLC coupling as evaluated by stimulation of IP3 levels. They also induced receptor internalization visualized by accumulation of fluorescence in endosomial vesicles. One or more of these new fluorescent hV1bR ligands promise to be useful tools for studying human V1b receptor localization or trafficking.

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P21824-134

Distribution of prolyl oligopeptidase in the mouse whole-body sections and peripheral tissues

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Prolyl oligopeptidase (POP) is a serine endopeptidase that hydrolyses proline-containing peptides shorter than 30-mer, including many bioactive peptides. The distribution of POP in the brain has been studied but little is known about the distribution of peripheral POP. We used immunohistochemistry to localize POP in mouse whole-body sections and at the cellular level in peripheral tissues. Furthermore, we used a POP activity assay to reveal the associations between POP protein and its enzymatic activity. The highest POP protein densities were found in brain, kidney, testis and thymus, but in the liver the amounts of POP protein were small. There were remarkable differences between the distribution of POP protein and activity. The highest POP activities were found in the liver and testis while kidney had the lowest activity. In peripheral tissues, POP was present in various cell types both in the cytoplasm and nucleus of the cells, in contrast to the brain where no nuclear localization was detected. These findings support the proposed role of POP in cell proliferation in peripheral tissues. The dissociation of the distribution of POP protein and its enzymatic activity points to nonhydrolytic functions of POP and to strict endogenous regulation of POP activity.

P21900-135

The rapid cytoplasmic entry of cationic cell-penetrating peptides

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In the past few years, our understanding of the cellular import of cell-penetrating peptides has evolved rapidly. The initial concept of cell entry by direct permeation of the plasma membrane, was followed by endocytosis as a major route of import at least for most cell-penetrating peptide-cargo conjugates. More recently, we and others observed a rapid cytoplasmic delivery of fluorescein-labeled analogs of the cationic CPPs nonaarginine and Tat-peptide at subtoxic lower

micromolar concentrations (1). This import originates from spatially confined zones of the plasma membrane and depends on the presence of heparan sulfate proteoglycans. These molecules have been proposed to interact polyvalently with the guanidinium groups of the arginine residues. Moreover, the threshold for the induction of this import could be lowered by inhibition of endocytic routes of entry. Recently, it was reported that the absence of serum in the medium lowers the threshold for this uptake process (2). In summary, these observations suggest that the concentration of peptide associated with the plasma membrane is a decisive trigger for this import process. Currently, it is not known, to which degree this cytoplasmic entry of labeled conjugates at higher concentrations is based on similar molecular mechanisms as the direct permeation of unlabeled conjugates that has also been observed at lower concentrations (3). Here we summarize the current knowledge on direct transport across the plasma membrane of cationic CPPs and present new data on the molecular events involved in this uptake process.

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P21900-136

Novel Intracellular Delivery System using pH-Dependent Fusiogenic Peptide

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Plasma membranes have numerous essential functions for maintaining cellular homeostasis. However, the membranes are also barriers to intracellular delivery of various therapeutic molecules. For improvement of their translocations, we developed a novel method using GALA peptide/cationic lipid complexes. GALA, a 30-residue amphipathic peptide with a repeat sequence of glutamic acid-alanine-leucine-alanine, was designed to mimic the function of viral fusion protein sequences that mediate escape of virus gene from acidic endosomes to cytosol (1). When attached with bioactive cargoes, the GALA peptide may thus serve as intracellular vector bearing efficient endosomal escape function. However, because of negative charges from glutamic acids (7-residues) in the GALA sequences, access of the peptide on negatively charged cell surface would be not so efficient. To overcome this problem, cationic lipid was employed as an gadhesive h for pasting the GALA peptide onto cell surface to accomplish efficient cellular uptake. We examined the ability of GALA peptide as a delivery vector using FITC as a model of membrane-impermeable low-molecular weight drugs. When FITC-GALA (1 *f*ÊM) was administrated to HeLa cells, co-addition of cationic lipid, Lipofectamine 2000 (LF2000), drastically increased the efficiency in uptake of FITC-GALA. In a time-dependent manner, the FITC-GALA escaped from endosomes, and diffuse fluorescent signals were observed in both cytosol and nucleus. Also the GALA/cationic lipid system was applied for the intracellular delivery of FITC-avidin protein (68 kDa). When FITC-avidin was mixed with biotinylated-GALA/LF2000 complexes, FITC-avidin (250 nM) was internalized into cells effectively. In the absence of these complexes, internalization of FITC-avidin was poor. These results suggest the usefulness of our approach for intracellular delivery using GALA peptide and cationic lipid.

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P21900-137

The membrane repair response masks membrane disturbances caused by cell penetrating peptide uptake

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Even though cell-penetrating peptides are able to deliver cargos of different sizes into cells, their uptake mechanism is still not fully understood and needs to be elucidated in order to improve their delivery efficiency. Recent studies have suggested that there might be a direct penetration of peptides in parallel with different forms of endocytosis. However, the direct penetration of hydrophilic peptides through the hydrophobic plasma membrane is highly controversial. Three proteins involved in target cell apoptosis - perforin, granulysin and granzymes share many features common in uptake of cell-penetrating peptides e.g. they bind proteoglycans on the plasma membrane. The uptake of perforin activates the membrane repair response, a resealing mechanism triggered in cells with injured plasma membrane, due to extracellular calcium influx. Upon activation of the membrane repair response, internal vesicles are mobilized to the site of the disrupted plasma membrane resealing it within seconds. In this study we present evidence that the membrane repair response is able to mask damages caused by cell-penetrating peptides when they internalize cells, thus preventing leakage of endogenous molecules out of the cell.

P21900-138

In vitro and *in vivo* antitumor effect of symmetric GNRH-III dimer derivatives

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The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH-I; GnRH symmetric dimer derivatives ([In vitro (cellular uptake and antiproliferative effect of the dimer derivatives) and in vivo (comparison of per os and intraperitoneal administration) antitumor effect of these symmetric dimers were investigated. The cellular uptake of dimer derivatives were studied by flow cytometry (BD LSR II) on MCF-7(human breast cancer) and HT-29 (human colon carcinoma) cell lines using carboxifluorescein labeled symmetric dimer derivative. The cells were treated with different concentration of synthetic dimers and after the treatment were analysed by flow cytometry in order to investigate their cellular uptake. The antiproliferative effect of symmetric GnRH-III dimers were studied on MCF-7, HT-29 and T47-D (human breast cancer) cell lines. HT-29 xenograft was applied for studying in vivo antitumor effect of GnRH-III dimers in different administration routes. The cellular uptake and the antiproliferative effect are cell type dependent as it can be found in the literature. We found that symmetric dimer derivatives of GnRH-III significantly decreased the tumor volume in vivo (40%).

P21905-139

Peptides Derived from Cytoplasmic Region of β_3 Integrin can Inhibit Platelet Aggregation

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Platelets play a significant role in the pathogenesis of acute coronary syndromes. Among all glycoproteins-receptors, integrin $\alpha_{IIb}\beta_3$ has gained the interest of investigators, since it plays the central role in the final step of platelet aggregation and thrombus formation. On unstimulated platelets, the platelet receptor $\alpha_{IIb}\beta_3$ is present in a closed conformation that prevents ligand binding. Upon platelet activation by several agonists, $\alpha_{IIb}\beta_3$ receives intracellular signals (inside-out signaling) that allow cytoplasmic proteins to interact with the cytoplasmic domains of $\alpha_{IIb}\beta_3$ subunits, resulting in platelet aggregation. The aim of this work is to inhibit platelet thrombus formation by specifically disrupting the inside-out signalling pathway using synthetic peptides based on the cytoplasmic region of β_3 subunit, residues 743-762. Peptide analogues derived from

$\beta_3$743-762 region ($\beta_3$743-750, $\beta_3$743-750(pTyr747), $\beta_3$743-756, $\beta_3$755-762 and $\beta_3$749-756) were synthesized in their free, palmitoylated and/or tagged with the Tat(48-60) signaling sequence and carboxyfluorescein-labeled in order to investigate their membrane permeability, as well as their inhibition potency on the platelet aggregation. From the biological assays in PRP and washed platelets we concluded that the modified peptides that carry either palmitoyl-group or the Tat(48-60) signaling sequence penetrate platelet membrane and inhibit human platelet aggregation, in contrast to the corresponding free peptide analogues.
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P21905-140

Influence of Cdc42 Derived Peptides Conjugated to a Cell Penetrating Sequential Carrier on Rearrangement of Actin and Weibel-Palade Bodies Secretion

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The Rho family GTP-hydrolising proteins (GTPases), Cdc42 Rac and Rho, act as molecular switches in signalling pathways that regulate cytoskeletal architecture, gene expression and progression of the cell cycle. Cdc42 and Rac transmit signals through binding to effector proteins in a GTP-dependent manner. Wasp, an effector protein, is postulated to link activation of Cdc42 directly to the rearrangement of actin. Furthermore, several studies have shown the involvement of Rho GTPases secretion of Weibel-Palade bodies, which are storage organelles in endothelial cells, via reorganization of the actin cytoskeleton. The aim of this work was to define the minimal conserved region of Cdc42 that induces the rearrangement of actin and its possible involvement in secretion of Wiebel-Palade bodies. Three peptides derived from the binding domain of Cdc42 (Cdc42(158-172), Cdc42(173-187) and Cdc42(181-187)) to Wasp were synthesized in their free state and conjugated to a recently developed Cell Penetrating Sequential Carrier (CF-Ahx-[Lys-Aib-Cys(X)]$_4$-NH_2, CF-Ahx-CPSC), where X represents one of the Cdc42 peptides. Direct labeling with carboxyfluorescein allowed the assessment of the internalisation of the conjugates. Preliminary results suggest that the CF-Ahx-[Lys-Aib-Cys(Cdc42(181-187))]$_4$-NH_2 conjugate strongly affects the rearrangement of actin. Acknowledgements to the GSRT and EU (Pened 03ÅÄ629) for the financial support.

P21924-141

A Modular Strategy for the Design of Apoptogenic Cell Penetrating Peptides

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Cell penetrating peptides (CPP) have mostly been utilized as inert vectors for the intracellular delivery of bioactive cargoes. However, our more recent studies have focused upon the identification and application of CPP with intrinsic biological activities. For example, mitoparan ([Lys^{5,8},Aib^{10}]mastoparan (MP); Aib = α-aminoisobutyric acid) is a potent mitochondriotoxic analogue of MP that demonstrates a significant intracellular co-localization with mitochondria. Through co-operation with a protein of the mitochondrial permeability transition pore VDAC, mitoparan specifically promotes apoptosis. In contrast, CytC$^{77-101}$, a cryptic fragment of human cytochrome c, is a moderately potent apoptogenic CPP that demonstrates a strong propensity for co-localization within the endoplasmic reticulum.
Using a sychnologic modular design, we have synthesized and evaluated a broad range of chimeric constructs combining apoptogenic CPP (*message*) and peptidyl *address* motifs. The overriding objective of these studies was to enhance cytotoxicity and/or develop target-selective drug delivery vectors. Address motifs that target both plasma membrane and nuclear envelope protein structures included i) the integrin-specific RGD sequence, ii) a Fas ligand mimetic WEWT, iii) heptagastrin (AYGWMDF) that targets a novel membrane binding site on high grade astrocytoma and, iv) a mimetic of FG nucleoporins (Nups). Incorporation of peptidyl address motifs, by simple N-terminal acylation or via a flexible aminohexanoic acid (Ahx) linker, produced chimeric constructs with enhanced cytotoxic potencies. Most significantly, LD_{50} values readily achievable *in vivo* were demonstrated by the modular apoptogenic CPP Z-Gly-RGDf-mitoparan, (LD_{50} = 1.4 μM) and Ac-NFKFGLSS(Ahx)CytC$^{77-101}$ (LD_{50} = 1.0 μM). In conclusion, target-specific modular design of apoptogenic CPP is a promising strategy for the study and therapeutic induction of apoptosis.

P22000-142

Phage display screening of Geminin-binding peptide and validation of its therapeutic use in tumors

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DNA replication is controlled by the stepwise assembly of a pre-replicative complex (pre-RC). Geminin is a component of the pre-RC and plays a role in preventing incorporation of the minichromosome maintenance protein complex into the pre-RC via binding and inhibiting Cdt1 function. It may be possible to design low-molecular-weight chemicals that would bind to Geminin to suppress the aberrant cellular proliferation in tumor cells.
A random 12-mer peptide phage library was used for the selections. Phage was selected by panning on immunotubes coated with recombinant Geminin. Positive clones were identified by a screening phage produced from single colonies for specific binding to GST-Geminin in ELISA. Fluorescent-labeled peptide linked to the SV40 nuclear localizing signal was synthesized by solid-phase synthesis, using Fmoc chemistry. By detecting fluorescein fluorescence to mark specific transfected cells, we examined the effects of the peptide transfection on the synthesis of new DNA in HCT116 cells.
By screening a random peptide phage library, we identified a certain peptide sequence bound to Geminin. Using a series of mutant Geminin, ELISA test revealed the amino acid residues 31-111 are responsible for the peptide binding. We found fewer BrdU positive cells following transfection of the Geminin-binding peptide than following that of the control peptide.
This study suggests that the chemical peptidomimetics of this peptide might form the basis for the development of drugs that could be used to prevent tumor progression.
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P22020-143

Synthesis and antimicrobial activity of Nva[CSNH]-FMDP

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It has been previously demonstrated that N3-(4-metoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) is a strong inhibitor of glucosamine-6-phosphate synthase, a potential target for antimicrobial chemotherapy. FMDP is transported into the cells when incorporated in a peptide chain and hydrolyzed by intracellular peptidase releasing free inhibitor as the "warhead" component, which can react with the target enzyme. The concept of utilizing of peptide transport system for delivery of toxic amino acids into the microbial cells is characterized by authors as "illicit transport". Unfortunately, peptides are not stable in physiological fluids, due to activity of peptidases. Analogs with modified amide backbone are more resistant towards enzymatic degradation. It has been shown that enzymatic hydrolysis of endothiopeptides is often significantly slower than natural peptides. In our studies we synthesized Nva[CSNH]-FMDP, an analog of Nva-FMDP, which is a strong antimicrobial peptide. Because of the fact that the "warhead" component in this peptide

contains an amide backbone, firstly we decided to synthesize free FMDP with thioamide backbone. The results of enzymatic kinetics studies showed that in this way modified compound is much weaker inhibitor of glucosamine-6-phosphate synthase than FMDP. It was the reason why we planned and carried out a multi-step synthesis in order to obtain a potential more resistant towards enzymatic degradation, antimicrobial peptide containing a thioamide backbone only between Nva and FMDP. Activity of Nva[CSNH]-FMDP Nva-FMDP against selected microorganism in medium containing blood serum was determined.

P22021-144

Neuroprotective effects of cortexin and cortagen in rats with focal brain ischemia and brain trauma

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There is a growing body of evidences that natural peptides and their smaller synthetic analogues have good outlooks for medical use. Three compounds were studied in this research – Cortexin, Cortagen and N-îleylcortagen. Cortexin is a balanced combination of oligo-peptides isolated from calf/porcine cortex. The number of non-clinical and clinical studies confirmed Cortexin efficiency as nootropic and neuroprotective drug. In spite of beneficial results the natural source of the drug implies some difficulties for it general use. To overcome this issue a number of putative Cortexin synthetic analogues were developed. Among them is tetrapeptide Cortagen. In order to improve the peptide transport to the brain and lipophility the hydrophobic oleyl radical was added to the peptide chain.
The aim of research was to study the effects of these peptides on recovery of conditional reflex in Active Avoidance paradigm after heavy brain trauma and neurologic deficit dynamics induced by focal brain ischemia in rats. 170 males albino rats were involved in experiment. Closed craniocerebral trauma was modeled by weight-drop method. Focal cerebral ischemia was induced by cortical phototrombosis. Experimental therapy started in 0.5 hours after mechanical action and continued for 7 days. Synthetic peptides were administrated i.p. once-a-day in dose of 10 - 1000 mcg/kg. Cortexin was used at the same schedule in dose of 150 mcg/kg.
Cortagen and Cortexin administration in dose of 10 and 150 mcg/kg respectively resulted in pronounced neuroprotective effect in rats with focal cerebral ischemia. The observed effects were proved by Limb-Placing Test. Cortexin experimental therapy resulted in earlier restoration of conditional reflex starting from the 3rd day after brain trauma. N-îleylcortagen had weaker beneficial effect with significant improvement in conditioned response recovery by the 4th day.

P22021-145

Bioactive Peptides From *Lactobacillus rhamnosus* GG Protects Intestinal Epithelial Cells From Apoptosis Induced By Camptothecin

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Probiotics such as *Lactobacillus rhamnosus* GG provide health benefits beyond their mere nutritive value, and are useful in the treatment and prevention of several diseases. Isolation and use of the anti-apoptotic factor(s) would ultimately culminate in the development of novel therapeutic agents in enteropathy resulting from chemo- and radio- therapy in the treatment of cancer patients, which could in turn be administered in a consistent and pharmacologically sound manner. In present study, intestinal epithelial cells were isolated from Wistrar rats (n=12), and were grown in DMEM medium supplemented with 5% foetal bovine serum at 37°C in a humidified 5% CO_2 atmosphere. After 72 h, these cells were treated with bioactive peptides isolated from LGG (10-100 ìmol/ Lit) for 2 h, and were incubated with camptothecin (20 ìmol/ Lit) for 2 and 4 h, and cell viability was measured by MTT assay. Caspase activity assay was carried out to determine caspase 3 and caspase 9 activity. Moreover, cell lysates were also pretreated with caspase 3 inhibitor (200 ìM). DNA fragmentation assay was carried out to determine the increase in DNA fragmentation. Bioactive peptides significantly prevented camptothecin induced apoptosis in rat intestinal epithelial cells. The treatment of intestinal epithelial cells with camptothecin for 4 h resulted in five-fold increase in DNA fragmentation, and resulted in three-fold increase in caspase 3 activity, compared with controls. Pre-treatment with bioactive peptides (10 ìmol/ Lit) significantly attenuated the Camptothecin-induced DNA fragmentation by 29% ($P<0.001$), and significantly inhibited caspase 3 and caspase 9 activity by 35% and 39%, respectively, after 4 h of camptothecin exposure ($P<0.001$). Hence, it was concluded that novel bioactive peptide from LGG were found to be potential anti-apoptotic agents and hence could be administered as therapeutic molecule for treatment of enteropathy resulting from chemo- and radiaton- therapy.

P22100-146

Opioid peptide-platinum(II) complexes: synthesis, characterization and in vitro antitumor activity

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Cisplatin belongs to the most powerful and useful anticancer agents. It 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 works against both types of cells, destroying cancer and normal type ones. Therefore more selective delivery system of platinum to cancer cells is still needed. Based on the evidence of the presence of δ- and μ-opioid receptor types in carcinoma cells we proposed to use opioid peptides as selective carriers for delivering platinum ions to the cancer cells. 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. Such molecule can serve not only as carrier for platinum, but also give a strong analgesic effect. As a result such hybride molecule should express analgesic properties and provide anticancer activity. We will present synthesis of a few opioid peptide-platinum(II) complexes, the binding affinity at the opioid receptors and effect on the proliferation of the human glioblastoma cells.

P22100-147

Addition of a cholesterol group to an HIV-1 Peptide Fusion Inhibitor dramatically increases its antiviral potency and simultaneously improves its in vivo half-life

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Peptides derived from the C-terminal heptad repeat region of the HIV fusogenic protein gp41 are potent inhibitors of viral infection, and one of them, T20 (enfuvirtide), is used for the treatment of therapy-experienced AIDS patients. The mechanism of action of these peptides is to interfere

with the fusion of the viral and target cell membranes, and it is known that HIV entry takes place in membrane microdomains ('lipid rafts') enriched in cholesterol and sphingolipids. Therefore we explored the advantage of targeting a peptide fusion inhibitor to membranes by addition of a lipid moiety, specifically a cholesterol group. Since derivatization with lipids is also known to improve the half-life of peptide therapeutics, we chose the antiviral peptide C34, which is more potent than T20 in cell-based assays, but unlike T20 has a very short half-life in vivo. We show here that attachment of cholesterol to C34 (C34-chol) dramatically increases its antiviral potency against a panel of HIV strains, including primary isolates: for strain HXB2, IC50 = 4 pM for C34-chol, versus 205 pM for C34, and 692 pM for enfuvirtide). Consistent with the anticipated mechanism of action, C34-chol accumulates at the site of action, since washing of the target cells after incubation with the peptide, but prior to triggering fusion, increases IC50 only 5-fold, relative to 400-fold for C34. Moreover, cholesterol must be strictly positioned at the C-terminus of C34, in line with the need of an antiparallel orientation of the C-peptide relative to N-peptide trimeric coiled-coil, present in the fusion-active conformation of gp41. In addition to boosting antiviral potency, derivatization with cholesterol has the expected beneficial effect on the peptide pharmacokinetics. We believe that these findings may be of general utility for viruses, which share with HIV the dependence on a type I fusogenic machinery.

P22100-148

Development of a vaccine against Multiple Sclerosis

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Multiple Sclerosis (MS) is an autoimmune demyelinating disease mediated primarily by CD4+ T cells of the Th1 subset. The design of peptide mutants of disease-associated myelin epitopes to alter immune responses offers a promising avenue for the treatment of MS. We designed and synthesized a number of peptide analogues by mutating the principal TCR contact residue based on MBP83-99 epitope. The synthesis of the linear peptide agonist MBP83-99, as well as of the cyclic analogue was carried out by the Fmoc/tBu methodology, utilizing the 2-chlorotrityl chloride resin. Cyclization was achieved using O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) and 1-hydroxy-7-azabenzotriazole, 2,4,6 collidine allowing fast reaction and high yield cyclization product. The purification was achieved using HPLC reversed-phase chromatography and the peptide purity was assessed by analytical HPLC and by mass spectrometry (ESI-MS). Agonist and antagonist (linear and cyclic) peptides were conjugated to reduced mannan. Immune responses were diverted from Th1 to Th2 in SJL/J mice and generated antibodies which did not cross react with native MBP protein. The peptides [A91]MBP83-99, [E91]MBP83-99 and [Y91]MBP83-99 gave the best cytokine and antibody reactivity profile with [Y91]MBP83-?-99being the most promising candidate peptide for immunotherapy of MS. Structural alignment of existing crystal structrures revealed the peptide binding motif of H2-IAs. Molecular modelling was used to identify H-bonding and van der Waals interactions between peptides and MHC (I-As).

P22100-149

Mass spectrometric and immunoanalytical characterization of the recognition structures involved in the complex formation between neuroprotective factor humanin and Alzheimer's β-amyloid(1-40) peptide

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Humanin (HN) is a linear 24 amino acids peptide recently detected in human Alzheimer's disease (AD) brain. HN has been shown to abolish in vitro neuronal death induced by exogenous β-amyloid (Aβ) peptides and by various amyloid precursor protein (APP) and presenilin (PS) gene mutations in familial AD (1). Due to its high efficacy and specificity, HN represents a potential lead to new therapeutic approaches of AD. However, the molecular mechanism(s) of HN function(s) are not yet fully elucidated. Wild-type HN and HN-derivatives were synthesized by SPPS and amino acid sequences and homogeneities of the RP-HPLC purified peptides ascertained by ESI- and MALDI-mass spectrometry (MS). The complex formation between HN and Aβ(1-40) was studied by affinity-chromatography and high resolution MS (FTICR-MS), as well as by immunoanalytical (ELISA) techniques. The binding sites between the two peptides were identified by applying proteolytic epitope extraction/excision procedures [2,3]. The recognition region determined for HN was further characterized by binding analysis of the mutant HN sequences towards Aβ(1-40), using affinity-MS and ELISA. The identified interaction region of Aβ(1-40) was further analyzed by a comparative ELISA using Aβ(1-40) and Aβ(1-40)-partial sequences as ligand molecules for HN and by competitive ELISA experiments in which the influence of different anti-Aβ(1-40) antibodies upon HN-Aβ(1-40) interaction was investigated. The experimental results show a significant affinity between HN and Aβ(1-40) and provide molecular information on the binding sites, yielding useful insights for understanding the biological function of HN.

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P22100-150

The Chiral Sequence of a Natural Peptide Inhibitor of HIV-1 Integrase Elucidated

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Integramide A, an efficient inhibitor of the coupled reaction of HIV-1 integrase, is a 16-mer linear peptide characterized by 9 C^{α}-methylated α-amino acids (5 Iva, isovaline, and 4 Aib, α-aminoisobutyric acid, residues) that was isolated from fungal extracts of Dendrodochium sp. The amino acid sequence was fully elucidated by the Merck group a few years ago (S. B. Singh et al., *Org. Lett.* **2003**, *4*, 1431-1434). On the other hand, the chiral sequence was only partially determined. In particular, the precise stereochemistry of the Iva^{14}-Iva^{15} dipeptide (known to contain one D- and one L-residue) near the C-terminus was not reported. To solve this unsettled issue and to assess integramide A primary structure-bioactivity relationship we performed by solution methods the total chemical independent syntheses of both L-D and D-L 16-mer diastereomers and compared their properties with those of the natural inhibitor. For an unambiguous, complete stereochemical assignment of integramide A we relied heavily on HPLC and NMR techniques. Our results clearly indicate that the chirality sequence of the Iva^{14}-Iva^{15} dipeptide of the natural product is L-D. The two integramide A diastereomers were also evaluated as inhibitors of HIV-1 integrase in the coupled reaction of proviral DNA into the host cell DNA.

P22100-151

Molecular Evolution of HIV-1-Blocking CCR5 Antagonist Peptides Derived from the N-Loop/β1-Strand of RANTES

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The development of novel classes of HIV-1 inhibitors is key for the control and prevention of the AIDS pandemic. Increasing evidence points towards CCR5 as a primary target for both systemic therapy and topical prevention of HIV-1 infection. RANTES, the most potent HIV-1 inhibitor among CCR5-binding chemokines (Cocchi et al 1995 Science 270:1811-5), represents an ideal scaffold for engineering novel anti-HIV-1 compounds (Vangelista et al 2008 Vaccine, in press). We have previously identified the N-loop/β1-strand region as a primary determinant of RANTES anti-HIV-1 activity, involved in CCR5 binding but not in its activation, and exploited such region to design increasingly potent peptides (Nardese et al 2001 Nat Struct Biol 8:611-5; Vangelista et al 2006 Biochem Biophys Res Commun 351:664-8; Lusso et al, in preparation). A prototype peptide, R11-29, spanning RANTES residues 11 to 29, contains two hydrophobic clusters of fundamental biological importance connected by a nonessential hydrophilic linker. R11-29 has been rationally modified to improve its CCR5 binding and its antiviral potency. NMR studies on the modified peptides revealed important similarities and differences with the three-dimensional organization of RANTES. Although these peptides are consistently more active as dimers, an increase in anti-HIV-1 activity of the monomeric forms was observed in parallel with their molecular evolution. In addition, no tertiary interactions could be detected by NMR, indicating an autonomous folding of the monomers also in the context of dimeric peptides. The most potent peptide designed so far, Rmax, shows anti-HIV-1 activity in the low nanomolar range (Vangelista et al, in preparation). Strikingly, a similar potency was observed in the same assay with T20, an HIV-1 gp41-derived peptide currently licensed for use in AIDS therapy. These results provide a rationale for the use of RANTES-derived peptides as new candidates for treatment and prevention of HIV-1 infection.

P22101-152

Macrocyclic phosphino dipeptide isostere inhibitors of β-secretase (BACE1) with improved serum stability

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10% of the population older than 65 years and 40% exceeding the age 80 years are affected by Alzheimer's disease (AD).(1) A factor in the pathogenesis of AD is the cerebral deposition of amyloid fibrils as senile plaque. BACE1 initiates the pathogenic processing of APP by cleaving at the N-terminus and the resulting C99 membrane bound C-terminal peptide can then be hydrolyzed by γ-secretase to form Aβ-peptide (Aβ40 or Aβ42). BACE1 is not only a promising target cause it is a key player in formation of Aβ but also because BACE1 knock-out mice are viable and free of the gross phenotypic changes. Our group was recently able to shown that the phosphino dipeptide (PDP) isostere is a suitable replacement of the hydroxyl ethylene isostere in OM00-3(2) resulting in pseudo peptidic inhibitors of about same potency.[2,3] In our search for conformationally restrained PDP isostere BACE1 inhibitors we speculated that the P1 and P3 cyclization would lock the active conformation of the cyclic linear pseudo peptidic inhibitor in the N-terminal region. First the detail analysis of the crystal structure of OM00-3 bound to BACE1(2), revealed that the ideal macrocyclus should consist of a 13-membered heterocycle. On this basis we developed a macrocyclic inhibitor with P1 and P3 cyclized side chains containing a PDP isostere with improved serum stability.

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P22105-153

Tumor selective targeting by branched peptides

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Specific tumor-targeting, via tumor-associated antigens (TAA), selectively expressed or over expressed on tumor cells, is the goal of modern cancer therapy aimed at overcoming non-specific toxicity of most anticancer drugs. The expression of TAA varies among different tumors and patients, resulting in highly variable response to tumor targeted therapies. Therefore, diagnosis should provide information on the expression of the targeted antigen in each patient, thus allowing to predict possible efficiency of a therapy mediated by targeting agents directed to the same tumor antigen. In this approach, the molecule used for tumor cell tracing should be as close as possible to that used for therapy. The use of peptides as tumor targeting agents was envisaged years ago with the finding that receptors for different endogenous regulatory peptides are over-expressed in several primary and metastatic human tumors, and can be used as tumor antigens (Reubi JC. J Nucl Med 1995;36:1825–35). In previous works, we demonstrated that peptides synthesized in a branched form, result in molecules that are resistant to proteolytic activity and can retain (or even increase, through multivalent binding) peptide biological activity. A branched peptide that targets neurotensin (NT) receptors, known to be over-expressed in a number of tumors, including colon, pancreas and prostate carcinoma (Reubi JC. Endocr Rev 2003;24:389–427) and was conjugated to effector units and proved to be stable and active in vivo (Falciani C, et al. Mol Cancer Ther. 2007;6:2441-8). Here, we created new NT-based molecular tools conjugated to different fluorophores and chemotherapeutics and demonstrated that branched peptides can be modulated either as tracers for measuring the presence of the specific target in primary tumor or metastasis on human surgical resections, or as specific drug-carriers, which use the same target to enter and eventually kill tumor cells in vitro and in vivo.

P22105-154

Design and Development of Immunogens for Vaccination of Farm Fish Against Infectious Pancreatic Necrosis Virus

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Aquaculture has become an increasingly important activity, as an immediate source of animal protein required for several countries growing population. Vaccination of fish against bacterial and viral diseases is one of the best strategy for controlling certain infectious

diseases in aquaculture worldwide, thus decreasing the need for antibiotics, and increasing cost-effectiveness and net profits. Infectious pancreatic necrosis virus (IPNV) is a pathogen of farm fish with a worldwide distribution. Peptides derived from IPNV Protein 2 (VP2), a major structural protein, could be used for development of effective vaccine against IPNV, According to a search of VP2 antigenicity we selected two peptide sequences, K^{19}PYVRLEDETPQG^{42} (VP2-19-42) and N^{91}FSLAEQPANETK^{103} (VP2-91-103), that are expected to be highly immunogenic. These peptide sequences were synthesized in their N-terminus iodoacetylated form and conjugated to Ac-(Lys-Aib-Cys)$_4$-NH_2, a cell penetrating sequential carrier (CPSC). Conjugation of four copies to Cys side chains of the carrier was realised by the chemoselective ligation method. The resulted constructs were purified by HPLC and characterized by ESI-MS. Immunizations are in progress in order to test their immunoprotective potency. Acknowledgements to the GSRT (Greece-Egypt project 266-å) for the financial support.

P22116-155

New integrin ligands based on isoDGR sequence

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For more then two decades, the RGD sequence is known to bind integrins, e.g., α5β1, αvβ3, and αIIbβ3 among many others. It is present in the natural ligands for these integrin receptors, as in Fibronectin, Vibronectin, or Fibrinogen. Recently, it was shown that Fibronectin in which the RGD sequence has been mutated into an RGE sequence (which is known not to be recognized by integrins) still has the ability to interact with its receptor. However, it forms FN fibrils only with a slightly different phenotype than wild-type Fibronectin (1). A hypothetical model for these unexpected results was proposed by Curnis et al. (2). The NGR (Asn-Gly-Arg) sequence, present at four positions in the fibronectin molecule, is able to undergo a rearrangement to isoDGR (isoAsp-Gly-Arg), which shows activity on αvβ3 and - with less potency - on α5β1. The mechanism of Asn-deamination is already known for a long time and has widely been considered to be a process of degradation, acting as a biochemical clock that limits protein lifetimes in vivo (3). Curnis et al. were the first to show that the deamination process increases protein function instead (2). These findings stimulated us to create a library of cyclic peptides containing the isoDGR sequence. A screening of this library showed various new peptides with high activity and selectivity towards the integrin receptor α5β1.

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P22124-156

The correlation between the intensities of muramyl peptides adjuvant effect and NOD2 activation.

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It is well known that essential activity of muramyl peptides - minimal structures of bacterial cell wall - are adjuvanticity. The comparison of the adjuvant effect of these compounds with the ability to activate NF-kB pathway through NOD2 was examined.
The adjuvant activity of di, tetrasaccharide peptides and stearoyl containing derivatives has at least two peaks in dose-response curves and greater of them correlates with respective dose-response data for NF-kB stimulation through NOD2. Introduction of stearoyl moiety, with the aim of improving muramyl peptide interaction with the cell membrane and subsequent intracellular delivery, influenced the corresponding activities in vitro, but did not correlate with improved effects in vivo experiments.
The comparison of the adjuvanticity in vivo and the NOD2 activation in vitro revealed clear correlation between two responses. These findings confirm the view that NOD2 pathway activation should account, at least in part, for the adjuvant effect of these compouds.
The correlations of the NF-kB pathway induced by muramyl peptides with the aim of enhancing their adjuvant activity were investigated.

P22129-157

Thymus gland - chemical and biological determination of its peptides components

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The thymus gland plays an important role in overall immunomodulation. The studies in early 1960s by several authors have established that thymus is necessary for the normal development of immune response. It is thought to be responsible for the development and regulation of T-cell immunity, acting through endocrine mechanism (1). It is known that thymus peptides play an important role in the development, maturation, differentiation, and activation of T-lymphocytes. Investigation of the function and properties of this gland shows that the thymus contains pharmacologically active components with immunological properties. When the immune system is challenged, thymic peptides seem to regulate the expression of various cytokine and monokine receptors on T-cells and induce secretion of IL-2, interferon alpha, and interferon gamma. Up to date several thymic extracts have been isolated using different biochemical methods of extraction, homogenization and purification. These are, usually, semipurified aqueous and lipid calf thymus extracts. The goal of this study was to determine the biological activity of peptide components, isolated from the calf thymus. Extract of calf thymus was prepared and fractioned into lipid and nonlipid (peptides) fractions. The nonlipid fraction was isolated and characterised by biuret, IR , NMR and HPLC methods. Analyses of IR and NMR spectra indicated the presence of charasteristic bands and peaks for peptides. Results estimated from HPLC analyse showed that molecular masses of isolated peptides were below 1500 daltons. Biological activity was accesed by using proliferation of thymocites and splenocites in vitro in the presence of mitogen, and obtained results showed significant imunomodulatory effect. Keywords: thymus, peptides, immunomodulators, thymocites, splenocytes, proliferation

P22200-158

Biological activity of food peptides and proteins – in silico approach

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According to contemporary knowledge about proteins, every protein can play the role of a precursor of biologically active peptides. Bioactive peptides isolated from food proteins display various activities, for example: antihypertensive, antithrombotic, immunomodulating, opioid and antibacterial. The database of protein and bioactive peptide sequences designed in Chair of Food Biochemistry (www.uwm.edu.pl/biochemia) contains information on proteins which are precursors of bioactive peptides. The database, BIOPEP, enables an evaluation of food proteins according to the following criteria: the frequency of the occurrence of fragments with the given activity in a protein chain, a potential activity of protein fragments, and profiles of a potential biological protein activity i. e. the type and location of a bioactive fragment in the protein chain. The BIOPEP database was used to determine the profiles of potential biological activity of food proteins and classify them into families and

subfamilies. Results that we obtained indicate that the greatest number of bioactive peptides can be released from milk proteins. We also analysed structural properties of bioactive fragments encrypted in protein chains which are predicted to be accessible for endopeptidases. The application of BIOPEP and MSBLAST software enabled us selection of a fragments with high degrees of identity to the celiac-toxic peptides in food proteins sequences. Based on the BIOPEP, we are able to design the processes of the release of bioactive peptides from protein sequence and with the use of mass spectrometry - to identify released peptides. The detailed results of authors in silico food proteins analysis will be presented. The computational methods and the above-mentioned tools can be applied for designing food with the special designed and desired properties (functional food) as well as production of nutraceuticals i. e. food with therapeutic properties.

P22200-159

Neurotensin analogs with high affinity and selectivity at human neurotensin receptor 1.

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Neurotensin (NT), pGlu[1]-Leu[2]-Tyr[3]-Glu[4]-Asn[5]-Lys[6]-Pro[7]-Arg[8]-Arg[9]-Pro[10]-Tyr[11]-Ile[12]-Leu[13]-OH, exerts numerous physiological actions in the central nervous system and in the periphery. Studies in animal models have suggested its involvement in the modulation of dopamine transmission, hypothermia, analgesia, locomotor activity, cardiovascular function, and others. At present, three receptors are known to bind NT; two of them are G-protein coupled-receptors (NTSR1 and NTSR2). The physiological effects of NTSR1 have been extensively studied but the functions associated with the NT binding to NTSR2 are less well defined. The C-terminal fragment of NT, Arg[8]-Arg[9]-Pro[10]-Tyr[11]-Ile[12]-Leu[13]-OH, has been long recognized as an essential and sufficient segment for the effective interactions of NT with the receptors; a short peptide, Arg[8]-Arg[9]-Pro[10]-Tyr[11]-Ile[12]-Leu[13]-OH, designated NT(8-13), displays binding affinity similar to that of the full-length NT at both NTSR1 and NTSR2. In this study, the role of Arg[8] in the interactions of NT(8-13) with the human NTSR1 and NTSR2 was examined through ligand structure-function studies. The side chain of Arg[8] was determined to not be critical for binding to hNTSR1 but essential for binding to hNTSR2. Several analogs of NT(8-13) are reported which are high affinity hNTSR1 ligands (IC_{50} = 0.1 to 3 nM) and more than 500-fold selective versus hNTSR2.

P22200-160

Stimulation of anti-angiogenic activity of PSA by peptides

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Human umbilical vein endothelial cells (HUVECs) form tubular networks when cultured on top of the Matrigel basement membrane preparation, reflecting the ability of the cells to form blood vessels. Using this model, we have previously shown that PSA (also known as KLK3) inhibits endothelial cell tube formation(1), indicating reduced angiogenic potential. Furthermore, we have shown that the anti-angiogenic activity of PSA is related to its enzymatic activity[1,2]. We have developed peptides that stimulate the enzymatic activity of PSA towards a small chromogenic substrate. These peptides also enhance the anti-angiogenic activity of PSA both *in vitro* and *in vivo*. This supports our hypothesis that enhanced PSA-activity by our peptides could be used to reduce tumor angiogenesis and, thus, to reduce tumor growth.

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P22200-161

Development of new molecules that compete with p53 for Binding to TFIIH

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In eukaryotes, activators enhance transcription through protein/protein interactions involving their transactivation domains (TADs). A wide range of protein targets for TADs have been identified and they include several general transcription factors (TBP, TFIIB, TFIIH), components of the mediator complex (MED15, MED25), histone acetyltransferase complexes (CBP/p300, Tra1) and components of ATP-dependent nucleosome remodeling complexes (Swi1, Snf5). Through these interactions, activators are able to enhance transcription at multiple stages of the transcription process, including nucleosome disassembly, pre-initiation complex formation, promoter clearance and/or transcription elongation.

The most extensively studied TADs are those that contain acidic TADs and one extremely important protein is the human tumour suppressor protein p53. Given the presence of repetitive stretches of acidic amino acids TADs are generally disordered in the free state and this has also been demonstrated for p53TAD. Using a combination of NMR spectroscopy, isothermal titration calorimetry and site-directed mutagenesis studies we have recently characterized the interaction of the p53TAD with the Pleckstrin Homology (PH) domain of the Tfb1/p62 (yeast/human) subunit of TFIIH. The NMR structure of the Tfb1/p53TAD complex demonstrates that p53 forms a short alpha-helix in complex with Tfb1 (1). This structure is an important step towards developing a sequence code for acidic TAD binding to the PH domain of Tfb1/p62. Such detailed structural information is essential to design molecules that modulate transcription activators such as p53.

We will present the structure of the p53/Tfb1 complex, structural and biophysical characterization studies with peptides designed to mimic acidic TADs and compete with p53 for binding to TFIIH.

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P22301-162

Cyclic peptides comprising constrained amino acids as inhibitors of integrin-ligand interaction

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Integrins are a family of transmembrane cell surface receptors, which mediate cell-cell and cell-matrix adhesion. The binding of integrins to their natural ligands is the molecular basis of physiological processes such as cell adhesion, migration and signal transduction of cells, as well as of patho-physiological processes. Thus, small molecules capable of interfering with this integrin-natural ligand binding process have pharmacological potential in the therapy of cancer and inflammatory diseases. The amino acid sequence RGD, present on many of the natural ligands, is a prominent recognition motif of integrin ligands. Synthetic

RGD-containing peptides are an excellent starting point for the identification, synthesis and development of selective integrin ligands.
The affinity and selectivity of the peptide ligands towards different integrins depend strongly on the secondary structure of the sequence and the overall three-dimensional shape. Cyclization is frequently used as a method to reduce the accessible conformational space. Additionally, the incorporation of non-natural conformationally constrained amino acids can greatly affect the secondary structure of the peptide, in such a way that the synthetic ligands prefer to adopt a particular conformation.
The aim of this investigation are small cyclic peptides containing the RGD motif and constrained amino acids (such as α-methylated amino acids, β-amino acids or dehydroamino acids) that exhibit well-defined conformational properties.
The present communication describes the synthesis of different cyclic RGD peptides with the general sequence *c*-(-Arg-Gly-Asp-Xaa-Yaa-) and the evaluation of their activity as ligands for the $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrins, present on human cells.
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P22305-163

Conjugation of RGD Analogs with o-Quinone Methides of Bis-naphthalene and their Biological Activity

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In recent years there has been increasing interest in the design of chemical agents capable of inducing DNA interstrand crosslinking (ISC), which, shutting down the DNA replication process, represents by far the most cytotoxic of all the alkylation events. Quinone methides (QMs) are interesting compounds that have been proposed as intermediates in a large number of chemical and biological processes. The asymmetry introduced by the presence of two electronically different substituents, carbonyl and methylidene, on the cyclohexadiene ring imparts a strong dipolar character to quinone methides not found in benzoquinones and quinodimethanes. QMs have been successfully used to accomplish nucleoside alkylation and DNA ISC by photochemical and fluoride-induced activation. Apart from their involvement in biological processes, o-QMs react with a variety of nucleophiles (Michael addition), resulting in substituted hydroquinones. They also function as efficient heterodienes in Diels–Alder reactions and undergo a variety of self dimerization reactions. Here we present the synthesis of a new series of synthetic o-QMs (bis-napthalene type) conjugated with RGD analogue peptides. The biological activity of these compounds is under investigation. Aknowledgements: We thank the European Social Fund (ESF), Operational Program for Educational and Vocational Training II (EPEAEK II) and particularly the Program PYTHAGORAS II for funding the above work.

P22307-164

Analysis of POI-PO Interaction using Fluorescent Labeled Analogs Designed based on gCamouflaging Substitution h

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Phenoloxidase inhibitor (POI)[1], found in the hemolymph of housefly pupae, is a unique DOPA containing and cystine-rich peptide[2] that potently and selectively inhibits phenoloxidase (PO) with a *K*i value of nanomolar range. Because of difficulties in steady-state kinetics due to strong interaction between POI and PO, the SAR of POI is still unclear. Moreover, there has been confusion in understanding physiological role of POI for lacking of reliable value for dissociation constant. Fluorescent labeling is useful for the extremely low concentration experiments such as this study, however, introduced probes may have significant effect to the parent molecule in terms of its hydrophobicity and balkiness. Cyanine dye family, well-known brilliant fluorescent molecule possess a linear structure and two sulfates. We have found this structure of the dye can mimic linear chain of some peptides containing (D/E)XEXXXX sequence. The N-terminal region of POI is that the cyanine group, Cy3, can almost perfectly camouflage the structure. Based on this idea, gcamouflaging substitution h, we synthesized POI analog that was substituted its N-terminal sequence with Cy3. In addition, four Lys residues of this analog were randomly labeled to prepare peptide sets for exploring the important part of POI-PO interaction. Using these peptides, we performed simultaneous measurement of both binding and inhibitory activities. The binding characters were analyzed by fluorescent resonance energy transfer (FRET) and fluorescence polarization, whereas inhibitory activities were assayed by measuring residual PO activities of the mixture for interaction assays. Quantitative analyses of these results revealed direct inhibition of PO by POI molecules and the SAR of POI.
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P22320-165

Linear and Cyclic Synthetic Analogs of the A2 Subunit (Sequence 558-565) of the Factor VIIIa Blood Coagulation

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Platelets aggregation causes clotting in the blood vessels during the blood circulation due to several reasons. Factor VIII (FVIII), a blood coagulation glycoprotein, is a key component of the blood coagulation system. FVIII in its activated form (FVIIIa) acts as a cofactor to the serine protease FIXa in the conversion of the zymogen FX to the active enzyme (FXa). The role of FVIIIa is to increase the catalytic efficiency of FIXa in the activation of factor X (FX). The target of this research is the synthesis of biologically active peptides, which are expected to inhibit selectively the maximisation of thrombin production depended on factor IX (FIX) and accordingly the additional activation of platelets. These peptides are based on the regions in which the FVIII interacts with FIX. Glycoprotein FVIII is composed of three distinct domain types in the arrangement, A1-A2-B-A3-C1-C2. The sequence 558-565 of A2 subunit is the following: Ser558 -Val-Asp-Gln-Arg-Gly-Asn-Gln565 This work covers the synthesis and biological evaluation of linear and cyclic head to tail peptides, analogs of the loop sequence 558-565 of the A2 subunit, aiming at the inhibition of interaction of FVIIIa with FIXa. Substitutions have been taken place at Asn564, which are related to the pharmaceutical groups at the side-chain, like Asp(R)564. All the synthesized analogs are purified (RP-HPLC) and identified (ESI-MS). The synthesized peptides analogs were investigated for their inhibitory activity and tested for clotting deficiency by measuring their activated partial thromboplastin time (APTT) in vitro. These results will be discussed in relation with their biological activity against the FVIIIa factor of blood coagulation.
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P22321-166

Synthesis and in vitro and in vivo characterization of NGF mimetic peptides.

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Nerve Growth Factor (NGF) is an homodimeric protein that binds two different cellular receptors: 1) the transmembrane tyrosine kinase receptor trkA, a member of tyrosine kinase family; and 2) p75, a member of the tumor-necrosis-factor receptor superfamily. Both receptors are involved in the activation of different intracellular signal transduction cascades. Small peptides retaining the most essential elements of NGF may be useful either as agonist or competitive antagonist in the treatment of several neurodegenerative desease and nerve injuries. The N-terminal fragment of NGF was previously demonstrated to be an important determinant for affinity and specificity in the binding to trkA. Crystal structure of NGF-TrkA complex, site-directed mutagenesis studies and substitution of individual amino acids, contributed to identify within the NGF molecule the most relevant domains for its biological activity in the loop-1 (29-35), loop-4 (92-98) and in the N-terminal region (3-18). We synthesized twenty peptides mimicking the loop1 and 4 linked together with aminoacid-based spacers (n glycines) or with two unit of 8-ammino-3,6-dioxaottanoic acid with or without the N-terminal region. L1L1 and L4L4 homodimeric loops, moreover L1 and L4 were also synthesized, as single loop, and their biological properties were compared to L1L4 and N-L1L4 activity . The N-L1L4 (HPIFHRGEFSVADSVSVWVGDCTDIKGKCTGACDGKQC) and L1L4 (CTDIKGKCTGACDGKQC) peptides showed a good NGF agonist activity at concentration as low as 3 uM. Both were able to induce differentiation of chick dorsal root ganglia (DRG) and to stimulate the tyrosine phosphorylation of TrkA but not of TrkB receptor. In addition the L1L4 peptide was able to induce the PC12 cells differentiation into sympathetic-like neurons. Moreover the L1L4 peptide was shown to reduce neuropathic behaviour and restore neuronal function in a rat model of peripherical neuropathic pain, thereby suggesting a potential therapeutic role for this peptide.

P22324-167

Effect of 4-R-hydroxy-L-proline (Hyp) on peptide conformation and SH3 affinity

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Short proline-rich peptides interact with SH3 domains, exhibiting little or no secondary structure before their binding to the cognate protein-targets. Under these conditions the binding process of a proline-rich peptide with the SH3 domain shows unfavorable binding entropy, likely resulting from a loss of rotational freedom on the formation of the PPII helix. With the aim of stabilizing the PPII helix conformation in SH3 binding motifs, in the previous years, we replaced the proline residues of the HPK1 proline-rich decapeptide, PPPLPPKPKF (**P2**), either with 4-R- (**Fp**) or with 4-S- (**fp**) fluoro-L-proline at different *i, i+3* positions. The interactions of the fluoroproline-peptides with the SH3 domain of cortactin protein were analyzed quantitatively by Non-Immobilized Ligand Interactions Assay by Circular Dichroism (NILIA-CD), whereas CD thermal transitions were measured to correlate their propensity to adopt PPII helix with their affinity for SH3. Results show that although the introduction of the **Fp** residue stabilizes the PPII helix conformation of peptides in a position-dependent manner, the induction of a stable peptide conformation does not increase the ligand affinity towards the SH3 domain of cortactin. To explore the effect of electron-withdrawing substituent in the Pro residue, the **Fp** residues of **P2** were replaced by the natural amino acid 4-R-hydroxy-proline (**Hyp**). Unexpectedly, NILIA-CD and CD thermal transitions results showed that the Hyp-containing peptides exhibit a stable conformation in aqueous buffer and K_d values lower than the corresponding **Fp** peptide-analogues. In particular, **Hyp3** peptide containing Hyp residues at *i, i+3* and *i+6* positions adopts the greater percentage of PPII helix conformation over the entire studied temperature range and shows a K_d value comparable to that of the parent **P2** peptide.

P22324-168

Survivin - An inhibitor of apoptosis as a target for peptide based therapy for human breast cancer

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A promising class of molecularly targeted drugs are inhibitory peptides able to interfere with intracellular functions of proteins crucial for cellular transformation. These short peptides can be selected for their ability to recognize and inhibit a functional domain of a given target protein. Survivin, a member of the family of the inhibitors of apoptosis, is an interesting target molecule in this respect. It is highly expressed in most human tumor cells, whereas its expression is hardly detectable in most differentiated normal tissues. Survivin has an anti-apoptotic and a cell-cycle promoting function. Inhibition of Survivin by interacting peptides could possibly induce apoptosis and interfere with cell-cycle progression in cancer cells. To show that Survivin expression is required to maintain growth and survival of breast cancer cells, we down-regulated the expression of Survivin mRNA with a lentiviral vector encoding a Survivin specific shRNA. Virally infected breast cancer cells showed a decreased level of Survivin expression resulting in the formation of multinucleated cells and an arrest in cell proliferation. We are now searching for inhibitory peptides able to reduce Survivin function in breast cancer cells. We take advantage of the observation that Survivin forms homodimers in vivo and have derived short peptides representing the dimerization domain. To enable intracellular expression and visualization of these short peptides, they will be fused to a fluorescent carrier protein. Alternatively, the peptides comprising the dimerization domain will be inserted into a scaffold protein. This allows the presentation of the peptides in a constrained and stabilized conformation. We will analyze the effects of lentiviral expression of these fusion peptides in cancer cells expressing high levels of Survivin. The peptides will be analyzed for their ability to inhibit proliferation, cell-cycle progression and/or to induce apoptosis in cancer cells.

P22326-169

Aggregation of the Human Cellular Prion Protein (PrP^C) is Quantitatively Inhibited by the Octapeptide NMHRYPNQ

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Pathogenesis of transmissible spongiform encephalopathies (TSE) is correlated with a conversion of the normal cellular form of the prion protein (PrP^C) into the abnormal isoform (PrP^{Sc}). This transformation is induced by contact of the normal prion protein to its abnormal isoform (PrP^{Sc}). Molecules that inhibit such contacts may serve as leads for drugs against TSE. Therefore, we screened a synthetic octapeptide library of the globular domain of the human cellular prion protein ($hPrP^C$) for binding affinity to PrP^C using surface plasmon resonance (SPR). Two fragments, i.e. ^{149}YYRENMHR^{156} and ^{153}NMHRYPNQ^{160}, were identified

with dissociation constants of about 21 μM and 25 mM, respectively. Inhibition of prion protein aggregation was analyzed in vitro. A 10-fold excess of peptide ^{153}NMHRYPNQ^{160} inhibits aggregation of the prion protein by 99%. The binding site of this ligand is located in the region of helix-3 of the prion protein as determined by ^{1}H,^{15}N-HSQC-NMR observing changes in chemical shifts upon binding of the peptide to prion protein. For this we expressed a construct of the ^{15}N labelled prion protein that stretches from ^{90}G until ^{230}S and represents the folded domain of PrP^{C}. MALDI-TOF-H/D-exchange experiments were used to confirm the location of the binding site determined by NMR spectroscopy. The data show that the octapeptide NMHRYPNQ can serve as a lead for higher affinity compounds that inhibit formation of aggregates of the prion protein.

P22328-170

Binding of Peptides to GPCRs – A Molecular Docking Approach with PSO@Autodock

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The growth hormone secretagogue (GHS) receptor is a G-protein coupled receptor (GPCR) involved in growth hormone (GH) release, control of food intake and energy expenditure. The receptor is one of the most constitutively active receptor know so far. Its spontaneous, ligand-independent signaling constitutes about 50% of the maximal signaling capacity of the native ligand GH. Recently a Substance P derivative has been determined as a high potency inverse agonist (1). Nevertheless, little is known on the detailed binding of this peptide to the receptor. With a model of the GHS receptor available (2), computational modeling methods might be a promising approach to reveal the underlying mechanisms. However, molecular docking of peptides is computational challenging because of the high flexibility of the ligands. Recently, we developed the novel docking program PSO@Autodock (3) for fast flexible molecular docking. It employs swarm intelligence implemented in various particle swarm optimization (PSO) methods and can efficiently dock highly flexible ligands with many rotatable bonds. In this study, we docked a variety of peptide sequences and its derivatives to the human GHS receptor model. The results will inspire new experimental studies on this receptor system.

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P22328-171

Interaction of intercellular adhesion molecule 4 (ICAM-4) with leukocyte integrin CD11c/CD18 studied with peptides

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CD11c/CD18 (αXβ2) is a member of the leukocyte integrin (CD18 or β2 integrin) subfamily of adhesion molecules and it is expressed on monocytes, macrophages and granulocytes. Its ligands include fibrinogen, iC3b, LPS, collagen type I and denatured proteins as well as intercellular adhesion molecules ICAM-1 and ICAM-4.

ICAM-4 is a red cell specific membrane glycoprotein that was first described as anerythrocyte blood group antigen LW (Landsteiner-Wiener) and later it was found to belong to the family of intercellular adhesion molecules. The first reported receptors of ICAM-4 were leukocyte integrins CD11a/CD18 and CD11b/CD18. Later it has been reported to bind to several other integrins as well. Latest reported interaction is to CD11c/CD18.

The binding of ICAM-4 to integrins expressed in macrophages might clarify some of the controversy concerning the recognition and uptake of senescent red blood cells in spleen. Another function of ICAM-4/macrophage integrin interactions could be in retaining the maturing red cells in bone marrow until they are ready to be released in the circulation.

Adhesion between ICAM-4 and integrins have also been found to be important in different pathological situations and inhibition of these adhesion events could be of important therapeutic value.

We have studied the interaction between ICAM-4 and CD11c/CD18 using peptides derived from both binding partners using PepSPOT method and synthetised peptides. The inhibitory or activating role of these peptides could beof clinical importance in pathological conditions where abnormal red cell adhesion is observed.

P22400-173

C-Terminal region of the third intracellular loop of relaxin receptor LGR7 is responsible for G_s protein and adenylyl cyclase activation

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The regulatory effects of relaxin in mammals realize via receptors of the serpentine type. In this work we studied the relaxin activating effect on the adenylyl cyclase (AC) activity in the rat tissues and muscle tissues of invertebrates using a peptide strategy. The strategy involved the synthesis of the peptides 619–629 and 615–629, as well as the palmitate-modified peptide 619–629, all corresponding to the C-terminal region of the third intracellular loop (C-ICL3) of the human type 1 relaxin receptor LGR7. The peptides 615–629 and 619–629-Lys(Palm) had a dose-dependent activating effect on the AC activity and GTP-binding in myocardium, brain and, to a smaller extent, skeletal muscles of rat. The 619–629-Lys(Palm) had a stronger AC effect than the longer peptide 615–629, because of the possibility to be anchored in the membrane by the hydrophobic radical and, hence, to interact with the G protein more effectively. In mollusks and earthworm muscles, the stimulatory effects of both peptides were weak. Competitive inhibition of the stimulating effects of relaxin by the LGR7-derived peptides indicates that the relaxin signal in myocardium and brain is transmitted to AC via LGR7 receptor. In skeletal muscles of rat and muscle tissues of invertebrates, the inhibition of AC and GTP-binding stimulating effects of relaxin by peptides was almost indiscernible. It can be concluded that relaxin controls the AC of these tissues via the receptor different from the relaxin receptor LGR7. The stimulating effects of LGR7-derived peptides also decreased in brain and myocardium in the presence of Ñ-terminal peptide 385-394 of mammalian $G\alpha_s$-subunit and after cholera toxin treatment. Thus, relaxin stimulates AC via LGR7 receptor and G_s protein in myocardium and brain, and the coupling between receptor and G_s protein is mediated by the interaction of receptor Ñ-ICL3 and Ñ-terminal segment of $G\alpha_s$.

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P22400-174

Synthesis and biological evaluation of novel peptides influencing angiogenesis

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Normal tissue function depends on adequate supply of oxygen through blood vessels. Angiogenesis is a fundamental process by which new blood vessels are formed and which is highly regulated in healthy individuals. However, many diseases are driven by unregulated angiogenesis. Excessive angiogenesis is associated with cancer, rheumatoid arthritis, psoriasis, while insufficient angiogenesis results in ischemia or artherosclerosis. Many new angiogenic modulators have been developed in the last years, mostly to inhibit angiogenesis but only few peptide-based angiogenic stimulators have been reported.

There are data in the literature suggesting roles of small peptides or basic-hexa peptides as angiogenic modulators like Ringseis et al. reporting effects on endothelial cell function (such as EC proliferation) and Fazekas et al. describing effects for the latter. Endostatin, an endogenous inhibitor of angiogenesis, among its proteolitic fragments contains both, an inhibitor and a stimulator of angiogenesis. We considered it possible that fragments of basic heptapeptide D-Phe-Cys(-)-Tyr-D-Trp-Lys-Cys(-)-Thr-NH_2 (TT-232), a strong antitumor agent, could also act as angiogenic modulators. The heptapeptide was developed in our laboratory and has been recently in a clinical trial (phase II).

We synthesised, characterized and tested partially protected di- and tripeptide fragments of it such as Boc-D-Phe-Cys(Acm)-Tyr-OMe, D-Phe-Cys(Acm)-Tyr-OMe, Boc-Tyr-D-Trp-cyclohexilamide and H-Tyr-D-Trp-2-adamanthylamide. The biological activity of the compounds was tested *in vitro* using an immortalized Kaposi Sarcoma cell line to determine their pro- anti-angiogenic character. To test the angiogenic potential of the best compound, the aorta ring assay was used, which by using intact vascular explants reproduces more accurately the environment in which angiogenesis occurs. In this study we report the synthesis and angiogenesis modulating effects of the peptides.

P22400-175

Effect of somatostatin derived peptides and peptidomimetics on neurogenic inflammation

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Somatostatin is a neuropeptide that regulates several functions of the endocrine and exocrine systems. It also affects cell proliferation and neurogenic inflammation through a family of G protein-coupled receptors. Neurogenic inflammation plays significant role in the pathogenesis of numerous inflammatory diseases (such as asthma, arthritis, allergy and migraine). Inhibitory effect of somatostatin on inflammation is well known but the pharmaceutical use of the native peptide is limited due to its broad spectrum of anti-secretory effects and short plasma half-life time. TT-232, a heptapeptide analogue of somatostatin (developed by our research group and it is in Clinical Phase II trials) has selective anti-tumour and anti-inflammatory effect without regulating other endocrine or exocrine processes.

Receptor-ligand binding experiments with various analogues of the somatostatin as well as TT-232 [D-Phe-c(Cys-Tyr-D-Trp-Lys-Cys)-Thr-NH_2] verified that the side chains of Tyr, D-Trp and Lys are important pharmacophoric groups for somatostatin-like biological activity. Linear, cyclic and branching derivatives were designed and synthesised using above amino acids to selectively inhibit inflammatory actions. Unnatural moieties also were applied to enhance their enzyme resistance. Linear and cyclic peptides consist Tyr and D-Trp, while ring closed ones contain Lys too. Branching peptidomimetics have the same, flexible core [tris(2-aminoethyl)amine] and three protected or unprotected amino acids situated in equal positions.

The biological activity of the compounds was evaluated by *in vitro* assay of substance P release and *in vivo* assay of plasma protein extravasation. The most potent agents strongly inhibited substance P release by 90 – 95 % and one of them showed strong anti-inflammatory activity when administered orally. The structure of the novel compounds and the relationship between their structure and biological activity will be discussed.

P22428-176

P5U and urantide modified at position 7 with TPI

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Urotensin II (U-II), a potent vasoconstrictor, is found in diverse species, including human. Several biological studies indicate that U-II is the most potent mammalian peptide vasoconstrictor reported to date, and it appears to be involved in the regulation of cardiovascular homeostasis and pathology. In order to elucidate the importance of Trp residue for receptor interaction and biological activity recently we have designed, synthesized new analogues where Trp7 was replaced with constrained analogues LTpi or DTpi (1,2,3,4-Tetrahydronorharman-3-carboxylic acid). The Tpi residue was replaced in both agonist P5U and antagonist Urantide sequences. On these new ligands we performed biological and NMR conformational studies. The new ligands will be used in further biological investigations of the UT receptor. P5U H-Asp-[Pen-Phe-Trp-Lys-Tyr-Cys]-Val-OH Urantide H-Asp-[Pen-Phe-DTrp-Orn-Tyr-Cys]-Val-OH New H-Asp-[Pen-Phe-Xaa-Yaa-Tyr-Cys]-Val-OH Xaa = Tpi, DTpi; Yaa = Lys, Orn

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P22500-177

Peptides from a Phage Display Library Recognize of Modified Nucleosides of Anticodon Domain of Human $tRNA^{Lys3}$

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The contribution of the natural modified nucleosides to RNA identity in RNA-protein interaction is poorly understood. However, studies of tRNA with its many modifications and known biochemistry have revealed that tRNA modifications are recognition determinants for aminoacyl-tRNAsynthetases (aaRS) and initiation and elongation factors [1,2]. We have demonstrated that 15-aa peptides selected from a random phage display library selectively recognized the anticodon domain of human $tRNA^{Lys3}$ (ASL^{Lys3}). An analogue of ASL^{Lys3} having two modified nucleosides s^2U_{34} and ψ_{39} has been used for the selection. Affinity of the selected peptides was characterized by fluorescence quenching of the peptides' tryptophans or tyrosines. Peptides bound ASL analogues in

micromolar range and exhibited the highest and similar binding affinity for singly modified ASL^{Lys3}-s^2U_{34} and doubly modified ASL^{Lys3}-s^2U_{34}, ψ_{39}. Unmodified ASL^{Lys3} was bound a few times weaker. CD spectra showed that peptides had a tendency to adopt a random coil conformation in aqueous solution. Thus, modifications contribute identity elements in peptide recognition of the anticodon domain of human $tRNA^{Lys3}$ and can be targets for selective recognition by peptides.

Acknowledgements: This work was supported by Polish Ministry of Science and Higher Education, grant no. 1542/B/H03/2007/33.

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P22521-178

GnRH analogs as carriers for targeted suicide gene delivery

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Suicide gene therapy represents one of the promising approaches to the cancer treatment. An application of herpes simplex virus thymidine kinase (HSVtk)/ganciclovir system possesses additional advantage due to bystander effect on neighboring cancer cells. Overexpression of GnRH receptors in the case of most adenocarcinomas creates the basis of GnRH analogs use as carriers for targeted suicide gene delivery. We investigated different manners of GnRH molecule modification; their influence on peptide/DNA complex formation and its penetration into cancer cells. Analogs, containing NLS from large antigen of SV40 were synthesized using combination of BOC- and Fmoc- chemistry. Depending on peptide structure (agonist or antagonist) NLS was attached via position 6 or 1 of the natural molecule. The competition experiments demonstrated that internalization of peptide/DNA complex into HepG2 cells is mediated by specific receptor binding. Moreover, NLS/DNA complexes, lacking GnRH moiety were unable penetrate cellular membrane. Subsequent studies permit to identify the influence of analog structure on in vitro efficiency of suicide gene therapy, followed by acyclovir treatment. It was shown that application of reference peptide gene delivery system damaged about 50% of tumor cells. Use of cationic peptide conjugated with RGDF sequence provides high efficiency of treatment, however can not ensure selective action on cancer cells. GnRH analogs completely suppress tumor growth in vitro due to specific interaction of peptide/DNA complex with correspondent receptor. The efficiency of suicide gene therapy depends on peptide structure and is in favor of agonists as compared to antagonists. Preliminary data of experiments in vivo on laboratory animals demonstrated practical utility of tested peptides in the course of intravenous administration. Thus, it was shown that GnRH analogs containing NLS moiety represent promising candidates for the delivery of HSVtk gene into the cancer cells.

P22607-180

Peptidomics of grape

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The structural-functional study of putative grape (Vitis vinifera) uncharacterized protein sequences was performed. We developed a special method of computer analysis (1) for this. This method has allowed to reveal new potentially active regulatory oligopeptide sequences yet not investigated experimentally. Information on grape amino acid sequences of public databases [2, 3], computer database EROP-Moscow (Endogenous Regulatory OligoPeptides) 4. containing the information on structure and functions of known natural oligopeptides and specially created computer programs were used for this. Protein amino acid sequences were compared with all known oligopeptide sequences in this method. As a result several tens of grape oligopeptide sequences were elucidated. The similarity of their sequences with the known oligopeptide structures of other biological species was the basis for the prediction their potential functional properties. It has been shown that grape contain putative regulatory oligopeptides possessing functions of antibacterial and antifungal agents, enzyme inhibitors, calmodulin binding structures, rapid alkalinization factors, etc. The primary structure similarity of grape sequences was found not only with plant species but with bacteria, fungi, and animals also.

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P22717-181

Engineering stabilized VEGF-A antagonists: Synthesis, structural characterization and bioactivity of grafted analogues of cyclotides

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Cyclotides are plant derived mini-proteins with compact folded structures and exceptional stability. Their stability derives from a head-to-tail cyclised backbone coupled with a cystine knot arrangement of the three-disulfide bonds. Taking advantage of this stable framework we developed novel VEGF-A antagonists by grafting a peptide epitope involved in VEGF-A antagonism onto the stable cyclotide framework. Antagonists of this kind have potential therapeutic applications in diseases where angiogenesis is an important component of disease progression, including cancer and rheumatoid arthritis. A grafted analogue showed biological activity in an in vitro VEGF-A antagonism assay at low micromolar concentration and importantly the in vitro stability of the linear epitope was markedly increased using this approach. In general, the stabilization of bioactive peptide epitopes is a significant problem in medicinal chemistry and in the current study we have shown the cyclotide framework is ideally suited for such stabilization. Cystine rich scaffolds are emerging as valuable templates in drug design and in the current study we have shown that the cyclotide scaffold, with the advantageous features of a knotted disulfide core and a cyclic backbone, has significant potential in stabilizing a wide range of bioactive peptide epitopes.

P22800-182

Molecular modeling of GnRH analogues in DMSO solution using nuclear magnetic resonance (NMR) and molecular dynamics (MD)

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Gonadotropin Releasing Hormone (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2, GnRH) plays a significant role in the controlling of gonadotropins and steroids hormones. A large number of linear GnRH analogues has been synthesized and tested for several medical uses. Leuprolide acetate (pGlu-His-Trp-Ser-Tyr-(D)Leu-Leu-Arg-Pro-NHEt, LPA) is a potent GnRH agonist and is used to treat a wide range of sex hormone related disorders, including prostatic cancer, endometriosis and precocious puberty. Despite its widespread use, only limited information based on spectroscopic evidence regarding the solution conformation of Leuprolide are known. Moreover, non crystallographic data is available for the receptor of GnRH (G protein-coupled receptor). The aim of this study was to characterize the conformation of Leuprolide and its modified linear analogue (pGlu-His-Trp-Ser-Tyr(OMe)-(D)Leu-Leu-Arg-Aze-NHEt) in DMSO solution (which simulates better the receptor environment) using Nuclear Magnetic Resonance (NMR) and Molecular Modeling techniques. By using both NMR and Molecular Modeling we have characterized the secondary structural preferences of these GnRH analogues.

P22807-183

Structural Comparison of mu-Opioid Receptor Selective Peptides and Peptidomimetics Revealed Four Important Conformational Parameters of Bioactivity

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Structural determinants of binding to the mu-opioid receptor - an important target in analgesia - attracts great scientific attention. Many natural and synthetic peptides and peptidomimetics were shown previously to bind to the mu-opioid receptor selectively but there is no consensus about what structure is responsible for such biological activity. No high resolution structure of this receptor is available and the binding site of ligands is not exactly known despite numerous site-directed mutagenesis studies. This suggests that the determination of structural aspects of mu-opioid activity should focus on the ligands. mu-Opioid ligands with similar affinity and selectivity should possess at least one common structural feature in which they differ from other ligands of different affinity and selectivity. Comparative structural analysis of such ligands, considering adequate representation of binding conditions may reveal key features of bioactivity. In this study ten mu-opioid receptor ligands, DAMGO, Tyr-W-MIF-1, morphiceptin, endomorphin-1 and 2 and their analogues, possessing different affinity and selectivity were examined using molecular dynamics. Conformational preference of these molecules was determined in aqueous and DMSO media which were meant to model different possible binding environments. No structural trend, correlating with previously measured bioactivities was observed in aqueous media. In DMSO it was found, that a preference for trans orientation of the Tyr1 side chain and gauche (-) orientation of the third aromatic side chain, the free rotation of the Phe4 side chain and a high propensity of bent backbone structure is favorable for high affinity binding to the mu-opioid receptor, while deviations from these criteria results in variable loss of bioactivity. Constellation of these four key conformational parameters may be a guiding principle in the future for the design novel mu-opioid receptor ligands.

P22811-184

In silico Design of the Novel Epitopic Peptides of the Thyroid Peroxidase

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Human thyroid peroxidase (hTPO) is a 933 amino acid protein. It is member of family myeloperoxidase. The primary structure of hTPO has most high homology with human myeloperoxidase (hMPO). The 3D structure of hTPO is not defined. It is formed by 3 domains – a myeloperoxidase (MPO)-like, a complement control protein (CCP)-like and an epidermal growth factor (EGF)-like domains. Bresson with collaborators have defined some epitopes on the surface of MPO-like and CCP-like domains hTPO ($TPO^{353-363}$, $TPO^{377-386}$, $TPO^{506-514}$, $TPO^{713-720}$, $TPO^{766-775}$) involving in binding with anti-TPO autoantibody.

Methods and Results. We have used BLAST algorithm for primary structure of hTPO and hMPO alignment. Then the program complex designed by the author was used to modeling epitopes on the surface regions of the hMPO which having high structural homology with hTPO. The potential epitope is formed by amino acid resides $Glu^{734}Gln^{738}Asp^{739}Lys^{741}$ closely spaced on the surface of hTPO molecule. Based on this we have proposed some peptides structures. They were designed, synthesized and *in vitro* investigated. Competitive ELISA experiments with peptides have shown that one of they inhibited binding of anti-TPO autoantibody with hTPO on 80% with peptide concentration 1mM.

Conclusions. Competitive ELISA experiments with *in silico* designing peptides have shown high ability to bind pathogenic autoantibodies in serum patients suffering from autoimmune thyroid diseases. Based on the designed compounds we are developed new immunosorbent, diagnostic tools and therapeutic peptides able to modulate immune responses.

P22829-185

A new library of cyclic peptides obtained via i-to-i+4 intramolecular side chain-to-side chain azide–alkyne 1,3- dipolar cycloaddition: CD and NMR conformational analysis

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CuI-catalyzed azide–alkyne 1,3-dipolar Huisgen's cycloaddition - prototypic "Click reaction"- is a recently developed synthetic procedure to obtain cyclopeptides carrying, as a rigid linking unit, the lactam bioisoster, 1,4-disubstituted [1,2,3]triazolyl ring (1). We have recently reported the synthesis and conformational analysis of the 1,4-disubstituted-[1,2,3]triazolyl containing cyclopeptide derived from the sequence of the potent i-to-i+4 side chain-to-side chain lactam-containing antagonist of parathyroid hormone-related peptide (PTHrP) 2.. The conformational properties of triazolyl-containg peptide were compared to those of the corresponding lactam analog. CD and NMR studies revealed that, despite a slight difference of the backbone arrangement, triazolyl containing cyclopeptide and lactam-containing cyclopeptide share a common orientation of the side chains (3). Here we present the structural study of a new library of disubstituted-[1,2,3]triazolyl containing cyclopeptides designed to obtain different cycle dimensions and different triazolyl-ring positioning. CD and NMR analysis in different solvent systems shows that both, the dimension of the cycle and the specific positioning of [1,2,3]triazolyl ring are critical to fully resemble the conformational properties of the potent lactam-containing antagonist of parathyroid hormone-related peptide (PTHrP).

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P22900-186

The potent antiproliferative activity of small hydrophobic peptides containing adamantyl group

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The somatostatin (SRIF) is a cyclic tetradecapeptide, which exerts inhibitory effects on the secretory processes in the endocrine and exocrine systems, and the cell proliferation through somatostatin receptors (SSTR1-5). SSTRs are distributed throughout human body, not only in normal cells but also in tumor cells. Most of the somatostatin analogues developed for clinical use, such as Octreotide which act longer than somatostatin are being used in the diagnosis and treatment of endocrine tumors. The use of these analogues as antitumor agents has been limited because of their antisecretory effects and poor oral bioavailability. TT-232 [D-Phe-c(Cys-Tyr-D-Trp-Lys-Cys)-Thr-NH_2], was reported by Keri et al. to have potent antiproliferative activity without antisecretory action 1.. Based on the above, we aimed to design and synthesize somatostatin analogues with more potent antiproliferative activity and high oral bioavailability.

We synthesized pyrazinone ring containing cyclic peptides (2) and linear peptides which are substituted at the C-terminus Lys with hydrophobic and rigid groups. Our focus was on the active sequence: Tyr-D-Trp-Lys. We also examined their antiproliferative activity and found that Boc/H-Tyr-D-Trp-1-adamantylamide exhibited the most potent antiproliferative activity higher than that of TT-232. Furthermore, on the best analogues we studied DNA fragmentation by FACS analysis and cellular morphology. The results demonstrated that these somatostatin analogues induced cell death by apoptosis.

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P22900-187

Synthesis and biological activities of endomorphin-2 analogues containing Pro mimics

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Background and aims: Endomorphin-2 (EM-2: H-Tyr-Pro-Phe-Phe-NH_2) has high affinity and selectivity for the mu opiod receptor (1). We focus on the Pro residue, which imposes strong restraints on the conformation of the peptide chain or induces cis-trans isomerization of X-Pro bonds. We substituted the Pro with 2-Azetidinecarboxylic acid (Aze) or Piperidinecarboxylic acids (Pip) to increase or decrease the conformational flexibility. In this paper, we deal with the synthesis of EM-2 analogues containing Aze and Pip and the evaluation of the biological functions of the analogues on the opioid receptors. **Methods:** The synthesis of peptides was achieved according to the procedure of Okada Y. et al. (2) The final products were identified by MALDI-TOF mass spectrometry and elemental analyses. The receptor binding activity of peptides was assessed by radio-ligand receptor binding assay using mu and delta opioid receptors from COS-7 cell membranes expressing each opioid receptors. For the evaluation of the biological function of peptides, the GPI and the MVD tests were performed. **Results and conclusions:** The substitutions of Pro in EM-2 (IC_{50}=3.23 nM) with Aze or Pip gave the mu receptor binding activity in a nanomolar range. All of them were moderate mu-selective agonist. Furthermore, the substitution of Pro in [Dmt^1]EM-2 (IC_{50}=0.15 nM) with Aze or Pip(2) yielded the mu-binding activity with the IC_{50} values of 4.04 and 0.41 nM, respectively. [Dmt^1, Aze^2]EM-2 was mu-/delta-agonist, although its biding activity was not higher than that of [Dmt^1]EM-2. The other hand, [Dmt^1, Pip^2]EM-2 was potent and selective mu-agonist. Those findings showed the substitution of Pro could control the biological profile of EM-2. The conformational analyses of [Dmt^1, Aze^2]EM-2 and [Dmt^1, Pip^2]EM-2 are now in progress.

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P30102-001

Microwave-Assisted Solid Phase Synthesis of Backbone Cyclic Glycopeptide Libraries

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The new analogue of CSF114(Glc) 1., [Pro^7,Asn^8(Glc),Thr^{10}]CSF114 is characterized by a type I β-turn around the minimal epitope Asn(Glc), and it was demonstrated to show the highest antibody affinity in competitive ELISA in Multiple Sclerosis (MS) patients' sera and thus it appears as a promising tool for the detection in patients' sera of specific autoantibodies. In previous studies, we synthesized and tested different fragments of this new glucosylated peptide, [Pro^7,Asn^8(Glc), Thr^{10}]CSF114, identifying the shortest sequence [Pro^7,Asn^8(Glc),Thr^{10}] CSF114(5-11) able to detect antibodies by ELISA in MS patients' sera. This heptapeptide is characterized by Thr in position 10 generating a characteristic N-glycosylation consensus sequence. According to the results obtained with the linear heptapeptide in MS, we applied the Backbone Cyclicization method to develop two backbone cyclic libraries of glycopeptides based on the sequence of the linear hepta active peptide. Backbone Cyclization is a method that allows obtaining cyclic peptides without changing the natural sequence or the chemical character of the amino acid residues, in order to enhance activity, stability to metabolic degradation, selectivity and bioavailability (2). The first library contains a Gly building unit at the C-terminus and is connected to the N-terminus by linker of various lengths (n=2,3,4,6). In the second library, His^9 of the heptapeptide is replaced by Gly building units with various alkyl chains (n=2,3,4,6) [Fmoc-N$^{\omega}$(N$^{\omega}$Alloc(n-alkyl))Gly-OH] that is connected to the N-terminus by linker of various lengths. Twenty cycloglycopeptides were synthesized, and screened by competitive ELISA s on MS patients' sera to select the most bioactive cyclic glycopeptide.

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P30114-002

New peptide nucleic acid analogues: Synthesis and application

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Peptide nucleic acids have become, arguably, one of the most interesting of DNA mimics. Owing to their high chemical stability and resistance towards nucleases and proteases, they are very attractive as antigene/ antisense agents, molecular biological tools and for genetic diagnosis. The lack of charge and polar groups in the backbone decrease their solubility in aqueous environment and their ability to cross cell membranes, reducing their performance in in vivo applications. In order to overcome these problems - to improve solubility, increase affinity and specificity of binding, a number of analogues were synthesized. This study describes the synthesis of PNA-monomers on the base of non-protein amino acids analogues of basic amino acids Lys and Arg. Nucleobases are the second residue we have selected. Studies will include replacement for example of the uracil, with the fluorinated analogue.

P30208-003

New Ribosylated Building Blocks Useful For Glycopeptide Synthesis

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Growing evidences indicate that N-glycosylation is a co-translational modification that, either native or aberrant, may play a fundamental role in a large number of biological events. In particular among post- and co-traslational modifications glycosylation plays a crucial role in the immune system. In fact, almost most of all the key molecules involved in the immune response are glycoproteins. There are growing evidences of defects in glycosylation with diseases that assets to pathway oligosaccharides as code words.
In previous studies, We demonstrated that the presence of a β-D-glucopyranosyl moiety on an Asn residue at position 7 of CSF114(Glc) is fundamental for auto-Ab recognition resulting the first multiple antigenic synthetic probe (MSAP) able to detect autoantibodies in MS patients' sera. Up to now we have investigated the carbohydrates influence and specificity in MS antibody recognition introducing several glycosilated building blocks in the MSAP sequence (i.e. Glc, Man, GlcβGlc, Gal, GlcNAc on the side chain of Ser, Thr, Asp, Glu, HyPro) 1.. Due to microheterogeneity and the extremely high specificity of carbohydrate-protein interaction we included in our library screening, the ribose. We report the synthesis of new Asn-derivatives bearing on the side chain ribose βRib and αRib linked by an N-glycosidic bond and protected for SPPS.These building blocks introduced in the CSF114 β-turn scaffold lead to the new ribosylated peptides contributing to the library of glycopeptides to fishing out families of autoantibodies specific for different autoimmune diseases.

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P30208-004

Supramolecular Structures Formed by Immobilized N-Lipidated Oligopeptides as Artificial Esterases

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We designed and prepared supramolecular structures formed from N-lipidated oligopeptides immobilized in the regular pattern on the cellulose surface which are able to specific binding of ligand molecule. Due to the conformational flexibility of the fragments forming the supramolecular structure, the shape and prosperities the binding cavities are adjusted the most effectively to requirements of the guest molecules. The previous studies documented that process of binding guest molecules is highly selective, reversible and competitive. Therefore, we supposed that under favorable circumstances the structures could operate as catalysts if suitable molecular fragment are included inside the binding pocket. In order to verify this hypothesis we prepared library of supramolecular hosts with catalytic triade: His Asp(Glu) Ser, incorporated into the binding pocket 1.. For the first generation library the rate of hydrolysis of p-nitophenyl esters of N-protected amino acids was measured by spectrophotometric determination of liberated p-nitrophenole in buffered, aqueous methanol and compared with appropriate data obtained in the absence of catalytic structures. The most active catalyst were selected from the library and their stability, selectivity and ability for re-use was studied. For the second generation of library the stereoselectivity of artificial esterase

was studied. It has been found that enzymes prepared from natural L-amino acids hydrolyzed p-nitrophenyl esters of L-dipeptides, but D-enantiomers were resistant towards degradation.

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P30216-005

Multi-component fluorescence labeling of peptide library for in-situ screening

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We present here a new technique for identifying very small quantity of peptide mixtures that are selected out from a peptide library in solution, by using multi-component fluorescence labeling. The technique is basically a modification of positional screening method associated with a 1:1 correspondence between the amino acid at the i-th position and the type of the fluorescence lebel at the N-terminal. 2-dimensional fluorescence spectroscopy was employed for identifying the fluorescence labels in the mixture of peptides that bound to target cells or target proteins. The results of the new screening method will be presented for peptides that specifically bind to human cancer cells.

P30305-006

Semisynthesis of proteins dimers by Expressed Protein Ligation

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Several proteins and peptides carry out their biological function in a dimeric form. For example, transcription factors regulate gene expression by binding DNA as dimers; antimicrobial peptides, such as Magainin 2, show increased biological activity in a dimeric form1. In recent years there have been significant efforts to obtain minimized versions of naturally occurring proteins such as dimeric DNA binding proteins which retain their function. The GCN4 basic region peptides were connected trough a disulfide bond to give a dimer which specifically bound the AP1-DNA sequence. Dimeric peptides and proteins were obtained also by non covalent interactions.2 In this work we propose a strategy for obtaining by Expressed Protein Ligation (EPL), one pot protein homodimers covalently connected at the C-terminus. The synthetic strategy was extended also to the synthesis of heterodimers. EPL is a protein engineering tool for the chemo and region-selective modification of proteins based on the use of intein containing constructs.3 In this work dimers were obtained by reacting a new bi-functional linker with carboxyl-activated polypeptides. We synthesized a linker containing two cysteines in a N-terminal-like position, separated by an ethylendiamine spacer, and obtained thioester proteins by intein mediated splicing reactions. This strategy affords chemically stable dimeric proteins. The linker can be easily modified at need, changing the lenght and rigidity of the spacer between the cysteines. This strategy has potential in biochemical and bioorganic applications, for obtaining minimized and/or modified natural proteins and for joining two different proteins at the C-terminus position. This technique will be extended to the synthesis of dimeric proteins mimicking the transcription factor AP-1.

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P30309-007

Semi-synthetic approaches to glycosylation of MOG as autoantigen in Multiple Sclerosis disease

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Post-translational modifications, as glycosylation and phosphorilation, can modulate proteins activity and expand the diversity and complexity of their biological functions. A distinct pattern of Multiple Sclerosis (MS) pathology involves an Ab-mediated demyelination.[1] Previous studies showed that CSF114(Glc),[2,3] a designed glycopeptide characterized by a β-D-glucopyranosyl moiety, can detect and isolate specific autoAbs in sera of a significant number of MS patients. This synthetic Ag could be considered a mimetic of aberrantly glycosylated myelin proteins triggering autoimmunity in MS. Myelin Oligodendrocyte Glycoprotein (MOG) is considered a putative autoAg in MS.[4] Our aim is to obtain MOG properly glycosylated to characterize the molecular mechanisms of Ab-mediated MS and to design new antigenic probes to detect autoAbs as biomarkers. Production of specific glycoproteins may benefit from a chemical approach, such as Expressed Protein Ligation (EPL) a protein engineering strategy useful to introduce noncanonical amino acids and biological probes into proteins.[5] EPL allows synthetic and recombinant polypeptides to be chemoselectively and regioselectively joined together. The recombinant $rMOG_{ED}$(1-97), obtained as C-terminal thioesther by protein splicing, will be ligated to the peptide fragment [Gly^{103},Asn^{104}(Glc)]MOG_{ED}(98-117) bearing a Cys residue at the N-terminus. An alternative strategy exploits the selective reaction between a glucosyl iodoacetamide derivative and the Cys free thiol of a protein.[6] A site directed mutation has been performed on rMOG to introduce a Cys residue at its native site of glycosylation. The semi-synthetic proteins will be tested by ELISA using MS patients' sera.

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P30617-008

ÄKTA™ oligopilot™ for Automated Solid Phase Peptide Synthesis Daniel Latassa, Ph.D. (Lonza AG, Visp-Switzerland), Ulf Tedebark, Ph.D. (GE Healthcare Bio-Sciences AB, Uppsala-Sweden)

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Automation is an identified goal in the Peptides R&D at Lonza. This approach would facilitate and accelerate peptide production. This is highly desired within Peptides R&D, where the need exists for rapid synthesis of peptides to fulfil Iso and GMP projects requirements. Automated peptide synthesisers are available on the market but many have limitations which make them inappropriate for Lonza (e.g., low scale, coupling systems limitation, pre-activation procedure at low temperature). Furthermore, there is no obvious standard instrument for scalable SPPS equipped with PAT. GE Healthcare provides scalable and complete solutions for automated solid-phase synthesis of oligonucleotides from small research amounts to full commercial production (1µmol–1mol) based on the ÄKTA, Oligopilot 400 and OligoProcess™ platforms. The UNICORN™ software provides control and monitoring of the processes. GE Healthcare synthesisers are designed around flow-through column reactors, giving faster kinetics and lower solvent and reagent

consumption compared to batch synthesisers. Based on experience with scale-up of oligonucleotides, GE Healthcare and Lonza are confident that the flow through-column technology can be scaled up for peptides to a cost efficient process. This is a strong argument that the potential of the ÄKTA platform as a peptide synthesiser should be explored. GE Healthcare was approached by Lonza to develop a peptide synthesiser based on the ÄKTA platform instrument to compete with the state of the art in the peptide field. The aim is to develop the fluidics, programming and chemistry methods of the ÄKTA system to Lonza's needs for routine production of 0.4g to 2g of crude peptide, and later scale up to 2 kg. This synthesiser, controlled by the UNICORNTM software, has the capability to perform synthesis using on-line mixing or pre-activation at different times and temperatures of amino acids, coupling reagents, solvents and additives. Results from peptides will be presented.

P30727-009

Identification of neuropeptide-processing proteases by activity-based proteomics

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Neuropeptides are produced from precursor proteins by selective cleavage at specific sites. Classical biosynthetic cleavage occurs at basic residues due to the activity of a small number of well-known proteases. However, with the discovery and characterization of new neuropeptides, a new non-classical pathway has been described with cleavage occurring at tryptophane, leucine and other residues. Neuropeptide-processing peptidases involved in this new non-classical pathway are completely unknown but essential for correct processing of certain neuropeptides.
Therefore, we are interested in identifying proteases involved in the non-classical pathway using activity-based proteomics. For this purpose, we first designed and synthesized some peptides based on the sequence the mature neuropeptides described in the literature but with an active functional group able to bind the active site of the proteases of interest. Bound peptides were labelled with biotin or rhodamine by click chemistry, and the brain and pituitary proteome was then characterized by in-gel analysis and multidimensional nLC-MS/MS.
Several proteases that may be involved in neuropeptide processing have been identified in this experiments. Further studies concerning cloning, protein expression and activity assays will confirm their role in biological tissues.

P30915-010

Post translational modified peptides to study autoantibody response in Primary Biliary Cirrhosis

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The incidence of autoimmune diseases continues to increase. Although an enormous research effort has been directed in understanding this increment, etiology of human autoimmunity remains enigmatic. Primary Biliary Cirrhosis (PBC) is a chronic cholestatic liver disease characterized by destruction of bile ducts and the presence in the serum of antimitochondrial antibodies (AMA positive in 90-95% of patients), directed against the E2, which is one of the three component enzymes of the 2-oxo acid dehydrogenase multienzyme complex family chiefly pyruvate dehydrogenase complex (PDC-E2). Environmentally induced co- or post-translational modifications of autoantigens are hypothesized to break immune tolerance leading to self reactivity in PBC 1.. In this context it is possible to take advantage of a unique technology that allows the monitoring of peptide epitope modifications that will lead to the identification of altered autoantigens that the human host will recognize as foreign, similarly to what recently reported in multiple sclerosis (2). Our approach will lead to new diagnostic tools that can be used in earlier stage patients and possibly to monitor disease activity. Herein we report the synthesis of lipoamide and glyco-peptides characterized by a β-hairpin structure with the modification on the tip of the β-turn as peptidomimetic of native antigens involved in PBC. In order to identify the best synthetic antigens and to clarify the role of the β-turn in exposing the modification we compared the antibody recognition in PBC sera by ELISA using the modified peptides in comparison with the proposed autoepitope of PDC-E2 [PDH(44-63)]. The synthetic antigens were able to detect by ELISA autoantibodies in 30% of AMA negative sera of PBC patients confirming that PTM-peptides are useful diagnostic/prognostic tools.

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P31100-011

Construction of β-Sheet Proteins Embedding Amyloid β Peptide (Aβ) Sequences and Inhibitory Activity of Aβ Aggregation

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Alzheimer fs disease is characterized by the abnormal accumulation of amyloid β peptide (Aβ) into extracellular fibrillar deposits known as amyloid plaques. Aβ can self-assemble into soluble oligomers, protofibrils, and amyloid fibrils, and all of these aggregated forms contain significant β-sheet structure. The core of Aβ (14-23 residues) containing hydrophobic region is a key to promoting Aβ aggregation. In the previous study, we constructed green fluorescent protein (GFP) variants which have the core part of Aβ sequence on the surface β-sheets. It has been demonstrated that the GFP variants inhibit Aβ aggregation 1.. In this study, we have utilized a small protein, insulin-like growth factor ‡U receptor domain 11 (IGF2R-d11) as a scaffold, and a part of Aβ sequence was incorporated into the β-sheet surface of IGF2R-d11. IGF2R-KK and IGF2R-KA were designed by substituting two Aβ derived sequences for some amino acids in IGF2R-d11 as parallel and anti-parallel β-sheet models, respectively, of the Aβ aggregates. These insoluble proteins expressed by E coli. were solubilized by denaturing buffer, and the denatured proteins were refolded in conditions permitting formation of native proteins. After refolding, the proteins were purified as monomers by size exclusion chromatography. We have investigated the interaction between Aβ and the designed protein variants by surface plasmon resonance studies. As a result, IGF2R-KK bound tightly to Aβ more than IGF2R-KA. Inhibitory activities of Aβ fibrillization in the presence of IGF2R-KK, IGF2R-KA, or wild type IGF2R-d11 were evaluated by thioflavin T (ThT) binding assay. It was demonstrated that IGF2R-KK inhibited Aβ fibrillization effectively.

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P31100-012

The Potential Stem-Forming Sequence Consists of The 2-stranded β-structure in Prion Proteins

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Prion diseases have become a serious social issue, which are known to form amyloids in cells of mammalian brains. Certain fragment peptides of prion were reported to form amyloids using the interaction between one β-strand in each peptide. However, it seems difficult to form aggregates by the interaction with only one β-strand in each peptide,

because of their high structural flexibility. On the other hand, a 2-stranded β-structure is presumed to be more stable than a single strand as a fibril forming intermediate. Therefore, the aggregation mechanism of prion protein remains to be further studied on their precursor structures. Recently, we have established a novel strategy to determine the amino acid sequence for amyloid formation, based on their essential interactions. A number of fibril forming peptides at positions from 160 to 230 in the amino acid sequence were obtained by our calculation method. In order to confirm whether aggregates of the candidate peptides consist of the 2-stranded β-structure, a series of peptides consisting of g10 residues-turn-10 residues h were prepared. Several candidate peptides, of which regions are 166-187, 168-189, 170-191, and 178-199, showed the typical enhanced fluorescence intensities in the thioflavin T-binding assay, suggesting the amyloid formation. IR spectra of these peptides showed the typical bands corresponding to the β-structure. In addition, the peptide (178-199) exhibited a shoulder band at 1654 cm^{-1} in IR spectrum, reflecting a turn structure. These results revealed that the amyloids of the peptide (178-199) are constructed with the 2-stranded β-structure, which may be formed by intra-molecular hydrogen bonds. In conclusion, the results obtained here indicate that the sequence from 178 to 199 could be a key region for the transition from a normal to an abnormal structural state.

P31100-013

Molecular Force Balance for the Investigation of Receptor-Ligand-Interactions on Living Cells

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Single-molecule force spectroscopy provides a powerful tool to investigate biomolecular interactions. A different approach measures forces required for breaking a bond in a differential format by comparison with a known reference bond of dsDNA (1). Here we apply this molecular force balance to the integrin αvβ3, which is over expressed in OVMZ-6 cells. This protein interacts with ligands containing an –Arg-Gly-Asp-(RGD) motive. Ligands containing the RGD sequence were made by peptide synthesis and linked to a GCN4-peptide using a PEG-spacer. For detection carboxyfluoresceine was introduced to a lysine side chain between both peptides. The GCN4-peptide can be recognized by specific anti-body fragments (2) and can act as a reference bond. These force balances was linked to a PDMS-surface, whereas the reference bond is attached to the surface and the RGD-peptide is accessible to the integrin. When the PDMS-stamp gets in contact with the OVMZ-cells, the interaction of ligand and receptor can occur. By applying a force at the stamp the force balance is stretched and the weaker bond brakes. Investigation of the fluorescence-level on stamp and cells enables the localization of the balance construct and thus an estimation of the bond force. First stamp-experiments on living cells showed that the GCN4-anti body interactions are too strong to act as a reference system. Since the binding forces of DNA are well investigated by AFM and small DNA-molecules were already used as force sensor, DNA can act as a more sensitive reference. Thus in a further approach the RGD-Peptide was linked to a biotinylated PEG. This enables the application of short double-stranded DNA as a reference bond via a fluorescence-labelled streptavidin. Using this system the force balance could be transferred to the αvβ3 integrines located in the cell membrane of OVMZ-cells.

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P31305-014

Characterization of Nanogap Chemical Reactivity Using Peptide-Capped Gold Nanoparticles and Electrical Detection

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Nanogaps, which allow making electrical contact to structures on the nanoscale, are increasingly used for the preparation of biosensors. The positioning of the synthetic or biological species inside the nanogap must be controlled to reach optimal electrical or detection properties.(1-3) In this context, the chemical properties of the layer between the electrodes is of prime importance, since they will impose the imbibition of the nanogap and permit the formation of chemical bonds between the molecules of interest and the substrate. Thin films can be characterized by a variety of physical or chemical methods. However, the characterization of the chemical properties of nanogaps is complicated because of the small size of the substrate delimited by the electrodes. In this context, novel experimental tools are needed for probing rapidly the chemical reactivity of nanogaps. We show here for the first time that a specific functional group in a 30-90 nm nanogap can be detected by combining peptide-capped gold nanoparticles and electrical detection.(4) A semicarbazide layer and semicarbazone chemoselective ligation was used in this proof-of-concept study, which thus required the preparation of stable peptide-capped gold nanoparticles modified by aldehyde groups and control GNPs derivatized by amide groups. The chemoselective insertion of gold colloids into the nanogaps led to current increases from 2 to 4 orders of magnitude, in accord with the number of gold nanoparticles in the nanogaps detected by scanning electron microscopy.

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P31316-016

Current based antibodies detection from human serum enhanced by secondary antibodies labelled with gold nanoparticles immobilized in a nanogap

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We present the electrical detection of immunoglobulin G (IgGs) from human serum using a nanogap-based biosensor. The detection method is based on the capture of IgGs by a probe immobilized between gold nanoelectrodes of 30 to 90 nm spacing. The captured IgGs are further reacted with secondary antibodies labelled with gold nanoparticles (GNPs). Insertion of GNPs into the nanogap resulted in increasing the conductance through the nanogap. The use of a chip with ninety nanogaps enabled the calculation of a quality factor for the detection which, coupled with a non-linear regression analysis of the data, easily discriminated specific and differential capture of human antibodies by arrayed probes. We obtained a 500-fold higher quality factor with protein A compared to goat anti-murine antibodies. This method can be applied, through these proof-of-concept experiments, to the detection of protein-protein interactions in biological samples.

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P31317-017

Mimetibodies™, A New Platform Technology for the Development of Biologically Active Peptides that Prolongs the Half-Life

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In the past several decades, hundreds of peptides have been identified which have specific biological activity and are highly potent in in vitro assays but lack the prerequisite pharmacokinetics to become efficacious human therapeutics. We have developed a novel antibody-based platform technology that provides an improved pharmacokinetic profile for biologically active peptides, resulting in a long duration of action. One such Mimetibody™, CNTO 528, is a novel erythropoietin (EPO) receptor agonist. Although CNTO 528 bears no sequence homology to erythropoietin, it is a potent erythropoietin receptor agonist, rescuing EPO dependent cells from apoptosis in vitro and stimulating erythropoiesis in vivo. Studies were done in normal rats to explore the pharmacodynamics and pharmacokinetics of CNTO 528 in normal rats and to demonstrate its efficacy in rat models of anemia. In vitro, CNTO 528 was approximately 10 fold less potent than rhEPO in stimulating the growth of UT-7EPO cells. Despite this lower in vitro potency, when compared to rhEPO and darbepoietin in normal rats, a single subcutaneous dose of CNTO 528 resulted in a longer-lived reticulocytosis and longer-lived increase in hemoglobin. Also, CNTO 528 caused only minor changes in red cell distribution width (RDW) or mean cell volume (MCV) and led to the release of mature reticulocytes. We have also shown that CNTO 528 was efficacious in rat models of anemia and in a rat model of pure red cell aplasia. Taken together, our data show that CNTO 528 is a novel stimulant of erythropoiesis in rats. This platform has been applied to other biologically active peptides as well and has proven to be a robust platform for enhancing the pharmacokinetics of peptides that would otherwise be rapidly cleared.

P31325-018

Association of S4(13)-PV cell penetrating peptide with cationic liposomes: a synergistic effect on the intracellular delivery of plasmid DNA

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Cell penetrating peptides (CPPs) have been recognized as promising tools for the delivery of different therapeutic molecules. Previous studies in our laboratory have shown that the S4(13)-PV peptide accumulates inside cells very efficiently through a rapid, dose-dependent and non-toxic process. Formulations based on the S4(13)-PV cell penetrating peptide presented great potential for the delivery of plasmid DNA, which may prove useful for gene-based therapies.
In the present work, we aim to 1) investigate the relevance of the Dermaseptin-derived sequence and of the nuclear localization signal to the efficiency of the overall process of plasmid DNA delivery by the S4(13)-PV and related peptides; 2) compare the potential of the S4(13)-PV peptide to mediate plasmid DNA delivery with that of the extensively studied Tat CPP.
A comparative analysis of the transfection efficiency mediated by the systems based on the S4(13)-PV, reverse NLS and scrambled peptides was performed in TSA and HeLa cells. In general, for both cell lines, the reverse NLS peptide mediated transfection at efficiencies comparable to those observed for the S4(13)-PV peptide. However, transfection mediated by the scrambled peptide was significantly less efficient than that obtained for the S4(13)-PV and reverse NLS peptides.
To compare the biological activity of the S4(13)-PV with that of the Tat peptide, we transfected TSA cells with various S4(13)-PV- and Tat-based formulations. Our results have shown that both peptides enhanced the activity of cationic liposome–based systems. Above a threshold peptide/cationic liposome/pDNA charge ratio (10/1/1), the enhancing effect was independent of the peptide used, although for the lowest charge ratios, this effect seemed to be more relevant in the case of the S4(13)-PV peptide.

P31413-019

Novel Nanoscaffolds Carrying Cell Adhesion Peptide Sequence

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The aim of tissue engineering is to replace failed organs with new functional tissue and organs. To realize this, materials are needed, which can direct the growth of cells to generate new tissue. To control and direct cell behavior, a defined biomimetic environments is needed, which surrounds the cells and promotes specific cell interactions. The RGDS sequence has been recognized as the cell attachment site of the natural extracellular matrix. The purpose of this study is to fabricate RGDS-carrying nanoscaffords towards cell adhesion using a self-assembling technique. Here we synthsize two types of RGDS-based materials, one of which is an amphiphilic triblock peptide composed of Leu and Lys (RGDS-L4K8L4) that has been revealed to self-assemble into *f*À-sheet nanofiber under specific conditions 1., and another one is a RGDS-ended polystyrene (PSt-RGDS) that is a typical artificial polymer. These peptide and peptidomimetic were prepared by solid phase synthesis using Fmoc-chemistry and by coupling Fmoc-chemistry and atom-transfer radical polymerization (ATRP) method, respectively. RGDS-L4K8L4 was found to self-assemble into *f*À-sheet-based nanofiber at pH 9.6, and by lowering pH the conformational change from *f*À-sheet to random coil structure was induced, giving no aggregation of the peptide. When mouse NIH/3T3 cells were seeded on the RGDS-L4K8L4 nanofiber-coated plate, successful cell adhesion and spreading were observed, but not for the RGDS-L4K8L4 random coil-coated plate, indicating the importance of the RGDS sequences densely and regularly located at the nanofiber surface. The utility of PSt-RGDS nanofilms will be discussed, in comparison with that of nanofibers.

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P31500-020

The role of deiminated protein antigens in the diagnosis of Rheumatoid Arthritis

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Autoantibodies directed against citrulline-containing peptide (ACPA) have high specificity of rheumatoid arthritis (RA). Citrullinated proteins are formed by posttranslational modification, namely by deimination of arginine residues in protein sequences by peptidylarginine deiminase enzymes (PADI). Autoantibodies to deiminated (citrullinated) proteins are the most specific serological markers of rheumatoid arthritis. Rheumatoid arthritis symptoms develop gradually, and it is difficult to precisely date the beginning of the disease. These antibodies are detectable already years before the first clinical symptoms of the

disease. The aim of our study was to identify the epitopes of vimentin and filaggrin derived-peptides targeted by RA specific antibodies to provide further information about the nature of the initial autoantigenic substance. We used conventional solid-phase peptide synthesis (Fmoc strategy) carried out on „MULTIPIN NCP" (Chiron Mimotopes Peptide System) non-cleavable kit. Identification of epitope structure of antigenic proteins represents one of the major applications of these technologies. The peptides were prepared in duplicates. Citrullinated peptides and the nonmodified counterparts containing arginine instead of citrulline residues were synthesized in order to compare their respective reactivities. In the "indirect" ELISA experiments the presence of ACPA was determined using serum samples of RA patients and healthy blood donors. This series of experiments efficiently identifies citrullinated epitopes of filaggrin and vimentin as a potential antigenic target for RA specific antibodies. The determination of epitopes of these proteins could be important for the development of appropriate diagnostics in this most frequent human systemic autoimmune disease.

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P31500-021

New selective substrates for detection of cysteine proteinases

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At the present time the human lysosomal enzymes cathepsins B and L, cysteine proteinases of C1 family, attract great attention. They not only take part in protein degradation but also appear to play role in other important physiological processes, for example, antigen presentation, caspase-independent cell death and involved in different diseases such as osteosarcoma, acute pancreatitis, tumor invasion and metastasis. But selective detection of these enzymes is difficult because determination of their enzymatic activity is carried out using substrates also correspond to specificity of trypsin-like enzymes.

The chemo-enzymatic synthesis of selective substrates of cysteine proteinases of C1 family was developed. These compounds are chromogenic Glp-Phe-Ala-pNA (I), Glp-Val-Ala-pNA (II) and fluorogenic substrates Abz-Phe-Ala-pNA (III), Glp-Phe-Ala-AMC (IV). Peptides were obtained in preparative quantities and were characterized by the data of amino acids analysis, HPLC, mass-spectrometry, spectrophotometry (I and II) and fluorimetry (III and IV).

The specific activities of cathepsins B, L and similar enzymes of plants papain, bromelain and ficain were determined using synthesized substrates and commercial available substrates Z-Phe-Arg-pNA, Z-Arg-Arg-pNA and Bzl-Arg-pNA. The specific activities of enzymes of other classes – serine (chymotrypsin, trypsin and subtilisin), aspartic (pepsin) and metalloproteinases (thermolysin) – were also defined with these substrates. It was shown, that Glp-Phe-Ala-pNA, Glp-Val-Ala-pNA and Glp-Phe-Ala-AMC are not detectable cleaved by enzymes of other classes.

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P31501-022

Electrochemical Glycopeptide Probes to Detect Autoantibodies in Multiple Sclerosis Patients' Sera

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The glycopeptide CSF114(Glc) is the first specific Multiple Sclerosis (MS) Antigenic Probe, active molecule of MS PepKit, diagnostic test developed to recognize specific autoantibodies in sera of a statistically significant MS patients' population1.. To implement a new electrochemical biosensor for autoantibody detection in MS we used CSF114(Glc) analogues, properly modified at N-*terminus* with ferrocenyl and ferrocenyl-thiophosphine derivatives, as "electrochemical probes" in cyclic voltammetry (2). The electrochemical properties of ferrocene, coupled to thiophosphine ability to build simple monolayers on gold surfaces, allow peptides to be anchored on the working electrode used for detection. In particular, 4-FcPhP(S)Abu organometallic amino acid was specifically designed to be used directly in solid phase peptide synthesis. The organometallic moiety introduced in the new glycopeptides did not affect autoantibody recognition as demonstrated both in SP-ELISA and in inhibition experiments. An electrochemical monitoring was able to detect interactions of the modified glycopeptides with isolated antibodies from MS patients' sera. We demonstrated a detection sensitivity comparable to ELISA method. Therefore, the new electrochemical probes can be proposed to characterize autoantibodies as biomarkers of Multiple Sclerosis by a simple, rapid, and reproducible cyclic voltammetry-based diagnostic methodology.

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P31505-023

Development and Biochemical Characterization of New Specific Reagents for the Detection and Isolation of Cardiac Troponins

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Troponin is a structural protein complex, located on the thin filament of the contractile apparatus. It is composed of three protein subunits: troponin I (24kDa), troponin C (18kDa), and troponin T (37kDa) and exists as isoforms specific to the cardiac and skeletal muscle cells, respectively. Cardiac troponins are released in the peripheral blood during irreversible cardiac muscle damage in a time-specific manner. Rapid troponin ELISA assays based on the production of specific antibodies against the whole complex or individual subunits have been shown to possess sufficient sensitivity and specificity for use in the emergency departments. However, their usefulness sometimes is limited by various factors, such as the selection of epitopes for antibodies production, derived from cardiac troponins representing high homology against the skeletal isoforms, interfering blood factors etc. Aiming to contribute in the field of developing highly sensitive and specific reagents for the detection and isolation of cardiac troponins in the sera of patients with cardiovascular diseases, we selected epitopes derived from the cardiac isoforms for production of antibodies, mainly based on their predicted immunogenicity, the minimum homology against the skeletal isoforms

and the lack of interferences of the produced antibodies with various blood factors. The selected sequences were conjugated to the tetrameric Sequential Oligopeptide Carrier (SOC_4), either by the classic solid phase step-by-step methodology or by chemoselective ligation reactions and the resulted conjugates were used as immunogens for releasing anti-troponin specific antibodies. The performed ELISA experiments revealed the high affinity and specificity of the produced anti-troponin antibodies against the native protein.

P31509-024

A chemical reverse approach for autoimmune diseases diagnosis

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An increasing number of individuals throughout the world is affected by autoimmune diseases, a large and diverse group of disorders that are categorized by tissue injury or pathology. Although the incidence and prevalence of individual autoimmune diseases are not high, the population burdens of the disease are large and underestimated. Thus, reliable diagnostic/prognostic tools are necessary for an early diagnosis and for monitoring disease activity. In this scenario, we proposed a 'Chemical Reverse Approach' based on the use of patients' sera to screen synthetic modified peptides. We showed that this approach could lead to the effective identification of specific probes able to characterize highly specific autoantibodies as disease biomarkers of highly relevant autoimmune diseases such as Multiple Sclerosis and Rheumatoid Arthritis [1,2]. Toscana Biomarkers is a R&D company involved in the application of the "chemical reverse approach" to different autoimmune conditions for the development of innovative diagnostic/prognostic tests. As a number of autoimmune diseases have been associated with post-translational modifications, which alter the function and immunogenicity of protein/peptide antigens, we are synthesizing and screening focused peptide libraries based on post-translational modified amino acid, conformational and minimal epitope diversity. Lead compounds can be thus used as antigenic probes for specific recognition of autoantibodies as biomarkers of diseases in the set up of diagnostic/prognostic assays (3).

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P31509-025

Peptide-based immunoassays: a challenge for high performance diagnostics & prognostics of autoimmune diseases

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Autoimmune diseases are considered now as a plague. In fact some autoimmune diseases previously considered rare are actually increasing their frequency because of an earlier diagnosis (e.g. celiac disease). Possibly also an environmental factor (bacterial and/or viral infection) should contribute to autoimmune diseases. The idea we have been investigating for years and more recently proposed by others, is that aberrant post-translational modifications, i.e. glycosylation, deimination etc., create neoantigens triggering autoantibodies. This could explain why proteins (both recombinant and isolated), components of target organs or tissues, are failing. Anyway, it is evident that one single biomarker will never enable to reach successful diagnostic & prognostic tools. On the contrary, synthetic peptides specifically modified (with sugars, citrulline, lipoyl moieties, etc.) are interesting tools to fishing out of patients' sera these autoantibodies. We have recently reported that this can be efficiently done following a "Chemical Reverse Approach" 1.. We successfully applied this strategy in the development of the first Multiple Sclerosis Antigenic Probe [MSAP]: an N-glucosylated peptide characterised by a β-hairpin structure exposing at the best the minimal epitope Asn(β-Glc) involved in antibody recognition (2). A wider application of our SAP is obtained by its citrullinatation and/or galactosylation (3) useful for rheumatoid arthritis or lipoylation for investigating primary biliary cirrhosis.

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P31510-043

Epitopes of potato virus Y coat protein recognized by monoclonal antibodies

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Potato virus Y (PVY) strain groups are based on host-response and resistance-gene interactions. The strain groups PVYO, PVYC and PVYN are well-established for the isolates infecting potato in the field (reviewed in Singh et al. 2008). In this study we mapped the epitopes of three commercially available monoclonal antibodies directed to PVY coat protein(CP) and provided by Adgen (Neogen) using the SPOT method (Frank, 2002). While the differences of the CPs are not fully correlated with the biological differences of PVY strains (Singh et al. 2008), detection with these MAbs will serve as a useful preliminary characterization of unknown isolates of PVY from the field to make predictions on how globally they recognize or distinguish strains and isolates of PVY. Such predictions will be increasingly feasible with use of sequence data accumulating from the CP-encoding sequence of PVY isolates. Our data suggest that the three MAbs are useful for distinguishing PVYN from PVYO and PVYC, or for detection of all strain groups, at the global level.

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P31516-026

Evaluation of peptide arrays in serodiagnosis using CelluSpot™ method

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The aim of this project is to develop a method for synthetic peptides in biochips used in point-of-care serodiagnostics. We show that CelluSpot™ peptide arrays are applicable to diagnosis of parvovirus B19 infections. In addition, CelluSpot™ array screen provided a new insight in the previously identified parvovirus early epitope reactivity detected by Spot method and used as point-of-care EIA-test. A model for development of dedicated peptide array platforms of epitope type specific diagnosis is proposed.

P31521-027

A point-of-care test using a four-branched peptide for diagnosis of human parvovirus B19

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Human parvovirus B19 is the causative agent of erythema infectiosum ("fifth disease") the typical symptoms of which are fever and rash. Among adult patients arthralgia and arthritis are common, and an infection during pregnancy may cause foetal hydrops or death. The icosahedral capsid of human parvovirus B19 consists of virus proteins (VPs) 1 and 2. It has been shown that IgG antibodies recognizing conformational epitopes of VP2 are synthesized for many years after B19 infection. However, we have identified within VP2, a linear heptapeptide target sequence (KYVTGIN) of the IgG-antibodies that is specific for the acute phase of infection.

Using a branched synthetic peptide containing the epitope specific for acute-phase IgG, we set up a rapid assay (POC) for B19-virus diagnosis. In addition to being able to show the viral infection from a serum sample within a few minutes, at the point of care, the POC assays are inexpensive, easy-to-perform and do not require special equipment or laboratory.

As antigen in the POC assay we used a 24-amino acid peptide in four branches of a lysine core, with the KYVTGIN sequence in the middle. The peptide antigen was conjugated to gold particles and absorbed to a fabric ribbon and dried. In the test, a serum sample is added together with buffer, and the solution dissolves the conjugate. A positive result is obtained when the antibodies together with the gold conjugate bind to anti-human-IgG on the nitrocellulose, and form a specific coloured line which can be detected in the test window.

Serum samples were collected from patients with acute B19 infection, and control samples were drawn many years after infection; additional control sera came from subjects devoid of B19 antibodies.

The assay was shown to be stable in accelerated stability study. The conditions for industrial scale manufacturing were evaluated and the sensitivity and specificity were addressed, whereby the assay proved to be highly specific for acute B19 infection.

P31521-028

Antibodies against myelin oligodendrocyte glycoprotein(MOG) and myelin basic protein(MBP) as predictors of multiple sclerosis

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Multiple sclerosis(MS) is a chronic inflammatory disease of the CNS. Although most patients with MS initially present with a clinically isolated syndrome(CIS), the course of the disease is unpredictable at its onset and requires long-term observation. The aim of this study was to understand whether the presence of serum antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) in patients with a CIS predicts the interval to conversion to clinically definite MS. 25 patients with a CIS, positive findings on MRI and oligoclonal bands in the CSF were studied. At first, serum samples were collected to test for anti-MOG and anti-MBP antibodies with Western blot analysis and the lesions detected by MRI were quantified. Neurologic examinations for relapse or disease progression (defined as conversion to clinically definite MS) were performed at base line and subsequently every month. Patients with anti-MOG and anti-MBP antibodies had relapses more often and earlier than patients without these antibodies. Only 2 of 25 antibody-seronegative patients(8%) had a relapse and the mean time to relapse was 40 B±12 months. In contrast, 18 of 25 patients (72%) with antibodies against both MOG and MBP had a relapse within a mean of 8.5 B± 4 months and 5 of 25 patients (20%) with only anti-MOG antibodies had a relapse within 14.6+/-9.6 months ($P<0.0001$ for both comparisons with antibody-seronegative patients). The adjusted hazard ratio for the development of clinically definite MS was 79.5(95 percent confidence interval, 20.6 to 284.6) among the patients who were seropositive for both antibodies and 33.6 (95 percent confidence interval, 9.5 to 104.5) among the patients who were seropositive only for anti-MOG antibodies, as compared with the seronegative patients. According to the results, antibodies against MOG and MBP in patients with a CIS is a rapid, inexpensive and precise method for the prediction of early conversion to clinically definite MS.

P31521-029

Structure identification and binding affinities of neuroprotective β-amyloid (Aβ) epitopes recognized by a single-chain llama anti-Aβ-antibody and the protease inhibitor cystatin C

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Alzheimer's disease (AD) is a chronic neurodegenerative disease characterized by a progressive loss of memory and cognitive decline, for which the aggregation and plaque-formation by the β-amyoild (Aβ) polypeptide has been identified as a key event. Recently, unpaired variable domains of llama single chain antibody (VHH) fragments against Aβ have been found to exert considerable therapeutic potential for AD. VHH represents the smallest antigen-binding unit with a molecular size of ~15 kDa, compared to Ig- heavy and light chain variable domains, Fab fragments and complete IgG antibodies. Human cystatin C (hCC) is a cystein protease inhibitor present in all human body fluids which has a propensity to co-associate with Aβ-plaques/fibrils, and has plaque-inhibitory properties. Using proteolytic extraction and excision of the llama-VHH-Aβ(1-40) immune complex (e.g., trypsin, Glu-C protease) in combination with electrospray ionization (ESI)- and MALDI- mass spectrometry, the Aβ- binding epitope was identified at the middle-carboxyterminal domain of Aβ, Aβ(17-28). An analogous mass spectrometric approach was employed for the identification of the binding epitopes of the hCC- Aβ - complex, using immobilized hCC and Aβ-affinity matrices. An almost identical minimal epitope to that of the VHH-anti-Aβ-antibody (Aβ(17-24)) was found, which binds to a specific C-terminal domain of hCC, hCC(101-117) 1.. The identified hCC epitope peptide was found to specifically inhibit Aβ-oligomerization in vitro, in agreement with the Aβ-epitope domain interefering with the Aβ-aggregation. The identified Aβ and hCC epitopes represent new lead structures for designing neuroprotective inhibitors of the Aβ-aggregation process, and for molecular AD diagnostics.

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P31600-030

Using peptide arrays to reveal mechanisms of apoptosis

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ASPP2 is a pro-apoptotic protein that stimulates the p53-mediated apoptotic response. The C-terminus of ASPP2 contains Ankyrin repeats and an SH3 domain ($ASPP2_{Ank\text{-}SH3}$), which mediate its interactions with apoptosis-related proteins such as p53 and Bcl-2. We have used a combination of membrane-bound peptide arrays and biophysical methods to study the protein-protein interactions of ASPP2 at the molecular level in order to reveal their possible role in apoptosis. Using peptide arrays, we have mapped the binding interfaces of ASPP2 with its partner proteins such as Bcl-2, NF-κB and other proteins that are involved in apoptosis. We then applied the peptide array results to study profoundly the interactions of ASPP2 with proteins from the anti-apoptotic Bcl-2 family. We found that $ASPP2_{Ank\text{-}SH3}$ binds to Bcl-2, Bcl-XL and Bcl-W at two homologous sites in all three Bcl proteins tested: (i) The conserved BH4 motif (ii) A binding site for pro-apoptotic regulators. Quantitative biophysical analysis of the interaction with the free peptides revealed that the binding was selective, and the BH4 domain of Bcl-2 binds tightest to ASPP2. We propose a mechanism in which ASPP2 induces apoptosis by inhibiting functional sites of the anti apoptotic Bcl-2 proteins. The array screening results also served as a basis for docking studies that resulted in binding model for the complex between the full length protein Bcl-2 and $ASPP2_{ANK\text{-}SH3}$. We conclude that the use of combinatorial methods such as peptide arrays, combined with quantitative biophysical techniques, can significantly contribute to better understanding of biological pathways.

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P31613-031

Micron-sized monodispersed polystyrene for synthesis, tagging and biological screening of small molecules

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The unique available in vivo high throughput screening system was proposed by Ruoshlati and Pasqualini 1.. The system is based on the use of a library of filamentous phages expressing at their surface a high number of degenerated peptide sequences. Each individual phage expresses only one peptide sequence. The selection and identification of peptide sequences on phages associated with a tumor or other tissue after IV administration of the library, permits identification of novel tumor/tissue targeted peptide sequences. However, this approach is intrinsically limited to peptides. The aim of our work is to develop a new chemistry-based technology to overcome the limitation of phage libraries, thus extending the scope of in vitro/vivo biological panning to non-peptide molecules using a synthetic "phage-like" system. In this presentation we have developed a micron-sized mono-dispersed polystyrene system with magnetic susceptibility on which special tags and candidate ligands can be tethered and/or directly synthesized and screened. As a first prove of concept we have synthesized several model peptides with high reproducibility using fluorescent intensity and hapten tagged microspheres suitable for FACS and ELISA analysis. The spheres were tested in vitro for binding to selected isolated proteins and in cellular PC-3 model for binding of libraries to cancer cells.

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P31613-032

Production of peptide arrays consisting of labeled structured peptide and glyco-peptide libraries for the construction of protein detection systems

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Since high-throughput protein-detection and characterization systems are urgently required, a novel concept had been proposed [JP WO2002/090985]. This involves visualizing the interaction between proteins and labeled peptides as a bar-code using fluorescent intensities to generate "protein fingerprints". The use of peptide arrays as a gprotein-chip h affords significant advantages for industrial production and applications, in particularly for practical manufacturing, storage and delivery, compared to arrays with antibodies or recombinant proteins. The following key areas have been successfully investigated: 1) High throughput syntheses and characterization of labeled peptides as capture molecules, 2) Development of novel chip-materials, 3) Use of novel surface chemistries and quality control methods, and 4) Deposition of labeled peptide solutions on to the chip surface. Peptide libraries consisting of several hundred structured peptides with α-helices, β-loops and β-sheets have been prepared in a high-throughput manner and used for protein recognition [reviewed by Nokihara, K., et. al.(2004) Kobunsi Ronbunshu, 61, 523-532]. The protein-protein interaction is based on the recognition of different structural motifs, thus the present biochip can discriminate the structure of different proteins in analytes. The library has recently been expanded to include O-linked glyco-peptides that have been prepared by Fmoc-SPPS using a building block strategy. This was considered desirable as glyco-proteins play an important role in bio-recognition. In fact, some toxicant proteins, such as Ricinus communis agglutinin, cholera toxin, Vero toxin, and lectins, bind carbohydrate. The present paper describes the use of prototype arrays to detect prion proteins and their related peptides, as well as the above toxins and biological fluids. The present peptide array may be useful for the detection of secreted proteins in plasma, especially for biomarkers characterizing particular diseases

P31624-033

CelluSpots™ peptide arrays for immunodiagnosis and kinase profiling

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Peptide arrays are useful tools to characterize antibodies, to determine sequence specificities of enzymes (e.g. kinases) or to find interaction partners to given peptide sequences. One popular format for such arrays is a cellulose sheet with hundreds of synthetic peptides bound to it. These SPOT-arrays have been used successfully in a broad range of applications since their invention 15 years ago 1.. A drawback is the use of large reagent volumes and the limited throughput with only one copy of the library. CelluSpots™ represent a new method [2,3] that allows the production of hundreds identical peptide arrays from a single synthesis run on individual membrane disks. The peptides are synthesized on a modified cellulose support which is dissolved in a cleavage-mixture after the synthesis. Resulting solutions of peptide-cellulose-conjugates are then spotted onto coated microscope slides by conventional spotting techniques. The identical arrays are useful tools for large, parallel sera screening projects.

Here we present the use of CelluSpots™ arrays for a screening of 200 human sera samples against overlapping peptides representing different borrelia antigens. Clustering results of positive and negative control sera show immunodominant epitopes that are of interest for diagnostic tests and vaccine development. As second application example we present results obtained by kinase incubations on arrays containing annotated kinase substrates and consensus sequences of known kinase targets. The arrays are compatible with detection methods like autoradiography, chemiluminescence or enzymatic color development which can be performed without expensive instrumentation.

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P31700-034

De-Novo Design of Proteins for Molecular Electronics Applications

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There is a great importance in biological macromolecules integration within electronic devices. This is since these molecules are expected to be suitable both as the active components of electronic devices, or as guides for bottom-up assembly of hybrid structures. The goal of this research is to study the assembly and electronic properties of novel devices that exploit synthetic protein molecules.
Here we show the fabrication of de-novo protein based diode-like devices. We have chosen coiled coil protein structures, which can adopt two conformational states that differ in their internal molecular dipole. The proteins have been equipped with surface binding groups, typically cysteine thiols, on both ends. Dithiol bridges at one side facilitated self assembly on gold surfaces. Protected Cys residues were placed on the other end that after deprotection will allow successive binding processes in order to complete the device assembly process. Characterization of the correct folding in solution has been achieved by HPLC and CD. The dependence of the dipole in the dimeric protein has been shown using large scale Kelvin probe and high resolution Kelvin Force Microscopy (KFM) measurements.
We believe that this work will contribute to the understanding of electronic processes in proteins and may serve as foundation for their exploitation in device configurations by making use of the ability to trigger conformational changes in order to control device activity.

P31918-035

Synthesis of new tetracyclic RGD peptides for specific tumor cell targeting.

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The integrin family of adhesion molecules participate in important cell–cell and cell–extracellular matrix interactions in a diverse range of biological processes. The aVb3 integrin is overexpressed in several types of cancer cells and play an important role in angiogenesis as well as in tumour cell migration by interacting with vitronectin on the extracellular matrix mainly through the recognition of the tripeptide sequence RGD. The search for highly selective ligands to target the endothelial cell integrin aVb3 is currently the focus of many research groups as they may represent new therapeutics or valuable diagnosis in a number of areas such as metastasis, angiogenesis, arthritis and retinopathy. To date, the cyclic (-RGDfX) peptides and related N-methylated analogues developed by Kessler's group are among the most active and selective compounds for the aVb3 integrin receptors. For instance, the use of c(-RGDf(NMe)V-) is actually evaluated in several clinical trials. Therefore, we designed a new class of cyclic tetrapeptides. RGDK peptides were first synthesized by solid phase synthesis then cyclized in solution through a urea bond using N,N'-carbonyldiimidazole. Using SkmeL28 and A549 cells, expressing and non-expressing aVb3 respectively, we demonstrate that one of our peptide showed a better internalization than the reference peptide cRGDfE. Our strategy allowed the coupling of the best cyclic recognition peptide through its extracyclic acidic group to any molecular moieties containing an amino group that makes him a great cadidate for easy multimerization. Along this line, different applications are currently developped in our group.

P32000-036

Design and synthesis of amino acyl esters of unprotected saccharides as potent inhibitors of ACE

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Angiotensin converting enzyme (ACE) regulates the blood pressure by modulating rennin –angiotensin system. Several synthetic drug and bio-molecules are available for inhibition of ACE and possess antihypertensive effect. Recent investigations have revealed that glycoconjugates possessing an ester bond between the bioactive moiety and one of the sugar hydroxyls are valuable tools in biochemical and medical research. Carbohydrate-amino acid conjugates are new compounds which might be useful in different aspects of this field. In this study, we described a simple method for aminocylation of unprotected saccharides with mildly activated amino acids esters as potential ACE inhibitory activity. As a model reaction, we investigated esterification of sucrose -" royal carbohydrate" with cyanomethyl ester of benzyloxycarbonyl-phenylalanine.The difference of reactivity between the eight hydroxyl groups is a key factor in chemical exploration. In polar solvent as DMF or DMSO the primary positions might react faster than secondary ones if the reaction is essentially sensitive to steric interactions. On the other hand, the reaction might be more sensitive to the activity of the alcohol functions. In this respects, 2g-OH has been shown to be the most reactive among eight hydroxyl groups of sucrose. After removal of benzyloxycarbonyl group, the products possess groups which can accommolated in the hydrophobic S1 and S2 subsites of angiotensin I converting enzyme. The free amino group in the amino acid- carbohydrate esters can also serve as good ligands for Zn2+ in the ACE active site. Carbohydrates possess both hydrophobic and hydrophilic groups in their structure and could also bind with enzyme subsites. The measurements of IC50 value of newly amino acyl esters of carbohydrates are in progress.

P32114-037

Water-soluble covalent conjugates of peptide epitops of VP1 protein of foot-and-mouth disease virus (FMDV) with anionic n-vinylpyrolidone copolymers and their immunogencity

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Synthetic polyelectrolytes (PE) have been widely used to modify proteins via complex formation and covalent attachment, increasing (or reducing) the immunoreactivity and/or immunogenecity of originally antigenic proteins and improving their in vivo stability with prolonged clearance times. Such conjugates seem to be great importance for medicine and immunobiotechnology in particular with respect to drug delivery and vaccine innovation. Synthetic peptides are promising candidate vaccines for the control of viral diseases. Previous studies with Foot-and-mouth disease virus (FMDV) have identified fragments of isolated VP1 protein

and synthetic peptides from VPI which stimulate antibody production, albeit of poor neutralizing activity, or are recognized by antivirus antibodies. FMDV is an attractive model with which to study the potential of peptide-based synthetic vaccines. In this study, we sought to evaluate the immunogenecity of a candidate containing 135-161 synthetic peptide epitops of VP1 capsid protein of FMDV. The immunogenic properties of the conjugates were also investigated and the relationship between immunogeneticity and structure formation in the solutions is analyzed. A new high immunogenic protein-polymer complex and conjugates with antibody production, processing relatively prolonged times was obtained. It was obtained that a single immunization of mice with PE-peptide bioconjugates without classical adjuvant increased the primary and secondary peptide-specific immune response to FMDV.

P32114-038

Molecular-weight distribution and structural transformation in water-soluble conjugates of poly(acrylic acid) and synthetic peptide

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Water-soluble and insoluble protein–polyelectrolyte complexes as functional biopolymer systems represent a specific class of polymer–protein compounds that have important applications in various areas. Polyacrylic acid (PAA) is a well known bioactive polymer (bioadhesive nano- and microparticles, pH-sensitive PAA grafted poly(vinylidene fluoride) membrane bags, ultrafine cellulose fiber surfaces grafted with PAA for enzyme immobilization, PAA–polyvinylpyrrolidone biopolymeric systems for the treatment of the dry eye, etc. PAA, their alkyl-esters and non-toxic copolymers with vinyl pyrrolidones are strong adjuvants for primary and secondary responses and that they are promising alternatives to the mineral oil-based adjuvants presently used in various veterinary vaccines. In this study, the interaction between peptide epitops of VP1 protein of foot-and mouth disease (FMDV) and polyacrylic acid (PAA) will be discussed on the basis of the experimental results obtained by the size-exclusion chromatography (SEC) with on-line quadruple detection system: UV absorption (UV), refractive index (RI), right angle light scattering (LS) and viscosity (VIS) detectors and the binding coefficient, i.e., the binding ratio of peptide to polyacrylic acid.

P32317-039

Human IgG-specific binding peptides to distinguish normal and abnormal conformers: Applications for IgG purification and detection

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In recent years, human immunoglobulin G (IgG) attracts attention as protein of the main format of the antibody medicine. In the purification of IgG, Protein A originated from bacteria is frequently used as a affinity ligand, but several problems such as the contamination of endotoxin and the deterioration of protein A by the repeated use were pointed-out in the use of protein A. On this account, the development of low molecular ligands and mimic peptides which can be used instead of protein A has been performed. We have searched for the peptides which specifically bind to human IgG using a T7 random peptide phage library and, as a result, succeeded in isolation of two kinds of peptides called Type I and Type II. Type I peptide recognize normal structure of human IgG and can be used for the purification and the detection of IgG. However, it not only binds to human IgG but also to mouse and rabbit IgG with comparatively high affinity. On the other hand, Type II peptide is extremely specific only for human IgG. A more important thing is that Type II peptide does not bind to IgG in serum, but get possible to bind to IgG antibody purified by a protein A column. From this result, we elucidated the abnormal structure of IgG is generated by acid condition used in the elution from a protein A column and Type II peptide recognizes this specific structure. In this presentation, we report the results that the type I peptides functioning as an affinity ligand instead of protein A can be applied for the purification system of the antibody by improvement by amino acid substitutions and chemical conversion. Furthermore, we also report the generation condition of an abnormal conformer of IgG structure by pH and the temperature and the effective removal of the generated specific conformer by the Type II peptide-immobilized column.

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P32607-040

LC-ESI-MS Characterization and Prep-RP-HPLC purification of FMDV Synthetic Viral Peptides

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Peptide synthesis has got more and more importance on the last decades. Synthesis is partly complicated job of the desired peptide sequence. There are different methodology for synthesis (t-Boc, F-moc, etc.). But the vital parts of the producing synthetic peptide is the characterization and purification. Because characterization and purification are an answer for the question of "What is the synthesis result?"

In this study we characterized FMDV (Foot and Mouth Disease Virus) synthetic peptides with LC-ESI-MS (Shimadzu) and purified them with Prep-RP-HPLC (Shimadzu). ESI (ElectroSpray Ionization) is one of the medhod for ionisation for MS analysis. The other medhods are API (Atmospheric Pressure Ionisation), APCI (Atmospheric Pressure Chemical Ionisation). We used TFA or Formic acid in LC-MS analysis. And the gradients for Prep-RP-HPLC and LC-MS are H_2O-Acetonitrile.

All the peptides were synthesised in our laboratory with Liberty Automated Microwave Synthesis Workstation (CEM), by using Solid Phase F-moc Chemistry. After synthesis they cleaved with appropriate cocktails. Peptide sequences are;

VP1 200-213 WDRHKQRIIAPAKQLQ
VP1 135-161 WSKYSTTGERTRGDLGALAARVATQLPA,
VP1 40-60 WVKINNTSPTHVIDLMQTHQHG

P32715-041

Synthesis and Evaluation of Peptide Analogues as functional Probes targeting Matrix Metalloproteinases

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The isolation and detection of low abundant enzymes and proteins is one of the most challenging tasks in bioanalytical and pharmacological fields, especially if information about their state of activity is required. For this purpose tailored solutions for addressing the members of a protein family are required. Peptide chemistry provides established methods to assemble building blocks to construct such molecular probes. We chose matrix metalloproteinases (MMP) for validation of such an approach. This protein family processes and degrades various extracellular matrix proteins and possesses a highly conserved catalytic site with a zinc ion in its centre. Most of the known and potent inhibitors are peptidomimetics containing zinc chelating hydroxamate groups (e. g. marimastat). Although it binds reversibly, it is potent and active against a wide range of metalloproteinases. It was chosen as synthetically available binding group to target the protein in its active state. It was modified by peptide

chemistry in order to introduce multiple functions. Depending on the purpose, different reporter groups, photoreactive or cleavage sites were chosen.
The probes were tested positively to inhibit various human recombinant MMPs using activity assays. MMP were isolated using Streptavidin coated magnetic beads and a biotinylated probe. Furthermore a photoreactive group was introduced to enhance the binding to the target protein. A covalent interaction was achieved by irradiation of a probe protein mixture, isolation with magnetic beads and cleavage from the beads.

P32900-042

Pyclock superior coupling reagent for biosensors construction based on peptides coupling onto solid phase polymer

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Electrochemically biosensors are valuable analytical tools for the monitoring of various biological/ chemical molecule levels, and tremendous research effort has been put into the development of such accurate analytical devices. One crucial aspect in the fabrication of a biosensor is the deposition of biological macromolecules in high amounts with retention of their specific activity. Among the various deposition methods of biomolecules, such as direct adsorption onto the surface, cross-linking, covalent attachment by carbodiimide chemistry is one of the few methods that allows a stable deposition into a biocompatible environment. Hence the urgent need for proteins coupling with high yield to solid surface in the aim of biosensors construction. However the use of carbodiimide as peptide coupling agents was found to be limited in various aspects. Thus, during the activation of hindered carboxylic components, such as those involved in bulky protein coupling reactions to solid phase fibers PyClocK was found to be very efficient for slow coupling reactions and it can be used in excess to assure a complete activation of the carboxylic function. In this study 6-Chloro-Benzotriazole-1-yl-oxy-tris-Pyrrolidino-Phosphonium Hexafluorophosphate Coupling Reagent (PyClocK) was shown to have better performance than 1-ethyl-[3-(dimethyl¬amino)propyl]-3-ethylcarbodiimide (EDAC) and hydroxysulfosuccinimide (NHSS) in peptides large sequence coupling to solid phase fibers.

P32627-044

New Strategy for Protein Identification - Improving Signal Intensity and Sensitivity of MALDI Mass Spectrometry by Specific Peptide Derivatization

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General approaches used in protein identification and characterization involve consecutive purification steps. Then the desired protein extract is submitted to mass spectrometry analysis (LC-MS/MS) after enzymatic digestion. Technical difficulties are involved in determining the PMF of a protein particularly in relative low abundance. A typical protein will give rise to at least twenty to thirty peptides after trypsin digestion. Not all of these peptides will appear in MALDI analysis. One factor that is believed to cause incomplete detection is competition for protonation during the ionisation process inducing ion discrimination. We have recently developed a new technology allowing specific labeling of lysine residues in proteins and easy MALDI-MS detection and identification of labeled peptides following protein hydrolysis(1). N-hydroxysuccinimide ester of α-cyano-4-hydroxycinnamic acid (CHCA) was used as a labeling reagent to increase MALDI signal of Lysine-containing peptides in Cytochrome C proteolytic mixture. This original approach enables to discriminate labeled peptides of interest among other abundant peptides. Herein, we report the optimization process to investigate the limits of this tool.

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P40000-062

A hexapeptide that blocks SARS-Coronavirus

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A defined receptor-binding domain (RBD) on the viral spike protein (S) mediates the attachment of SARS-Coronavirus to its cellular receptor, angiotensin-converting enzyme 2 (ACE2). We have synthesized peptide libraries for identification of RBD binding epitopes by surface plasmon resonance (SPR) and saturation transfer difference (STD) NMR spectroscopy. Three dodecapeptides were identified to have significant affinity to ACE2; the best of them had a KD = 85 µM . Further refinement yielded a hexapeptide from Y438 to L443 (YKYRYL) of S that bound ACE2 at KD= 46 µM. STD NMR spectroscopy reveals close contacts of the aromatic tyrosine residues to the receptor.
The peptide was also analyzed for antiviral activity using an in vitro assay that measures SARS-CoV infection of Vero cells. At a concentration of 7 mM virus replication was reduced about 100 fold. No replication occurred at peptide concentrations above 10 mM. The peptide blocks the binding site on ACE2 that is necessary for the virus to infect the cells. It can be used to design peptidomimetic compounds as entry inhibitors for SARS-CoV. To exclude other effects that were due to unspecific inhibition of viral replication, the peptide was tested during an alpha virus infection of Vero cells. No significant inhibition was observed. However, for the human Corona Virus NL63 that causes severe colds in humans and that uses the same receptor a clear inhibition could be observed comparable to the results obtained for SARS-CoV.
Additionally, we have synthesized a hexapeptide library with amino acid substitutions of the chemical lead YKYRYL and measured their binding affinity to ACE2 via SPR to analyze the importance of the individual amino acids. SPR studies indicate an important role of R441.

P40100-001

Development of N-acyl-amino acid bisphosphonate amide derivatives as potent bone metastatic prostate anti-cancer agents

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Prostate cancer is one of the most common malignancies in men and is responsible for more deaths than any other cancer, except for lung cancer. In the last decade we have developed bradykinin antagonist peptides (B10234, B10238), peptide dimer (B9870, Suim-(D-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-D-Igl-Oic-Arg)2, Suim: suberimidyl; Hyp: *trans*-4-hydroxyproline; Igl: α-(2-indanyl)glycine; Oic: 2S,3aS,7aS-octahydro-1H-indole-2-carboxylic acid) and four generations of our small molecule, BKM-570 which showed high inhibition against lung cancer *in vitro* and *in vivo*. Some of the compounds also showed inhibition against prostate cancer. It is well known that prostate cancer metastasizes into bones. While prostate cancer itself has many treatment options, no drugs currently exist for the treatment of bone metastatic prostate cancer. We modified our potent anti-cancer small molecules by incorporating an aminobisphosphonate group to target these compounds to bone. BKM-570, F5c-OC2Y-Atmp (F5c: 2,3,4,5,6-pentafluorocinnamoyl; OC2Y: (O-2,6-dichlorobenzyl)-tyrosine; Atmp: 4-amino-2,2,6,6-tetramethylpiperidine) is the first generation of our small molecules. This compound consists of three parts: A, an acyl group; B, a tyrosine amino acid residue and C, an amide group. We introduced aminobisphosphonate or aminobisphosphonate derivatives at position C, and the new N-(*bis*-phosphonatoalkyl)amide small molecules had anti-cancer activity against prostate cancer metastases in mice. One of these compounds has been shown to be effective against the growth of pre-established human prostate tumors in mouse skeleton through the induction of apoptosis and blockade of survivin expression. The synthesis of these aminobisphosphonate small molecules, the structure relationship studies and the biological activity of these compounds in cultured human prostate cancer cells and animal models will be presented.

P40100-002

Novel anti AIDS Lead Compounds that Inhibit the viral Integrase Protein

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The HIV-1 Integrase protein (IN) mediates the integration of the viral cDNA into the host genome. IN is an emerging target for the design of novel drugs against AIDS, and the first IN-inhibitor, Raltegavir, was approved by the FDA in October 2007. Our research in the past few years focuses on the development of peptide inhibitors of IN. We designed inhibitors using two approaches: (1) Rational design based on protein-protein interactions of IN: Peptidic sequences derived from IN-binding sites of IN-binding proteins are already optimized by nature to bind IN and have the potential to inhibit it. Using this approach we developed several IN inhibitors: two peptides derived from the IN-binding loops of the cellular binding partner of IN, LEDGF/p75 (LEDGF 361-370 and LEDGF 401-412), and two peptides derived from the HIV-1 Rev protein, which we found to bind IN; (2) A peptide selected using combinatorial library screening using the yeast-2-hybrid system, revealed a 20-mer peptide, termed by us IN(1) All five peptides bound IN with low micromolar affinity and inhibited the DNA-binding of IN as well as its enzymatic activity in vitro. These peptides penetrated cells and consequently blocked HIV-1 replication in infected cultured cells due to the lack of integration. The most potent peptides in vitro and in cells were LEDGF 361-370 and Rev 13-23. These two peptides significantly inhibited HIV-1 infection in mice model. We conclude that the five peptides, particularly LEDGF 361-370 and Rev 13-23, are promising anti-HIV lead compounds.

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P40100-003

Novel, potent and selective Angiotensin IV short analogues

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The hexapeptide angiotensin IV: H-Val-Tyr-Ile-His-Pro-Phe-OH (Ang IV) mediates a wide range of physiological actions, including control of blood flow and cognitive enhancement. It exerts its effects by binding to AT4 receptors, which are widely distributed across tissues. The AT4 receptor has been identified as the insulin-regulated aminopeptidase or IRAP. It has been proposed that Ang IV exerts its action by inhibiting the catalytic activity of this enzyme. We have reported that the β-homo amino acid containing analog H-β^{2}hVal-Tyr-Ile-His-Pro-β^{3}Phe-OH (AL-11) is a potent, selective and stable Ang IV antagonist, in which the β^{2}hVal is responsible for stability and the β^{3}hPhe for selectivity.[1] In this study we report the new Ang IV short analogues. The incorporation of *erythro*-βMePhe^6 or Tic^6 resulted in analogues which have great ability to inhibit the hydrolysis of Leu-p-nitroanilide by IRAP or by AP-N. Our data showed that the full Ang IV sequence is not necessary for high potency. Peptides with Pro deletion containing Tic or *erythro*-βMePhe were even more potent than full Ang IV sequence. Moreover a peptide

lacking His-Pro residues and stabilized by introduction of β²hVal resulted in an analog H-β²hVal-Tyr-Ile-Tic-OH (AL-35) that was very selective for IRAP versus AP-N and AT(1) .

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P40100-004

Design and synthesis of a non – peptide PAR1 thrombin receptor antagonist, using cyclohexane as template

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Receptors that mediate thrombin action are attractive drug discovery targets because of their involvement in cardiovascular pathophysiology (dysregulation of platelet aggregation and endothelial cell function). The cellular actions of thrombin are, in large part, caused by the activation of Proteinase-Activated Receptors (PARs) 1, 3 and 4 (Pharm. Rev. 54:203). The serine proteinase thrombin cleaves and activates cellular PAR1 in many pathophysiological settings associated with hemostasis, tissue injury and the proliferation of vascular smooth muscle and tumor cells. In the present study, we synthesized a novel non-peptide PAR1 mimetic, based on a conformational analysis of the S_{42}FLLR46 tethered ligand sequence of PAR1 in order to inhibit the cellular actions of thrombin. The rational design, based on NMR constraints and Molecular Dymamics, led to compound **1** (Fig.1) containing the spatially closed key pharmacophoric guanidyl and phenyl groups, attached to cyclohexane as a template. Compound **1**, inhibited both TFLLR-amide (10 μM) and thrombin (0.5 and 1 U/ml)-mediated calcium signaling in a cultured human HEK cell assay (J Pharm. Exp Ther. 288:358).

P40103-005

Synthesis and folding of the circular cystine knotted cyclotide cycloviolacin O2

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The cyclic cystine knot motif, as defined by the cyclotide peptide family, is an attractive scaffold for protein engineering (1). However, to date the utilization of this scaffold has been limited by the inability to synthesize members of the most diverse and biologically active subfamily, the bracelet cyclotides. Here we describe the synthesis and first direct oxidative folding of a bracelet cyclotide, cycloviolacin O2, and thus provide an efficient method of exploring the most potent cyclic cystine knot peptides (2). The linear chain of cycloviolacin O2 was assembled using Fmoc solid phase peptide synthesis and cyclized by thioester-mediated native chemical ligation, and the inherent difficulties of folding bracelet cyclotides were successfully overcome in a single step reaction. The folding pathway was characterized and included predominating fully oxidized intermediates that slowly converted to the native peptide structure.

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P40111-006

Search for modulators of VEGF-KDR interaction: Hydrocarbon-bridged VEGF derived peptides

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Angiogenesis, the process of new blood vessel formation, is important in both physiologic and pathologic situations, and its inhibition can be useful in the fight against several diseases, such as tumor development, diabetic retinopathy, etc. One of the key factors in promoting angiogenesis is the Vascular Endothelial Growth Factor (VEGF), which exerts its biological activity through interaction with specific receptors, VEGFR-1 (Flt-1), VEGFR-2 (KDR, Flk-1) and VEGFR-3 (Flt-4). VEGF is over-expressed in all examples of pathologic angiogenesis, and its effects on tumor growth are mainly mediated by KDR. Our approach to novel anti-angiogenic drugs is centered on the search for inhibitors of VEGF-KDR interaction. Directed mutagenesis studies allowed the identification of a surface of VEGF recognized by KDR, with residues Arg^{82}, Ile^{83}, Lys^{84}, His^{86} and Glu^{89}, located in a β–hairpin of loop 3, identified as essential for the interaction. Most residues in this region are also located in the interface of interaction between VEGF and some none-humanized phage-displayed antibodies with potent anti-angiogenic activity, suggesting a binding to VEGF similar to that of VEGF receptors. Starting from $VEGF_{81-91}$ fragment (MRIKPHQGQHI), we have designed different hydrocarbon-bridged analogues to preserve the β–hairpin native structure. This communication will describe the solid-phase synthesis of olefin-bridged (C=C) peptides and their saturated (C-C) analogues. Considering that the β-turn of native $VEGF_{81-91}$ is slightly distorted, due to the presence of an extra amino acid residue, in addition to the bridged undecapeptides, two series of decapeptide analogues have also been prepared, just by removing the residues Glu^{87} or Gly^{88}. The conformational behavior of linear and bridged-peptides has been analyzed by NMR (NOE, ^{1}H and ^{13}C chemical shifts). The ability of these peptides to adopt the native VEGF β-hairpin structure will be compared with their anti-angiogenic activities.

P40121-007

Rational Design of Highly Active and Selective α5β1 Integrin Antagonists

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Integrins constitute a family of heterodimeric, transmembrane cell adhesion receptors which connect cells to the scaffolding proteins of the extracellular matrix. The pioneering observation that integrins - especially αvβ3 and α5β1 - are hallmarks of metastatic cancer and seriously involved in the process of tumor angiogenesis turned them into attractive targets for cancer therapy. Out of that, the inhibition of integrin function is a major challenge in medicinal chemistry. Potent ligands are currently in different stages of clinical trials for the antiangiogenic therapy of cancer and age-related macula degeneration (AMD). Especially the subtype α5β1 has recently been drawn into the focus of research due to its genuine role in angiogenesis.(1) In here, we describe the rational design and the synthesis of high affinity α5β1 binders and the optimization of their activity and selectivity against αvβ3 by means of extensive SAR-studies and docking experiments.(2) Starting from a tyrosine scaffold(3) we succeeded in getting compounds with affinities in the low and even sub-nanomolar range and selectivities of 400 fold against αvβ3. The insights about the structure-activity-relationship

gained from the tyrosine based ligands could then be successfully transferred to ligands bearing an aza-glycine(4) scaffold to yield α5β1 ligands with affinities of even sub-nanomolar range and selektivites exceeding 10.000 fold.

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P40128-008

Modeling studies and biological activities of a non-peptide AT_1 receptor Angiotensin II antagonist

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The octapeptide Angiotensin II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) is the major factor of the Renin–Angiotensin System (RAS) and plays a significant role in the regulation of arterial blood pressure. In the present study, we have modeled a Losartan analogue, the non-peptide Angiotensin II AT_1 antagonist, 5-butyl-1-hydroxymethyl-1-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl} imidazole (**V8**). Structure Activity Relationship (SAR) and Molecular Modeling Studies indicate close proximity of hydroxymethyl and tetrazole pharmacophoric groups of antagonist **V8** and Losartan. Conformational Analysis was performed using a Grid Scan Search in order to derive all the possible conformations from which six energy local minima (syn and anti) were extracted after a Cluster Analysis. Furthermore, these different conformations were superimposed with Losartan, where a spatial correlation among the pharmacophoric groups is observed. Antagonist **V8** showed similar potency with Losartan in our *in vivo* model with anesthetized rabbits and *in vitro* binding studies to AT_1 receptor. At specific concentrations compound **V8** showed higher affinity compared to Losartan indicating that reorientation of butyl and hydroxymethyl groups on imidazole template allows a better binding to AT_1 receptor.

P40300-009

Fast Automated Conventional FMOC Solid Phase Peptide Synthesis with HCTU

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1H-Benzotriazolium 1-[*bis*(dimethylamino)methylene]-5-chloro-,hexafluorophosphate (1-),3-oxide (HCTU) is a non-toxic, non-irritating and non-corrosive coupling reagent [1-2]. Seven biologically active peptides (GHRP-6, $^{65-74}$ACP, oxytocin, G-LHRH, C-peptide, $hAmylin_{1-37}$, and $\beta\text{-amyloid}_{1-42}$) were synthesized with reaction times reduced to deprotection times of 3 minutes or less and coupling times of 5 minutes or less using HCTU as the coupling reagent. No expensive coupling reagents or special techniques were used. Total peptide synthesis times were dramatically reduced as much as 42.5 hr (1.8 days) without reducing the crude peptide purities. It was shown that HCTU can be used as an affordable, efficient coupling reagent for fast Fmoc solid-phase peptide synthesis.

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P40300-010

Fast Conventional Synthesis of Chemokine SDF-1α (1-68) on the Symphony®

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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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P40417-011

Semi-automated microwave solid-phase peptide synthesis

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Despite the development of new coupling reagents and solid supports, SPPS is still often faced with difficulties in the assembly of long or "difficult" sequences, e.g. due to aggregation and steric hindrance giving rise to incomplete reactions. The use of convenient and precise heating with microwaves for SPPS has gained in popularity as it for many syntheses has provided significant improvement in terms of speed, purity, and yields, maybe especially in the synthesis of long and "difficult" peptides. Thus, precise microwave heating has emerged as one new parameter for SPPS, in addition to coupling reagents, resins, solvents etc. We have previously reported on microwave heating to promote a range of solid-phase reactions in SPPS.

Here we present a new, flexible semi-automated instrument for the application of precise microwave heating in solid-phase synthesis. It combines a slightly modified Biotage Initiator microwave instrument, which is available in many laboratories, with a modified semi-automated peptide synthesizer from MultiSynTech. A custom-made reaction vessel is placed permanently in the microwave oven, thus the reactor does not have to be moved between steps. Mixing is achieved by nitrogen bubling. Washing steps are automated, however the activated amino acid derivatives have to be added manually. First, we developed optimized protocols for short cycle times in semi-automated SPPS with general Fmoc chemistry. We utilized a microwave-compatible temperature probe for exact temperature measurements during microwave heating. Then we developed protocols for on-resin reductive amination for anchoring of the first amino acids to a BAL handle. Finally, we used the new instrument and the optimized protocols to assist in the synthesis of a range of difficult and long sequences. We believe that these successful syntheses demonstrate that this semi-automated instrument and the methods developed for it, can be an efficient starting point for SPPS with microwave heating.

P40423-012

Investigation of polyelectrolyte-antigenic peptide conjugates by fluorescence spectroscopy

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Fluorescence techniques have recently been used to study peptide-PE conjugates. From the fluorescent emission shift of tryptophan residues

in proteins or peptides, it is possible to localize the interaction between protein and PE at certain protein domains.
We investigate covalent binding mechanism of synthetic peptides which include tryptophan residue in the peptide sequence with copolymers of acrylic acid and N-vinylpyrolidone (VP\AA) depending upon the weight concentration ratio of components by fluorescence method. Antigenic peptides are synthesized by microwave assisted solid phase peptide synthesis method. Characterization of these peptides are performed with LC-MS. Subsequently these crude peptides are purified by Preparative HPLC system.
Peptide-polymer covalent conjugation are performed in organic and PBS media. Covalent conjugation are carried out by carbodiimide method. Carbodiimide is used for the activation of carbonile groups of synthetic polymer which is the binding area of peptides that includes free amino groups.
From the analysis of Peptide-PE conjugates, it is possible to discuss the mechanism of the conjugate formation and structure of forming particles.
According to the fluorescence analysis results, free peptide which containing Trp residue, gives fluorescence spectrum. However, after the conjugation reaction it is observed that products λmax decreased and it is characterized as blue shift of λmax. This indicates that when conjugates formed, Trp is completely isolated from aqueous solution.

P40511-013

Antiangiogenic Thrombospondin Type 1 Repeat Analogs: Synthesis, Structure, and Biological Activity

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The thrombospondin type 1 repeat (TSR) has been shown to inhibit angiogenesis and tumor growth in a number of *in vivo* models of cancer, but the details of this activity, including structure-function relationships, are not well understood. We explore these TSR elements in further detail via structural and biological evaluation of a series of TSR analogs.
The TSR domain has a characteristic fold consisting of a two-stranded β-sheet and a third 'rippled' strand. Three tryptophans on the 'rippled' strand form cation-π stacking interactions with two arginines on the adjacent β sheet, forming an extended cation-π network. In addition its structural importance, it has been postulated that the surface formed by this interaction is a key site for protein-protein recognition. A synthetic approach to the generation of novel TSR analogs, utilizing SPPS and native chemical ligation, has allowed us to rationally introduce unnatural amino acids in an effort to probe the structural and functional landscape of this clinically relevant protein domain.
We have synthesized analogs of the second TSR domain of human thrombospondin-1 (TSR2) designed to probe the importance of the cation-π stack. The arginine residues have been replaced by ornithine and citrulline, and thermal denaturation experiments by circular dichroism have been used to estimate stability differences between the mutant TSR2 and the native. Taken as a set, these thermodynamic measurements provide insight into the stability afforded by a cation-π interaction in the context of a native protein fold.
Synthetic access to the TSR2 domain also provides the opportunity for the introduction of various modifications, including the introduction of aminooxy groups as chemical handles, PEGylation for *in vivo* stability, and biotinylation to create an affinity caputure reagent for identification of protein-protein interaction partners.

P40518-014

Improving Pharmacokinetic Properties of Radiolabeled Bombesin Analogues by Incorporation of Polar Groups

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The tetradecapeptide bombesin (BBS) has a high affinity for the Gastrin-Releasing Peptide (GRP) receptor. These receptors are overexpressed in human tumors such as breast and prostate cancers. Therefore they can serve as targets for *in vivo* imaging and therapy of these tumors with ^{99m}Tc- and ^{188}Re-radiolabeled BBS analogues, respectively.
For the radiolabeling, a chelator is attached to the *N*-terminus of the BBS analogues. Our group developed the (N^{α}His)Ac chelator, which is easy to synthesize and has a very high affinity for the ^{99m}Tc- and ^{188}Re-tricarbonyl complexes.
However, an important part of the injected radiolabeled BBS analogues accumulated in healthy organs, such as liver and kidneys. To improve the pharmacokinetic properties, a BBS analogue was glycated via the Lys side chain of the spacer using the Maillard reaction. Unfortunately, during glycation, overalkylation on the secundary amine of the chelator was observed.
Therefore, two alternatives for this glycation were examined. The first one was based on the chemoselective reaction between a hydroxylamine (incorporated into the spacer) and an aldehyde (glucose). The second alternative was the Cu(I) catalyzed cycloaddition between an alkyne (incorporated into the spacer) and an azide (azido-glucose). These approaches for carbohydration circumvented the problem encountered during glycation via the Maillard reaction.
Glycation by the Cu(I) catalyzed cycloaddition was, by far, the easiest way to incorporate the glucose moiety. Moreover, after ^{99m}Tc labeling, this analogue showed the best biodistribution and the best diagnostic properties of all glycated analogues.
An alternate approach to improve the pharmacokinetics consisted of including different polar spacers such as β^3hGlu, β^3hAsp, β^3hLys, β^3hSer and -NH($CH_2CH_2O)_2$CO- between the (N^{α}His)Ac chelator and the bombesin sequence. In particular the analogues with a negative charge (β^3hGlu, β^3hAsp) in the spacer showed a favorable effect on the pharmacokinetics.

P40523-015

Determination of binding ratio of hydrofobic peptide-polymer conjugates by using fluorescamine assay

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Non-fluorescent 4-Phenylspiro-[furan-2(3H),1-phthalan]-3,3'-dione (fluorescamine) reacts readily with primary amines in amino acids, peptides and proteins to form stable, highly fluorescent compounds (fluorophors). Fluorescamine has been used to detect free amino groups on peptides or completion of coupling reactions in solid phase peptide synthesis and not only in aqueous solution but also in organic solvents and on solids.
In this study, peptide epitops of VP1 capsid protein of Foot-and-Mouth-Diseases Virus 40-60 amino acid residues (Val-Lys-Ile-Asn-Asn-Thr-Ser-Pro-The-His-Val-Ile-Asp-Leu-Met-Gln-Thr-His-Gln-His-Gly) were synthesized by Sigma. We synthesized the covalent conjugate of 40-60 amino acid residues with copolymers of acrylic acids and N-vinylpyrolidone (VP\AA) at different ratio of components (npeptide/nPolymer) and investigated the mechanism of condensation reaction by using different physicochemical analyses as HPLC, Fluorescence Spectroscopy and Fluorescamine assay.
For determination of binding ratio of hidrofobic peptide-polymer congutages, fluorescent measurements were performed in the presence of fluorescamine at the wavelengts of excitation 390 nm and emission 475 nm.
When conjugation reaction completed, the amount of free amino groups had decreased and observed that Fluorescence Intensity had decreased and the estimated degree of primary amino group after conjugation, calculated from fluorescence spectrums.

P40523-016

Determination of Conjugation Degree of Synthetic Peptide-Polymer Conjugates

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Fluorescamine (4-Phenylspiro-[furan-2(3H),1-phthalan]-3,3'-dione) which heterocyclic dione is a reagent for the detection of primary amines on peptides or completion of coupling reactions in the picomole range. Its reaction with amines is almost instantaneous at room temperature in aqueous media, organic solvents ect . Fluorescamine reacts with primary amines (in peptide, protein ect.) to form highly fluorescent product (fluorophors) whereas the reagent and its degradation products are nonfluorescent (1). This is the basis of a fluorescent protein assay [1,2]. Fluorescamine is used in many sensitive detection methods, e,g., characterization of poly-L-lysine (pLL)/DNA complexes post-modified with a multivalent hydrophilic polymer (3), or synyhetic peptide-polymer conjugates. In this study, Foot-and-Mouth-Diseases Virus VP1 capsid protein's synthetic peptide epitope which containing tryptophane (Trp), 135-161 (P1) amino acid residues (Try-Ser-Lys-Tyr-Ser-Thr-Thr-Gly-Glu-Arg-Thr-Arg-Thr-Arg-Gly-Asp-Leu-Gly-Ala-Leu-Ala-ala-arg-Val-Ala-Thr-Gln-Leu-Pro-Ala) were synthesized by using the solid-phase methods. We synthesized the covalent conjugate of copolymers of acrylic acids and N-vinylpyrolidone (VP\AA) with 135-161 amino acid residues at different npeptide/nPolymer ratio and investigated the mechanism of condensation reaction by using Fluorescence Spectroscopy and Fluorescamine assay. In the fluorescamine assay the conjugate solution was measured on a PTI QM-2003 Steady State Fluorescence Spectrometer with an excitation wavelength of 390 nm and emission at 475 nm. After the conjugation reaction, the amount of free amino groups had decreased because of binding carboxyl group and observed that Fluorescence Intensity had decreased. Determination of free amino group degree after conjugation, calculated from fluorescence spectrums.

P40600-017

Newly developed high strength and chemically stable silica gel based preparative reversed phase packing materials

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A new high strength silica gel and a bonding technology based on preparative bulk packing materials for HPLC have been developed to provide improved recovery, selectivity, and longer life time for the preparative peptide separations. The novel preparative silica particle was successfully prepared by the new generation process, which allows the higher gel density than typical silica gel and the particle size distribution would be practically mono-dispersed character. For the effective reversed phase peptide separations, pore size and pore volume of these new particle were optimized depending on the molecular weight of peptides. To enhance chemical stability and selectivity under the typical peptide purification conditions, the combination of chemical bonding method and functional group density was optimized for maximum performance. By repeated packing and unpacking of this synthesized gel with large dynamic axial compression column, it was demonstrated that no fine has appeared and no back pressure increasing has occurred comparing to commercially available packing materials. Also cost effective peptide purification with high loadability, productivity, and recovery was achieved with significant small and large peptides.

P40601-018

Peptide Antagonists to the Calcitonin-Gene Related Peptide Receptor (CGRP1) with Higher Receptor Affinity and Increased Plasma Stability.

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Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide produced by tissue-specific alternative mRNA splicing of the calcitonin gene. The CGRP peptide signals through a seven transmembrane G protein-coupled receptor (GPCR) belonging to the secretin receptor family. Co-expression of the calcitonin-like receptor (CLR) with receptor activity modifying proteins-1 (RAMP1) forms a mature CGRP receptor on the cell membrane surface. CGRP is widely distributed in the peripheral and central nervous systems. In the later, CGRP is expressed in trigeminal ganglia nerves and when it is released has potent dilator effects on cerebal and dural vessels. Through this mechanism, CGRP is involved in the regulation of blood flow to the brain and pain-sensitive meninges. The pathology of migraine has been associated with the vasodilation effects of CGRP on cerebal circulation. It has been reported that the CGRP peptide consist of an alpha-helical N-terminal region, a flexible center region, and two putative C-terminal beta-turns. Truncation of the first seven residues in CGRP results in antagonists of the CGRP1 receptor (IC50 4 nM), however, CGRP(8-37) is rapidly degraded in plasma. Here, we report the iterative design and synthesis of new high affinity CGRP antagonists with significantly increased plasma stability, and higher affinity for the human CGRP1 receptor.

P40700-019

Synthesis of conformationally restricted analogues of GnRH-I and III and studies on prostate cancer cell proliferation

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Mammalian Gonadotropin Releasing Hormone (GnRH-I) and the sea lamprey Gonadotropin Releasing Hormone type III (GnRH-III), belong to the class of conserved gonadotropin releasing hormone peptides. In addition to the classic hypophysiotropic action of GnRH-I, it has been shown that many malignant cells, such as prostate cancer cells, secrete GnRH-I and express the GnRH-I receptor/s. GnRH-III has no endocrine activity in mammals even at high doses, but has been shown to suppress directly the growth of breast and prostatic cancer cells in vitro. In a continuation of our previous work and in order to study the effect of modifications in positions 4 and 6 of Leuprolide, an agonist of GnRH-I, on prostate cancer cell proliferation, we synthesized ten new conformationally restricted analogues. D-Leu6 of Leuprolide was substituted by D-Lys, D-or L-Glu and Ser4 by N–Me-Ser. We also synthesized five new analogues of GnRH-III and studied their effect on prostate cancer cell proliferation. Asp in position 6 of GnRH-III was substituted by Asn and Glu, Pro9 was substituted by á-aminoisobutyric acid (Aib) and Trp3 and/or Trp7 by D-Trp. Peptides were synthesized by the solid phase methodology and Fmoc/But chemistry in high yields. Results show that the inhibitory effect of GnRH-I analogues on the proliferation of human prostate cancer cells depends on the nature of the substituted amino acid in position 6. Incorporation of D-Trp in positions 3 and 7 of GnRH-III preserved its antiproliferative activity, whereas all other modifications reduced its activity.

P40700-020

Antimicrobial properties of new mastoparan analogues

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Mastoparan (MP) is an antimicrobial cationic tetradecapeptide with following primary structure INLKALAALAKKIL. This amphiphilic α-helical peptide was originally isolated from the venom of wasp *Paravespula lewisii*. However some MP analogues (tetradecapeptides with different amino acids sequence) have been also isolated from the venom of hornets, yellow jackets and paper or solitary wasps. They are rich in hydrophobic amino acids such as Leu, Ile, Ala and commonly posses Lys residues.

MP shows a variety of biological activities such as inhibition of the growth of Gram-positive bacteria, activation of mast cell degradation and histamine release, activation of phospholipase A2 and C or erythrocyte lysis. MP is also turned out to enhance the permeability of artificial and biological membranes and activate GTP-binding regulatory proteins by a mechanism analogous to that of G protein-coupled receptors.

Nowadays many mastoparan analogues have been synthesized. Some of them contains in primary structure the mastoparan sequence but they loss some of the properties of MP, such as transportan (a cell penetrating peptide, consisting N-terminal fragment 1-12 of galanin and mastoparan at the C-terminus linked with Lys residue) or its truncated analogue, transportan-10.

In present study we have designed and synthesized several new chimeric mastoparan analogues composed of MP and other biologically active peptides (*e.g.* galanin, RNAIII inhibiting peptide) and containing natural or unnatural structures. Next we examined each of these hybrid constructs as well as natural peptides for they antimicrobial activities.

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P40700-021

Synthesis and antioxidative activity of cinnamic acid amides of oxazole containing amino acids

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Cinnamic acid derivatives as esters, amides and glycosides are known to have antibacterial, antiviral, antiinflammatory, antiproliferative, immunostimulatory etc. properties. Some of them (amide and ester analogues of caffeic acid with natural amino acid esters) exibit stronger antioxidative activity. In particular thiazole, oxazole and imidazole amino acids that may play a key role in biological activities of unusual peptides are also important intermediates for natural product synthesis and peptidomimetics. Here we report the synthesis of novel cinnamic acid amides with oxazole containing glycyl-methyl ester as potential antioxidants. The amides were synthesized from sinapic, coumaric, ferulic acids and the corresponding glycyl-methyl ester hydrochloride form using N-ethyl-N-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 4-N,N-(dimethylamino)-pyridine (DMAP). Their antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl DPPH (1,1-diphenyl-2-pycrylhydrazyl) test.

P40702-022

Positional scan libraries of α-conotoxins targeting neuronal nicotinic acetylcholine receptors

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The venoms of marine cone snails contain a complex mixture of peptide neurotoxins known as conotoxins (1). They are a diverse class of biomolecules that exhibit exquisite selectivity and potency for a wide range of pharmacological targets in the central nervous system, making them valuable tools for studying the mechanisms of neurotransmission and pain. Despite their diversity, conotoxins have evolved from a relatively small number of rigid disulfide bonded frameworks that give rise to a series of intervening loops of amino acids that project outwards from the framework and interact with the receptor binding site. Receptor targets are generally determined by the shape of the framework, with the amino acids within the framework influencing receptor subtype specificity. This work aims to use positional scan libraries (2) based on 4/3 α-conotoxin frameworks to study the interactions of amino acids with the binding site of neuronal nicotinic acetylcholine receptors, and to discover new ligands that possess greater potency and selectivity for different subtypes these receptors. The disulfide bond frameworks were formed by oxidizing each mixture in dilute aqueous buffer and their correct formation monitored by CD spectroscopy. An á7 nAChR functional assay was used to screen each mixture to determine the activity of each mixture and the results of this assay were used to design a series of individual candidates for use in further structure activity relationship studies.

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P40719-023

Melittin effect on the sensory neurons prelevated from double transgenic vs. normal mice

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Melittin is a 62 AA peptide. It is the active compound of bee venom and a powerful stimulator of phospholipase A2. Its antimicrobial effect was extensively studied in the literature, but little is known about its neuroactive properties. Melittin increases the cell membrane permeability in excitable and non-excitable tissues, as a result of its interaction with the negatively charged phospholipids. Our interest was focused on the algesic properties of melittin and on its potency against the electrophysiological parameters of sensory neurons from dorsal root ganglia. In our study, we have performed patch-clamp studies, by the whole-cell configuration. Primary cell cultures were obtained from double transgenic mice TCR-HA+/INS-HA+ with type I diabetes. This animal model is well-suited for the study of neuropathic pain and it enables us to test the analgesic effects of melittin (0.1-5 μM). Our recordings indicate that the active-peptide modifies the algesic profile of the sensory neurons, in particular by acting against the capsaicin-activated ionic currents. In addition, we have monitored the action of melittin on the I_h currents, Na^+ voltage -dependent currents, delayed rectifier K^+ currents. A particular interest was focused on the recordings of Ca^{2+} voltage-dependent currents. The maximal effect was obtained at a dose of 1 μM melittin, and at higher doses the active-peptide strongly disturbs the lipid membrane order. In conclusion, the algesic profile of the sensory neurons from double transgenic mice is significantly modified by the neuroactive melittin in comparison with the profile of neurons from Balb/c mice. These electrophysiological data are important, and are very well corroborated with the increased thermal and mechanical hypersensitivity induced by melittin itself and the bee venom extract, that have been proved their efficiency in behaviour tests .

P40719-024

The toxic effects of AMPs tested on mammalian cell cultures

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The AMPs (antimicrobial peptides) is a class of molecules that belongs to the innate immunity. The ability of some of these peptides to penetrate the

microorganism's external membrane and to induce their death suggests that this class of molecules may represent a new generation of antibiotic pharmaceuticals. In our work, we focused on the AMPs side effects on mammalian cells. The target was to characterize the toxicity of several AMPs evaluated on *in vitro* cellular cultures. The following AMPs were used in our experiments: melittin, magainin I and II, cecropin A and mastoparan. The viability of cells in cultures was assessed by MTS test on Chinese hamster lung fibroblasts V79 and on human lymphocytes primary cultures. Concentrations in the range of 0.1 – 100 μM were used. The IC50 value for each type of AMP was evaluated from the viability *versus* concentration curves. Haemolysis assays were also performed for these active-peptides. Based on IC50 values, melittin has a stronger citotoxicity than magainin I and magainin II. On the other hand, magainins appears to posses higher haemolytic properties than melittin. The less cytotoxic peptide seems to be mastoparan. These types of results are very useful to characterize the ability of AMPs to induce toxic effects in human body during the treatment.

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P40720-025

Analgesic effects of MIF-1's analogues

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The melanocyte-inhibiting factor (MIF-1), which is the tripeptide Pro-Leu-Gly-NH2, was isolated in the conventional way from bovine hypothalamus tissue. MIF-1 represents a class of naturally occurring opiate antagonists with varying activities in independent situations. The purpose of the present study was to investigate the analgesic activity of MIF-1 and its analogues modified at position 2 with unnatural amino acids Cav, sLys, sLeu, sIle and sNle. The experiments were carried out on male Wistar rats (180-200 g). The changes in the mechanical nociceptive threshold were measured by the paw-pressure test using an analgesiameter (Ugo Basile). MIF-1 and analogues (all in dose 1mg/kg) were administered intraperitoneally (i.p.). MIF-1 exhibits a weak analgesic effect and selective affinity for the μ-opioid receptor. MIF-analogues - MIF-Cav, MIF-sLeu, MIF-Ile and MIF-Nle were found to have a naloxone-reversible analgesic effects.

P40720-026

Structure-activity studies of the N-terminal segment of PACAP

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PACAP (pituitary adenylate cyclase-activating polypeptide) exists in two biological isoforms, i.e a 38-amino acid peptide and a shorter form of 27 residues corresponding to the N-terminal part of PACAP38. Previous structure-activity relationships studies revealed that the N-terminal segment is essential for the activation of the specific and selective type I PACAP receptor (PAC1). In order to clarify the molecular requirements for PAC1 activation, we initiated structure-activity investigations of the N-terminal part of both PACAP isoforms. In particular, an important library of analogs focusing on the first seven residues (His1-Ser2-Asp3-Gly4-Ile5-Phe6-Thr7) was rationally developed and pharmacologically evaluated for their capacity to bind the PAC1 receptor and to induce Ca2+ mobilization in Chinese hamster ovary (CHO) cells stably expressing the human PAC1 receptor. Ala scan demonstrated that the carboxylic acid function of residue Asp3 and the benzyl group of Phe6 are essential feature for proper PAC1 receptor activation. The inversion of chirality of residues Ile5, Phe6 and Thr7 caused a loss of affinity whereas the incorporation of D-amino acids in positions 1, 2 or 3 did not affect the binding properties of PACAP. Moreover, using a N-methyl scan, we showed that the N-terminal domain of PACAP is not tolerant to back-bone modifications. However, replacement of Ser2 by Pro did not decrease significantly the potency of the peptide while substitution of this same residue by D-Pro totally inhibited the biological activity of PACAP. Furthermore, other structural constraints reduced the potency of PACAP, suggesting that the N-terminal domain needs flexibility to bind and activate the PAC1 receptor. Finally, residues 28-38 of C-terminally extended peptides played a favorable role for the binding affinity towards the PAC1 receptor as PACAP38 analogs demonstrated highest binding affinity compared to their PACAP27 counterparts.

P40720-027

Neutrophil elastase-dependent synthetic host defence pro-peptides for the treatment of bacterial infections in cystic fibrosis patients

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Chronic infection and inflammation play a major role in the pathophysiology of lung disease in cystic fibrosis (CF). Besides Pseudomonas aeruginosa, there is increasing evidence that other multidrug-resistant organisms, such as methicillin-resistant Staphylococcus aureus (MRSA) are implicated in the activation of the inflammatory response in CF patients. Cationic host defence peptides, multifunctional mediators of the innate immune system, have been recognised as promising candidates for the development of novel antimicrobial agents (1). Most of these macromolecules are produced as inactive prepropeptides and are proteolytically activated to release a C-terminal cationic peptide with antimicrobial activity. We thought to mimic this natural mechanism to target CF pathogens with synthetic host defence propeptides. Salt-resistant, all-D cationic antimicrobial peptides (2) were conjugated at their N-termini to a polyglutamic acid sequence to compensate the net positive charge of the parent peptide, through a linker selectively degraded by a disease-associated enzyme (neutrophil elastase). In Vitro effects of the all-D P18 peptide candidate on planktonic and biofilm forms of Pseudomonas aeruginosa and Staphylococcus aureus clinical isolates were assessed. Reactivation studies of the propeptide were performed in presence of purified neutrophil elastase and in physiologically relevant conditions, using bronchoalveolar lavage fluids from CF patients. The NMR structures of the propeptide and active P18 sequences were also compared. These studies confirmed that the propeptide remains inactive in the absence of neutrophil elastase and that the latter enzyme can potentiate its antimicrobial activity.

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P40720-028

Synthesis and biological activity of a series of azaβ3-pseudopeptides related to 26RFa, the endogenous ligand of GPR103

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26RFa, a novel neuropeptide of the RFamide family, is the natural ligand of the previously orphan receptor GPR103. Both 26RFa and GPR103 mRNAs are highly expressed in hypothalamic nuclei of rodents, and icv injection of 26RFa induces a potent orexigenic effect in mice. Recently, it has been shown that GPR103-deficient mice suffer from osteopenia. Analysis of the 26RFa precursor reveals that it may generate several additionnal RFa-peptides including an N-terminally extended form (43RFa) and a truncated form (26RFa(20-26)). 26RFa and 43RFa increase dose-dependently $[Ca^{2+}]i$ in GPR103-transfected cells while 26RFa(20-26) is about 100 times less potent than 26RFa. Molecular modeling under NMR constraints of 26RFa shows that the N-terminal region encompasses an α-helix and the C-terminal region adopts a γ-turn in DPC micelles. The C-terminal peptide 26RFa(20-26) exhibits major distortions of this turn that may be responsible for its weak potency. The aim of this work was to introduce an azaβ3 residue in 26RFa(20-26) in order to stabilize the γ-turn. Sequential azaβ3-counterpart substitution of amino acids at positions 20 and 21 enhanced by 2 and 7 folds the potency of 26RFa(20-26), respectively. The azaβ3-Phe^{22} analog was 2 times less potent than 26RFa(20-26). Replacement of the native Ser^{23} by the azaβ3 surrogate of (HO)homoThr (azaβ3-Hth) to favor hydrogen bonding, generated an analog that was 2 folds more potent than 26RFa(20-26). In contrast, substitution of residues 24 to 26 led to analogs totally devoid of effect on calcium mobilization. As the γ-turn is centered on the Ser^{23} of 26RFa, we can assume that the azaβ3-Hth^{23} moiety partially mimics the γ-turn in the 26RFa(20-26) sequence. These data constitute the first step towards the development of new GPR103 analogs that could prove useful for the treatment of feeding disorders and/or osteoporosis.

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P40721-029

Development of Biotin-Tagged Cyclicdipeptide Based Anti-Microtubule Agents and Tubulin Photoaffinity Labeling

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It is well known that microtubule targeting agents (MTAs) are used in clinical treatments as anticancer agents. Recently, it has become clear that some MTAs also function as gvascular disrupting agents (VDAs), which induce tumor-selective vascular collapse. As one of such candidates, a natural cyclicdipeptide, phenylahistin (PLH) exhibiting colchicine-like anti-microtubule activity, has been our focus. We have succeeded in synthesizing PLH, and performed the structure activity relationship study of its derivatives. From the biological evaluations, According to these results, a highly potent derivative NPI-2358 (IC50 = 15 nM, HT-29) was selected, which is now in Phase I clinical trial as an anticancer drug in the US. Furthermore, we have established the synthetic route of NPI-2358 and its derivatives. About 100 analogs were prepared so far and screened by HT-29 citotoxicity assay. As a result, several highly potent derivatives ware developed. Although NPI-2358 and its derivatives are believed to be recognized around the colchicine binding site on tubulin, the three-dimensional structure of NPI-2358 could not be superimposed over that of colchicine. In order to understand the precise binding mode of NPI-2358, we developed a highly potent cytotoxic derivative, KPU-244 (IC50 = 3.9 nM, HT-29 cells) with a benzophenone structure. Because benzophenone is recognized as a photo-reactive group, we synthesized biotin-tagged KPU-244 derivative as a photoaffinity probe. Since this probe has biological activity enough to function to tubulin, photoaffinity labeling was performed to analyze the binding site. As a result, irradiation-time-dependent labeling towards tubulin was observed. The labeling was also dose-dependently inhibited by colchicines addition, suggesting that the probe specifically recognizes around the colchicine binding site on tubulin. Chemical biology study for determining the precise binding site is now in progress.

P40728-030

Conformational analysis of the new temporin analogues: Gln3TA and Pro3TL.

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Temporins are antimicrobial peptides (AMPβ€™s) isolated from the skin of Red European frog Rana temporaria. They are active particularly against Gram-positive bacteria, Candida species, fungi. They have the ability to bind and permeate both artificial and biological membranes. We have recently investigated by spectroscopic means two members of this AMP family Temporin L (FVQWFSKFLGRIL-NH2) and Temporin A (FLPLIGRVLSGIL-NH2) (1). Based on the NMR results, we developed two new analogues of these peptides named Pro3TL (FVPWFSKFLGRIL-NH2) and Gln3TA (FLQLIGRVLSGIL-NH2). Biological data indicate that Pro3TL has a higher antimicrobial activity and a lower hemolytic activity than the native peptide TL. In contrast, Gln3TA more hemolytic than the parent peptide TA. The conformational behavior of the new analogues was investigated in membrane mimetic environment (SDS and DPC micelles) by spectroscopic and computational methods. Diagnostic NMR parameters observed for Glu3TA and Pro3TL indicate a conformational propensity toward helical structure. For Glu3TA, B-turn structures are also observed along the N-terminal fragment of the peptide. We are currently studying the conformational behavior of the four peptides also by molecular dynamics simulation in explicit solvated dodecylphosphocholine and sodium dodecylsulfate systems. These simulations would provide a realistic picture of the interactions between the peptide and models of bacterial and mammalian membranes.

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P40729-031

New, All Non-Aromatic Vasopressin V1a Receptor Agonists

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We have recently reported potent and V1a receptor selective analogues of the neurohypophyseal hormone $[Arg^8]$vasopressin (AVP) (1). The compounds, of general structure $[Xaa^2,Ile^3,Yaa^4,Zaa^8]$VP, with X being an aromatic side chain amino acid, exhibited high selectivity versus related receptors (V2R, V1bR and OTR). We present here a series of new analogues of AVP of the general structure $[Xaa^2,Ile^3,Yaa^4,Zaa^8]$VP where Xaa was selected form a collection of cyclic and acyclic non-aromatic amino acids. The peptides were prepared by standard SPPS methods using either the Fmoc or Boc strategy and tested in vitro for their agonistic potency and efficacy at the V1a and the related receptors. Unlike AVP, which has aromatic amino acids in positions 2 and 3, these analogues contain only non aromatic residues, but are still potent V1a agonists. Compounds with cyclic aliphatic residues (e.g. Cha) in position 2 were found to be generally more potent V1a agonists than the ones containing acyclic residues. The Ala^2 analogue ($[Ala^2,Ile^3,Dab^8]$VP) was totally inactive (no significant V1aR agonism up to 1000 nM) as

reported previously for [Ala^2]AVP (2). The Cha^2 containing peptides are slightly less potent as V1aR agonists than their Phe^2 counterparts, but the overall in vitro pharmacological profile of the two classes of compounds is similar. Selected new analogues were also tested in vivo in a rat pressor model. The compounds were found to be effective in raising arterial blood pressure and appeared to be longer acting in vivo when compared to AVP and related compounds, as demonstrated by slower disappearance of their pressor effect at equieffective doses. Detailed experimental procedures, the results of biological testing, and SAR discussion will be presented.

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P41013-033

Enhancement of anti-tumor properties of TRAIL by targeted delivery to the tumor neovasculature

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anti-cancer agent with tumor-selective apoptotic activity. TRAIL plays a role in the innate and adaptive immune response, autoimmune disease, and may also be involved in hepatic cell death and inflammation. For these reasons, chronic exposure to TRAIL may have deleterious side effects in patients as a cancer therapeutic. In this study, we have improved the anti-tumor activity of TRAIL by targeted delivery to the tumor vasculature, leading to dramatic enhancement of its therapeutic properties. TRAIL was fused to the ACDCRGDCFC peptide (named RGD-L-TRAIL), a ligand of ¦ÁV¦B3 and ¦ÁV¦B5 integrins. Biological activity was evaluated in vitro and anti-tumor efficacy was investigated in vivo as a single agent and in combination with irinotecan hydrochloride (CPT-11). The fusion protein RGD-L-TRAIL, but not TRAIL or RGE-L-TRAIL, specifically bound to microvascular endothelial cells in a dose-dependent manner and showed enhanced apoptosis-inducing activity (caspase-3 and caspase-8 activation) in ¦ÁV¦B3 and ¦ÁV¦B5 integrin positive cancer cells. In addition, RGD-L-TRAIL was more effective in suppressing tumor growth of COLO-205 tumor-bearing mice than an equivalent dose of TRAIL. The anti-tumor effect of RGD-L-TRAIL was further enhanced by combination with CPT-11 in both TRAIL-sensitive COLO-205 and TRAIL-resistive HT-29 tumor xenograft models. Our findings suggest the novel fusion protein RGD-L-TRAIL can directly target tumor endothelial cells as well as ¦ÁV¦B3 and ¦ÁV¦B5 integrin positive tumor cells. The tumor-targeted delivery of TRAIL derivatives, such as RGD-L-TRAIL, may prove to be a promising lead candidate for cancer therapy.

P41128-034

The impact of lithium cations on the peptide bond

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Lithium ions play an important role in some biological processes, e.g. in the treatment of neuronal dysfunctions and some metabolic pathways. However, the mechanisms for these actions are not known up to now. Recently it has been shown that Lithium cations influence the isomerisation state of peptide bonds which is essentially pronounced in the case of the amino acid proline. Such an isomerisation goes hand in hand with conformational changes possibly resulting in altered folding and structuring. Thus, Lithium cations might essentially influence the biological function of peptides and proteins.

To shed light on the underlying mechanism we carried out detailed studies on several model peptides. Thus, the isomerisation as well as the kinetic properties of the peptides have been determined employing various techniques of NMR spectroscopy. Besides, quantum chemical studies on Li^+-peptide complexes were performed to get insight into the structural aspects of the cation-peptide interaction.

P41217-035

Enhanced screening and understanding of hypolipidemic peptides assisted by informatics

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Hypolipidemic peptides targeting the bile acid to inhibit cholesterol absorption in intestine has been indicated by several naturally obtained short peptides. These bile acid-binding peptides are known to disrupt the micellar formation of bile acid for capturing intestinal cholesterol, and lead the aggregates pass the intestine without absorbance. Such effect also disrupts the hepatic circulation of bile acids, which accelerates the conversion of stored cholesterol into lacking bile acids. However, traditional extraction procedure of peptides form natural products is time-consuming. Therefore, to enhance the screening of such functional short peptides, we have combined the array-based screening strategy with bioinformatics strategy, to design effective experiments by prediction of physicochemical peptide structures. As a result, we resulted in significantly higher screening efficiency and high bile acid affinity compared to random screening. We hare report the introduction of one of supervised learning algorithms, fuzzy neural network, and unsupervised algorithm, hierarchical clustering scheme for such functional peptide screening.

P41218-036

Analogues of the kinin B1 receptor antagonist R-954 bearing N-terminal lipid moieties.

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We previously reported the identification of a potent peptide antagonist of the kinin B1 receptor (B1R) namely the R-954 (Ac-Orn-Arg-Oic-Pro-Gly-α-MePhe-Ser-D-β-Nal-Ile-OH) (Neugebauer et al., Can. J. Physiol. Pharm., 80, 287-292, 2002). In an effort to further improve stability and pharmacokinetic properties of R-954 while retaining its high antagonistic potency, the latter peptide was extended by a lipid moiety at its N-terminal region. N-acylating lipidic moieties comprising either, a ω-amino fatty acids (C-6, C-8, and C-11) or cholic acid were coupled on the resin during the solid phase synthesis of the antagonist sequence. Antagonist potencies (IC50) of derived analogues were determined by classical in vitro rabbit B1R bioassay using the isolated rabbit aorta and compared to the parent peptide R-954. The results showed that the analogue N-190 with 6-amino caproyl acyl component was slightly more potent (IC50: 10.5±0.8 nM) than R-954 (IC50: 14.8±0.8 nM) while its counterpart comprising no ω-amino group, the peptide R-958, was significantly less active (IC50: 35.5±0.9 nM). All synthetic analogues were FITC-labelled at their N-terminal to probe surface B1R expression by fluorescent techniques. On human B1R-transfected HEK-293 cells, both FR-954 and FN-190 showed a cell membrane staining by confocal microscopy; however, an intracellular labelling was also noticed with the FN-190. A positive fluorescent signal with FN-190 as opposed to FR-954 was observed in untransfected HEK-293 cells using fluorescence-activated cell sorting (FACS) technique. Similar results were obtained with ω-amino fatty acyl and cholyl derivatives. These results can be explained by an increased membrane permeability of the lipopeptides that is unrelated to the presence of B1R. We developed more potent R-954 analogue by extending N-terminally peptide with 6-amino caproyl acyl. The lipidic moieties used; can be use as permeability vehicles to other peptides.

P41407-037

Multifunctional Peptides. Analogues Of Tyr-MIF

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Multitarget medicines are a new, very promising avenue of drug development. Interestingly, most of the endogenous neuropeptides function through interaction with several receptors and/or regulatory pathways. Tyr-MIF (Tyr-Pro-Leu-Gly-NH2) is one of the best examples of such multifunctional molecules. Tyr-MIF interacts with specific receptors and blood-brain-barrier transporter that recognizes Tyr-Pro motive. In addition, this molecule expresses weak opioid properties. Structure-activity studies suggest that Tyr-MIF that does not play direct function as endogenous analgesic (opioid) is an important, not fully understood, regulatory component of neurohomeostase of pain and stress signal. In contrast to opiate alkaloid tyramine moiety of the N-terminal tyrosine in neuropeptides exists mainly in conformation that does not fit to opioid receptor binding site. Therefore, for high opioid activity, additional conformational elements should exist that enhance receptor-peptide complex formation. In recent years, several tyrosine analogues that much more effectively simulate “opioid” tyramine have been developed, including 2,6-dimethyltyrosine (DMT) or 6-Hydroxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (6Htc). To enhance opioid function with preservation of all other functional components, the analogues of Tyr-MIF with such “opioid enhanced” tyrosine residue have been developed, synthesized and tested in chronic pain. Acknowledgements: This work has been financially supported by European Grant “Normolife” LSHC-CT-2006-037733.

P41500-038

Adrenomedullin Analogs : Potential Diagnostic Tools for Pulmonary Circulation Imaging

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Adrenomedullin (AM) is a 52-amino acid peptide that shares structural similarities with regulatory peptides belonging to the calcitonin family (calcitonin, CGRP and amylin). AM is known to produce a potent vasodilation via the coupling to the calcitonin receptor-like receptor (CRLR) associated with a chaperone protein, the receptor-activity modifying-protein (RAMP). Binding studies performed in mammalian tissues revealed that the largest distribution of AM binding sites was found in the heart and lungs. In fact, the pulmonary blood vessels display a vast abundance of AM binding sites that act as clearance receptors. Pulmonary arterial hypertension (PAH) is a condition associated with obliteration of pulmonary arterioles which carries a very poor prognosis, in part because of delayed diagnosis due to the lack of specific non-invasive diagnostic tools. Likewise, there currently exists no molecular imaging agent to diagnose pulmonary embolism, a pathological condition that occurs when a blood clot blocks a lung artery. Therefore, due to the high density and incidence of AM receptors in the cardio-respiratory system, AM could represent a key-target for diagnosis with radiolabeled AM-related drugs. In this study, we looked at structure-activity relationships and synthesized various AM fragments exhibiting high binding specificity for AM receptors but reduced biological activity. Moreover, we introduced different chelating moieties into these AM peptides to evaluate the possibility of labelling those molecules for imaging purposes. Using cell binding assays, we observed that a cyclic moiety combined with the AM(22-52) sequence is able to maintain a good affinity for AM receptors. Furthermore, we showed that the incorporation of a 4-amino acid sequence into the peptide chain was the best chelating moiety that allows high efficiency labelling with 99mTc. Hence, our study strongly suggests that AM derivatives are promising leads for lung specific imaging.

P41518-039

^{99m}Tc-labeled Tetraamine-Derivatized Cyclic Nonapeptides with Pansomatostatin-like Properties: Synthesis and Comparative Biological Evaluation

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Aim: A part of our current research is focused on the development of novel ^{99m}Tc-radiotracers with expanded sst_{1-5} affinity profile. We report herein on two pansomatostatin-like analogs Demopan 1 (N_4-des-Tyr^0-KE108) and Demopan 2 (N_4-KE108), and their respective ^{99m}Tc-labeled radioligands (KE108 = Tyr-c[(D)Dab-Arg-Phe-Phe-(D)Trp-Lys-Thr-Phe]). **Materials & Methods**: Demopan 1 and Demopan 2 were synthesized on the solid support. The affinity profile of Demopan 1 and Demopan 2 for the $hsst_{1-5}$ was studied by receptor autoradiography using [^{125}I][Leu^8,(D)Trp^{22},Tyr^{25}]SS-28 as radioligand and for the $rsst_2$ by competition binding in AR4-2J cell membranes using [^{125}I-Tyr^3]octreotide as radioligand. Induction of sst_2 and sst_3 internalization by Demopan 1 and Demopan 2 was assessed by immunofluorescence microscopy. Internalization of the respective ^{99m}Tc-radioligands was studied in AR4-2J cells. Biodistribution of [^{99m}Tc]Demopan 1 and [^{99m}Tc]Demopan 2 was performed in Lewis rats bearing AR4-2J tumors. **Results**: Demopan 1 and Demopan 2 showed high affinity binding for all $hsst_{1-5}$. Significantly higher affinity for the sst_1 and the sst_2 was exhibited by Demopan 2 (IC_{50}= 0.84 nM and 2.5 nM) than by Demopan 1 (IC_{50}= 5.9 nM and 10.7 nM), concordant with results for the $rsst_2$ (Demopan 1 IC_{50}= 3.4 nM; Demopan 2 IC_{50}=0.57 nM). The peptides induced sst_2 and sst_3 internalization to a lesser extent than native SS-28. At tracer level, [^{99m}Tc]Demopan 1 and [^{99m}Tc]Demopan 2 failed to internalize in AR4-2J cells. After injection in AR4-2J tumor bearing rats, [^{99m}Tc]Demopan 2 specifically accumulated in the tumor and in sst-expressing organs while [^{99m}Tc]Demopan 1 failed to target any sst-positive tissue. **Conclusion**: ^{99m}Tc-labeled analogs with pansomatostatin properties may lead to clinically useful radiotracers and further search for analogs displaying improved biological profile is warranted.

P41518-040

Introduction of Hydrophilic Asp-Residue(s) in [^{99m}Tc]Demotate 1: A Structure – Activity Relationships Study

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Aim: In this work we present a series of [^{99m}Tc]Demotate 1 ([^{99m}Tc-N_4^0]Tate) derivatives, generated by systematic introduction of hydrophilic, negatively charged Asp residue(s) at the N- and/or C-terminus of the peptide chain. The impact of these modifications on the affinity profile and the in vivo characteristics of resulting (radio)ligands were compared. **Materials & Methods**: Demotate 1 ([N_4^0]Tate), 2 ([N_4^{-1},Asp^0]Tate), 3 ([N_4^{-2},Asp^{-1},Asp^0]Tate), 5 ([N_4^0,Asp^1]Tate), 6 ([N_4^0,Asp^8]Tate) and 7 ([N_4^{-1},Asp^0,Asp^8]Tate) were synthesized by SPPS. Their affinity profiles for the human (h) sst_{1-5} were studied by receptor autoradiography using [^{125}I][Leu^8,(D)Trp^{22},Tyr^{25}]SS-28 as radioligand,

and for the rat (r) sst_2 by competition binding assays in AR4-2J cell membranes with [^{125}I-Tyr^3]octreotide as radioligand. After ^{99m}Tc-labeling, radiopeptide internalization was studied in AR4-2J cells. Biodistribution of [^{99m}Tc]Demotates was studied in healthy Swiss albino mice and for [^{99m}Tc]Demotate 1, 2, 3 and 6 in AR4-2J tumor bearing mice. **Results**: Demotate 1 showed the highest affinity binding for the $hsst_2$ and the $rsst_2$, while introduction of one Asp linker(s) at the N-terminus diminished the affinity for the $hsst_2$ and the $rsst_2$ and led to lower internalization rates. Substitution of Thr^8 by Asp^8 in Demotate 6 resulted in good affinity for the $rsst_2$ and lower affinity for the $hsst_2$. Single (D)Phe^1-substitution/5 or Thr^8 substitution by Asp^8 combined with Asp introduction at the N-terminus/7 led to inferior binding affinity, poor internalization capacity and poor uptake of the respective radiopeptides in target-organs and, in the case of [^{99m}Tc]Demotate 1, 2, 3 and 6, in AR4-2J tumors in mice. **Conclusion**: Introduction of Asp residues in the original [^{99m}Tc]Demotate 1 chain exerted a negative effect on receptor affinity, internalization capacity and targeting of somatostatin binding sites in mice precluding their use as hydrophilic pharmacokinetic modifiers.

P41600-041

Design, synthesis, and characterization of Candida albicans secreted aspartic proteinase (SAP) inhibitors

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The family of secreted aspartic proteinases (SAPs), which are encoded by ten distinct genes, is an important virulence factor of the human pathogen Candida albicans.(1) Due to an increase in the number of Candida strains that are resistant to the drugs currently used in therapy, these proteinases are highly promising new drug target candidates. Based on the knowledge of SAP2-substrate specificities(2)and X-ray structural studies of SAP2 in complex with inhibitors,(3) we designed and synthesized a series of SAP inhibitors by modifying the structure of the pentapeptide pepstatin A, which is a potent yet non-selective aspartic proteinase inhibitor. These inhibitors were synthesized manually via a SPPS methodology using a 2-Cl-tritylchloride resin and Fmoc-protected amino acids. In addition, the key residue of the designed peptide inhibitors, the γ-amino acid statine (Sta), which was prepared according to a slightly modified literature procedure,5. was further protected at its hydroxyl group as a silyl ether for the purpose of avoiding competitive side reactions during amino acid couplings. We prepared 9 new inhibitors by varying the pepstatin A structure at the P3, P2, or P2' position. All variants showed efficient inhibition when screened against the isoenzymes SAP1, SAP3, and SAP6, and some of them exhibit IC50 values similar to or even lower than that observed for pepstatin A.

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P41617-042

Multi-component Fluorescence labeling method for Efficient Positional Screening of Peptide Library

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Peptide library is a general technique for screening specific peptides that bind to a target protein. But, in a standard screening method, peptide libraries need to be fixed on large carriers like phage, beads and so on. The large carriers would affect the binding between peptides and the target protein. In this work, we develop a new method for screening peptide libraries without carriers. We attached a variety of fluorescent amino acids to peptide libraries and the peptides that bind to target proteins were identified through 2-dimensional fluorescence spectroscopy in aqueous solution. In this presentation, 36-different fluorescent amino acids were synthesized or purchased. Of those amino acids, we newly synthesized 30 fluorescent amino acids. 2-D fluorescence spectra of those amino acids were measured. They were different in fluorescence excitation/emission wavelengths. Excluding those of weak fluorescence intensities, 15 fluorescent amino acids are selected. Those 15 fluorescent amino acids are mixed in methanol/water (pH7.4) (=1/1(v/v)) and the 2-D fluorescence spectrum was measured. Then, concentrations of the component amino acids were evaluated by a linear least-squares method. The results suggested that all fluorescent amino acids can be quantified. The set of fluorescent amino acids combined with the 2-D fluorescence spectroscopy technique will be a powerful tool for screening peptides and other drug compounds without using any carriers.

P41620-043

N-methyl phenylalanine-rich peptides as potential blood brain barrier shuttles

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Several peptide families containing *N*-methylated amino acids were designed and synthesized using solid-phase peptide synthesis (SPPS). The permeability of these compounds was studied by Parallel Artificial Membrane Permeability Assay (PAMPA) and Immobilized Artificial Membrane chromatography (IAMC) so as to select the best peptides in terms of length, terminal groups and amino acid replacement to be used as carriers that pass through a model of blood-brain barrier (BBB) by passive diffusion for non-permeating agents. Furthermore, their enzymatic stability in human serum and their cell viability were tested by MTT assay. These peptide families showed great stability and non-toxicity. The three best peptides were coupled to levodopa and assessed. These peptides transferred levodopa through an artificial membrane and transformed it from a non-passive permeating drug into a compound able to cross the membrane by passive diffusion.

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P41729-044

Solid-phase synthesis of europium-labeled human INSL3 as a novel probe in development of a high-throughput receptor binding assay

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The structure-activity study of insulin-like peptide 3 (INSL3) requires the design and synthesis of various analogues. In order to test these for their receptor binding affinity, a high-throughput receptor binding assay is needed. We have therefore developed an efficient solid phase synthesis protocol to prepare specifically mono-labeled human insulin-like peptide 3 (INSL3) for the study of its interaction with its G-protein-coupled receptor, RXFP2. A commercially available chelator, diethylene triamine pentaacetic acid (DTPA), was coupled to the N-terminus of solid-phase bound INSL3 A-chain and then a coordination complex between Eu^{3+} and DTPA chelator was formed. After combination of the purified A- and B-chains together with sequential formation of the three insulin-like disulfide bonds, the labeled peptide was purified at high yield using high-performance liquid chromatography with high pH buffer to prevent the liberation of europium from chelator. Saturation binding assays were undertaken to determine the binding affinity (pK_d) of labeled INSL3 for RXFP2 in HEK 293 stable cell line expressing RXFP2. The binding affinity of DTPA-labeled INSL3 (9.05 ± 0.03) was comparable to that of ^{125}I-labelled INSL3 (9.59 ± 0.09). The efficient solid-phase synthesis has provided a novel lanthanide-coordinated, DTPA-labeled INSL3 with excellent sensitivity, stability and high specific activity and which is superior to the traditional ^{125}I-INSL3. This labeled peptide can be used in a high-throughput screening of INSL3 analogues in structure-activity studies.

P41815-045

Multivalent bombesin analogues conjugated to DOTA-based chelators for targeted multimodal imaging of GRP-receptor positive tumors

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Multimodal tumor imaging using MRI and PET/SPECT provides comprehensive diagnostic information as it combines anatomical information (by MRI) and functional information (by PET or SPECT). Development of DOTA-derived imaging probes is advantageous for multimodal imaging as it accommodates many metal ions employed in different imaging modalities including Gd(III) for MRI. However, high relaxivity and enhanced specific up-take at the targeted site is important for the Gd-based MRI contrast agents. The DOTA-based prochelators required for the multivalent vectorization of targeting ligands were synthesized by functionalizing cyclen or DO2A-tert-butyl ester with modified glutamic acid. Multivalent conjugation of bombesin peptides, which specifically target tumors expressing gastrin-releasing peptide (GRP) receptors, yielded the corresponding mono-, di-, and tetravalent bombesin analogues. The conjugates showed excellent chelating properties with Gd(III) (for MRI applications) and also with ^{111}In, ^{177}Lu and ^{68}Ga (for radiopharmaceutical applications). The ^{111}In and ^{177}Lu labeled divalent conjugates showed rapid internalization and slower externalization rate compared to their corresponding monovalent analogues as studied with prostate tumor cell lines. The gadolinium complexes of monovalent and divalent conjugates showed significant relaxivities at 60 MHz ranging from 9.3 to 19.2 mM-1s-1 at 25°C. The values represent the 2 to 4 fold enhancement of relaxivity compare to clinically employed Dotarem® (Gd-DOTA). Further, the relaxivities increased significantly from monovalent to divalent conjugates. This is highly promising as we would expect much higher relaxivities in case of tetravalent conjugates, which are currently under investigation. In conclusion, the work demonstrates the development of high relaxivity multivalent bombesin analogues, which could be employed for the multimodal imaging such as PET/MRI or SPECT/MRI with improved tumor targeting capabilities.

P41815-046

A new highly potent DOTA-conjugated bombesin antagonist for GRPr-positive tumor targeted imaging

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Peptide receptors are very promising targets for tumor imaging. The somatostatin receptors were successfully targeted with peptides labeled to γ-emitters (^{111}In, ^{67}Ga and ^{99m}Tc) and positron emitters (^{18}F, ^{68}Ga, ^{86}Y) for diagnostic imaging. For tumor targeting, radiolabeled agonists were developed as they usually trigger the internalization of the peptide-receptor complex. We have recently shown that somatostatin-based radiolabeled antagonists may not only have a higher tumor uptake than equipotent agonists but also a longer lasting tumor uptake. Among the most promising receptors to be targeted, the bombesin receptors are of great interest as they are overexpressed in major human tumors such as prostate and breast. The aim of this study was to develop a radiolabeled bombesin-based antagonist conjugated to the macrocyclic chelator DOTA which allows efficient and stable labeling with a variety of two- and three-plus charged radiometals for imaging (PET, SPECT). We compared its in vitro pharmacologic properties and biodistribution in the PC-3 mouse model side-by-side with the potent agonist [^{nat,111}In]–DO3A-CH_2CO-G-4-aminobenzoyl-Q-W-A-V-G-H-L-M-NH_2 (AMBA). In(III)-AMBA was shown to be a potent agonist by Ca^{2+} flux and immunofluorescence studies and In(III)-RM1 was efficiently antagonizing the activity of In(III)-AMBA. The pharmacokinetics showed a distinct superiority of the radioantagonist with regard to the high tumor uptake as well as to all tumor to normal tissue ratios. [^{68}Ga]-RM1 showed similar pharmacokinetic than [^{111}In]-RM1 with a lower initial kidney uptake and a faster wash out from the kidney. The PET/CT scintigraphic studies of [^{68}Ga]-RM1 in the animal model confirm the significant high tumor uptake and tumor to background ratio. As we found for somatostatin receptor-targeting radiopeptides,bombesin-based radioantagonists also appear to be superior to radioagonists for in vivo imaging and potentially also for targeted radiotherapy of GRPr-positive tumors

P41815-047

Cell Penetrating Peptides Delivering Intracellular Targeted Agents for Molecular Imaging

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Cell penetrating peptides (CPP) are a special class of peptides that possess the property to traverse the formidable barrier of the plasma membrane and deliver cargos into cells. Using CPP as vectors and DNA, mRNA or proteins/enzymes as potential intracellular targets, a new generation of intracellular contrast agents (CAs) can be developed. These agents have prospective use for molecular imaging (both optical and magnetic resonance imaging) by targeted labeling of cells. Aiming to image the presence of specific mRNAs or enzymes, two mRNA

targeting (contains a PNA sequence antisense or non-sense to the target mRNA of DsRed) and one enzyme targeted (contains a unit cleavable by β-galactosidase) CAs were tested for their activity in the presence and absence of respective targets.
The antisense targeting CA, their nonsense derivative and the enzyme targeted CA were taken up efficiently into cells by an exclusively endosomal mechanism as observed by fluorescence microscopy. Cell free binding assays proved a specific interaction with a synthetic target for the antisense but not for non-sense CA. Magnetic Resonance studies showed a higher uptake in transgenic DsRed expressing cells than the parent cells. However, no difference was observable for antisense versus non-sense CA in DsRed cells, due to the vesicular entrapment which is preventing the specific interaction between CA and cytosolic target. Since a comparable cellular distribution was visible for the enzyme targeted agent, a specific accumulation in β-galactosidase containing cells is also unlikely.
The results show that even though the designed CAs were efficiently taken up into cells, they can interact specifically with the target only if colocalization is achieved. However, a lack of specificity is caused by the endosomal entrapment. Further modifications are required to achieve the release from endosomes or a direct uptake into the cytosol.
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P41819-048

The influence of PEGylation on the tumor accumulation of FROP-1, a tumor specific peptide identified by phage display

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The pool of natural peptides suitable for the tumor targeting is limited and therefore novel lead structures identified by screening of phage-displayed libraries are highly warranted. However, transfer of the novel peptide sequences to clinical application is often difficult. We had shown that the coupling of DOTA to FROP-1, a peptide identified in phage display libraries resulted in a fundamentally improved in vitro binding capacity. However, a biodistribution study revealed that the slow binding kinetics FROP-DOTA (H-Glu-Asn-Tyr-Glu-Leu-Met-Asp-Leu-Leu-Ala-Tyr-Leu-Lys(DOTA)-Cys-NH2) allowed the excretion to forestall a significant tumor accumulation. The aim of this study was to investigate whether the conjugation of PEG to FROP-DOTA results in a derivative with a prolonged residence time in the blood. FROP-1 bearing a C-terminal DOTA residue to allow labeling with In-111 and a cysteine to attach a maleimido-modified 2000 Da PEG oligomer was obtained by solid phase synthesis. The conjugate was purified by size exclusion chromatography. Several attempts to couple different PEG derivatives on the solid support did not proceed satisfactorily and therefore the PEG residue was attached in solution. The breast cancer cell line MCF-7 showed a relative low accumulation of the PEGylated peptide in the cells. In contrast, biodistribution studies of the labeled conjugate in mice bearing human FRO82-2 showed a time dependent increasing uptake of the PEGylated peptide with a high retention (at 24 h p.i. 76% of the maximal activity concentration persisted in the tumor). The highest uptake values were determined at 120 min. p.i. reaching 2.3 %ID/g tumor as compared to 0.06% %ID/g observed for the non-PEGylated derivative at 135 min p.i. Apparently PEGylation provides a substantially improved stabilization in the circulation which allows a stable tumor accumulation.

P41820-049

In vivo SPECT/CT imaging of intracranial human glioblastoma xenografts with 111indium-labeled homing peptide.

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Phage display libraries provide a powerful tool to identify new biologically active peptides that bind specifically to their target molecules. Different tumors express a distinct range of molecular markers on their vasculature providing a possibility to use peptides for targeting. Tumor-homing peptides offer an opportunity to target, image and destruct the target tissue. Glioblastomas represent the most aggressive form of brain tumors. We have identified a novel peptide, CooP, using an ex vivo / in vivo phage display screen. This peptide homes specifically to the early stage astrocytoma model. In addition, the peptide homes to the U87 human glioblastoma xenografts (Enbäck and Laakkonen, unpublished data). We have studied the biodistribution and tumor homing properties of Indium-labelled peptide in mice bearing intracranial human U87 glioblastoma tumors using SPECT/CT imaging. CooP peptide was conjugated with the DTPA (diethylenetriamine pentaacetic acid) via amino terminus and labeled with 111Indium. Imaging was performed in four different time points (15 minutes, 1 hour, 2 hours & 24 hours), and the organ and tissue specific radioactivity was measured after the scarification of the animals. Intravenously injected 111In-labeled CooP peptide accumulated in the U87 braintumors. The amount of the peptide increased with time and a clear radioactive accumulation was seen in 1 /3 animals at 1 hour and in 5 / 6 animals at 2 hours after injection. Due to the small size of the DTPA-peptide conjugate the majority of the molecules were secreted via the kidneys during the first 15 minutes. At 2 h timepoint the tumor-to-brain tissue ratio was 11,5. We report here that the CooP peptide, which specifically homes to the brain tumor vasculature and tumor cells, strongly accumulated in vivo to the xenografted human glioblastoma tumors in mice. This is the first time when a synthetic peptide has been used in SPECT imaging of brain tumors in animal mode.

P41821-050

Failure of the development of a novel peptide tracer for molecular imaging of cancer therapy response

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Han, Z. et al. Nat Med 14, 343-9 (2008) have shown that fluorescently labeled derivatives of the HVGGSSV motif accumulated in tumors undergoing therapy. This peptide motif had been identified by the in vivo screening of phage-displayed peptide libraries. The ability of the peptide to differentiate between responding and nonresponding cancers after treatment could be utilized for the development of promising tracers to monitor cancer therapy. The goal of this study was to investigate whether radiolabeled derivatives of the HVGGSSV motif can be used for the noninvasive determination of therapy response in vivo. Therefore three different conjugates were synthesized by solid phase synthesis using Fmoc chemistry. The first peptide was N-terminally modified with the chelator DOTA (DOTA-GGGHVGGSSV-CONH2) to allow radiolabeling with metallic radioisotopes such as 111In or 68Ga. A glycine linker was placed at the N-terminus to separate the peptide motif from the chelator. In order to obtain derivatives accessible to radioiodination a tyrosine was introduced at the N-terminus (H2N-YGGGHVGGSSV-CONH2) of the second peptide. The third peptide was biotinylated at the N-terminal amino group and bound to radioiodinated streptavidin (SA-Biotin-GGGHVGGSSV-CONH2). The biodistribution was monitored by scintigraphy in mice bearing A431-tumors treated with a VEGF receptor-specific inhibitor (SU5416). This study revealed that the 111In labeled hydrophilic DOTA conjugate shows a rapid renal clearance, the

iodinated peptide accumulates mainly in the liver and kidneys. All of the peptide derivatives do not specifically accumulate in treated tumors after i.v. injection. In contrast to the promising results shown by Han Z. et al. our study shows that the HVGGSSV motif can not be easily adapted to radiolabeling approaches with clinical relevance. In conclusion, the peptide motif is not attractive for further clinical evaluation as new diagnostics for the imaging of cancer response.

P41900-051

A Novel Approach To Improve Cellular Delivery Of 5-Aminolaevulinic Acid: New ALA-containing Peptide Prodrugs For Photodynamic Therapy.

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Photodynamic therapy (PDT) is a binary therapeutic modality which is currently under investigation for the treatment of several kinds of malignancies. It relies on the interaction of two individually harmless components: a photosensitiser and an external radiation. The interaction of the photosensitiser with light of the appropriate wavelength and molecular oxygen results in the generation of cytotoxic species, namely singlet oxygen and/or radicals, and localized destruction of tumours or infected tissue. In 5-aminolaevulinic acid photodynamic therapy (ALA-PDT), exogenous administration of ALA is employed to generate elevated intracellular levels of the natural photosensitiser protoporphyrin IX (PpIX), via metabolism through the haem biosynthetic pathway. However this approach suffers from several drawbacks associated with ALA's lack of stability at physiological pH and its highly hydrophilic nature, which prevent it from crossing biological membranes and hence limit tissue penetration. ALA delivery with synthetic peptide prodrugs is a promising way to address these problems. In this work we report the synthesis and characterisation of a series of peptide prodrugs of general structure Ac-Xaa-ALA-OR, where Xaa is an alpha amino acid, chosen to provide a prodrug with appropriate lipophilicity and water solubility. The uptake of the compounds and metabolism to PpIX in PAM212 keratinocytes, relative to ALA is evaluated by fluorescence spectroscopy, and further quantified by recovery and chemical derivatisation of intact/partially metabolised prodrugs. In a parallel study, we have also explored the possibility of coupling of ALA and ALA prodrugs to cell penetrating peptides (CPP). The conjugation of one or more molecules of ALA to a CPP sequence represents an interesting approach to enhanced topical delivery of ALA. Preliminary results of these studies are reported herein.
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P41900-052

BioShuttle as a carrier for temozolomide transport into prostate cancer cells

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If metastatic prostate cancer gets resistant to antiandrogen therapy, there are few treatment options, because prostate cancer is not very sensitive to cytostatic agents. Temozolomide (TMZ) as an oral applicable chemotherapeutic substance has been proven to be effective and well tolerated with toxicity especially for brain tumors. Unfortunately TMZ was inefficient in the treatment of symptomatic progressive hormone-refractory prostate cancer. This may have different reasons like the short plasma half-life of TMZ, a non adapted application schema and as a result, an insufficient bioavailability. To improve the specificity, we built our so called TMZ-BioShuttle-construct with cathepsin B (CTSB) mRNA specificity. This complex combines, a transmembrane transporter molecule connected via a disulfide bridge to an antisense-peptide nucleic acid (PNA) against a docking site in exon 1 of the CTSB mRNA. Furthermore this part is connected via a CTSB cleavable peptide substrate to a nuclear localization sequence coupled with TMZ. Inside the target-cell, the PNA recognizes the cytoplasmic CTSB mRNA and after annealing (the PNA/RNA hybride is not a substrate for RNAse H) results in a cell-specific retention of the TMZ in the cytosol especially of CTSB-expressing cells. Then, after the cathepsin B-mediated cleavage in the cytoplasm, the NLS-sequence is separated and activated for an RAN/importin-mediated transport of the TMZ-cargo into the nucleus of the target cells. This BioShuttle-mediated TMZ transfer could be a step forward to a successful therapy with higher specificity, avoiding adverse reactions and circumventing the previous therapy limiting situation.

P41900-053

Design, Synthesis and Properties of a New Cell Penetrating Sequential Carrier

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The intracellular delivery of protein segments and other bioactive molecules using membrane -permeable peptides has been investigated in multiple aspects. Most of the currently recognized Cell Penetrating Peptides (CPP) are of cationic nature and derived from viral, insect or mammalian proteins endowed with membrane translocation properties. These peptides enter the cell by a receptor independent mechanism, which is poorly understood and carry only one epitope. Our target was to achieve a cell-penetrating carrier able to transport more than one bioactive molecule. To this aim we designed a Cell Penetrating Sequential Carrier (CPSC) formed by the repetitive –Lys-Aib-Cys- moiety, which incorporates the cysteine residue for anchoring the bioactive molecules through a thioether bond. The lysine free side chain possesses the cationic nature of the construct while the á-amino isobutyric moiety could induce a 3_{10} helicoid peptide backbone with amphipathic characteristics. To test the ability of the CPSC to penetrate the cell membrane we synthesized the carboxyfluorescein -labelled CPSC, CF-[Lys-Aib-Cys(-CH_2CONH_2)]$_4$-NH_2, while the CF-Tat-Cys(-CH_2CONH_2)-NH_2 analogue, which is a small basic peptide that has been shown to deliver a large variety of cargoes into the cells, was used as a positive control. The results indicated that CPSC had a homogeneous distribution into the cytoplasm suggesting that CPSC may provide a new powerful biological tool for drug transportation.
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P41913-054

Amphipathic Pro-rich peptides as intracellular drug delivery systems

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Recent advances in genomics and proteomics have led to numerous interesting new drug candidates, such as short oligonucleotides, plasmids, peptides or proteins. Unfortunately, these compounds are unable to cross the cell membrane, unless specific mechanisms have evolved with this objective. Thus, carrier delivery methods have to be developed. One hopeful strategy is the use of peptides able to translocate the cellular membrane, named cell-penetrating peptides (CPPs). Positively-charged amino acids, hydrophobicity and amphipathicity are common features

shared among many of the known CPPs. In 2004 our laboratory described a novel group of CPPs: amphipathic proline-rich peptides. The principal advantages of these compounds are non-cytotoxicity, non-viral origin and high solubility in aqueous media. The best candidate for CPP among the amphipathic Pro-rich peptides was SAP (Sweet Arrow Peptide), $(VRLPPP)_3$.(1) Derivatives with hydrophobic moieties, such as fatty acids or silaproline, have shown highly improved internalisation efficiency; an all D-amino acid version of the CPP SAP was shown to be completely protease resistant and was evaluated in a preliminary in vivo study. CD and TEM studies regarding the self-assembly properties of this family of peptides highlight the possible role of aggregated species in the internalisation process. Finally, these CPPs have shown to be internalised via caveolae or lipid-rafts mediated endocytosis, which circumvents the lysosomal route of degradation. In order to test a challenging cargo for SAP, gold nanoparticles were conjugated to SAP. Thereafter, the transport of Au Np by SAP in HeLa cells has been studied by TEM. While Np alone are not internalised, Np-C-$(VRLPPP)_3$ are clearly uptaken. Studies with QDs (quantum dots) and EGFP as SAP cargo are currently being done in our laboratory. 1. Pujals, Sílvia; Giralt, Ernest. Proline-rich, amphipathic cell-penetratingpeptides. ADDR (2008), 60, 473-484.

P41915-055

Novel Cysteine-rich Cell Penetrating Peptide: Efficient Uptake and Cytosolic Localization

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Introduction: Crossing the plasma membrane is a prerequisite for intracellular targeted drug delivery. Cell penetrating peptides are actively used as the delivery tool for intracellular delivery of various cargos. However, confinement of biomolecules into endosomes limits their use for intracellular targeting. Therefore, there is a need for vectors capable of transferring cargo molecules directly into the cytoplasm. Herein, we focus on the development of a novel CPP (derived from polypeptide Crotamine 1.) which shows an efficient uptake at low concentrations (≤2.5 μM) and cytosolic distribution along with vesicular uptake.
Methods: Series of peptides were synthesized by Fmoc strategy, introducing mutations in $Cro_{(27\text{-}39)}$ (proposed CPP sequence in Crotamine). All were N-terminally labeled with fluorescein isothiocyanate for optical imaging. Structure Activity Relationship (SAR) studies were done by substitution and/or deletion of amino acid residues in the sequence observing the uptake behaviour by fluorescence spectroscopy and microscopy.
Results: Amongst 60 synthesized peptides, one of shorter length showed the best intracellular delivery and cytosolic distribution at lower concentration (2.5 μM) when compared to other CPP. Replacing or deleting cysteines had negative impact on internalization. Results also displayed the involvement of tryptophans in cellular uptake indicating, along with cationic amino acids, the importance of each residue in this optimized sequence.
Conclusions: SAR studies identified a novel cell penetrating peptide showing, besides of endosomal uptake, also an efficient delivery into the cytoplasm at low concentrations. Thus, this peptide might prove useful for efficient transmembrane delivery of agents directed to cytosolic targets.
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P41925-056

CPP or Cholesterol Conjugated Antisense PNA for Cellular Delivery

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Peptide nucleic acid (PNA) is a DNA mimic consisting of the four common bases of DNA on a pseudopeptide backbone that makes it extremely stable in biological fluids. Antisense PNA is targeted against mRNA in cytoplasm in a sequence specific manner. However, the main hindrance to the effective use of PNAs has been their relatively poor uptake by cells. Endosomal release or direct uptake into cytosol of agents is mandatory for attaining mRNA based targeting. There are reports on the cell penetrating peptide (CPP) based delivery system. It has also been reported that conjugates of cholesterol and siRNAs facilitate cellular import (1). The aim of this study was to synthesize different sequences of cholesterol coupled antisense PNA and to compare its uptake characteristics with a CPP-PNA conjugate.
The synthesis of PNA (anti-dsRed PNA (agcgcctgtacc), specifically targeted to mRNA of dsRed, a red fluorescent protein) conjugated to CPP (d-Tat) or cholesterol was performed in fully automated synthesizer (Prelude, Protein Technologies, Inc.) using continuous solid phase chemistry. To increase the solubility in water, linkers (AEEA) and additional charged amino acids were coupled or the sequence of peptide, PNA and cholesterol was changed. All compounds were labelled with FITC to confirm the cellular uptake by fluorescence microscopy and spectroscopy.
Cell uptake studies showed that the CPP bound PNA was located predominantly in vesicles indicating an endosomal uptake mechanism and subsequent entrapment in vesicles. Cholesterol bound PNA was also efficiently internalized. However, it was also located inside vesicles without detectable cytosolic distribution.
PNA-Cholesterol has fewer synthetic steps than PNA-CPP. However, it was also located inside vesicles restricting its applicability for mRNA targeting. The efficient uptake might make it a promising cellular delivery agent after further improvements.
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P42000-057

Design and Synthesis of Novel Bifunctional Peptides as δ/μ Opioid Receptor Agonist and NK-1 Receptor Antagonist

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Novel bifunctional peptides which are designed to act on both opioid receptors and NK-1 receptors have been prepared and tested. Common aminoacid residues of the known pharmacophores were overlapped to achieve bifunctionality within a single molecule. Most peptide ligands showed agonist activities for δ and μ opioid receptors (15.1 ~ 3930 nM) and some of the ligands showed NK-1 antagonist activity up to nM range (Ke = 280 nM).

P42000-058

Chimeric opioid-neurotensin ligands as a new prospective analgesics in chronic pain

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Opioids are gold standards in pain treatments. Unfortunately, fast development of tolerance and dependence creates limitations in application of these drugs in chronic pain treatment. Over twenty years ago we have proposed development of multitarget medicines as a new avenue of drug discovery. Identification of numerous endogenous components that participate in the formation, transmission, modulation and perception of pain signals offers various strategies

for the development of new analgesics. Neurotensin is an endogenous neuropeptide that play important regulatory role of pain transmission. Therefore, it was interesting to develop chimeric analogues that hybridize opioid and neurotensin active components. In such chimeric compounds the possible structural interference may significantly influence on interaction of both components with target receptors and/or biological barriers transport systems. In this communication we present synthesis, pharmacological profile and structural analysis of new potent chimeric compounds.

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P42000-059

Artificial ribonucleases based on short peptides. Synthesis and anti-influenza activity

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The synthesis of molecules that are capable of nonrandom RNA cleavage has found a variety of important applications in molecular biology. For instance, such molecules are used as structural probes for nucleic acids in solution, or in rational design of novel anti-infectives, since RNA is the genetic material of many pathogenic viruses. Here we represent design and synthesis of peptide-like molecules mimicking the catalytic site of natural ribonucleases (A and T1): Series F AA1 – AA2 – AA3 – Phe – OAlkyl; AA1 – Glu/Lys, AA2 – Thr/Ser/Lys, AA3 – Glu/Lys/Thr/Arg; Series R Glu – X – Arg – Gly – OAlkyl; Series K Glu – X – Lys – Gly – OAlkyl; X – Gly, β-Ala, 4-aminobutyric acid, 6-aminohexanoic acid, p-aminobenzoic acid; Series L AA3 – AA2 – AA1 – L1 (L2)– AA1 – AA2 – AA3 , L1 - 4,9-dioxa-1,12-dodecanediamine, L2 - 1,12-diaminododecane; AA1-AA3 – Glu, Lys, Ser, Arg. Ability of artificial RNases to RNA cleavage was shown in experiments with 96-mer RNA HIV-1. The efficacy of RNA cleavage depends on artRNases structure (for example, on location of negative and positive charged amino acids (Glu, Arg или Lys) in peptide). The efficacy of the most active compounds was 60-98 % at 18 h incubation. Anti-influenza activity in vitro of 12 such artRNases was investigated by inhibition of reproduction virus A/Hong Kong/1/68 (H3N2) in tissue cultures of chorioallantoic membranes (CCM) of 12-14 days age chick embryos.

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P42000-060

Esters of purine nucleosides (abacavir) with natural and unnatural amino acids -synthesis and antiviral activity

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Abacavir (Ziagen)–(1S,cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene -1-methanol is a synthetic carbocylcilic nucleoside analogue with inhibitory activity against HIV. Serious and sometimes fatal hypersensitivity reactions have been associated with abacavir. A possible way to increase the side effects is by modifying the known antiviral drugs with various amino acids. The aim of this study was to design and to synthesize of new amino acids and peptide (Gly, Gly –Gly) and thiazole containing (Gly, Gly –Gly) esters prodrugs of abacavir and to explore their activity on the HIV.

P42000-061

Chemical stability of some purine analogues

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In the search of new prodrugs effective against herpes simplex virus, series of acyclovir-9-[(2-hydroxyethoxy)methyl]guanine (ACV) esters with peptidomimetics, as well as abacavir–(1S,cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol derivatives have been synthesized and tested for antiviral activity. The chemical stability of some of them is studied at pH 1 and 7.4 and temperature of 37°C. A high-performance liquid-chromatografic (HPLC) method was developed for quantification of the unchanged ester concentration.

P42007-063

The Development of a FAP Activated Promelittin Pro-toxin: Targeting Cancer's Reactive Stroma

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Cytolytic peptides represent an attractive avenue for cancer drug development. The mode of action of cytolytic peptides is independent of the rate of cellular proliferation and such peptides are less prone towards drug resistance, since they target the lipid bilayer of the cell. One of the most studied cytolytic peptides is melittin. Melittin, a 26 amino acid amphipathic peptide, is the main toxic component in the venom of the honeybee Apis milefera. The ability of melittin to induce the lysis of prokaryotic and eukaryotic cells has been well documented. Melittin is synthesized in the honeybee as promelittin, containing a 22 amino acid N-terminal pro-domain rich in the amino acids proline and alanine. Promelittin represents a readymade cancer pro-toxin; the prodomain inhibits cytolytic activity and the sequence of the prodomain could be manipulated into a substrate for activating proteases. Here we present the development of a novel promelittin pro-toxin that can be activated by the serine protease fibroblast activation protein (FAP). FAP is over expressed on the surface of reactive stromal fibroblasts present in the stroma of human epithelial tumors. FAP is not expressed by tumor epithelial cells or by fibroblasts in other tissues, thus making FAP a pan-tumor target for pro-toxin development. Peptides containing truncated pro-domain sequences were tested on erythrocytes to determine the optimal prodomain length for inhibiting cytolytic activity. Once the length was optimized, modified promelittin peptides were generated that contained previously identified FAP substrate sequences in the prodomain. These peptides were digested with FAP and tested in vitro against FAP+ and FAP- cell lines in order to determine their FAP cleavage potential and toxicity. Our lead promelittin peptide was found to be efficiently activated by FAP and selectively toxic to FAP+ cell lines with an IC50 value in the low micromolar range that is similar to that of melittin.

P42014-064

Synthesis of Hepatitis B Surface Antigen Peptide Bioconjugates with Polyelectrolytes by Microwave Energy

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In this study, we aimed to develop a synthetic vaccine prototype for Hepatitis B disease. Hepatitis B is a viral disease. There are capsid particles on the virus outer side these particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigen HBsAg (Hepatitis B Surface Antigen). We synthesized the 95-109 WLVLLDYQGMLPVCPL

region of highly hydrophobic and the antigenic part of HbsAg by using microwave assisted Solid Phase Peptide Synthesis (SPPS). Tryptophan at the N terminus of the sequence was added by our research group for Fluorescence dedection analysis. This peptide was conjugated with synthetic polyanions. Different conjugates comparised each other. The synthesized bioconjugates analysized by choromatographic and fluorometric methods. The final product peptide was characterized by LC-MS and purified by RP-HPLC. The bioconjugates were synthesized using different activation mechanisms and the different initial molar ratios (npeptide/npolymer = 1, 3, 5, 7, 9) of the polyanions and the peptide. Also physicochemical properties of the bioconjugates were investigated. The structure and characterization of the synthesized conjugates was analyzed by various choromatographic and fluorometric methods such as HPLC, GPC and Fluorescence Spectrometer.

P42017-065

Study of spillover tritium reaction with insulin

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The reaction of high temperature solid-state catalytic isotope exchange (HSCIE)(1) between insulin and spillover tritium was studied. HSCIE reaction with the solid mixture containing 1 mg recombinant human insulin was performed for 10 min at 120o C using 5% Pd/BaSO4 catalyst. The product of the reaction was purified by HPLC on Waters Delta-Pak C4 300A 3.9 x 150 mm column. Tritium labeled insulin with specific radioactivity 40 Ci/mmol and radiochemical purity of more than 98% was obtained. Performic acid oxidation of cysteine residues and subsequent acid hydrolysis were used to analyze the distribution of tritium. It was demonstrated that specific radioactivity of À and B chains amounted to 8.2 and 31.8 Ci/mmol respectively. All the amino acids contained tritium and its content in His residues of the B chain was equal to 45%. Experiments for the evaluation of the biological activity of tritium labeled insulin were performed on CD-1 6-8-week-old awake male mice. Activity of labeled insulin was compared with the activity of standard insulin. Insulin was injected subcutaneously at a dose of 0.8 U/kg. Twofold statistically significant lowering of glucose level was observed 40 min after injection (4,9 + 0,4 and 4,3 + 0,3 mmol/l for standard and tritium labeled insulin, respectively). Lowering of the glucose level in animals obtaining labeled insulin was the same, as in animals obtaining standard insulin. Thus, HSCIE reaction of insulin with tritium doesn't change its hypoglycemic activity.

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P42018-066

The targeting properties of cell penetrating peptides

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Cell penetrating peptides (CPPs) offer the ability to penetrate across the plasma membrane of mammalian cells and to carry cargoes such as proteins, oligonucleotides and liposomes. Due to this, CPPs have gained high attention to improve the cellular uptake of the delivery of 'biologicals'. However, most of the research that has been performed with CPPs restricted to in vivo studies. Preclinical investigations are rare and the clinical validation of potential of CPPs is required. A series of CPPs were synthesized by solid phase peptide synthesis (SPPS) on an ABI 433A synthesizer. The stability of these peptides in human serum was determined. Subsequently, the uptake was studied in the following cell lines: SW 1736, PC-3, MH wt, HNO 97, MCF-7 and HCT 116 cells. The DOTA-conjugated peptides Poly Arginine (DOTA-RRRRRRRRR), SynB1 (DOTA-RGGRLSYSRRRFSTSTGR), Model Amphipathic Peptide [MAP] (DOTA-KLALKLALKALKAALKLA), the Membrane translocating sequence of kaposi fibroblast growth factor (DOTA-AAVALLPAVLLALLAP), the Nuclear Localization Sequence NLS (PKKKRKVK å(DOTA)), Penetratin (DOTA-RQIKIWFQNRRMKWKK), PreS2-TLM (DOTA-PLSSIFSRIGDP), pVEC (DOTA-LLIILRRRIRKQAHAHSK) Tat (DOTA-GRKKRRQRRRPPQ) and Transportan 10 (AGYLLGKå(DOTA)INLK ALAALAKKIL) were obtained in high yields. The stability determination in human serum revealed long half lives. The values ranged from 1 h (Penetratin) to > 72 h (Transportan). The in-vitro studies revealed high accumulation rates (e.g. 224% of the applied dose per million cells for the model amphipathic peptide [MAP] after 30 min incubation with the SW 1736 cell line) for most of the peptides studied. The in vivo biodistribution studies revealed the peptides are rapidly excreted and do not show specificity for any organ including PC-3 tumor cells. In summary, the in vitro uptake studies did not reveal great differences that might have revealed a tumor type specificity. In vivo studies revealed neither a distinct tissue uptake or tumor specificity.

P42021-067

Design and synthesis of a tripartate paclitaxel prodrug for melanoma therapy

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Therapy of melanoma continues to be a challenge since, regardless of the treatment used, long-term survival is quite uncommon. In an attempt to improve the effectiveness and decrease the toxicity of anticancer chemotherapy, one useful approach might be to administer a prodrug that specifically releases the active cytotoxic drug at the tumor site. In this work, we describe the synthesis of a peptide conjugate of paclitaxel potentially useful in the treatment of human melanoma, and characterized by the simultaneous presence of three functional domains: a "targeting domain", an "activation sequence", and the antitumor drug paclitaxel. The "targeting domain" of the prodrug is represented by an RGD-containing cyclic peptide, able to bind selectively to alpha-V beta-3 integrin, which is known to be highly over-expressed by both metastatic human melanoma cells, and endothelial cells of tumor vessels. The "activation sequence", responsible for the selective release of the drug, is a short peptide which is cleaved specifically by cathepsin B, a protease highly up-regulated in malignant tumors. The results of NMR conformational studies, as well as those of biological experiments aimed at evaluating the plasma stability of the prodrug and its ability to inhibit alpha-V beta-3–mediated tumor cell adhesion to vitronectin, will be also presented.

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P42024-068

Neuroprotective effects of Cortagen, Cortexin and Semax on glutamate neurotoxicity

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Cortexin is polypeptide (up to 10 kDa) brain extract accepted for clinical use in several countries including Russia due to its positive effects on memory, attention, and cortical processes. A synthetic peptide analog of Cortexin, Cortagen (Ala-Glu-Asp-Pro), was recently developed. The heptapeptide Semax (Met-Glu-His-Phe-Pro-Gly-Pro) is an analogue of the ACTH(4-10) fragment, which is completely devoid of any hormonal activity associated with the full-length ACTH molecule. Both Cortexin and Semax are currently effectively used for treatment of brain hypoxia and ischemia with comparable clinical benefits. However, the cellular and molecular mechanisms underlying the action in the brain are mainly unknown. In present work we studied effects of Cortagen, Cortexin, Semax, its analogue Pro-Glu-Pro (PGP) on glutamate-induced neuronal death and intracellular Ca2+ homeostasis deterioration in cultured cerebellar granule cells. To explore Glu-induced [Ca2+]i changes cultured neurons were loaded with fluorescent Ca2+ probe Fura-2FF. Cell viability was estimated using MTT-test or vital fluorescent dyes (propidium iodide or Hoechst 33258). In control cultures glutamate (100 ìM, 0 Mg2+, 10 ìM glycine) induced the development of delayed Ca2+deregulation (DCD) and 35% neuronal death (24 hours following glutamate challenge). Pretreatment of neurons with these peptides resulted in considerable decrease of the neuronal death after glutamate treatment and number of neurons exposed to DCD. Studied compounds are worth to be deeply investigated because of their promising role for therapy.

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P42026-069

Pharmaceutical drugability of peptides

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Peptide drugs gain increased interest as a new supra-group of therapeutics between the classic-organic, small-molecule drugs and the large biotechnology-derived bio-drugs. They are used in different therapeutic areas like allergy, anti-infection, oncology, obesity, etc… Due to their particular structure and biochemical origin, pharmaceutical development of a peptide drug poses special challenges which will be discussed here, exemplified by own research as well as literature data. Currently, there are about a dozen classical peptides described in pharmacopoeia, excluding the derived peptidomimetics like ACE-inhibitors. These pharmacopoeial peptide-drugs, together with the approved marketing authorisations of new peptidic entities, are a good starting point to look at the desired characteristics. Last, the regulatory developmental guidelines are in a general way seldom taken peptide drugs into account, leaving an interpretational gap between the two current main supra-groups of therapeutics. After the initial active pharmaceutical ingredient (API) synthesis, analytical characterisation is aimed at integrity and purity evaluation of the API, which is also required for biomedical experiments. In this analytical characterisation, sample treatment issues like solubility and adsorption are considered as well. The chemical and plasma-metabolic stability, a critical parameter for peptides, is to be assessed to obtain kinetic and mechanistic information. Functionality is tested in vitro using cell- and organ-based protocols, including ligand binding studies, as well as in vivo encompassing ADME and target-organ confirmation like brain. The pharmaceutical drugability information thus obtained allows further development decisions including required API modifications and proof-of-principle drug delivery formulations.

P42100-070

Lysine dendrimers and starburst copolymers as new carriers of anticancer drugs based on the complexes of platinum and gold.

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Though the complexes of metals such as platinum and gold are very promising compounds for the treatment of different types of cancer, the low solubility complicates their application as medicines. In this work a series of lysine dendrimers of different structure and starburst copolymers of lysine and glutamic acid was studied as solubilizing carriers of anticancer drugs based on the complexes of platinum (cisplatin) and gold. CD analysis showed the small changes in the structure of the polymers upon metal binding. The ESEM study revealed that the presence of the amino groups on the surface of the carriers is crucial for the metal ligation. The highest content of metal bound was found in the samples with high amount of lysine residues. While the content of gold was rather low in all the samples (about 2%), the copolymer of lysine and glutamic acid with the ratio 2:1 as well as lysine dendrimer of fourth generation had 7 to 10% of platinum linked to the carrier. The absence of the bromine in the samples in the case of gold complex allowed to assume the covalent bond between metal and polymer. In the same time all the conjugates were highly soluble in water. The peculiarities of the synthesis and the anticancer activity in different cell lines will be discussed.

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P42100-071

Prospects for the Development of Synthetic Peptide Vaccines against Hepatitis C

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Hepatitis C presents a serious threat to the human population health. No vaccinoprophylaxis of this disease or cardinal treatment means has been developed till now because of the problems produced by high genetic variability and heterogeneity of hepatitis C virus (HCV) isolates, lack of both HCV infection models and efficient expression systems for the virus and its components. The problem of anti-HCV vaccine development can be solved via a principally new approach, the creation of artificial synthetic immunogenic constructs with the help of bioinformatics and high-throughput screening technologies. Two approaches exist towards the development of anti-HCV peptide-based vaccines: one is to develop vaccines that stimulate cytotoxic T-cell response (peptides loading dendritic cells; the so-called "cell vaccines") and the second is to construct immunogenic peptides raising protective antibodies. However, the first approach may lead to the massive hepatocyte death and the development of the heavy hepatic injury during HCV infection. In order to develop peptide immunogenic constructs able to raise antibodies against whole native HCV envelope proteins analysis of amino acid sequences of these proteins belonging to different HCV genetic variants has been performed. This analysis has allowed to choose the most conserved and presumably functionally important protein fragments. Immunogenicity of these fragments in the whole protein molecules has been determined and interaction sites for one of the putative HCV receptor, heparansulfate, have been revealed with the help of peptide scanning. Despite of the low immunogenicity of the most fragments inside the whole protein, antipeptide antibodies against them have been obtained via immunizing by conjugates of the peptides with

certain carriers. Two synthetic peptide immunogenic constructs have been developed that have raised antibodies against whole HCV envelope proteins.

P42100-072

Synthetic immunoactive fragments of endogenous proteins: Selection and application for diagnostics and immunotherapy

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We have selected and synthesized the immunoactive fragments of four endogenous proteins: nucleophosmin, survivin, prion and alpha-7 subunit of acetylcholine receptor (AChR). Nucleophosmin and survivin are overexpressed in tumor cells and exhibit an antiapoptotic activity. Their detection in tumor cells could be used for tumor differential diagnostics and selection of optimal chemotherapy scheme. Accumulation of pathogenic isoform of prion protein in brain causes the neurodegenerative prion diseases and antiprion antibodies as known could be used for immunotherapy of prion disorders. Alzheimer's disease (AD) development is accompanied by an accumulation of β-amyloid peptides (βA) in brain and destruction of neurons . One of the AD development hypotheses assumed that βA when binding to the alpha-7 AChR forms a complex that penetrates into the cells, resulting in the neurons death. We have proposed that alpha-7 AChR could be a new target in the AD therapy and antibodies against alpha-7 subunit could prevent its binding to βA. Selected synthetic fragments of these four proteins were able in a free nonconjugated to a protein carriers state induce antibody response in experimental animals. Obtained rabbit antibodies against fragments of nucleophosmin and survivin were affinity purified. Antibodies against nucleophosmin detected mono- and oligomeric forms of this protein in immunoblotting of HeLa cells lysates. Antibodies against survivin were able to detect this protein in immunohistochemical test of the breast cancer tumor samples. Rabbit antiprion antibodies interfered with patigenic prion isoform in ScN2a neuroblastoma cell culture. It was shown that synthetic alpha-7 AChR fragments induced the antibodies formation in mice with symptoms of AD which penetrated the blood brain barrier, regenerated the spatial memory and normalized the beta-amyloid level in animal's brain.
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P42119-073

Peptide conjugates of new *in silico* identified drug candidates and first/second line antituberculars – design, synthesis and antimycobacterial effect

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Prolonged chemotherapy of tuberculosis could lead to poor compliance, which associates with relapse, spread of infection and emergence of drug resistance. New promising drug candidates effective on crucial enzymes of the pathogen and *in vitro* cultured *Mycobacterium tuberculosis* were identified by high performance *in silico* screening method. Considering that elimination of *M. tuberculosis* from infected phagocytes could be more efficient with target cell directed delivery of antituberculars we have prepared tuftsin and scavenger receptor's specific peptide conjugates. In these conjugates the *in silico* identified drug candidates or isoniazid (INH) or *p*-aminosalicylic acid (PAS) were used as drug moiety. The new conjugates were chemically characterized by ESI-MS, RP-HPLC and amino acid analysis. The Minimal Inhibitory Concentration (MIC) and Colony Forming Unit (CFU) of the free drugs, carrier peptides and the new drug-conjugates were determined on *M. tuberculosis* $H_{37}Rv$ and *M. kansasii* cultures. The cytostatic effect of the compounds was tested at concentrations equal to and higher than the MIC value by MTT assay on human hepatoma (HepG2) cell line and periferal blood monomorphonuclear cells (PBMC).
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P42120-074

Investigation of the antineoplastic properties chimaeric peptides, containing fragments of the P16INK4A and cell-penetrating sequence of the antennapedia

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The purpose of the work was investigate cytostatic and cytotoxic properties of the cell-penetrating chimaeric peptide are constructed from parts of two molecules: fragment of cyclin-kinase inhibitor p16INK4a and cell-penetrating sequences Antp. The action of peptide has been investigated on cell lines 293, A549, MCF-7 and HCT-116; on the shot-term cultures of human tumor cell received from surgical material. The concentration of peptides varied from 10 to 40 mkM in experiments with cell in vitro. The level of apoptosis and changes of a cell cycle was investigated by method of flow cytometry. In vivo experiments (nude mice) with tumor received by subcutaneous inoculation of 10 6 cells A549 or HCT-116 have been executed. Administration of the peptide starts when the tumors became palpable in quantity of 0,1 mg and 0,2 mg hypodermically into area of tumor. We observed that peptide pAntp_ p16INK4a in concentration of 40 mkM delay of transition G1-S phases of cells-cycle in cells for all investigated cell lines in vitro. The specificity of effects was proved by investigation pRB phosphorilation and expression Cycline D and A on mRNA level. The concentration peptide 10 mkM and higher induce concentration-dependent apoptosis. The MCF-7 cell line was less sensitive to proapoptotic action of peptide. The shot-term cell cultures of renal cancer were more sensitive then the breast, bladder, gaster cancer and normal skin cells. The experiments with nude mice has been shown, that the injection of pAntp_p16INK4a in tumor node leads to decrease of tumor growth and death rate of animals. Thus, it has been shown, that investigated peptide has anticancer properties in vitro and in vivo and is a promising antitumor agent.

P42123-075

Peptide-based cyclin A inhibitors: new tools to understand and to regulate the cell cyle-apoptosis signalling pathway

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The knowledge of the aetiology of cancer has increased considerably in the last two decades. The objectives have moved from unspecific cytotoxic chemotherapeutics to the identification of small molecules (and antibodies) with a well defined molecular target. Proliferation of eukaryotic cells is under control of a series of concerted molecular mechanisms defined as the cell division cycle whose progression is tightly governed by members of the cyclin-dependent kinase family.

The protein-protein complexes formed between different cyclins and cdks (CDKs) are central to cell cycle regulation. These complexes have been object of extensive research in cancer programs. Considerable effort has been focused on the development of ATP-competitive small molecule inhibitors of CDKs. However, in general, these compounds have several alternative protein targets that compromise their demanded selectivity. Cdk-2 was recently suggested to be dispensable for cell proliferation, although this does not appear to be the case for cyclin A, which therefore is defined as a more appropriate target for drug design. We have identified an hexapeptide (NBI1) that inhibits the kinase activity of the cdk2-cyclin A complex through selective binding to cyclin A (1). The characterization of the inhibitory mechanism revealed that the hexapeptide does bind neither to the ATP site nor to the cyclin recruitment site. A cell permeable derivative of the NBI1 peptide induces apoptosis and inhibits proliferation of tumor cell lines. We believe that the current structural and mechanisms of action studies on NBI1 will be useful for characterizing and controlling the important relation between cell cycle and apoptosis signalling pathways.

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P42313-076

Size and Zeta Potential Analysis of Synthetic Peptide-Carrier Protein Conjugates Depend on the Time

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The average size and size distributions of protein, polymer and peptide-protein or polymer-protein mixtures (complexes) and conjugates was investigated using photon correlation spectroscopy with a Zetasizer Nano ZS instrument. The zeta potential measurements of this complex and conjugates were carried out in the folded capillary cell of the Zetasizer Nano ZS instrument the same as used for dynamic light scattering measurements. In this study we investigate that size and zeta potential of synthetic Peptide-Carrier protein covalent conjugates. Synthetic peptides are small antigenic molecules and not strong immunogens because of their small size for this reason, synthetic peptide must be coupling to a suitable carrier proteins or polymers (BSA, KLH, OVA) for increasing their immunogenity. Most of the vaccine development studies are focused on the preparation of a good effective adjuvant. In this study we prepare carrier-protein (Bovine Serum Albumin Protein)- Synthetic peptide conjugates whit carbodiimide method by using 1-ethyl -3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). For one protein molecule we studied with different ratios of peptides (npeptid/nBSA) and synthesized the conjugates at these ratios. Conjugates are purified by using different column systems. Purified conjugates are characterized and the mechanism of the binding process was investigated comparatively with synthetic peptide, BSA and BSA-synthetic peptide physical mixture by using Zeta-Sizer Nano ZS depent on the time.

P42324-077

An analysis of deletion mutants of the PLD1 D4 domain defines short regions within the PLD1 interacting with PED/PEA15: implications for the development of peptides-specific antagonist.

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Phosphoprotein enriched in astrocytes 15 or in diabetes (PEA15/PED) is a small protein widely produced in different tissues and conserved among mammals (1). Several studies have revealed that it regulates cellular functions by binding components of intracellular transduction pathways. Recent reports also evidenced that it binds to phospholipase D (PLD1) and enhances its stability, resulting in increased intracellular levels of diacylglycerol(2), deregulating protein kinase C signalling and generating resistance to insulin action on glucose transport(3). Thus, disrupting the interaction between these proteins by a cell-penetrating compound represents a novel strategy for improving insulin sensitivity in target cells. The expression of D4 domain, (the shortest PED-interacting region with PLD1) in L6 skeletal muscle cells stably overexpressing PED, reduces the interaction of this protein with PLD1 (4), suggesting that this region could bind PED preventing its interaction with the whole PLD1 and restoring insulin action. Aim of this work is the identification of D4 crucial residues for PED interaction and the development of specific antagonists. We expressed three soluble truncated D4 domains, determining whether binds to PED like the D4 wild type, by carrying out dose-response ELISA. Only one of these regions exhibited an efficacy similar to D4-wt and functional cellular data support this evidence. To further investigate the D4 residues involved in PED binding, we prepared a set of overlapping peptides covering the D4-region identified. Our results suggest that the N-terminus region of D4 encompassing residues 762-801 is involved in PED/PEA15 recognition and, currently, cellular experiments on peptides are underway.

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P42324-078

Synthesis and biological activity of new linear and cyclic SHP-1 N-SH2-ligands

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The tandem SH2 domain (N-SH2, C-SH2) containing protein tyrosine phosphatase SHP-1 regulates multiple signal transduction pathways through the dephosphorylation of its specific pY-substrates. In the native state, however, the enzyme is inactive because of a N-SH2-PTP (catalytic phosphatase domain) interaction. The occupation of the N-SH2 domain with a pY-containing ligand leads to the dissociation of the N-SH2-PTP complex and subsequently to the activation of the enzyme (1). The sequence specificity for most SH2 domains is dictated by the amino acids surrounding the pY-residue (position 0) and the C-terminal positions relative to pY (2). In contrast, the SH2 domains of SHP-1 in addition to pY+1 and pY+3 depend on position pY-2 (3). It was further demonstrated that beyond this minimal consensus sequence the positions pY+4 and pY+5 also significantly affect the binding affinity and specificity of the SHP-1 SH2 domains (4). For the investigation of SHP-1 mediated signaling pathways, inhibitors of this phosphatase are of great interest. Therefore, we focused our research on linear and cyclic peptide ligands based on the previous studies [3-5]. The new ligands were C-terminally prolongated according to the recognition determinants for pY+4 and pY+5. Except, an additional motif designed to occupy a basic gap on the surface of the PTP-domain was introduced. The latter was predicted to impair the N-SH2-PTP dissociation process. The influence on SHP-1 phosphatase activity as well as binding affinities to the N-SH2 domain of these ligands will be discussed.

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P42414-079

EW–Peptide Family. One Family, Two Drugs.

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Two dipeptide analogs – L-Glu-L-Trp (1) and γ-D-Glu-D-Trp (2) – had been registered in Russia as immunomodulator Thymogen (1) and immunosuppressor Thymodepressin (2).
These two drugs possessed reciprocal activities in vivo [1, 2]. The individual peptides were initially separated by preparative HPLC from the crude thymus homogenate and sequenced. Dipeptide L-Glu-L-Trp was the most active in the majority of in vitro and in vivo tests.
In the process of SAR studies, the "signal" role of L-Glu-L-Trp in the immune response was discovered, as well as the critical role of the indolyl-side chain of Trp. Two particular enantiomeric structures – L-Glu-L-Trp (γ-L-Glu-L-Trp) and D-Glu-D-Trp (γ-D-Glu-D-Trp) – possessed reciprocal immunological effects in vivo. D-isomers cause a blockage of the immunocompetent cell proliferation, thus suppressing immuno- and hemopoesis in vitro and in vivo. Structure–functional in vitro studies were performed in different models to determine the biological target of these peptides.
Manifestation of in vitro effects as well as the influence of precise chemical and optical structures on biological activity of different EW-peptide analogs will be discussed. The experiments were conducted on blood neutrophiles, monocytes, thymic epithelial cells, lymphocytes, thymocytes, endothelial cells of human blood vessels and newborn blood cord. We evaluated the peptide effects on the phagocyte activity of cells, on the expression of functionally important membrane molecules and cell activation markers, and on the capacity of cells for mutual adhesion.
The dose dependence data of 1 and 2 on such activities as cytokine production, the processes of stimulation and suppression of apoptosis and proliferation of immunocompetent cells will be presented.

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P42600-080

A newly developed hydrophilic polymer-based ion exchange chromatography column for biomolecules

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Ion exchange chromatography (IEC) is widely used for analysis and purification of biomolecules. We have newly developed polymer-based IEC column, named YMC-BioPro, specially designed for separation of proteins, peptides and nucleic acids. YMC-BioPro IEC columns are based on 5 micron @porous and non-porous hydrophilic polymer beads with low nonspecific adsorption, and they show higher binding capacity and higher recovery of biomolecules compared to conventional IEC columns. The completely spherical and monodispersed beads, with optimal packing technology, provide high theoretical plate number and symmetrical peak shape. Excellent resolution is achieved from the high column efficiency coupled with the excellent selectivity of QA and SP ion exchangers. In this poster, we will show benefits of YMC-BioPro IEC columns and some example cases of superior separation of important biomolecules, such as monoclonal antibody and DNA.

P42613-081

Ultra-Fast Separations of Peptides and Proteins using Large Pore, Sub-Two Micron Columns

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The analysis of proteins and peptides by RP-HPLC is important in the development of well-characterized biotechnology pharmaceuticals. Since polypeptides interact only slightly with the stationary phase after desorption, short columns are suited for fast separations. Also, by decreasing the particle size of HPLC packings, column efficiency is increased. We demonstrate the use of short HPLC columns packed with 1.5um, large pore C18 silica for ultra-fast biomolecule analysis. Due to the short column format, high flow rates and optimal separations are possible, with moderate backpressures (< 3000 psi), using a conventional high-pressure gradient, binary pump system. Narrow-bore LC and LC-MS analyses were performed on a binary or a quaternary HPLC system, with detection by UV or MS (AB/MDS Sciex Q TRAP). The solvent system comprised of ACN/water or n-propanol/water plus one or two ion pairing agents (formic acid, TFA, HFBA) at 0.01 to 0.2%. A flow rate of 0.8 mL/min (1200 cm/h), 4x higher than typical for a 2.1 mm ID column, allowed for ultra-fast one minute separations of proteins with broad physicochemical characteristics. Closely related insulin variants (bovine, sheep, human) may be separated in under 1.5 minutes. While the typical HPLC run time for the separation of hemoglobin chains on conventional columns ranges from 25 to 60 minutes, runs under two minutes may be realized with the large pore, sub-two micron column. Accordingly, five different hemoglobin samples (from different animal species) may be compared after a total of only 14 minutes (includes run and equilibration time). 10 highly reproducible runs of a synthetic peptide mixture can be completed in < 20 minutes, including equilibration with 20 column volumes between runs. Intact IgG in sheep serum may be separated from albumin in < 4 min. using a n-propanol/water solvent system containing 0.1% TFA.

P42811-082

New peptides and triterpene derivatives as dimerization inhibitors of HIV-1 protease

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Peptidic dimerization inhibitors (DIs) of HIV protease (PR) are derived from the terminal segments of PR. They either prevent the formation of the dimeric and enzymatically active PR within the precursor polyprotein, or disrupt already formed PR into inactive monomers. Good DIs are lipopeptides, e.g. palmitoyl-YEL, Ki ~5 nM (Schramm HJ (99) Biol. Chem. 380, 593. Dumond J (02) Biochem. Pharm. 65, 1097). D-form amino acids can replace some of the amino acids. Triterpenes also show activity (Quéré L BBRC (96) 227, 484). By computer modelling, new modified peptides and triterpenes have been identified as possible DIs. The triterpene inhibitors have 2 carboxyl groups 10.4 A apart (at C4 and C28: medicagenic acid, quillaja acid, presenegine, gypsogenine, hederageneine). Modification of the 3-OH group is possible, e.g. attachment of amino acids through dicarboxylic acid linkers. The C28 carboxyl group should be free. The triterpene scaffold binds to a hydrophobic cleft formed by the re-arranged PR terminal segments; there are three attachment points: 3-OH (+ 2-OH) to R8, 4–COO- to R87 (+ T26), 28-COO- to P1, H69. The "maturation inhibitor" PA-457 of Panacos (betulinic acid modified at 3-OH) should also act as a DI! FDA approved PIs (darunavir, tipranavir) have recently been reported (Koh Y (07) JBC 282, 28709) to show DI activity in addition to competitive inhibition. This explains their activity against therapy mutants. It has already been shown that DIs act in synergy with active site inhibitors and may be valuable constituents of anti-AIDS cocktails.

P50107-001

Protein-oligopeptide fragmentomics

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The substantiation and definition of the term "fragmentomics" is given. Within the framework of this scientific direction the theoretical structural-functional analysis of all possible fragments of protein molecule can be performed with the purpose of determination of its sites which could be potential sources of the regulatory oligopeptides. The data on the primary structure of proteins from public protein databases, information from the EROP-Moscow database (1) that contains data on the structures and functions of the natural oligopeptides, and special computer program complex were used for it. As a result the natural regulatory oligopeptides both representing exact structures of protein fragments and containing protein fragments were found. The method has allowed also to reveal new potentially active sites of protein amino acid sequence yet not investigated experimentally. It was shown that different fragments of the food protein molecules are involved in amino acid sequences of many natural antimicrobial oligopeptides, toxins, neuropeptides, and hormones. These results confirmed deep relationship between the basic regulatory systems. In connection with the obtained data, the process of oligopeptide biogenesis, the possibility of natural formation of regulatory oligopeptides from different protein molecules, formation of exogenous oligopeptides pool, and correspondence of the obtained results with the conception of oligopeptide continuum are discussed. A possible practical importance of active fragments of proteins in regulatory processes of a living organism is noted.

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P51100-002

The binding of peptides containing tyrosine residue to β-cyclodextrin

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Cyclodextrins form inclusion complexes with various organic molecules. Aromatic amino acid residues bind to β-cyclodextrin with deep penetration of the cyclodextrin cavity. In case of oligopeptides binding depends on the peptide conformation. The formation of β-cyclodextrin inclusion complexes with the tyrosine residues within three peptides was investigated using steady-state fluorescence spectroscopy and molecular dynamic simulations. The free energy along the reaction pathway delineating the inclusion of tyrosine's aromatic ring into β-cyclodextrin was computed using umbrella sampling molecular dynamic simulation. The association constant and the corresponding association free energy were derived by integrating the potential of mean force over a representative ordering parameter. The three peptides studied consist of eighteen amino acids and the tyrosine residues are located at the position 1, 2 or 4. Selected sequences are fragments of NOTCH receptors. (NOTCH1=YKIEAVQSETVEPPPPAQ, NOTCH2 =TLSYPLVSVVSESLTPER, NOTCH3=PYPLRDVRGEPLEPPEPS). Out of three peptides only NOTCH3 binds strongly to β-cyclodextrin with binding constant similar to that of AcTyrNHMe. The binding of cyclodextrins with phenolic compounds involves nonspecific van der Waals and hydrophobic interactions and depends on accessibility of tyrosine sidechain.

P51107-003

Role of carboxyl groups in the secondary structure and function of sturgeon gonadotropin

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Free negatively charged carboxyl groups were selectively modified (neutralized) in sturgeon (Acipenser güldenstädti Br.) gonadotropic hormone (GTH) α and β subunits. 11 carboxyl groups, 3 in α and 8 in β subunit, were neutralized by the reaction with glycine ethyl ester. Investigation of re-associated α–β dimers (recombinants) comprising one or both modified subunits showed that specific hormonal activity was completely lost while immunoreactivity was lowered in comparison with that of the standard α–β dimer. CD-spectroscopy of the modified subunits did not indicate considerable changes in their spatial structure. Conclusion was made that free COOH-groups of GTH are important as bearers of the negative charge necessary for the hormone activity on the level of the hormone-specific membrane receptors.

P51317-004

Coiled Coil Peptides as Entities for Molecular Computation

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Living cells can process rapidly and simultaneously multiple extra-cellular input signals through the complex networks of evolutionary selected biomolecular interactions and chemical transformations. Recent approaches to molecular computation have sought to mimic or exploit various aspects of biology. A number of studies have adapted nucleic acids and proteins to the design of molecular logic gates and computational systems, while other works have affected computation in living cells via biochemical pathway engineering. We described recently the graph structure and experimental analysis of a self-organized synthetic peptide network (1-3). The system was designed rationally to operate in neutral aqueous solutions based on sequence selective auto- and cross-catalytic template-directed coiled-coil peptide fragment condensation reactions. Here we show that such de novo designed synthetic networks can also mimic some of the basic logic functions of the more complex biological networks. Consequently, we describe experimental and simulation studies that highlight the possibility of segments of the networks to express all sixteen two-input Boolean logic functions (4, 5).

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P52200-005

Antiparallel and Parallel Double Helix Patterns in α-Peptides

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Many studies deal with the folding of double helices in nucleic acids. In contrast, much lesser attention is given to double helices in peptides. Nevertheless, the investigation of peptides with alternating L- and D-α-amino acids, like Gramicidin A, shows various double helix patterns with antiparallel and parallel arrangements of the strands. In this study, we want to give a systematic overview on the possibilities of double helix formation in α-peptides on the basis of ab initio MO theory. According to general principles, double helices with characteristic intermolecular hydrogen bonding patterns were generated and verified as minimum conformations at the Hartree-Fock level employing the 6-31G* basis set. The calculations show several types of antiparallel and parallel double helices with different stability, which is strongly influenced by backbone substituents.

P52200-006

Investigation of substrate interactions with dengue virus proteases by using proteochemometric analysis

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The dengue virus causes a spectrum of clinical symptoms, ranging from mild dengue fever to the severe forms of dengue hemorrhagic fever and dengue shock syndrome. There are four dengue virus serotypes (DEN1-4). The dengue virus NS3 protease is an attractive target for development therapeutics against dengue.
The aim of the study was to investigate the prime side specificity of dengue virus NS3 protease substrates using proteochemometrics, a new technology for drug target interaction analysis. A set of 48 internally quenched peptides were designed using statistical molecular design (SMD), synthesized and assayed with proteases of four subtypes of dengue virus for Michaelis (K_m) and cleavage rate (k_{cat}) constants. The obtained data were subjected to proteochemometrics analysis, concomitantly modeling all peptides on all the four dengue proteases, which yielded highly predictive models for both activities. The interpretation of the models suggested that considerably differing physico-chemical properties of amino acids (AA) contribute to K_m and k_{cat} activities. It was found that for k_{cat} activity only P1' and P2' prime side residues played important role, while for K_m all four prime side residues, P1'-P4', were important. High cleavage rate (characterized by k_{cat}) was obtained for peptides having small AA (Ser, Gly, Ala) at the P1' position and small or flexible AA at the P2' position. For high affinity (low K_m), at the P1' position AA should be small and moderately hydrophilic, while acidic residue is highly unfavorable. For the P2' position, the most favorable was Trp; however, this position allowed a broader diversity of AA. For the P3' position, Cys was more beneficial than the AA present in native cleavage sites of the dengue polyproteins. For the P4' position, the best affinity would be obtained with Ala, Gly or Cys.
The models may be used to modify each P' substrate position to optimize separately substrate affinity and cleavage rate for DEN1-4 proteases.

P52212-007

Identification of novel bioactive peptide sequences from human proteins for the development of potential therapeutics

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Many protein-protein interactions are facilitated by the binding of peptides recognition modules to short linear motifs within the target proteins's primary sequence. Therefore, synthetic peptides based on parent sequences of human proteins containing functional motifs are useful tools to uncover protein signaling and interactions. To date, peptide studies typically derive sequences from a single identified protein or (at the other extreme) screen random combinatorial peptides, often without knowledge of the signaling pathways targeted. The objective of the novel bioinformatic approach presented here was to determine whether rational design of oligopeptides specifically targeted to potentially signaling-rich juxtamembrane regions could identify modulators of human platelet function (1). The aim of this project was to identify peptides that span residues strongly conserved in the corresponding proteins in other species (orthologues), but that differ from those in related human proteins (paralogues). Synthetic selection rules to avoid unfavorable amino acid combinations and excessively hydrophobic peptides were devised to reduce the risk of by-products during synthesis, postsynthetic degradation and solubility problems. High-throughput in vitro platelet function assays of fatty acid-modified cell-permeable peptides corresponding to these regions identified many agonists and antagonists of platelet function. The combined bioinformatic and experimental screens of human protein subsequences can therefore be used to validate functions of candidate proteins and provide templates for the development of potential therapeutics.

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P52225-008

Recognition of ligands by similar peptide fragments from unrelated protein structures

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Many small molecule ligands (or parts thereof) with important roles in the biochemistry of the cell are recognized by different proteins with different cellular functions. These proteins do not necessarily share a common fold since their three-dimensional structures can differ completely such that they are considered to be unrelated proteins. Surprisingly, we see on numerous occasions that similar ligands are often recognized in a very similar way by means of a common structural motif formed from main-chain elements of several consecutive amino acids within the ligand-binding site of the proteins. These short polypeptide segments can be found in many protein folds, and variations in their structure can often explain different elements of protein function. In particular, we have characterized the presence of a nucleotide binding motif present in more than 30 protein folds that function in part to recognize the adenine ring system in many essential biological ligands (e.g. ATP, NAD(P), FAD, FMN, CoA, etc.). Furthermore, a study of unrelated folds among pyridoxal phosphate dependent enzymes led to the characterization of a CαNN structural motif for protein recognition of phosphate ions common to 104 fold-representative protein structures that belong to 62 different folds. Interestingly, amino acid side chains play little or no role in ligand recognition, explaining the evolutionary independence of such binding mechanisms (i.e. the motifs are found in unrelated folds), while possibly reflecting the types of interactions that were characteristic of the earliest protein-ligand interactions during early stages of molecular evolution.

P52228-009

Bioinformatics meets medicine: Structure-based peptide design as a basis for drug development

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Here, we present different structure-based approaches on the design of peptides mimicking the whole surface, a specific region of a protein or even tumor markers. The potential of such methods stretches from the development of peptide-derived drug candidates to immunological screenings.
Mimicry of binding sites:
We have developed SUPERFICIAL, a program that proposes peptide

libraries representing the entire surface or just regions of proteins. These peptides can be synthesised, e.g. using the SPOT-synthesis technique, and tested for their ability to mimic linear as well as non-linear binding sites. As a proof of principle, a library of peptides representing the surface of lysozyme was generated starting from a crystal structure of a lysozyme-HyHEL-5 complex. Adjacent sequence segments were linked by spacers to conserve local conformations. Disulfide bonds were generated to tether the peptides. Binding assays against the HyHEL-5 antibody identified several peptides representing the non-linear recognition site of the lysozyme.
Translation of a tumor marker into peptides:
The tumor-associated carbohydrate antigen GD2 is an established target for immunotherapy in neuroblastoma. We proposed peptidic mimics of this ganglioside for active immunisation against the tumor marker GD2 using molecular modelling. The ability of these peptides to mimic the carbohydrate moiety was verified with in silico docking experiments. In a next step they were successfully tested as mimotopes in a mouse model.
Design of switchable peptides: Photo-switchable compounds are becoming increasingly popular for a series of biological applications. Goal is the reversible photo-control of structure and function of biomolecules. A required death domain for the binding of proapoptotic proteins (e.g. Bak) to the hydrophobic groove of anti-apoptotic proteins is the BH3 helix. Inserting the photo-reactive compound hemithioindigo into this short peptide, stabilization towards proteolytic degradation is achieved.

P52300-010

Restoring antigen binding for mutated antibodies by using Spot technology

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The identification of peptides that bind to antibodies is an important step in characterizing antibody specificity in order to study molecular recognition. Numerous approaches have been developed to select peptides that bind with desired specificity and affinity to antibodies of interest. In our study peptide arrays produced by Spot technology were used to develop and optimize binders to antibody derived by mutations from the anti-p24 (HIV-1) single chain Fv antibody, CB4-1. Three mutations were introduced in the complementarity determining region 3 of the light chain being in close proximity with epitope-homologous peptide. This mutated antibody had completely lost its affinity for the epitope-homologous peptide. A substitutional analysis of epitope-homologous peptide was performed on cellulose, in which all positions of the peptide sequence were substituted by each of the 20 naturally occurring amino acids. The peptides representing the binding activity in Spot membranes were synthesized using solid phase synthesis and their binding activity was confirmed by fluorescent polarization method. A substitutional analysis indicated that binding to mutated antibody could be restored and improved by combining favourable substitutions at the N-and C-terminal residues of the epitope-homologous peptide. This approach has allowed us to generate binders from non-binders to mutated antibody in high micromolar range.

P52422-011

Cryptic Signal: A Novel Signaling Mechanism Involving Cryptides

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It is well known that physiological regulatory peptides that act as hormones and neurotransmitters are produced by the specific cleavage of their precursor proteins that per se have no biological functions. During these processes, many fragmented peptides are also produced from the same precursor proteins. It is expected that they may have various unexpected biological activities, but their biological functions have not been investigated in depth. The neutrophil-activating peptides we recently purified turned out to be the peptides that are cleaved from mitochondrial proteins by proteolysis, suggesting that fragmented peptides produced by maturation and degradation of functional proteins may also have various biological functions [1,2]. Therefore, we named such functional cryptic peptides hidden in protein sequences cryptides, and those cryptides that are derived from mitochondrial proteins “mitocryptides” in particular (3). We then identified many mitocryptides by the combined investigation with “dry” and “wet” experiments, i.e., the sequences of mitocryptides that activate G proteins were predicted based on the distribution of charged and hydrophobic residues and their activities were examined on granulocytic cells. Receptors for these peptides were also characterized by the direct cross-linking experiments between peptides and their targeted proteins. Moreover, it is demonstrated the presence of a novel signaling mechanism involving mitocryptides. The comprehensive identification of cryptides is expected to lead to the elucidation of various cryptic signaling mechanisms.
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P60105-001

Photoinduced macrocyclizations on helical Bpa/Met oligopeptides

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The 4-benzoylphenylalanine (Bpa) residue is widely used as a photoaffinity label for the study of intermolecular (peptide) ligand-protein (receptor) interactions, where it is thought to function by hydrogen-abstraction from Met residues followed by covalent C-C bond formation of the resulting radical pair. We are carrying out detailed studies of this reaction in a series of five, structurally rigid, hexapeptides of general sequences Boc-$U_xBU_yMU_z$-OMe and Boc-$U_xMU_yBMU_z$-OMe, where B = L-Bpa, U = Aib, M = L-Met, and $U_x+U_y+U_z = 4$, with a view to determining the effects of spacer length (U_y = 1-3) on the rate of the intramolecular excited state reaction and the chemical and 3D-structures of the resulting products. The triplet state lifetimes of the Bpa residues in the compounds, determined in a deoxygenated, dilute, acetonitrile solution by laser flash photolysis, vary in the following order: UBU_2MU, τ = 60 ns; UMU_2BU, τ = 190 ns; $UBUMU_2$, τ = 350 ns; $UBMU_3$, τ = 430 ns; and UBU_3M, τ = 920 ns. In addition to the information these data provide on the structural requirements for intramolecular excited state quenching in the molecules, they also serve to define the conditions necessary for optimal intramolecular reaction in preparative photolysis experiments. Accordingly, the products resulting from UBU_2MU and $UBUMU_2$ have been prepared in high yields, isolated, and structurally characterized by HPLC, mass spectrometry, NMR, and CD techniques. For each of the two photoinduced macrocyclizations, two diastereomers, arising exclusively from the Bpa diradical regioselective attack on the Met S-methyl group, were found.

P60111-002

Molecular dynamics study of β-peptides

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During the past decades, the dependence of the secondary structure of peptides on their amino-acid sequence has been the focus of numerous investigations. In addition to naturally occurring peptides based on α-amino acids, peptides based on β-amino acids, have raised particular interest because of their potential for pharmaceutical use. These peptides often have high folding propensities and it has been found that peptides with as few as four residue may fold into a stable secondary structure.
β-peptides offer the possibility to investigate the folding-unfolding equilibrium in the context of very small systems, therefore they have also been the subject of a number of investigations based on atomistic molecular dynamics (MD) simulations which revealed that the folding-unfolding equilibrium typically occurs on time scale of nanosecond to tens of nanoseconds.
How the backbone bound heteroatoms (OH, NH_2,F) influence the structure of a peptide is little known by now, both experimentally as well as theoretically. We performed several MD studies on the conformational behavior of centrally placed βHAla(α-Met) fluoro and hydroxy analogues, βHAla(α-F) and βHAla(α-OH), as function of chirality and level of substitution to investigate the influence of these factors on secondary structure formation and stability. All β-peptides were simulated in methanol solution at temperature of 340 K and a pressure of 1 atm using the GROMOS96 biomolecular simulation software and the GROMOS 45A3 force field. The ensembles of trajectory structures were analyzed in terms of conformational space sampled by the peptide, folding behavior, structural properties such as hydrogen-bonding and in terms of the level of agreement with the available experimental NMR data, NOE's and ^{3}J-coupling constants.

P60111-003

Synthesis and conformational analysis of doubly spin-labelled β-peptides based on the nitroxide bearing POAC residue

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The nitroxide spin-labelled $\beta^{2,3}$-cyclic amino acid POAC was synthesized, resolved and its absolute configuration assigned (K. Wright et al., *Tetrahedron*, **2008**, *64*, 4416-4426) in order to be used as a spin-probe to evaluate the 12-helical β-peptide secondary structure. A series of β-hexapeptides, Boc-ACPC-POAC-POAC-ACPC-ACPC-ACPC-OMe, Boc-ACPC-POAC-ACPC-POAC-ACPC-ACPC-OMe, Boc-ACPC-POAC-ACPC-ACPC-POAC-ACPC-OMe, and Boc-ACPC-POAC-ACPC-ACPC-ACPC-POAC-OMe, based on the (3R,4R)-POAC enantiomer, combined with (1S,2S)-ACPC for configurational homogeneity of the amino acid components, was designed. In these hexapeptides two POAC residues are incorporated at positions i, i+n (n = 1-4) to observe conformation-related spin-spin interactions. The peptides were synthesized by N- to -C chain elongation of N^{α}-Boc protected peptide segments in solution. Their conformational analyses, performed by CD, FT-IR absorption and ESR spectroscopic techniques, will be also described.

P60111-004

Design and synthesis of asymmetric β-Sandwich Proteins

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Computational design has been successful in creating new proteins. We focus on the design of a β-sandwich protein which is challenged by general problems as H-bonds between neighboring strands being dependend on a tightly packed hydrophobic core and aggregation. An improved version of our program ProPac was tested to repack the backbone of several natural protein structures with high reproducibility. Starting with backbone coordinates we found amino acid sequences by packing amino acid side chains with defined conformations (rotamers) into the core of β-sandwich proteins. In a first approach we have assembled four synthetic β-hairpins on a cyclic peptide template (TASP) to form a β-sandwich with two identical four-stranded antiparallel β-sheets. Improvement of surface residues reduced the aggregation of the proteins. Spectroscopic analysis shows a fold with a free energy near -25 kJ/mol and confirms the β-structure by CD and FTIR. We improved the design to a partial asymmetric β-sandwich assembled from three different β-hairpins. The synthesized molecule was the first β-sandwich molecule showing a defined fold in 2D-NMR. This data and results from the symmetric betabellins (1) suggest that symmetrical sheets do not fold into defined structures. To synthesize completely asymmetric sheets we simplified the synthesis of β-sandwiches by directed coupling of two four-stranded antiparallel β-sheets. The computed proteins were selected for tight packing of the hydrophobic core and analyzed for a stable fold during dynamic simulations. One of our goals is to find a correlation between the experimentally determined protein stability and the parameters used for the selection of the residues in the hydrophobic core and at the surface.

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P60329-005

Biocatalyst-catalyzed peptide synthesis using inverse substrates AS acyl donor

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Previously we reported that the *p*-amidinophenyl esters behave as specific substrates for trypsin and trypsin-like enzymes. In these esters the site-specific group (charged amidinium) for the enzyme is included in the leaving group portion instead of being in the acyl moiety. Such a substrate is termed an inverse substrate (1). Inverse substrates allow the specific introduction of an acyl group carrying a non-specific residue into the trypsin active site without recourse to a cationic acyl moiety characteristic of conventional substrates. We also showed in an earlier study that inverse substrates were applicable to enzymatic peptide synthesis. Therefore, a general method for the preparation of a variety of inverse substrates would be valuable. We designed two series of new type inverse substrates, *p*-(amidinomethyl)phenyl and *m*-(amidinomethyl)-phenyl esters derived from *N*-(tert-butyloxycarbonyl)aminoisobutylic acid, were prepared. We also analyzed the kinetic behavior of trypsin towards these synthetic esters (2). They were found to be readily coupled with an acyl acceptor such as L-alanine *p*-nitroanilide to produce dipeptide. The optimum condition for the coupling reaction was studied by changing the organic solvent, pH, and acyl acceptor concentration. The optimum standard condition was selected as follow: acyl donor (inverse substrate), 1 mM; acyl acceptor (L-alanine *p*-nitroanilide), 20 mM; enzyme, 10μm; 50% DMSO-MOPS (50 mM, pH 8.0, containing 20 mM CaCl2); 25 Ž. An α-aminobutyric acid containing dipeptide was obtained in high yield. Streptomyces griseus trypsin was a more efficient catalyst than the bovine trypsin. It was found that the enzymatic hydrolysis of the resulting product was negligible.

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P60700-006

Peptides derived from NK-2 selective for phosphatidylserine as novel cancer agents

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Chemotherapeutic agents, commonly used as anti-cancer drugs, have severe side effects, also affecting healthy human cells. Natural antimicrobial peptides, and their derivatives, have gained interest as potential anti-cancer agents. Under normal conditions, due to the asymmetric distribution of plasma membrane lipids across the bilayer, mammalian cells comprise phosphatidylserine (PS) only in the inner leaflet. In the case of malignant transformation inner leaflet PS can move to the outer leaflet and act as a surface marker. The surface exposure of negatively charged PS on various tumour cells makes these cells susceptible to killing by cationic membranolytic peptides such as NK-2 (1).The aim of this study is to develop short peptide sequences still acting selectively towards PS exposed on cancer cells without damaging healthy cells. In order to use this new strategy with PS-specific peptides it is necessary to analyze the lipid composition of mammalian cancer and non cancer cell membranes. Further as a basis for peptide activity studies the biophysical characteristics of cancer cell membranes and healthy counterparts with respect to lipid composition were determined by investigation of liposomal mimics composed of phosphatidylcholine and/or phosphatidylserine by DSC and X-ray. These model systems were also used to screen the activity of a series of peptides derived from NK-2. Fluorescence spectroscopy was applied to test the release of fluorescence marker molecules from liposomes composed of solely PS, PC or PC/PS mixtures in the presence of various concentrations of peptides. Data revealed that some NK-2 derived peptides have a high affinity towards PS causing significant leakage of liposomal content, whereas healthy mammalian cell mimicking PC liposomes were not affected. Optimized peptides resulting from these experiments will be used for in-vitro studies on prostate cancer cell lines.

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P60711-007

NMR solution structure analysis of the active and inactive fragments of pheromone biosynthesis-activating neuropeptide (PBAN) from the silkmoth *Bombyx mori*

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In most moths, the sex pheromone production is regulated by pheromone biosynthesis-activating neuropeptide (PBAN), a 33-34 amino acid neuropeptide that is released from the subesophageal ganglion into the hemolymph in response to physiological and environmental cues. PBAN exerts its pheromonotropic effects by binding to PBANR, a member of the rhodopsin-like family of G protein-coupled receptors (GPCR) that is predominantly expressed in the pheromone-producing cells of the female pheromone gland. The structure-activity relationship studies for *Bombyx mori* PBAN have revealed that the shortest peptide with pheromonotropic activity is the C-terminal pentapeptide-amide, PBAN(29-33)-NH_2 (FSPRL-NH_2), and that the C-terminal amide group is required for the pheromonotropic activity of PBAN.

In this study, we have analyzed the solution structures of the C-terminal decapeptides derived from *Bombyx mori* PBAN with an amidated and a free C-termini by two-dimensional NMR. The pheromonotropically active decapeptide-amide, PBAN(24-33)-NH2 (SRTRYFSPRL-NH2), and its inactive counterpart, PBAN(24-33)-OH (SRTRYFSPRL-OH), were dissolved in three kinds of solvents: (1) 50 mM sodium phosphate buffer (pH 6.0)/100 mM NaCl/0.02% NaN3 in 90%(v/v) H2O/10%(v/v) D2O (buffer A), (2) 500 mM dodecyl phosphocholine (DPC)-*d*38 in buffer A, and (3) 30%(v/v) 2,2,2-trifluoroethanol (TFE)-d_3 in buffer A. The NMR data indicated that these decapeptides did not adopt specific conformations in buffer A, but they adopted specific conformations in the presence of DPC micelles or TFE. These peptides exhibited different chemical shifts for some protons in all the solvents used, and they took similar but different conformations in the presence of DPC micelles. The conformational difference between these peptides may reflect their difference in pheromonotropic activity.

P60712-008

Structuring and membrane interactions of the human antimicrobial cathelicidin LL37

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Cathelicidins are a family of vertebrate Host Defence Peptides with both a direct capacity to inactivate microbes and to modulate components of the innate and adaptive immune systems. They are characterized by a conserved pro-region carrying individual, highly variable antimicrobial sequences, that become active only after proteolytic release, and with quite different structural and aggregational features that lead to differential biological effects on prokaryotic or eukaryotic cells. LL-37 is the only human Cathelicidin, and displays a broad-spectrum, medium sensitive antimicrobial activity in vitro that is accompanied by some cytotoxicty towards eukariotic cells at antimicrobial concentrations,

and a strong capacity to modulate host immune and healing processes at lower concentrations. These activities likely all involve interaction with biological membranes at some point, and are related to its amphipathic, helical structure and strong tendency to aggregate in specific conditions. The latter is an evolved feature absent in some primate orthologues, whose activities appear more limited to a direct antimicrobial action. The structural and aggregational behaviours of LL37 and selected primate orthologues were systematically investigated by biophysical and biochemical methods. These included CD spectroscopy in different buffers, SDS micelles or anionic or zwitterionic LUVs, transmission FTIR spectroscopy, dye release from liposomes, and ATR-FTIR spectroscopy on supported lipid monolayers, followed by atomic force microscopy to probe morphological effects on lipid order. Data was also collected from antimicrobial, cell lysis and cytofluorimetric assays, giving a more complete picture of the possible mode of action of these helical peptides, and highlighting the importance of biochemical parameters such as structuring and dimerization/oligomerization processes in the selective interaction with biological membranes, leading to cytotoxic or immunostimulatory effects.

P60712-009

Oligomeric Structure of Fowlicidin-1, an Antimicrobial/ Anti-endotoxic Peptide from the Family of Cathelicidin, in Lipopolysaccharide Bilayer

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A recurring theme in the structure-function correlation studies of the cationic antimicrobial peptides (AMPs) is that the formation of oligomeric structures in lipid environments by AMPs. Self-assembly of AMPs may result disruption of the membrane (outer/inner) structures of the microorganisms by forming pores or ion channels. However, high-resolution oligomeric structures of AMPs in lipid environments are difficult to obtain. To-date, oligomeric structure at atomic resolution of any antimicrobial peptide has not been reported. Secondary structures of AMPs are usually obtained in perdeuterated SDS or DPC micelles, as a mimic to the inner cytoplasmic membrane, by NMR spectroscopy. Cathelicidins comprise a major family of host-defense antimicrobial peptides in vertebrates. These peptides are synthesized as a part of large precursor proteins containing a well conserved cathelin domain and an extremely variable, in terms of length and amino acid compositions, C-terminal region. The C-terminal part of the cathelicidins is bestowed with antimicrobial and LPS neutralizing activities. Here, we report a tetrameric structure of a 22-residue active fragment of fowlicidin-1 or VK22, one of the five cathelicidins found in chicken, in lipopolysaccharide by NMR spectroscopy. The tetrameric structure of the VK22 determined from trNOE is highly helical. A large number of trNOE cross-peaks were observed connecting residues far apart in the sequence. Calculated structures reveal an anti-parallel arrangement of four helices. The interface of the tetramer appears to be rich in polar or charged residues delineating a plausible pore or channel. Most of the non-polar residues are found to be exposed indicating plausible interactions with non-polar fatty acyl chains of LPS or with membrane in general. The oligomeric structure of VK22 peptide may be useful to understand structure/activity relationship, in particular pore formation, by the helical antimicrobial peptides.

P60712-010

The Interaction of the Antimicrobial Peptide NK–2 with different Lipid Systems

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Antimicrobial peptides are important components of the natural defense system of most living organisms against invading pathogens. These are relatively small (below 10kDa), cationic and amphipathic peptides of variable length, sequence and structure. In this study, the antimicrobial peptide NK–2, which is a 27 amino–acid residue derivative of the cationic core region of NK–Lysin, has been used. The used membrane mimetic model systems have different dimensionality: Two–dimensional (monolayers at the air–liquid interface) as well as three–dimensional (vesicles, micelles) systems of different phospholipids.
The aim of this study was to investigate the influence of peptide–lipid interactions on the lipid structure and vice versa on the secondary structure of the peptide. The peptide secondary structure was measured by CD (Circular Dichroism spectroscopy) in bulk and IRRAS (Infrared Reflection–Absorption Spectroscopy) at the air–liquid interface. The structure of lipid Langmuir monolayers has been examined by numerous techniques such as pressure–area isotherm measurements, fluorescence and Brewster angle microscopy and X–ray techniques.
Charged as well as zwitterionic phospholipids have been used to study the adsorption of NK–2. The peptide adsorbs at the air–buffer interface due to its amphiphilic character. It also inserts into uncompressed phospholipid monolayers. The peptide reorients from random coil in bulk to α–helix lying flat at the interface. The incorporation of NK–2 into a DPPG monolayer leads to a different orientation (either α–helix with an oblique orientation or random coil) and to the fluidization of the aliphatic chains. NK–2 influences also the structure of ordered DPPG domains. To assess the location of the peptide NK–2 in the lipid matrix, specular X–ray reflectivity studies were carried out.

P60712-011

Membrane-fusion properties of various antimicrobial and cell-penetrating peptides

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Antimicrobial peptides (AMPs) constitute an essential part of innate immunity and act against an external microbial invasion where as cell-penetrating peptides (CPPs) can carry a biologically functional molecule through the cell membrane and are relevant in drug delivery developments. Fusogenic peptides (FPs) are active on membrane-interfaces and are instrumental in fusion of membranes which is vital for various fertilization and viral processes. Membrane fusion properties of short AMPs and CPPs have been seldom tested and have therefore eluded the attention of most investigators, instead only their cytotoxicity is investigated. There is general lack of understanding if these peptides are fusogenically active. We have investigated the ability of AMPs and CPPs to trigger membrane fusion. As an example, HIV-1-fusion peptide FP23 is used as benchmark to compare fusion activity of various AMPs and CPPs. Most FPs are believed to be unstructured or conformationally flexible (α-helix or β-sheet); therefore the secondary structure of the peptides before and after the fusion reaction is investigated and correlated to their respective ability to execute membrane fusion. Our results show that various AMPs and CPPs which are capable to switch their secondary structure are also able to promote fusion to an extent that is even higher than the known HIV-1 fusion peptide FP23. These results will be presented in the poster.

P60712-012

Interaction of the minimal active peptide sequence of human growth hormone releasing factor with negatively charged liposomes

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The most bioactive peptides such as hormones and neurotransmitters do not exist in an ordered structure in aqueous solution. The conformation of the peptide changes from this flexible unordered structure into an inherent one, only when it reaches a biomembrane. That's why it is important to investigate the such peptides like growth hormone-releasing factor (GHRF) in the presence of lipid bilayers. Human

GHRF is an amidated peptide consisting of 44 amino acids residues. A lot of studies has show that the residues N-terminal part are the active core of the peptide. We studied the interaction of human GHRF (1-29) with phospholipid membranes. The interaction of GHRF (1-29) with the liposomes was investigated fluorescence spectroscopy. To detect a fluorescence signal the peptide were labelled at first, 15th, 24th and 29th position with Dansyl. The fluorescence intensity of GHRF (1-29) at different lipid-peptide-ratios were analysed using a model, which includes the hydrophobic and the electrostatic interaction between the peptide and the phospholipid membrane. The hydrophobic binding constant of GHRF(1-29) and its effective charge were determined using this model. Further the peptides position in the membrane were investigated using spin labelled phospholipids, which are able to quench fluorescence over large wavelength arrays. The quenching efficiency will decrease by increasing the distance between flourophore and spin probe. The use of 15 mol% Tempo-PC, 5-Doxyl-PC, 10-Doxyl-PC and 16-Doxyl-PC in the phospholipid composition of the liposomes allows the determination of the peptides membrane penetration depth. All results indicate, that the binding of GHRF (AA 1-29) to phospholipid membranes is increased strongly by increase of the surface charge density of the liposomes. The peptide is arranged parallel to the membrane surface and is localized in membrane-water-interface of the liposomes.

P60728-013

Folding propensity and biological activity of selected antimicrobial peptides

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The innate immunity of multicellular organisms relies in large part on the action of antimicrobial peptides (AMPs) to resist microbial invasion. Crafted by evolution into an extremely diversified array of sequences and folds, AMPs do share a common amphiphilic 3-D arrangement. This feature is directly linked with a common mechanism of action that predominantly (although not exclusively) develops upon interaction of peptides with cell membranes of target cells. It is generally agreed that AMPs are essentially unstructured in the aqueous phase and fold upon contact with the membrane, adopting an amphiphilic fold. This favours absorption of peptides onto lipid bilayer and their subsequent integration into the membrane with expansion of the outer leaflet, which in turn leads to membrane thinning and permeabilization. However, recent observation suggest that things might be more complicated than previously believed. Indeed, MD simulations coupled to CD spectroscopy, studies with model membranes, and antimicrobial assays, have shown that, at least for some peptides, a significant correlation exists between the conformation adopted by the peptide in solution, i.e. before the interaction with membranes, and its antimicrobial activity. The linear peptides we have studied following this approach include two members of the frog skin-derived temporin family, namely temporin A and temporin L, and two members of amphibian bombinins H, i.e. bombinins H2 and H4. We observed that the presence of a partially folded structure in water solution may facilitate, both thermodynamically and kinetically, the peptide folding in the microbial membrane, and thus favour biological activity. Looking for such built-in conformational characteristics could well help to rationalize the different spectrum and level of activity recorded for cationic alpha-helical AMPs on membrane-enveloped targets, and assist the design of improved analogs and biomimetic synthetic peptides with antibiotic properties.

P60912-014

Investigation and computational modelling of antimicrobial peptides

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The uprising resistance of pathogenic bacteria against treatments with conventional antibiotics emerged an acute search for alternatives. One class of promising alternatives are naturally occurring antimicrobial peptides. We present a comparative study of computational modelling of peptide properties with structural characterization of the interaction and antibacterial or haemolytic activity of three peptides (NK-CS, NKCS-[LP] and NKCS-[AA]). We compared computational interaction models with measurements of antibacterial and haemolytic activity, Small Angle X-Ray and Surface Plasmon Resonance data, and structure predictions by Circular Dichroism (CD). All peptides were active against Escherichia coli (Gram negative) and Staphylococcus carnosus (Gram positive) bacterial cultures, but the haemolytic properties against human red blood cells were found to be poor and indicated the peptides' selectivity. CD studies of the peptide secondary structure confirmed the prediction of peptide helicity. The antibacterial activity can be correlated with a change of the hexagonal phase transition temperature of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) as determined by Small Angle X-ray Scattering (SAXS). The calculated peptide membrane affinity is not related in linear way with the antibacterial activity. The reason for this might be aggregation as shown by surface plasmon resonance. The inverse hexagonal phase transition temperature was increased by the peptides and this promotes a positive curvature of the membranes. We assume that this curvature finally leads to the disruption of the model membranes. In summary an over all helical structure, electrostatic and hydrophobic parameters as well as strong amphipaticity are good measures to describe antibacterial peptide interaction.

P61100-015

An End-cap for Enhancing β Hairpin Stability

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Over the last decade, β-hairpins have emerged as excellent model systems for probing the intrinsic stabilities of portions of β-sheet structure and for studying the sequence dependence of the folding dynamics of β-strand association and alignment. For many decades, it has been established that peptide helices can be substantially stabilized (3 – 7 kJ/mol) by starting the sequence with an N-cap (Acetyl, Asp or SEDE). No comparable strategy has appeared for reducing the end-fraying of β-hairpins. Fully folded models of hairpins have typically been constructed by conversion to a cyclic system: inserting another turn favoring sequence (e.g. D-Pro-Gly, pG) at the end of the hairpin strands. We now report a set of favorable through-space interactions that can serve to cap β-hairpins. The discovery followed from NMR studies of Ac-W-IXGK-WTG (X = p, N). These studies indicated a favorable face-to-edge (FtE) W1/W6 interaction which also allows for a G8-HN to W6 indole ring H-bond and the sequestration of the Thr hydroxyl by H-bonding to the Acetyl. These interactions produce spectroscopic diagnostics: a CD exciton couplet together with extreme upfield shifts for He3 of the C-terminal Trp (1.5 – 2.1) and HN of the C-terminal Gly (2.7 – 3.6 ppm). These feature disappear when X = L-Pro and the Gly-HN shift returns to its coil value (as in Ac-WTG) upon removal of the N-terminal Ac. In its most favorable application, CH3CH2CO-WIpGLWTGPS, we were able to establish that DGU at 280K was 8.9 kJ/mol (97.8% folded) by backbone HN H/D exchange protection measures. We now report that these interactions are retained in longer hairpins. Peptides of the general formula, Ac-W-(ZZ)nINGK(ZZ)n-WTG (n = 1, 2, or 3) display enhanced fold stability. In each case, the Ac is essential to prevent end-fraying and to elicit the full set of chemical shift diagnostics of the "β-cap".

P61100-016

Investigation of backbone conformational heterogeneity in polyproline peptides by fluorescence resonance energy transfer

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The unique structural features of polyproline peptides to adopt an extended, relatively rigid polyproline II (PPII) backbone conformation in aqueous solution, has led to their widespread application as a "molecular ruler" in biological and biophysical investigations. In the left handed PPII helix the peptide bonds show the all-*trans* conformation resulting in an interterminal distance, which increases by 2.8 to 3.1 Å per proline residue. The same backbone conformation has been identified in important proline rich protein-protein recognition motifs involved in the regulation of physiological processes like transcription, cell growth, cytoskeleton rearrangement and postsynaptic signal transduction via modular SH3, WW or EVH domains. To investigate the structural features of polyproline and proline rich sequences by fluorescence resonance energy transfer (FRET) and other spectroscopic methods, these peptides were labelled *N*- and *C*-terminally with different fluorescent dyes. For synthesis of homopolymeric polyproline peptides with a sequence length of more than 20 amino acids a fragment condensation strategy has been applied to prevent the occurrence of shorter side products which can perturb distance measurement by FRET. Spectroscopic analysis of these peptides indicated that even for short polyproline sequences (< 5 residues) there is a remarkable deviation from the expected PPII conformation. FRET measurements revealed that polyproline peptides show an increase of the mean interterminal distance of about 1.7 Å per proline residue. Single molecule FRET measurements of polyproline peptides with a length of 21 amino acids point to a heterogeneous ensemble of different conformations caused by randomly distributed *cis* conformations resulting in diverse species with different interterminal distances. Similarly the structural consequences of the integration of non-proline amino acids in a polyproline sequence have been investigated by FRET.

P61100-017

The unusual helix stability of a VEGF mimetic peptide

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Understanding helix stability and formation is a prerogative to elucidate mechanism of protein folding and design helix peptide with specific activity. Peptide helix is a simple model system in which various contributions to helix formation can be dissected and understood qualitatively. Many strategies have been pursued to design peptide helices and notable results have been achieved even with very short sequences, but mainly these methods rely on the use of non natural amino acids or introducing constraints. In this communication, we report the stability characterization, via CD, NMR and MD studies, of a designed, α-helical, 15-mer peptide, composed only of natural amino acids, which activates the VEGF-dependent angiogenic response (1). This peptide shows an unusual thermal stability whose structural determinants have been determined. Two factors, the N-terminal region and an hydrophobic interaction i, i+4, are found as playing a mayor role of this remarkable stability(2). These results could have implication in the field of protein folding and in the design of helical structured scaffolds for the realization of peptides to be applied in chemical biology.

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P61100-018

Oligomerization of Adiponectin

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Adiponectin, a cytokine secreted by adipose tissue, which has been shown to affect lipid and glucose metabolism, attracts interest because it is a target for therapeutics in the metabolic syndrome. The molecule has a tendency to associate to form various multimers. Although this multimer formation could modulate its biological function, the details of this mechanism are still unclear. Thus studies on this system from the aspect of molecular assembly are interesting. The molecule consists of three domains, i.e. a variable (V), a collagen-like (C) and a globular (G) domain. The structure of the G domain determined by X-ray crystallography showed that it exists as a trimer but the remaining C and V domains have not been well characterized.

We synthesized various parts of the molecule, C, G, VC, CG, and full length VCG in E. coli. The CD spectra of CG and VCG showed a positive peak around 230nm which is the hallmark of collagen structure. This may be attributed to the repeats of typical collagen type triplet, X-Y-Gly. The temperature dependency of these CD spectra showed the three states conformational changes of these peptides. The lower transition, where the positive peak disappeared corresponds to the melting of collagen like structure and the higher one corresponds to that of globular structure of the G domain. The apparent molecular weights determined by ultra-centrifugal analysis showed that these peptides exist in trimeric form at the intermediate state. However, neither C nor VC showed such transitions. These results showed that, contrary to our expectations, the contribution of the triplet of X-Y-Gly is not the dominant one for stabilizing the trimeric state.

In order to explore the stabilizing factor, we are carrying out various experiments, such as mutation analysis, titration of Ca^{++}, redox reaction of disulfide bonds and so on. One result is that Ca^{++} was shown to be a factor for increasing the thermal stability of the trimer.

P61100-019

Are V57 mutants of amyloidogenic protein - human cystatin C more or less resistant to denaturation conditions?

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Cystatins are natural inhibitors of cysteine proteases - enzymes widely distributed in animals, plants and microorganisms. Human cystatin C (hCC) has been also recognized as an amyloidogenic protein directly involved in formation of pathological fibrillar aggregates which deposit in the brain arteries of elderly persons causing cerebral amyloid angiopathy (1). Our studies were performed to explore possibilities of preventing this lethal disease.

The overall 3D architecture of monomeric human cystatin C is not known but its fold could be anticipated from the structure of the dimer which has been already determined (2). The dimeric cystatin C is created through the exchange of 'subdomains' between two molecules (3D domain swapping) and consists of two identical subunits in great extent reconstituting the fold of the monomeric chicken analogue of hCC. It is possible that similar mechanism is involved in cystatin C oligomerization

and fibrilization process.
The most significant structural changes during dimerization process are observed in the L1 loop (55-59, QIVAG). This loop occurred to be a hinge region in the 3D domain swapping event. With the aim to check implications of greater or decreased stability of this loop for dimerization and aggregation propensity of human cystatin C, we designed and construct hCC L1 mutants with Val57 residue replaced by Asp, Asn or Pro, respectively. The structural studies of these mutants and their thermal denaturation process have been performed by means of CD and FTIR spectroscopies. Results of these studies will be presented.
Acknowledgements: This work was supported by Polish Ministry of Higher Education grant No 4233/H03/2007/32.
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P61100-020

Azobenzene-mediated photomodulation of a collagen triple helix monitored by IR spectroscopy

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Following previous studies on the use of photoresponsive molecular switches to induce conformational transitions in model peptides (1), our most recent efforts were addressed to the design and synthesis of a photoswitchable collagen triple helix. By replacing in suitable positions of the Ac-(Gly-Pro-Hyp)$_7$-NH$_2$ a Pro and Hyp residue with (4*S*)-mercaptoproline, respectively, a side chain-to-side chain crosslinking of the collagen peptide was afforded with a purposely designed azobenzene derivative. As expected from modeling studies, self-association of the modified collagen model peptide into a stable triple helix was observed with the *trans*-azobenzene clamp, while its photoisomerization to the *cis* isomeric state leads to unfolding processes as well assessed by NMR structural analysis (2). Unfolding pathways can be studied by comparing FTIR difference spectra induced by temperature or light. We found the photomodulation of the triple helix to be reversible and the efficiency of the photoisomerization increased with temperature reaching a maximum value shortly below the melting point. IR spectroscopy was thus used to identify the optimal temperatures required for structure destabilization at sufficient extents to enable unfolding by the weak driving force of the azobenzene clamp. The results confirmed the correctness of the design and the ability of the azobenzene switch to photocontrol this complex tertiary structure, thus allowing for time-resolved monitoring of the triple-helix unfolding process.
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P61100-021

Synthesis and Characterization of the Collagen Model Peptides containing 4(*S*)-hydroxyproline

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Studies on various collagen model peptides (X-Y-Gly)$_{10}$ where X and Y are often imino acids, Pro or Hyp^R (4(*R*)-hydroxyproline), have shown that Hyp^R at the Y position plays an important role in stabilizing the collagen triple helix. However substitution of non-natural 4(*S*)-Hyp (Hyp^S) at both positions decreases the thermal stability of triple helix as (Pro-Hyp^R-Gly)10 exists in a stable triple helical state, whereas (Hyp^S-Pro-Gly)10 and (Pro-Hyp^S-Gly)10 are in a single coil state. Similar effects on the stabilities of triple helices are provided by the substitution of fluoroproline. However there is an exception that (fPro^S-Pro-Gly)10 takes a triple helix at 4oC whereas the counterpart, (Hyp^S-Pro-Gly)10, is in a single coil state. Although the difference could be explained by the steric hindrance of hydroxyl group in *S* configuration, it is still controversial how much this hindrance could obstruct the triple helix formation. Therefore, even though apparently the tripeptide with the Hyp^S-Pro-Gly sequence is not capable of triple helix formation, we could expected that (Hyp^S-Pro-Gly)$_n$ forms a triple helix as *n* increases. The transition temperature of (Pro-Pro-Gly)$_n$ was shown to have the chain length dependency. Here, we synthesized (Pro-Pro-Gly)$_n$ and (Hyp^S-Pro-Gly)$_n$ (*n*=10,15) and investigated their thermal stabilities. The CD spectra of (Hyp^S-Pro-Gly)15 showed the conformational transition from collagen-like triple helix to random coil with the melting temperature at 21oC. The apparent molecular weight, determined by the sedimentation equilibrium method, showed that (Hyp^S-Pro-Gly)15 exists as trimer at 4oC and as monomer at 37oC. The melting profile was also clearly detected by DSC. @Thus, it is concluded that the existence of Hyp^S at the X position is not essential to interfere the triple helix formation. We are on course to investigate the stabilizing mechanism of the collagen structure.

P61100-022

The influence of O-glycosylation on the folding and stability of α-helical coiled coil peptides

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Glycosylated proteins have been shown to play a key role in many biological events like cell-cell communication, immune response, cell adhesion, intracellular targeting, protease resistance, and many other processes. Recently, there is a growing interest in the effect of glycosylation on the secondary structure of proteins, because of the association with the so called conformational diseases that arise from the dysfunctional aggregation of proteins in not native conformations.
In this study we used α-helical coiled coil based peptides as model systems to investigate the effects of serine-linked β-galactose on both the secondary structure and amyloid formation tendency. The α-helical coiled coil structural motif consists of two to seven α-helices which are wrapped around each other with a slight superhelical twist. Its simplicity and regularity have made it an attractive system to explore fundamentals of protein folding.(1) The importance of the coiled coil motif is obvious with the amount of 5% of all amino acid residues in the Protein Data Bank being part of a coiled coil motif. At first we examined to which extent and at which positions a glycosylation is possible without destroying the coiled coil structure. Therefore, we systematically incorporated one to six serine-linked β-galactose units into several solvent exposed positions of a 26 amino acid long coiled coil peptide. The hydrophobic dimerization face was not modified. The preformed L-Ser(Ac$_4$-β-D-Gal)-OH building blocks were introduced by convenient solid-phase synthesis following the Fmoc-strategy. Folding and stability of the glycopeptides were monitored by CD spectroscopy. The amyloid formation tendency was investigated by a ThT fluorescence assay.
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P61100-023

Nanomolar aggregation of amyloid beta peptides covalently and site-specifically modified by cholesterol oxidation products

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Aging, associated with decreasing protein homeostasis (proteostasis) capacity and increasing oxidative stress, is a prominent risk factor for amyloid diseases. Alzheimer fs disease (AD), which is one of the most common amyloid diseases, involves intra- and extracellular amyloid formation by the amyloid bata peptide (Abeta). However, how Abeta can aggregate in vivo even though its physiological concentration (PC) is much lower than its critical concentration (CC) for aggregation is a mystery. We have proposed that covalent modification of Abeta by small molecule oxidation products can explain how Abeta can form amyloid at PC. The aldehyde-bearing cholesterol oxidation product 1(2), which can modify Abeta by Schiif-base formation, is an example of the products that could affect AD onset. However, significant questions about the modification of Abeta by 1(2) persist, including: Does modification by 1(2) lower the CC of Abeta aggregation into the PC range? And, is Abeta modified by 1(2) able to aggregate at low concentrations on a biologically relevant time scale? In this study, these questions are answered by studying chemically synthesized analogs of Abeta that are site-specifically modified by 1(2) at Asp1, Lys16, or Lys28. Modification at the different sites has a similar effect on the thermodynamic propensity for aggregation. In contrast, the effect of metabolite modification on aggregation kinetics depended strongly on the modification site. Abeta modified at Lys16 formed amorphous aggregates fastest and at the lowest concentrations. In contrast, the appearance of thioflavin-T positive aggregates at higher concentration was fastest for Abeta modified at Asp(1). The influence of modification site on the nature of the aggregates suggests that amorphous aggregation and fibrillization place different conformational demands on Abeta. Furthermore, these studies may partially explain how Abeta can aggregate at nM PCs when the CC of unmodified Abeta is in the *f*ÊM range.

P61112-024

Activation of phospholipase A_2 by co-aggregation with an antimicrobial peptide

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Antimicrobial peptides are ubiquitous in nature and consist of several families of cationic amphiphilic peptides. They partition into lipid membranes and promote the segregation of anionic phospholipids (2, 3). We have shown several antimicrobial peptides, e.g. temporin B and L, indolicidin, and magainin 2 to activate secretory phospholipase A_2 (PLA_2) (1, 4) and we concluded that this could represent synergistic action of these peptides/proteins in defense against microbes. The fact that the sequences of these AMPs are very different suggest rather non-specific mechanism to be involved. To pursue the latter in more detail we used Förster-type resonance energy transfer (FRET) between labeled PLA_2 and temporin B. Interestingly, FRET coincides with concentration dependant activation of PLA_2 hydrolysis of dipalmitoylphosphatidylcholine (DPPC) liposomes. Accordingly, temporin B and PLA_2 interact forming a supermolecular complex terminating into amyloid-like fibrils (4). Homo-oligomeric fibers are formed on a slower time scale when PLA_2 interacts alone on DPPC liposomes (5). A general mechanism of peptide induced PLA_2 activation forming heterooligomeric cofibrils is suggested.

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P61112-025

Human islet amyloid polypeptide forms lipid-encased amyloid fibrils on supported lipid membranes

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Islet amyloid polypeptide (IAPP) forms fibrillar amyloid deposits in the pancreatic islets of Langerhans of the patients with type 2 diabetes mellitus and its misfolding and aggregation are thought to contribute to β-cell death. Increasing evidence suggests that IAPP fibrillization is strongly influenced by lipid membranes and, *vice versa*, the membrane architecture and integrity is severely affected by amyloid growth. We performed direct fluorescence microscopic observations of the morphological transformations accompanying IAPP fibrillization on the surface of supported lipid membranes. Within minutes of application in submicromolar concentrations, IAPP caused extensive remodelling of the membrane including formation of defects, vesiculation, and tubulation. The effects of IAPP concentration, ionic strength, and the presence of amyloid seeds on the bilayer perturbation and peptide aggregation were examined. Growth of amyloid fibrils was visualized using fluorescently labelled IAPP or thioflavin T staining. Two-colour imaging of the peptide and membranes revealed that the fibrils were initially composed of the peptide only, and vesiculation occurred in the points where growing fibres touched the lipid membrane. Interestingly, after 2-5 hours of incubation IAPP fibres became “wrapped” by lipid membranes derived from the supported membrane. Progressive increase in molecular level association between amyloid and membranes in the maturing fibres was confirmed by Förster resonance energy transfer (FRET) spectroscopy. The possible role of lipid wrapping in stabilization of IAPP amyloid *in vivo* is discussed.

P61112-026

Fibrinogen-derived peptides that mediate cell adhesion: structure and activity studies

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Highly conserved sequences on the C-termini chains of fibrinogen (~20AA), termed Haptides, induce attachment (haptotaxis) of different matrix-dependent cell types when bound to matrix (1-3). When soluble, they accumulate in cells and enhance transfection of micro-particles and liposomes into them (2). Here we studied the structural basis for Haptides activity. Circular dichroism (CD) showed that two Haptides that are unstructured in solution gain a helical conformation in DPC, which serves as a model for a membrane-like environment. We propose that Haptides-induced transfection into cells and cell adhesion to Haptides-coated matrices may both occur through direct interaction of Haptides with the cell membrane, resulting in a random coil to helix conformational change, followed by a possible insertion of the formed helix into the membrane. In the full-length fibrinogen chains, Haptides form anti-parallel beta sheets with another conserved fragment of the protein. To mimic the natural structure of the Haptide we have *de novo* designed a single peptide mimicking the beta-sheet based on the sequence of the Haptide preCã and its anti-parallel conserved sequence. The new peptide was active at least as the original haptide, and with

improved stability. CD showed that this new peptide also displayed a random coil to helix transition when transferred from aqueous solution to DPC environment. We propose that Haptides activity with cells is based on their transition to helix upon contact with membranes. Based on our results, novel peptides may be designed to mediate cell adhesion for tissue engineering and trans-membrane trafficking agents.

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P61112-027

Interaction of human islet amyloid poly peptide with phospholipid membrane vesicles

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Amylin, also known as Human Islet Amyloid Polypeptide (hIAPP), is a 37-residue peptide, suspected to play a major role in the malfunction of insulin secretion in diabetes mellitus type II. Co-secreted with insulin in the beta-cells, hIAPP, in higher rates destroys the barrier function of the beta-cells, leading to a failure in insulin production. Because of its amyloidogenity, aggregates of fibrils can be observed in the islands of Langerhans to indicate its overexpression. We studied the physico chemical properties of hIAPP by observing changes in its structure depending on time and the surrounding media using MALDI-TOF-MS, ATR FT-IR- spectroscopy. To understand the process of penetration and toxicity to cells, we performed leakage measurements of carboxyfluoresceine containing phospholipid large unilamellar vesicles by means of fluorescence spectroscopy. Moreover we determined membrane binding of dansyl-labeled hIAPP. At physiological pH value, hIAPP is positively charged and thus negative charges at the phospholipid membrane surface accelerate the process of peptide folding. Being random coil as initial state, a mixture of anti-parallel beta-sheet and alpha-helices emerges in time. In the presence of negatively charged phospholipids, hIAPP aggregates can be seen within a few minutes after titration. Also in absence of any free charges, as seen in water, fibrils grow and after an incubation for 24 hours at 37°C, some alpha-helices are twisted and after two weeks, no random coil is detected anymore. Titration of hIAPP to carboxyfluoresceine filled liposomes, showed different results concerning equilibrium time and maximal extent depending on age and preparation of the peptide. In particular the composition of the vesicles seems to determine their stability in the presence of hIAPP.

P61113-028

Nanoparticle induced folding and fibril formation of a coiled coil peptide

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Nanoparticles present large surface areas and are capable to catalyze fibril formation of peptides.(1). One assumes that the nature of surface controls which of the peptides will interact with the nanoparticles and that an enhanced local peptide concentration reduces the lag-time for aggregation. In previous studies, we reported the design of a coiled coil peptide that can adopt a random coil, α-helical structure, and β-sheet folding in dependence of pH and peptide concentration.(2) Here, we expose this peptide to Au nanoparticles that are either negatively or positively charged. The interaction of peptide and nanoparticle was investigated by CD and UV/Vis spectroscopy, gel electrophoresis, and transmission electron microscopy. We found that electrostatic interactions with Au nanoparticles can affect the peptide folding resulting in more than one secondary structure, namely, a competition between the α-helical and β-sheet structures. The latter folding is very likely a consequence of the high local peptide concentration on the surface of nanoparticle that facilitates a β-sheet fibrillation of peptide. Moreover, several factors such as pH, peptide concentration, and size of the nanoparticle have a strong influence on the nanoparticle-mediated folding. These results provide valuable information on pharmaceutical applications of nanoparticles and will help to reduce possible adverse effects.

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P61123-029

Vascular Endothelial Growth Factor (VEGF) and its receptors: key regulators of angiogenesis

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Angiogenesis is a remodeling process characterized by the sprouting of new blood vessels from pre-existing ones. It occurs during embryogenesis and to a limited extent in the adult. VEGF is a homodimeric protein and has been characterized as a prime regulator of angiogenesis and vasculogenesis; when cells lose the ability to control the synthesis of VEGF, angiogenic disease ensues(1). In vitro studies show that VEGF is a potent and specific angiogenic factor involved in the development of the vascular system and in the differentiation of endothelial cells(2). VEGF biological function is mediated through binding to two receptor tyrosine kinases: the kinase domain receptor (KDR) and the Fms-like tyrosine kinase (Flt-1), which are localized on the cell surface of various endothelial cell types. This binding activates signal transduction and can regulate both physiological and pathological angiogenesis(3). VEGF and its receptors are overexpressed in pathological angiogenesis, making this system a potential target for therapeutic and diagnostic applications(4). The extracellular portion of VEGF receptors is comprised of 7 immunoglobulin-like domains; deletion studies have shown that the ligand binding function resides within the first three domains of Flt-1 and in domains 2 and 3 of KDR. Actually, no structural data are known on the extracellular portion of these receptors except for the second domain of Flt-15.. So, our aim is the cloning and the expression of part of extracellular domains of both VEGF receptors for structural characterization and to be used in interaction studies with peptide ligands or small organic molecule.

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P61124-030

Structural Studies on the Membrane-Reconstituted Transmembrane-Juxtamembrane Region of ErB2/ Neu(647-693)

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We are interested in an activation mechanism of receptor tyrosine kinases (RTKs), especially, how the transmembrane (TM) and the

intracellular juxtamembrane (JM) region couple ligand binding to tyrosine phopsphorylation. One of the RTKs that we are working on is Neu. Neu (ErbB2) is one of the four members of ErbB receptor family. This RTK with a mutation in the transmembrane region (V664E) has been recognized as a potent transforming oncogene product. Structure of the TM region dimer and its structural difference between the wild type and V664E mutant have been reported by Smith et.al. (1), providing structural constraints for modeling the TM dimer. Recently, we have performed a structural study on a TM-JM region of EGFR (ErbB1) showing that the TM helix breaks at the membrane interface and the unfolded JM region binds electrostatically to the membrane. We also have shown that Ca2+ complex with calmodulin (Ca2+/CaM) binds to the positively charged JM region and pulls this portion off the membrane. These results are consistent with an electrostatic engine model, postulated by McLaughlin and coworkers (2), for the autoinhibition and activation of the EGFR. In this research, we are revisiting the structure of TM-JM region of Neu to see if we can apply the electrostatic engine model to Neu and to see if there is a structural difference between the wild type and the mutant TM-JM sequence. Here we describe structural studies on the TM-JM sequence, Neu(647-693), reconstituted into bilayer vesicles. A combination of solid state NMR, infrared and fluorescence measurements are used to draw conclusions that the unfolded JM binds electrostatically to the membrane and binding of Ca2+ - calmodulin to the positively charged JM sequence can, under some conditions, reverse its charge and release it from the membrane.

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P61126-031

^{19}F-NMR Study of Collagen Model Peptides containing 4(*R*)-fluoroproline

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@The collagen triple-helix consists of three polypeptide chains, each of which takes the poly-L-proline II form in a left-handed helix and undergoes a transition to a single coil state as the temperature increases. This characteristic structure is ascribed to the unique amino acid sequence X-Y-Gly, where X and Y are commonly Pro or 4(*R*)-hydroxyproline (Hyp^R). @So far, various NMR studies have been done to explain the mechanism of folding and the thermal stability of the triple helical structure by using model peptides rich in imino acids. However, the complexity of NMR spectra imposed severe limitations on detailed analysis. The spectrum is complicated because of a large number of conformational isomers due to cis/trans isomerization around each imino acid causing various chemical shifts with small differences in 1H-NMR spectra. It has been demonstrated that the site directed 15N enrichment allows real time NMR monitoring of the folding of residues at specific locations of collagen model peptides (1). @To approach this problem, we have applied 19F-NMR study on various collagen model peptides containing 4(*R*)-fluoroproline (fPro^R). Compared to 1H and 13C, 19F chemical-shifts are extremely sensitive to small environmental changes around the nuclei and show a wide dispersion of chemical shifts. As a result, we not only distinguished the signals from the triple helix and unfolded states, but also differentiated the signals at intermediates states in 1D 19F-NMR spectrum of (Pro-fPro^R-Gly)$_7$. @Here, in this study, a combination of 2D NMR experiments including EXSY, HOESY, and TOCSY has been used to investigate detailed equilibrium properties of collagen model peptides. Especially, in the 2D ^{19}F-EXSY spectra, we successfully observed many exchange cross peaks at high temperature. The ^{19}F-NMR method shown here provides a clue to analyze the conformational transition, dynamics and stability of collagen triple-helix.

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P61126-032

α-helix-induced secondary structure formation in β- and γ-amino acid containing foldamers

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By contrast to well-folded natural peptides, oligomers consisting of non-natural building blocks, so-called foldamers, are highly stable towards proteolytic degradation which is a key feature in the development of peptide-based drugs. Aside from the fact that foldamers consisting of β- and γ-amino acid building blocks are able to display different secondary structures, the combination of different homologous building blocks to hybrid peptides has recently shown some promising results.[1,2] The ab initio MO theoretical studies have shown that β/γ-hybrid peptides composed of alternating β- and γ amino acid building blocks might be very well suited to mimic α helical conformations.(3) Here we report the first examples of α-helical coiled coil formation with synthetic foldamers. Based on the computational studies, we synthesized heterogeneous peptides by automated Solid Phase Peptide Synthesis (SPPS). Purification was carried out by HPLC, and products were confirmed by ESI-ToF-MS. Furthermore, temperature-dependent CD spectroscopy was applied to investigate the stability of hetero-oligomers formed between, either β/γ- or α/β/γ-hybrid peptides with a natural α-helical coiled coil peptide. In addition, the interaction potential with their natural α-helical counterparts in aqueous solution were investigated by CD, and Förster resonance energy transfer (FRET).

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P61200-033

Development of novel antimicrobial peptides with improved activity

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Antimicrobial peptides are synthesized by all living organisms as a part of their innate immune system. Those molecules are endowed with a broad spectrum of activity against pathogens. They can be divided into two classes differing in the mechanism of killing: membrane disruptive antibiotics cause a dysfunction of the membrane and subsequent cell lysis; non-membrane disruptive peptides are focused on the intracellular targets. In both situations the initial stage consists in the interactions with the cytoplasmic membrane, in most cases without the exploitation of any receptors. A non-receptor type of interactions decreases the possibility of development of microbial resistance and makes peptides a very potent alternative to conventional antibiotics. In the face of the reduced efficiency of traditional drugs there is an urgent need for the design of new therapeutic agents with the optimized activity. Monte Carlo simulations of peptide– membrane interactions can be one of the strategies. That method takes in the account biophysical properties of a membrane as well as structural and physicochemical nature of peptides. In our work we are focused on the development of new analogues of NKCS, a derivative of potent antibiotic peptide NK-2. In the present study the outcome of Monte Carlo simulations is compared to

experimental results of antibacterial tests performed against *Escherichia coli*. The insight into the molecular mechanism of peptides activity is obtained *in vitro* using SAXS method and artificial systems mimicking a bacterial cytoplasmic membrane. The results indicate that Monte Carlo modelling is a good tool to predict the peptide – membrane interactions and can be very useful for the design of novel antibiotics.

P61207-034

Aβ16-35 peptide: structural features in membrane mimicking systems

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β Amyloid (Aβ) peptides, the hallmark of Alzheimer disease, depending upon conditions, undergo conformational transition from soluble monomers to the highly toxic β-sheet oligomers which form the mature fibrils. Conformational and biological analyses were carried out on several different Aβ fragments, to understand the role of the single residues in the fibrillation process. Aβ25-35, is considered the shortest fragment exhibiting large β-sheet aggregates and retaining the toxicity of the full-length peptide. We recently reported the conformational analysis of the synthetic Aβ25-35 under several solution conditions, and analyzed the modulation of the conformational behaviour of Aβ25-35 peptide, by interaction with nicotine-like molecules. Aβ16-35 is the 20-mer Aβ peptide, composed of the Aβ25-35 fragment, and additional N-terminal residues, known to be endowed with fibril disaggregating activity. Aβ16-35 encompasses part of hydrophobic (1-28) and hydrophilic (29-35) Aβ- regions. Due to the amphipathic character of this fragment, common to the full Aβ1-42 and Aβ1-40, a tossicological mechanism involving Aβ-peptide-membrane interaction may be hypothesized. On these bases we decided to perform the structural investigation of the fragment Aβ16-35 in membrane mimicking systems including micelle and vesicles aggregates, differing for composition and structural complexity. High resolution three dimensional structure was solved by NMR spectroscopy in negatively charged SDS and in DPC/SDS mixed micelles. Fluorescence and EPR spectroscopies were used to monitor the peptide lipid interaction. The data agree on the critical role played by the membrane composition on the stabilization of different conformers, potentially driving to different oligomeric and/or polimeric toxic species.

P61207-035

Model peptide LAH_4 and its interaction with phospholipid bilayers

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Charge and hydrophobicity are important determinants of membrane activity of antimicrobial peptides. The model peptide LAH_4 whose charge can be easily controlled by pH of the medium (1) is a very convenient tool to study the relationship between physical properties of peptide molecules and their membrane activity. It was shown that the changes in the charge of LAH_4 molecules result in changes in their orientation with respect to phospholipid bilayers (1). In the present study, effects of LAH_4 on the properties of phospholipid bilayers at different pH values are studied by several methods. Its influence on the lateral mobility of lipids within an SPB and on the stability of suspensions of large unilamellar vesicles were characterized by fluorescence correlation spectroscopy, showing evidence of peptide induced aggregation of lipids at basic pH. Changes of hydration and local viscosity within the bilayer following changes in orientation of peptide molecules were measured by solvent relaxation technique. The effect of phospholipid charge was also taken into account in each case.

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P61207-036

Determining the location of antimicrobial peptides inside lipid bilayers by combined fluorescence spectroscopy and molecular dynamics simulations

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Several bioactive peptides, such as antimicrobial, cell-penetrating or fusogenic peptides, exert their biological function by interacting with cellular membranes. Therefore, structural data on the location of these molecules inside lipid bilayers are very important for a detailed understanding of their mechanism of action. Fluorescence spectroscopic methods are particularly suited to the study of peptide-membrane association, but give only low-resolution information on peptide position in the lipid bilayer. Molecular dynamics simulations, on the other hand, can provide a very detailed picture of the peptide-membrane interaction, but need to be validated by quantitative comparison with experimental data. We applied several fluorescence approaches, together with MD simulations, to the investigation of two antimicrobial peptides: the lipopeptaibol trichogin GA IV, and PMAP-23, a member of the cathelicidin family. To perform the spectroscopic studies, a variety of peptide analogues containing a single fluorophore were synthesized. Fluorescence spectra, depth-dependent quenching experiments, and peptide-translocation assays were employed to determine the location of the two peptides inside lipid bilayers, in particular as a function of peptide/lipid ratio. Molecular dynamics simulations were performed by a "minimum bias" approach, starting from a random mixture of water, lipid and peptide, and following the spontaneous self-assembling of the lipid bilayer. The final membrane-bound 3D-structure is in quantitative agreement with the position of the fluorescent labels determined by depth-dependent quenching experiments. For both peptides investigated, the atomic details of MD simulations provide new insights on the mechanism of membrane destabilization. Acknowledgements: With the support of the Ministry of Education, University and Research, and of Foreign Affairs of Italy.

P61213-037

Conformation of peptide neck domains for cosmetic applications

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Surfactant Proteins (SP-) are found in lungs of mammalians. Most surfactant proteins have a carbohydrate binding domain (CRD) and neck domain. CRD have defense function in mammalian mucosa's, by eliminating microbes, due to strong binding to sugars in the cell walls. Neck domains are found to order phospholipids allowing the exchange of oxygen between the alveoli and blood. X-Ray structures indicate that neck domains are alpha-helixes, but circular dicroism indicates that in the presence of phospholipids, those peptides present a disordered structure. Neck domains with 20 residues from natural sequences of SP-A, SP-B, SP-C, SP-D have been tested to be delivered in lipophilic media. Only disordered structures would have the ability to cross the lipid barriers. The results obtained here indicated that neck domains possibly can be used as carriers to deliver molecules across skin and hair.

P61217-038

Fraternal Twins ! γ-Peptides and oligoureas are isosteric, isostructural foldamers endowed with yet distinct biomolecular recognition properties.

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In the field of peptidomimetics, there has been a sustained interest towards the design of non-natural oligomeric backbones with new folding patterns. Over the past 12 years, the amide linkage has become the quintessential motif to elaborate folding oligomers. Aliphatic and aromatic oligoamides (peptoids, β-, γ-peptides) have provided numerous helical-folded structures, many of which have shown interesting biological activities (1). Interestingly, substituting urea for the CH_2-CO-NH units in the γ^4-peptide backbone represent a spectacular case of isosteric and iso-structural replacement. Detailed NMR studies of the resulting oligoureas revealed a helical fold very similar to that reported for the cognate γ^4-peptides. Definitive confirmation of this isostructural relationship came with the recent X-ray structure determination of the canonical *2.5*-helix of oligoureas. How such isosteric and isostructural oligoamide and oligourea backbones compare in biomolecular recognition events is an interesting question that we attempted to address in the present work. Notably the two systems were compared for their antimicrobial activity, membrane interaction and disruption properties. Both γ-peptides and oligoureas designed to mimic globally amphiphilic alpha-helical host-defense peptides have been synthesized and tested. The results showed a surprising dichotomy in bactericidal activity between the two isostructural systems and enlightened the unique antibacterial of amphiphilic oligourea helices. To question whether this functional difference results from differential membrane disruption activities, we have undertaken detailed physicochemical investigations using negatively charged phospholipid membranes as model systems.

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P61219-039

Understanding of the cellular uptake of an amphipathic cell penetrating peptides complexed with siRNA

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The efficiency of delivery of macromolecules into living cells is very important for therapeutic purposes. The discovery of a class of peptides, known as cell-penetrating-peptides (CPPs), with the ability to mediate translocation of various cargoes both in vitro and in vivo has provided new perspectives in the field of delivery. We have designed a new secondary amphipathic CPP, Caddy, which can transfect siRNA. Many studies have tried to elucidate CPP internalization mechanisms and there is currently still much controversy between endocytosis phenomena and direct interaction with membranes. However, elucidating the interactions between peptides and lipid membranes is essential to understand how Caddy delivers cargoes in cells. To highlight these interactions, we have applied biophysical method to phospholipids monolayer and bilayer as model plasmic membranes. Regarding the increase of the phospholipids monolayer surface pressure in presence of Caddy, it is obvious that this peptide have high affinity with lipids. While cholesterol presence does not induce anything in peptide insertion on a phospholipids monolayer, the cargo and a widely studied partner of the internalization: Heparan Sulfate of the GAG' s family, do not induce the same behaviour. The intrinsic probe of Caddy, tryptophan residues, and the FITC probe bound to the cargo allowed to follow and identify interactions between these two entities by fluorescence spectroscopy. Adding a bilayer vesicular solution unsettle these interactions, tryptophan residues interact with phospholipids while FITC probe are less embarrassed by peptides. Caddy alone in solution is not structured but CD measurements show a typical spectrum of helical conformation in presence of phospholipids vesicles. And when the peptide complex its cargo, CD spectrum show a contribution of the siRNA relevant of a conformational change inside both partners interacting together.

P61221-040

High membrane coverage as the basis of antimicrobial peptide activity

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The interaction of the antimicrobial peptides (AMPs) omiganan (H-ILRWPWWPWRRK-NH_2) and BP100 (H-KKLFKKILKYL-NH_2) with model bilayers was characterized. The activity and selectivity of these peptides could be attributed to a strong preference towards anionic membrane model systems, which mimetize bacterial membranes. Regarding the interactions with bacterial membrane models, there were marked differences in the interaction patterns, as well as in functional properties of the peptides at high peptide:lipid ratios. These differences occurred for both peptides, despite their being unrelated in sequence and in occurrence in nature. Such events at high membrane coverage could represent the equivalent at the molecular scale of the conditions at which the antimicrobial activity of the peptides is triggered. Although the lipid: peptide ratios at these transitions are lower than 10 phospholipids per peptide molecule, the plausibility of this hypothesis was demonstrated taking into account an estimate of the amount of lipid per bacterium, and the bacterial concentration in minimum inhibitory concentration (MIC) assays. According to the partition constants obtained towards bacterial membrane models, these peptides are expected to reach, at the MIC, precisely those high concentrations in the membrane. In addition, surface charge neutralization was shown to occur in these conditions. Activity at high membrane coverage is thus likely not only for these peptides but also for any peptide displaying high membrane affinity and micromolar MICs, which is common amongst AMPs.

P61229-041

Kinetic Study of Liposome Adsorption to a Peptide-modified Au Electrode for Applications in Biomembrane Sensors

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Biosensors based on artificial membrane system that permits the functional reconstitution of transmembrane receptors have been studied for the past decade. Immobilized liposome encapsulates intracellular chemicals in the internal water cavity and provides a potential advantage over supported planar lipid membrane. To prepare a stable liposome adlayer, we have proposed an immobilizing method based on small-peptide modification of solid surface. In the present study, we investigated kinetics of liposome adsorption on the surface-bound synthetic peptides which have several alanine, lysine and tryptophan residues as amphiphilic segment with a cysteine at terminus. The quartz crystal microbalance studies showed that the peptide sequences can be divided into two groups according to liposome-size dependence of Langmuir adsorption constant. While the initial adsorption processes could be satisfactorily described by simple Langmuir adsorption kinetics, the amounts of liposome adsorbed irreversibly were increased as time advances. The results from AFM reveal that most of the liposomes are bound to peptidic surfaces as single flattened particles without fusion together for several hours. We

next performed the detection of ganglioside GM1-lectin interaction on the liposome surface. All the binding constants and binding amounts, as observed by QCM, were fairly consistent with each other and and showed the effectiveness of our approach.

P61928-042

How do cell penetrating peptides translocate across cell membranes? *A theoretical model and its experimental validation*

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Recently we have proposed theoretically an energy independent pathway for the uptake of cell penetrating peptides (CPPs) that challenges fundamental concepts associated with protein membrane interactions, *H. D. Herce and A. E. Garcia, PNAS, 104, 20805 (2007)*. This mechanism involves strong interactions between CPPs peptides and the phosphate groups on both sides of the lipid bilayer, the insertion of charged side chains that nucleate the formation of a transient pore, followed by the translocation of CPPs peptides by diffusing on the pore surface. This mechanism explains how key ingredients, such as the cooperativity among the peptides, the large positive charge, and specifically the arginine amino acids, contribute to the uptake. We will describe the details of this mechanism and present novel experimental results that directly validate the model.

P61929-043

A comparative studies on lipid affinity of cell penetrating peptides in presence or absence of cargo

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A growing number of natural and /or synthetic peptides with cell membrane penetrating capability have been identified and described in the past years. These molecules have been considered as targeting structures for the delivery of bioactive compounds into various cell types. Although the mechanism of uptake is still unclear, it is reasonable to assume that the relative contribute of each proposed mechanism could differ for the same peptide, depending on experimental protocol and cargo molecule composition. In this work we try to connect the capability to interact with model lipid membrane of CPP and their structural and chemical characteristics in order to obtain a biophysical classification that predicts the behavior of CPP-cargo molecule in cell system. Indeed, the interaction with cell membrane is one of the primary step in the interaction of CPP with cells, and consequently the studies on model membrane could become important for understanding peptide-membrane interaction on a molecular level, explaining how CPPs may translocate a membrane without destroying it and how this interactions come into play in shuttling CPPs via different routes with different efficiency. We analyzed by fluorescence spectroscopy the binding properties of six different CPPs (KFGF, Antp and Tat derived peptides, and oligoarginine peptides containing 6, 8 or 10 residues) in absence or presence of the same cargo peptide (the [392-401]pTyr^{396} fragment of HS1 protein). The binding properties were correlated to the conformational and chemical characteristic of peptides, as well as to the cell penetrating properties of the CPP-cargo conjugate. Results show that even if certain physico-chemical properties (conformation, positive charge) govern CPP capability to interact with the model membrane, these cannot fully explain cell-permeability properties.

P62100-044

Effect of flavonoids on Aβ(25-35) /DLPC vesicle interaction

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Great deals of data show that Alzheimer's pathology lead to the loss of neuronal functionality and synaptic plasticity. These effects seem to be the consequence of a toxic effect of Aβ peptides related to their ability to modify cell membrane homeostasis. In particular, Aβ peptides, as full length or in fragments, being in oligomeric or polymeric form, could alter membrane fluidity and compromise its functionality. We have previously demonstrated that the 25-35 fragment of Aβ peptide Aβ(25-35) is able to penetrate into the outer leaflets of the membrane. Furthermore, it is able to alter membrane fluidity changing membrane response to cholesterol, and thus affecting its ability to form low fluid membrane regions named lipid rafts. Aβ(25-35) is considered the shortest fragment exhibiting large β-sheet aggregates and retaining the toxicity of the full-length peptide. Flavonoids are a group of naturally occurring, benzo-γ-pyrone derivatives, ubiquitous in plants. They are endowed with tumor prevent activity and They act as antioxidants through a membrane mediated molecular mechanism involving the membrane ion transport. On the basis of the common ability of flavonoids and Aβ peptide of altering membrane fluidity, we carried out an EPR spectroscopic analysis of several flavonoids – specifically quercetin, naringenin, rutin and naringin - in 1, 2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) vesicles. The modifications of the DOPC physio-chemical environment were analyzed in presence of flavonoids and beta-amyloid fragment Aβ(25-35). Our results indicate that the addition of flavonoids induces a decrease in membrane fluidity. This effect is retained in presence of Aβ(25-35). Thus flavonoid compounds could be able to antagonize the effect induced by amyloid peptide on membrane fluidity and in this respect they could be therapeutically useful as neuroprotective agents.

P62100-045

Spin-label aided investigation of feline immunodeficiency virus Gp36 derived peptide in presence of membrane models

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P59, a 20-mer peptide modeled on the membrane-proximal external region (MPER) of the feline immunodeficiency virus (FIV) gp36 ectodomain, has potent antiviral activity. The lipoylated analogue, lipo-P59, displays a similar activity, which is preferentially retained by cellular substrates. A mechanism has been proposed recently in which the peptide, being positioned on the surface of the cell membrane, inhibits its fusion with the virus; the lipophilic chain of lipo-P59 is thought to insert into the membrane interior, thus anchoring the peptide at the surface. Here we present a NMR and EPR combined investigation, aimed to

analyse lipid−peptide interactions of P59 and lipo-P59 with membrane mimicking represented by micelles and vesicles. NMR structures of P59 and lipo-P59 in mixed SDS/DPC micelles are reported; the positioning of P59 and lipo-P59 peptides with respect the lipidic surface is analyzed using 5-doxyl stearic and 16-doxyl stearic acid. EPR analysis is carried out in the zwitterionic dimyristoyl phosphatidylcholine and the anionic dimyristoyl phosphatidylglycerol vesicles using several different lipid spin labels. Both peptides bind to lipid bilayers and Trp residues and lipidic chain of lipo-P59 show a important role in the process of peptide adsorption onto the membrane, to exert its antiviral activity.

P62112-046

HIV fusion inhibitor peptides interaction with biomembranes and their model systems

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Enfuvirtide (T-20) was the first HIV-1 fusion inhibitor peptide approved for clinical use. T-1249 is another HIV-1 fusion inhibitor peptide under clinical trials. Their interaction with biological membrane model systems (large unilamellar vesicles) was studied by fluorescence spectroscopy to evaluate a possible role of lipid bilayers in their mode of action at the molecular level. Both peptides partition extensively to the liquid-crystalline zwitterionic POPC and locate at the interface of the membrane. When other lipid compositions are used (POPC and cholesterol, gel phase and negatively charged bilayers) partition decreases. Fluorescence resonance energy transfer analysis show that T-1249 adsorbs to cholesterol-rich membranes. Studies on human cell membranes were necessary to further establish the role of membranes in these peptides mode of action. This interaction was assessed by evaluating the effects that these peptides have on the membrane dipole potential of human erythrocytes, using the fluorescence probe Di-8-ANEPPS. In the presence of enfuvirtide or T-1249, a decrease in the di-8-ANEPPS fluorescence excitation ratio dependent of peptide concentration was observed. These results show that T-1249 has ten times more affinity to the erythrocyte membrane than enfuvirtide, a factor that can be associated with the adsorption of T-1249 on cholesterol rich membranes observed on the studies with membrane model systems. Moreover, as a fraction of HIV associates with erythrocytes in vivo, these cells can have a role in delivering these peptides to the viral surface. The improved clinical efficiency of T-1249 relative to enfuvirtide may be related to its higher partition coefficient and ability to adsorb to rigid lipid areas on the cell surface, where most receptors are located upon membrane fusion. Moreover, adsorption to the sterol-rich viral membrane helps to increase the local concentration of the inhibitor peptide at the fusion site.

P62311-047

Fluorescence Spectroscopy as a Tool for Studying Protein-Peptide Interactions: Evaluation of Talin Affinity for β_3 Integrin Derived Peptides

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The platelet receptor $\alpha_{IIb}\beta_3$ plays a critical role in the process of platelet aggregation and thrombus formation. Upon platelet activation its conformation changes leading to an increased affinity for fibrinogen, which forms bridges between adjacent platelets and assembles them into an aggregate. The $\alpha_{IIb}\beta_3$ activation is regulated by “outside-in” and “inside-out” signaling. Among the protein-protein interactions, which contribute to «inside-out» signaling, that of talin with the β_3 cytoplasmic tail is the most important. It has been recently suggested that talin-mediated $\alpha_{IIb}\beta_3$ activation relies on the cooperative interaction of the membrane proximal (MP) and the membrane distal (MD) β_3 regions with talin F3 domain and that the $N^{744}PLY^{747}$ motif of β_3, which can be phosphorylated at Y^{747}, plays a critical role in this process. To evaluate the interaction of talin with the α_3 tail of integrin we designed and synthesized two peptides corresponding to the MD and MP parts of β_3 in their carboxyfluorescein-labeled form (MD: CF-R^{736}AKWDTANNPLYKE^{749} and MP: CF-K^{716}LLITIHDRKE^{726}). Emission and anisotropy fluorescence spectroscopy was used to quantitatively assess the affinities of these peptides for talin. Furthermore, to challenge the role of the Y^{747} phosphorylation in talin-$\alpha_{IIb}\beta_3$ interaction we also studied the binding of talin to the modified analogue of MD, CF-R^{736}AKWDTANNPL(pTyr)KE^{749}. Our experiments revealed that the MD and MP parts of β_3 bind tightly to talin and that Y^{747} phosphorylation has an inhibitory effect on this binding. Finally, Circular Dichroism studies of all peptides in aqueous solutions and mixtures with TFE were also performed in order to characterize structure-affinity relationships.

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P62326-048

Assignment of the Binding Epitope of V3-Glycopeptides of the HIV 1 GP120 and the Human Coreceptor CCR5 by Saturation Transfer Double Difference (STDD) NMR and Surface Plasmon Resonance

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The infection of human cells with HIV requires two initial steps: binding of the viral glycoprotein GP120 to the receptor CD4 followed by the interaction between the V3 loop of GP120 and a seven helix transmembrane coreceptor (CCR5 on macrophages or CXCR4 on T cells). The N-type glycosylation at Asn301 of the V3 loop is assumed to play a crucial role in this process. In order to understand the interaction in detail, it is important to analyze the binding of V3-glycopeptides to the membrane integrated coreceptors on an atomic scale.

Here, we present binding studies between multiple V3-peptides and –glycopeptides and the coreceptor CCR5 by STDD NMR and Surface Plasmon Resonance (SPR).

SPR experiments were carried out by immobilizing the (glyco)peptides on an SPR chip by amide coupling. Several concentrations of CCR5 overexpressing human osteosarcoma (HOS-R5) cells were passed over the sensor surface. The role of individual amino acids in the binding process to CCR5 can be analyzed by using different V3-peptides and -glycopeptides.

Additionally, the observation of interactions between the glycopeptide ligands and the receptor proteins embedded in liposomes is possible by using the Saturation Transfer Double Difference (STDD) NMR technology. STDD NMR allows removal of all unwanted signals resulting from native binding processes in cells.(1). We used CCR5 liposomes derived from HOS-R5 cells. Substraction of an STD spectrum of CCR5 liposomes from an STD spectrum of the same concentration of liposomes incubated with a ligand allows recording of clean STDD NMR spectra presenting only signals of protons of the ligand interacting with the transmembrane receptor.(2) K_D values of the ligand with respect to CCR5 have been determined in series of experiments with varying ligand concentrations.

We could show that the binding epitope between HIV and CCR5 is formed by the carbohydrate at Asn301 as well as the peptide. This knowledge is important for the design of new inhibitors.

P62328-049

Temperature dependant methionine proximity assays highlights conformational variations occurring through the mechanism of peptidergic GPCR activation

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G protein coupled receptors are invaluable for cell signal transduction mediated by external stimulus. Pharmacological efforts towards these targets are of primordial importance albeit few efforts have resulted in structural characterisation, although rational drug design necessitates such information. Recent advances in crystallisation of the β-adrenergic receptor have been highly insightful and such a structure corroborates our results on the human Angiotensin II type 1 receptor (hAT1) using the Methionin Proximity Assay (MPA) in identifying ligand receptor contact points. Unfortunately, physical methods such as crystallography are still far from routine procedures and are limited to a static picture of a given receptor. In the present contribution we propose a more accessible method that permits analysis conformational variations of different receptor states through an energy landscape, spanning from low energy conformations to conformations at physiological temperatures. Photolabelling, MPA mutants constructed on the WT receptor, the constitutively active receptor (CAM) and a corresponding non-activable mutant across the temperature range reveal activation-status dependent labelling patterns. As an example, position 256 becomes significantly less accessible in the CAM receptor compared to the WT receptor. Ligand accessibility can be classified from easily accessible (low temperature photolabeling) to less accessible residues (higher temperature photolabeling). This labelling pattern can be associated to the activation status of the receptor and may allow quantifying and identifying structural changes occurring during receptor activation. Such structural understanding is crucial for future endeavours in rational drug design.

P62617-050

Capillary electrophoresis for difficult characterization of hardly soluble polypeptides

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Recently, we have designed and synthesized polypeptides able to stimulate the natural plant defenses. These homopeptides were obtained by ring opening polymerization of N-carboxyanhydride (NCA) with several initiators. Ratio NCA/initiator is determinant for the length of the polymers. On the bases of elicitor activity, we identified a leader, called LAPP6, which results from L-Ala-NCA polymerization initiated by alaninol. The mixture of poly(alanine) with different lengths is difficult to analyze considering important solubility problems. MALDI-tof confirmed polymerization despite limitation due to molecular discrimination during ionization. NMR in deuterated TFA was possible but only afforded the number-average degree of polymerization (DP). To obtain more detailed characterizations, we developed separation by capillary electrophoresis in new solvents mixtures based on hexafluoroisopropanol (HFIP) and water. This technique allowed us to separate the oligomers with baseline resolution. Different parameters influencing electrophoretic separation were investigated and optimized conditions were established. This technique enabled a full characterization of the polymer distribution, with determination of number-average DP, weight-average DP and polydispersity index. The pertinence and the reliability of capillary electrophoresis for characterization of non-water soluble polypeptides have been confirmed, with analysis of short and long peptide chains.

P62800-051

Weakly polar interactions support polypeptide structures

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Although the sequence of a polypeptide appears to determine the secondary and tertiary structure, weak inter-residue interactions such as between aromatic side chains and other aromatic side chains, aliphatic side chains and the peptide backbone may contribute significantly to the stability of the final folded structure. Recent advances in structural bioiformatics and computational chemistry made it possible to study precise strurctural features and energetics of these interactions in model peptides and miniproteins such as TC5b, aPP and c-VHP. The interaction energies of the weakly polar interactions are of the same order as of the hydrogen bonds which occur in biopolymers (15 - 65 kJ/mol). Furthermore, these interactions not only stabilize local secondary structures but also entire tertiary folds of miniproteins.

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P62900-052

Lysozyme aggregation behavior in membrane environment

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A number of so-called conformational diseases including neurological disorders (Parkinson's, Alzheimer's and Huntington's diseases), type II diabetes, spongiform encephalopathies, systemic amyloidosis, etc., are associated with the deposition in tissue of highly ordered aggregates of specific peptides and proteins. Despite the main structural elements of amyolid fibrils (particularly, cross-β-structure) are well-characterized, the mechanisms of fibrillogenesis remains poorly understood. Accumulating evidence substantiates the idea that formation of fibrillar structures can be initiated and modulated by peptide/protein-lipid interactions. Membrane-related determinants of fibrillization are thought to involve conformational changes of the peptide/protein, increase of its local concentration at lipid-water interface, specific orientation of aggregating species, neutralization of the peptide/protein surface charges by anionic lipid headgroups, particular arrangement of the inserted and solvent exposed segments of the peptide/protein molecule, etc. The present study was undertaken to explore the formation of lysozyme (Lz) amyloid-like fibrils in the model lipid-protein systems. Lipid component of the model system was represented by liposomes prepared from zwitterionic phosphatidylcholine (PC) and anionic phosphatidylglycerol (PG) lipids in the molar ratio 4:1. Fluorescence microscopy studies performed with fluorescein-labeled and rhodamine-labeled Lz showed the presence of long Lz fibers (length >80 ìm). The To elucidate the nature of the events preceding Lz self-assembly into amyloid-like structures, the protein adsorption onto PC:PG vesicles was examined by monitoring fluorescence changes of fluorescein-labeled lysozyme. The observed sigmoidal shape of the adsorption isotherm is strongly suggestive of oligomerization of membrane-bound protein. It seems highly probable that such oligomers serve as nuclei in the membrane-assisted lysozyme fibrillogenesis

P62926-053

Structure and dynamics of photosystem II light-harvesting complex revealed by high-resolution FTICR mass spectrometric proteome analysis

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Structure and dynamics of membrane-bound light-harvesting pigment-protein complexes (LHCs), that collect and transmit light energy for photosynthesis and thereby play an essential role in the regulation of photosynthesis and photoprotection, were identified and characterized using high-resolution FTICR mass spectrometry. LHCs from photosystem II (LHCII) were isolated from the thylakoid membrane of Arabidopsis thaliana leaves after light stress treatment using sucrose density gradient centrifugation, and separated by gel filtration into LHCII subcomplexes.

Using reversed phase high performance liquid chromatography and two-dimensional gel electrophoresis, the LHCII proteins, Lhcb 1-6 and fibrillins were efficiently separated, and identified by FTICR-MS proteome analysis. Some of the LHCII subcomplexes were shown to migrate from photosystem II to photosystem I as a result of short term adaptation to changes in light intensity. In the mobile LHCII subcomplexes, decreased levels of fibrillins and a modified composition of LHCII protein isoforms were identified compared to the tightly-bound LHCII subcomplexes. In addition, FTICR-mass spectrometric analysis revealed several oxidative modifications of LHCII proteins (1). A number of protein spots in 2D-gels were found to contain a mixture of proteins, illustrating the feasibility of high resolution mass spectrometry to identify proteins that remain unseparated in 2D-gels even upon extended pH gradients.

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P70100-001

Novel phosphinic dipeptide inhibitors of leucine aminopeptidases

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Phosphinic analogue of the homophenylalanyl-phenylalanine dipeptide was demonstrated to be a potent, competitive inhibitor of cytosolic leucine aminopeptidase (LAP, E.C.3.4.11.1), mimicking the transition state of the reaction catalysed by the enzyme. Exhibiting *K*i value at low nanomolar range it was ranked among the most potent inactivators of LAP reported so far (1). Recently, the compound has been also successfully employed to inhibit leucyl aminopeptidase of *P. falciparum*, a potential target protease for the development of new antimalarials (2). Thus, its structure represented an attractive lead for the design and construction of next generations of modified analogues. They were targeted towards both LAP as well as a related metalloprotease – microsomal leucine aminopeptidase (APM, E.C.3.4.11.2). These achievements, including recent results of studies on synthesis and activity, will be summarized here.

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P70100-002

Formation of supramolecular complexes (fibres and spherulites) from some L-Valine derivatives in aqua solutions

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Formation of fibrous supramolecular complexes from L-Val derivatives and their arrangement in spherulites was studied applying spectrophotometric approach and electron-microscopic observations. Experiments show that boiling one and the same compound with different initial concentrations in water to completely dissolving and posterior cooling to room temperature lead to formation of stable supramolecular complexes with different sizes and properties. They behave like polymer molecules which specific numbers of monomers depend on the initial concentration of the boiling solution, moreover at higher supersaturations they crystallize in sperulites which is typical for crystallization of polymer molecules.

P70100-003

Metal – organic gels based on the self-assembly of peptidomimetics and Cu (II) ions

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Peptidomimetics constructed from L-Val and nicotinic/isonicotinic acid are good low molecular weight gelators and their supersaturated solutions in a number of solvents turn into gels. They make complexes with some metal ions. Mixing of pure solutions of the peptidomimetic and some Cu (II) salts lead to immediate gel formation in some solvents. This kind of compounds, named metal-organic frameworks (MOFs), are new class of nanoporous materials and are very promising ones for applications in catalysis, pharmaceutical industry, etc. The stability and molecular structure of these complexes in some solvents is in the process of investigation.

P70101-004

Synthesis and Evaluation of RGD Peptide Analogs Cyclized by Coordination of Oxorhenium and Oxotechnetium Cores for Molecular Imaging of Cancer

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Peptide metalloconstructs often possess particular conformations and hence display increased activities and metabolic stabilities. We investigated new RGD peptide analogs cyclized through oxorhenium / oxotechnetium coordination. The RGD sequence is known to bind specifically to 10 of the 25 known integrins, a family of integral proteins that plays an important role in tumor neoangiogenesis, development and proliferation. Several cyclic RGD pentapeptides bearing an exocyclic Tc-99m oxotechnetium core have been proposed for molecular imaging, however few of them have displayed attractive selectivities and metabolic stabilities. Structure, biological activity and metabolic stabilities of metallated peptides cannot be predicted. Therefore, we preferred a combinatorial approach to generate a panel of tracers that may be evaluated by tumor imaging in mice. Tracers were constructed from a RGD model of general formula : NS2-X1–X2–X3–RS where X1,2,3 are respectively arginine, glycine and aspartic acid analogs and R is a series of linkers that feature various lengths and geometries (NS2 is a N-bis(ethylthio) moiety). First attempts for synthesizing these peptides by the versatile Ugi multicomponent reaction did not yield the peptides with sufficient purities. A representative library of 64 peptides was obtained by standard parallel peptide synthesis and purification of all members. Peptides were metallated either with rhenium or technetium using standard procedures. Their resistance towards mice serum, glutathione and tumor extracts was evaluated and showed that compounds containing an aminoethanethiol linker displayed higher stabilities. Influence of the chemical environment on isomers ratios of the metallated peptides was also investigated. Finally, oxorhenium peptide coordinates were assayed as specific ligands of integrins. Their oxotechnetium equivalents were evaluated for tumor imaging in mice.

P70106-005

Degradation products of desmopressin in phosphate/ citrate buffer

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MINIRIN and OCTOSTIM Nasal Spray RTS® are formulations of desmopressin (Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH2, cyclo 1-6) in phosphate/citrate buffer, pH 5.0. On storage degradation products are formed. Five major degradation products are identified. All with the same monoisotopic mass, m/z 1070.5 (+1 Da compared to desmopressin). Four of them are the expected hydrolysis products [Glu4]-, [Gly9-OH]-, [Asp5]- and [β-Asp5]desmopressin. The fifth major degradation product is identified as [seco-3/4,pGlu4]desmopressin. It is assumed to be formed via an imide intermediate, [pGlu4]desmopressin, in analogy with the formation of [β-Asp5]desmopressin via a cyclic aminosuccinimide derivative. The [seco-3/4,pGlu4]desmopressin is assumed to be formed by cleavage of the amide bond between Phe and pGlu in [pGlu4]desmopressin. In another formulation of desmopressin without buffer and pH 4, [seco-3/4,pGlu4]desmopressin is not observed.

P70111-006

A new stable Copper(III)/Cyclopeptide complex: structural characterization by XANES and EXAFS studies

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We recently described the synthesis of some cyclic tetrapeptides bearing imidazole side chains and we analyzed, in detail, their copper(II) binding properties in aqueous solution.[1] The copper(II) species obtained are of interest in relation to copper-protein active-site biomimetics. In particular, a 13-membered ring cyclic tetrapeptide *c*(Lys-DHis-βAla-His) (DK13) was synthesized by the solid-phase peptide synthesis method and its copper(II) coordination properties were studied.[2] Surprisingly, all collected data strongly support the presence, at alkaline pH, of a stable peptide/copper(III) complex that is formed in solution by atmospheric dioxygen oxidation. In order to clarify the mechanism of the copper oxidation through the average Cu-N bond distance and to get information on the local geometry around copper, depending on the peptide sequence, we have collected experimental XANES and EXAFS spectra. The investigated cyclopeptide/copper complex shows pre-edge peak energy position and integrated intensity consistent with those of Cu^{3+} model compounds. Also, edge energy is consistent with the presence of trivalent copper. Then, calculations performed on the EXAFS measures should give information on the local geometry, confirming the square planar geometry proposed by us for this DK13/Cu(III) complex.

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P70305-007

Improved expressed protein ligation method for consecutive coupling of polypeptide fragments

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In the post-genomic era, to support the structural and functional studies of proteins, there is a growing demand for novel methods with which a wider range of selective protein modifications achievable. Expressed protein ligation (EPL) can be the method of choice for the preparation of unique protein derivatives not available by recombinant methods when the protein fragments extend the size accessible by solid phase peptide synthesis, and when extra chemical information (i.e. a special covalent modification) is introduced into a well-defined part of the sequence. However, this method is not generally applicable, especially in the case when the target protein derivative is assembled from three or more polypeptide fragments. We demonstrate here an improved EPL method that facilitates the effective ligation of large fragments in a consecutive way. The method employs a novel protection-deprotection scheme. Supported by OTKA F049222 and János Bolyai fellowship (Cs.T.).

P70517-008

Protein ligation using protein trans-splicing

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Protein splicing is a post-translational modification in which an intein catalyzes ligation of flanking peptide sequences and the concomitant self-excision from the precursor protein. Protein splicing can also take place in trans, thereby ligating two separate polypeptide chains by a split intein. Thus, protein trans-splicing enables protein ligation of diverse protein fragments of interest by fusing them to halves of a split intein. We have engineered a naturally split intein, DnaE intein from Nostoc punctiforme (Npu), in order to facilitate protein ligation using synthetic peptides. Highly efficient fluorescent labeling of proteins has been demonstrated in a site-specific manner by protein ligation using the newly designed intein. This approach could be applied for site-specific modification of proteins in vivo because protein trans-splicing is an autocatalytic process requiring no additional cofactor. Moreover, protein trans-splicing approach could be extended to dual site-specific modification using an additional intein.

P71100-009

Spectroscopic characterization of doppel peptide fragments and their complex species with Cu (II)

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Doppel (Dpl) is a glycosylphosphatidylinositol-anchored protein that exhibits 26% sequence homology with prion protein but lacks the octarepeat region. The role of prion is not yet completely known but PrPc seems to be involved in Cu (II) trafficking from synaptic clefts and in preventing neuronal oxidative damage. Doppel is expressed in heart and testis and has been shown a regulator of male fertility. Therefore, despite a high sequence homology and a similar three-dimensional fold, it's possible to hypothesize that the function of the two protein is not correlated. In the literature is reported that both PrPc and of Dpl are able to bind Cu (II) but the characterization of the complex species is well defined only for PrPc. Several binding sites of Dpl are involved in the complexation with Cu (II). [1, 2] In the present work we have focused our attention to the third alpha-helix of the Dpl which is the preferential binding site for the metal ion. We have synthesized two peptide fragments relative to the 122-140 sequence of the Dpl. The first peptide has the native sequence whereas in the second fragment the Asp 124 was replaced by a Asn residue. This substitution was performed to understand the role played by the carboxylate group of the Asp residue in the complexation of Cu (II). CD ad NMR spectra were carried out on the two peptides in absence and presence of Cu (II) to investigate conformational features upon Cu (II) binding. Finally, the complex species formed were completely characterized by using spectroscopic (NMR, CD, UV-Vis, EPR) and thermodynamic measurements (potentiometric titrations).

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P71100-010

The impact of human cystatin C hinge loop L1 on its dimerization and oligomerization propensity

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Three-dimensional (3D) domain swapping of proteins is a phenomenon associated with formation of oligomers and fibrils of several amyloidogenic proteins (1). The propensity of a particular protein to undergo domain swapping depends on factors like changes in environment or presence of specific mutations, but also on some topological determinants. It was proposed that domain swapping-prone proteins have some common "hot spots" in their structures that facilitate the process. One of these motives are so called "hinge loops" - turns showing enhanced propensity for unfolding due to conformational constraints (2).
Cystatin C (hCC), main inhibitor of cysteine proteases in human body was shown to dimerize (3) and oligomerize (4) through domain swapping. hCC contains highly conserved throughout the cystatin family loop L1, connecting two beta strands which, together with the only helix in the protein fold create a structural unit that undergoes the 3D process.
In order to confirm important role of distortions in L1 loop, which are centered around valine residue in position 57 in the dimerization/oligomerization process of hCC, we designed point mutations which should inhibit or promote aforementioned processes. As it was expected, substitution of Val57 with either asparagine or aspartic acid residues abolished dimerization process almost completely. In contrast, V57P mutant shows enhanced dimerization propensity. Above observations are in good agreement with theoretical studies, performed on entire hCC and its fragment encompassing the hairpin structure centered on loop L1.

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P71100-011

Study of the inhibition of polyglutamine aggregation using a pH-dependent morpholino-derived switch

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The expansion of the CAG trinucleotide that produces polyglutamine (polyQ) segments in several proteins is responsible for at least eight neurodegenerative diseases. A possible therapeutic approach would be to inhibit the polyQ self-assembly process using disrupting agents. Although, screening studies have identified several polyQ aggregation inhibitors, their mechanism of action remains unknown. This is due to the poor aqueous solubility and fast aggregation of uncharged polyQ peptides, which complicate their study. The most common strategy to resolve these problems is to produce polyQ stretches flanked with charged residues. However, this strategy was found to modify the aggregation behaviour of polyQs, their aggregate structure and their affinity for disrupting molecules. To circumvent these problems, polyQ peptides containing morpholine moieties, whose charge is pH-dependent, were produced using Fmoc-based chemistry on SPPS. Following an acid treatment, the designed peptides were soluble in acidic aqueous solutions and their aggregation rate could be controlled by pH variations and followed by dynamic light scattering. Furthermore, aggregation initiated at physiological pH provided uncharged fibrils allowing the evaluation of the effects of charged disrupting agents. The actions of known polyQ aggregation inhibitors such as polyQ-binding peptide 1 (QBP1), trehalose and Congo red were studied.

P71101-012

2-alkyl-2-carboxyazetidines as reverse turns inducers when incorporated at (i+2) position of model peptides

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Peptide-protein and protein-protein interactions play key roles in most biological processes, and therefore represent valuable targets for drug discovery. An approach in the search of new chemical entities able to interfere with these interactions is the use of small molecules able to mimic or to induce precise aspects of specific peptide secondary structures. Among the secondary structure elements, reverse turns have been shown relevant in many biomolecular recognition events. Thus, different efforts have been directed to the design of turn mimetics or inducers. In this sense, 2-substituted azetidine-2-carboxylates, synthesized by our group through a versatile procedure from amino acids, possess the φ dihedral angle restricted to approximately 70º or -70º, depending on the absolute configuration at the asymmetric carbon. These values are similar to those reported for the central residues of main types of β- and γ-turns, and in fact, recent studies have shown the ability of the 2,2-disubstituted azetidines to induce γ-turns when incorporated at the i+1 position of model peptides. To know if this conformational behavior is distinctive of these restricted amino acids, we now investigate the influence of the incorporation of these amino acids at the i+2 position of model peptides. With this aim, we have synthesized a series of simplified tetrapeptide models, RCO-L-Ala-Azx-NHMe, in which the RCO and NHMe groups are simplifications of the i and i+3 residues of the turn, respectively. For comparative purposes, dipeptide derivatives incorporating azetidine-2-carboxylate, Pro and α-MePro have also been prepared. The turn inducing capacities of all these model tetrapeptides have been analyzed by molecular modelling, ^{1}H RMN, FT-IR and X-ray methodologies. The results have shown the importance of the α,α-disubstitution at the heterocyclic amino acid for stabilizing reverse turns, and the influence of the ring size on the induced turn type

P71128-013

Influence of position of dehydroamino acid in peptide chain on conformation of dehydropeptides containing two dehydroamino acid residues

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It is known that presence of C^{α} -C^{β} double bound in dehydroamino acid influences on dramatic limitation of conformational space, not only side-chain but also main-chain [1-2]. According to the peptide's length and neighboring amino acid residues, dehydroamino acid exerts S-shaped, β-turn or helical conformation in peptides [3-7].

It shows, that introduction of unsaturated residue to the peptide chain could be useful tool to design bioactive compounds with desirable structure [8-9]. Therefore full knowledge about relation between presence of dehydroamino acid and peptide's conformation is necessary to predict biological proper and to design newdrug. For that reason we have undertook conformational investigations of numerous peptides containing two dehydroamino acid residues (Δ^ZPhe, Δ^EPhe, ΔAla) in peptide chain, in different position. The investigations were based on NMR measurements (standard 2D techniques and 1D experiments, typical for detection of hydrogen bonding) and theoretical calculations. Conformational preferences of investigated systems were obtained on base of ROESY and NOESY experiments and calculations by use of X-PLOR and quantum chemical calculation

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P71128-014

Angiotensin-I/Captopril competitive interaction studies with 46-residues catalytic site maquettes of angiotensin-I converting enzymes through NMR studies

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Angiotensin-I converting enzyme (ACE) somatic form bears two catalytic domains with two Zn metal ions, while the testis form bears remarkable similarity to the aminoacid sequence of ACE C-catalytic domain and only one Zn metal ion. However, both exhibit their hydrolytic activity to vasoactive peptides such as angiotensin I (AngI) and bradykinin (BK), though with different efficacy. In order to study experimentally the possible interaction and binding events between the ACE active site(s) and known ACE substrates or inhibitors, we constructed peptide-based catalytic site maquettes (CSM). 46-residue peptides were synthesized through solid-phase peptide synthesis having the aminoacid sequence that correspond to the ACE Val380-Ala425 N- domain segment and to the Val978-Ala1023 C-domain segment and enzyme's active sites were reconstructed through addition of zinc metal ion in solution. The resulting complexes have the metal ion bound in a native-like mode, as manifested by previously reported NMR data. The reconstituted peptides were titrated separately by Captopril and Angiotensin-I with a molar ratio ACE:Captopril/AngI 1:15 and 1:5, respectively. Titration was followed by ^{1}H NMR spectroscopy and spectral changes (chemical shifts, line broadening, etc.) were recorded and analyzed. After the end of AngI titration the solution of interacting peptides was titrated with Captopril and the titration was followed by ^{1}H NMR spectroscopy. At the end of each titration 2D homonuclear ^{1}H-^{1}H TOCSY and NOESY spectra were recorded. Analysis of high-resolution NMR data probes the conformational variation of ACE CSM and peptide substrates upon interaction, and in the presence of Captopril in ACE-AngI solutions.

P71300-015

Self-Assembling Ureido Di-Peptide: Novel Building Blocks For Nanotechnology

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Contemporary development in nanotechnology is fabrication of nanostructured materials using the peptide-based self-assembly. Importance of peptide as monomeric components is their capacity to be engineered to mediate spontaneous supramolecular self-assembly and their inherent chemical nature that facilitates chemical and biological recognition process. Tubular self-assembled peptide nanostructures are of special interest since these can serve in various applications including 'bottom-up' fabrication of molecular scaffolds, nanoelectromechanical systems and nanomachines.

An important discovery by Gazit group revealed that the simple di-peptide molecule of phenylalanine which is core recognition motif of the Alzheimer's β-amyloid polypeptide efficiently self-assembles into well-ordered nanotubular structure (1) which could be controlled by relatively simple chemical modifications at its termini.

Based on these observations and our interest in the synthesis of soft-materials for nanotechnology, we have successfully synthesized novel monomeric units of di-peptides with ureido functional group at N-terminal of various hydrophobic L-amino acids. These derivatives have been prepared by using solid phase peptide synthesis protocol with cost effective process retaining the chirality of molecule. These ureido di-peptide formed nanostructures with high yield under mild and controlled condition in aqueous media. To fully understand the molecular and supramolecular mechanism guiding the formation of nanostructures we have selected a multidisciplinary approach by combining spectroscopic studies with advanced electron microscopic techniques. The morphology of nanostructures can be controlled by using variety of L-amino acids.

These novel ureido di-peptides nanotubes can be used in fabrication of biocompatible peptide-based nanostructures and they will undoubtedly have nanotechnological applications.

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P71300-016

Screening of peptides that specifically bind to fine structured peptide nanofibers

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Biomolecules are excellent in hierarchically organizing a characteristic structure by self-assembly. The spontaneous assembly of biomolecules has advanced a bottom-up approach for fabrication of nanostructured materials. We have developed fabrication of controlled nanofibers through self-assembly of simple and short *de novo* designed β-sheet peptides with unique sequences [1, 2]. The single straight nanofiber with high regularity constructed by β-sheet FI peptide (sequence: PKFKIIEFEP) was functionalized with proteins by biotinylated peptides (3) and using functional anchors that have binding and functional groups. Conjugation of self-assembled peptide nanofibers with biomolecules such as peptides and proteins will expand their potentialities of the nanofibers for various applications. To identify peptides binding to the self-assembled FI peptide nanofiber, a phage display combinatorial screening using a random peptide library was performed. After five rounds of biopanning, phage pools with highly specific affinities to FI nanofibers were screened. As a result of DNA sequencing after phage cloning, 12 phage clones were identified. Binding affinities of these clones were quantitatively investigated by an enzyme-linked immunosorbent assay. These clones showed specific affinities to FI nanofibers, as compared with FF and VI nanofibers having slightly differences of amino acid sequences.

Affinities and specificities were dependent on the sequence of each peptide, indicating that different amino acid sequences contributed to peptide interactions with nanofibers. Infrared spectroscopy studies by using chemically-synthesized peptides showed that the peptide binds specifically to the FI nanofiber with structural transitions.

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P71314-017

Control of duplex formation and columnar self-assembly with Heterogeneous amide/urea macrocycles

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Owing to their diversity in size and shape, easy access, and biocompatibility, peptides represent versatile units for the construction of H-bonded tubular assemblies and other biomimetic materials with potentially useful applications. So-called peptide nanotubes (PNTs) have been obtained through multiple and complementary approaches (1). Originally designed from α-peptides made of D- and L-amino acids (2), flat macrocyclic systems forming cylindrical β-sheet like assemblies have diversified to include oligoamides made from higher amino acid homologs as well as peptide hybrids (e.g. α,γ-peptides). Tubular sheet-like assemblies however are not restricted to oligoamides. The urea group which shares a number of features with the amide linkage, i.e. rigidity, planarity, polarity, and hydrogen bonding capacity is an interesting surrogate. We and other have demonstrated that macrocyclic biotic and abiotic N,N'-linked oligoureas have a unique propensity to self-organize into polar H-bonded nanotubes (3). Partial peptide backbone N-methylation has been introduced as a general strategy to generate truncated stacks (i.e. H-bonded dimers) useful to gain access to the thermodynamics of nanotube formation (1). Herein, we describe biotic macrocyclic amide/urea hybrids with partially N-alkylated backbones as new candidates for the formation of H-bonded dimers.

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P71314-018

Cysteine branched PGA for application in drug controlled release

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A cysteine branched PGA (polyglutamic acid) hydrogel is being investigated for therapeutic peptide and protein controlled delivery. Entrapment of hGH as a model protein in this hydrogel has been carried out in order to check its later release in different aqueous buffered media and stability. Different physico-chemical analysis (by MALDI-TOF, SEC-MALS-RI, 1H-NMR, CD, …) of the released hGH showed no effect on destabilization of the protein. Controlled release systems of proteins involve important challenges such as maintaining the protein integrity. Researchers need an exhaustive understanding of protein stability issue in drug delivery formulations. Common degradative reactions in proteins involve: oxidation, deamidation, stability of disulfide bonds and aggregation, which may affect secondary and tertiary structure of proteins and thus, their biological activity. The high water content which imbibes the polymer network of hydrogels enables a good accommodation for proteins. Polyamino acids have been already studied in some biotechnological applications, however, in spite of their potential, they have barely been taken into account for the development of controlled release microparticles. We show that proteins could be easily loaded into Cys cross-linked PGA hydrogels. The protein hGH has been loaded and released preserving its physico-chemical integrity. Thus, these hydrogels are promising for their use as therapeutic protein delivery systems.

P71314-019

Totally synthetic collagen-like gels by intermolecular folding of designed peptides

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Our goal is to develop artificial collagens that can use as safer and functional biomaterials. Here, we report the development of collagen-like gels by means of the intermolecular folding of chemically synthesized peptides. The peptides are disulfide-linked trimers of collagenous Gly-X-Y triplet repeats with self-complementary shapes. The self-complementary peptides are able to form elongated triple-helix through spontaneous intermolecular folding. Upon cooling of the peptide solutions, hydrogels of peptide supramolecules formed. The gel-sol transition was appeared to be reversible, and the transition temperatures were found to be tunable by the design of the peptides. Our strategy for the totally synthetic collagen will offer possibilities for the development of innovative biomaterials.

P71317-020

Bio–assisted conformation control of inorganic materials by Peptide Engineering

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Recent advance in biotechnology enables us to find the peptides with affinity for nonbiological materials and with function of mineralizing inorganic materials. The use of the functional peptides is attracting a growing interest for bottom-up fabrication approaches of nanoscale devise. Zinc oxide (ZnO), a semiconductor with a wide direct band gap, possess unique optical, acoustic, and electronic properties, so that it is one of most widely studied metal oxides for solar cells, ultra violet nanolaser, blue light-emitting diode and so on. This wide variety of applications requires various fabrications of morphologically and functionally distinct ZnO nanostructures. Here, the peptides which are selected from the phage-displayed peptide library with a 12-mer on the surface, can bind ZnO particle but not other metal oxides particle, and further, the ZnO-binding peptides play an important role on the crystal growth of ZnO in its synthesis. The anti-ZnO peptide can assist the synthesis of ZnO nanoparticles from a $Zn(OH)_2$ solution even at 4 ºC, and further, the peptide leads to the self-assembly of synthesized ZnO particles to flower-type morphologies. We describe the biomimetic ZnO synthesis using the artificial peptide with affinity for ZnO.

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P71419-021

Peptide-grafted nanodiamonds: preparation, cytotoxicity and uptake in cells

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The design of nanoparticles suitably functionalized for applications in biology or medicine is an ongoing challenge for both chemists and biologists. Nanoparticles such as quantum dots, gold nanoparticles or carbon based-materials offer, in addition to their remarkable physical properties, the possibility to be functionalized by biomolecules, making them suitable for sensing, detection, diagnostic, and/or therapeutic applications. Recently, quantum dots have been designed for biological imaging owing to their unique size-dependent fluorescence properties. However, their plausible toxicity still remains a major concern for in vitro and in vivo applications. Nanodiamonds, (NDs) are also candidates for biomedical applications considering their intrinsic or induced fluorescence and biocompatibility. The work will describe: i) the functionalisation and the characterization of ≤ 35 nm non-fluorescent cNDs and ii) their use in toxicity and transport studies in living cells after the grafting of a fluorescent model peptide. We chose to introduce a non-permeant fluorescent peptide for the tracking of the nanoparticles on or inside the cells. Nanodiamonds (cNDs, ≤ 35 nm) coated by silanisation or with polyelectrolyte layers have been grafted with a fluorescent thiolated peptide via a maleimido function, leading to aqueous colloidal suspensions stable for months. For each step of the preparation, the diameter of the NDs measured by dynamic light scattering (DLS), the zeta potential, the amount of amino groups grafted onto the surface, as well as the stability of the different NDs suspensions No cytotoxicity was observed up to 72 hours incubation with CHO cells with any of the prepared (40 μg/mL). Their capacity to enter mammalian cells, and their localisation inside CHO cells, have been ascertained by confocal microscopy, reflected light and fluorescence.

P71421-022

Microwave assisted Polyelectrolytic bioconjugates of Hepatitis B surface antigenic polypeptides and their immunological properties

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Hepatitis B Virus is one of the most common problem and potential danger of all over the world and also in our country. It is known that the use of proteins and peptide antigens as vaccines has several potential advantages over whole viral or bacterial preparations. To elicit the maximum immunogenic response from synthetic peptide antigens ,it is generally necessary to bind the peptide to carrier protein. Moreover, to realize the full potential of synthetic peptide antigens protein-peptide conjugate will have to be used with an adjuvant. We have developed new approches for obtaining highly immunogenic peptide conjugates-synthetic polyelectrolytes (PE) were used for the conjugation with peptide molecules in which PE carry out the carrier and adjuvant roles simultaneously. This concept drive us to create new immunogenic bioconjugates of Hepatitis B surface antigenic polypeptides (HbsAg) with synthetic PE. In this study we used the region 95-109 of the s gene. The synthesis of peptides was performed by Explorer PLS ® Automated Microwave Synthesis Workstation (CEM), by using F-moc Chemistry. Copolymers of acrylic acid with different monomers were used as a PE for the conjugation with polypeptides. PE-peptide conjugates was synthesized by microwave assisted method. Composition and structure of bioconjugates werw characterized by HPLC, Spectrofluorometry and different spectrophotometric methods.

P71623-023

Analysis of protein-protein interaction sites without natively folded protein samples: fiction or fact?

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Protein-protein and protein-ligand interactions are among the few essential cell processes whose understanding provide us insights into fundamental events of the life cycle. Aside from *in silico* methods, natively folded proteins are an absolute prerequisite in all current biotechnological tools used for the study of these interactions. However, the recently developed IANUS peptide array has a potential to evolve in to a protein-free method for detection of protein-protein interaction sites. Using this assay, protein-protein interactions could be represented by and investigated as peptide-peptide interactions using peptide pairs immobilized on a solid support.(1) .
Two main obstacles had to be overcome for successful implementation of this array: (i) the library size and (ii) identification of interacting peptide pairs. If, for example, interactions between two small proteins of only approx. 100 amino acids each should be analyzed, a library of ca. 2500 peptide pairs would be needed to cover all possible combinations of overlapping peptides derived from these proteins. Although libraries of several thousand peptides were already successfully synthesized, the library size could be reduced drastically if the binding pocket of one protein is already known or if the protein-ligand interactions are studied. Up to date, two methods for the identification of interacting peptides in a library have been developed in our group. Both methods will be demonstrated in studies of protein-protein and protein-ligand interactions.

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P71724-024

Is concentration overload or volume overload the best strategy for synthetic peptide purification?

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As the complexity of synthetic peptides increases so the demands on the synthesis and purification increase. Whilst improvements in synthesis resins and techniques enables higher purity peptides to be produced, 100% purity is not achieved. For many applications post-synthesis purification is still required. Methods for the purification at the mg level can be relatively straightforward but where there is a need to develop methods which may be used for larger scale production there are a number of additionly considerations. The method developed must be scaleable and the economics of the process must be compatible with the final product costs. When looking to develop a purification method the loading will be critical to the throughput and final production costs. The selection of the purification media and the purification conditions are two of the major influences on loading as these determine capacity and resolution. However, it is also important to consider the the physical properties of the pepitide - especially its solubility. As part of the purification method development a loading study must be performed - increasing the amount of peptide loaded onto the column. The most common way of doing this is to increase the concentration of the sample and keep

the injection volume constant, concentration overload. But this does require that the peptide is readily soluble in a solvent compatible with the HPLC purification conditions. Alternatively volume overload can be used where the concentration of the peptide solution is kept constant and the volume purified increases. The most commonly used method is concentration overload. The work presented in this poster compares the two overload strategies, concentration overload and volume overload, for the purification of synthetic peptides. The suitability of the two strategies for large scale manufacture will be explored.

P71900-026

Expansion of human stem cells by passive transmembrane transfer of homeoproteins

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The HOXB4 homeoprotein was known to promote the expansion of mouse and human hematopoietic stem cells (HSCs) and progenitors of the myeloid lineages. However, the putative involvement of HOXB4 in lymphopoiesis and particularly in the expansion of early lymphoid progenitor cells remained elusive. Based on the ability of the HOXB4 protein to enter cells by way of a passive transmembrane transfer process (Derossi et al, J Biol Chem 269:10444; 1994), we previously designed a long-term co-culture procedure of human CD34+ immature hematopoietic cells with stromal MS-5 cells engineered to actively secrete the HOXB4 protein. This procedure allowed ex vivo expansion of primitive human hematopoietic cells (Amsellem et al, Nat Med 9:1423; 2003). More recently, we used the same method to investigate whether HOXB4 could support expansion of cells originating from CD34+ cells and committed at various levels toward the lymphoid lineages. We provided evidence that HOXB4 protein delivery does promote the expansion of primitive hematopoietic progenitor cells (HPCs) that generate lymphoid progenitors. HOXB4 acts on lympho-myeloid HPCs and committed T/NK HPCs but not on primary B-cell progenitors (Haddad et al, Stem Cells 26:312; 2008). Moreover, HOXB4 improves the ex vivo generation of human NK-cell progenitors, resulting in high numbers of functional mature NK cells (Haddad et al, Leukemia 21:1836; 2007). These results clarify the effect of HOXB4 in the early stages of human lymphopoiesis, emphasizing the contribution of this homeoprotein to the intrinsic lympho-myeloid differentiation potential of defined lymphoid progenitor subsets. Finally, this supports the potential use of HOXB4 for HSC and HPC expansion in a therapeutic setting, by means of direct transmembrane protein transfer.

P71921-025

Tumor selective delivery by cell-penetrating peptides

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Systemic delivery of therapeutic agents used for cancer chemotherapy today lead to undesired side effects and toxicity. Increasing the selectivity of the anti-cancer agent will not only facilitate lower dosage for equal therapeutic effect, but also decrease the frequency of drug resistance. We have studied two different targeting approaches both based on cell-penetrating peptides. The first approach is a chimera between cancer homing peptides and a CPP the other is a enzyme activated prodrug design. The cyclic peptide cCPGPEGAGC (PEGA) is a homing peptide that has previously been shown to accumulate in breast tumor tissue in mice. PEGA peptide does not cross the plasma membrane per se; however, when attached to the cell-penetrating peptide pVEC, the conjugate is taken up by different breast cancer cells in vitro. Additionally, the homing capacity of the PEGA-pVEC is conserved in vivo, where the conjugate mainly accumulates in blood vessels in breast tumor tissue and, consequently is taken up. Matrix metalloproteinases (MMPs) are over-expressed in a variety of tumor tissues and cell lines, and their expression is highly correlated to tumor invasion and metastasis. To exploit these characteristics, we designed a tumor cell-selective prodrug, by constructing modified version of the already shown to be functional MTX-YTA4 conjugate. It is an inactive pro form of a cell-penetrating peptide (NOPE) conjugated to the cytostatic agent metothrexate (MTX), selectively cleavable and thereby activated by MMPs.

P72123-027

Characterization of hantavirus envelope structure

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Hantaviruses are zoonotic viruses carried by different rodent species and if transmitted to man cause two severe diseases, the hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome to which there is no specific therapy. The hantavirus envelope consists of spike structures formed by surface glycoproteins Gn and Gc which anchorage to the viral lipid bilayer. The quaternary complexes formed by glycoproteins have not been solved. To begin with, it is important to understand the structure of virus particle in order to treat infection for example by prevention of membrane fusion. Using PepSpot technique the interaction site between Gn and Gc proteins was mapped to peptide level. To interpret interaction mapping results three dimensional (3D) models of Gc protein were created. The structure of hantavirus was also investigated in this study by cryo-electronmicroscopy (cryo-EM). According to our results, hantavirus spike consists of either four or eight glycoprotein units and the spike would consist of equimolar associations of both glycoproteins. Overall it seems likely that the glycoproteins of hantavirus form a heterodimeric base unit analogous to Semliki Forest virus E1-E2 glycoprotein complex, where the interaction between E1-E2 proteins hides the fusion peptide in E1 protein and thus prevents premature fusion.

P72124-028

Isolation of a novel 24 kDa protein from ginger rhizomes having anti-fungal and anti-proliferative activity

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Medicinal properties of ginger have been known for long. Ginger has been used in traditional Indian and Chinese medicine and is effective on a wide range of ailments including diarrhea, nausea, respiratory disorders, inflammatory diseases, arthritis etc. A number of constituents and active ingredients are present in ginger. Recent studies have shown the role of ginger extract in the modulation of biochemical pathways involved in chronic inflammation and thus providing evidences for the anti-inflammatory role of ginger. Mainly the medicinal properties and anti-proliferative activity of the ginger is because of the presence of certain pungent vallinoids, viz. 6.-gingerol and 6.-paradol, as well as some other constituents like shogaols, zingerone etc.
We have identified and purified a novel anti-fungal and anti-proliferative protein with a molecular mass of 24 KDa from the crude extract of ginger rhizobium (Zingiber officinales), belonging to the Zingiberaceae family. The isolation procedure involved ion exchange chromatography using DEAE-cellulose and affinity chromatography using affi-gel blue gel. Crude extract was loaded on the DEAE-cellulose column equilibrated with 10 mM Tris-HCl buffer (pH 7.0). The unadsorbed protein fraction from DEAE-cellulose was further loaded on Affi-gel blue gel column equilibrated with 10 mM Tris-HCl buffer (pH 7.0), from which the elution of adsorbed protein was done with the same buffer. The purified protein of 24 kDa exhibited a potent anti-fungal activity against the mycelial growth in different fungal species, for example Aspergillus fumigatus. In addition, the antifungal activity is also seen against important fungi, viz, Fusarium and Candida species. Further, the purified protein also showed 60 % inhibition of cell proliferation at 10 µM concentration. The anti-proliferative activity was checked on human oral cancer (KB cells).

P72216-031

Search for native interacting partners of fluorinated amino acids using phage display

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The introduction of fluorine has proven to be a successful concept for improving the biological and pharmaceutical properties of drug candidates. The unique properties of fluorine as well as its absence from the pool of canonical amino acids make fluorinated amino acids a promising tool in the development of peptide based drugs.(1) However, the application of fluorinated amino acids for rational protein design requires a comprehensive knowledge of their properties within a native protein environment. Our research focuses on the effects caused by single substitutions by fluorinated amino acids within a polypeptide environment.(2) Based on a parallel, heterodimeric α-helical coiled coil peptide we applied phage display technology (3) to screen for preferred interaction partners of fluorinated building blocks within the pool of the twenty canonical amino acids. Three fluorinated amino acids were introduced either at an a- or a d-position into one of the coiled coil monomers. The second coiled coil monomer was randomized at the four positions that represent the direct interaction partners of the fluorinated amino acid within the dimerization domain. Coiled coil pairing selectivity was used to determine the best binding partner out of the library.

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P72300-029

Regulation of the HIV-1 integrase activity by the viral Rev protein: The effect of cell permeable peptides derived from the integrase and Rev proteins

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The Human Immunodeficiency Virus (HIV) has caused the death of over 25 million people, worldwide, since 1981. Today 33.4 to 46 million people are infected with HIV and the numbers are rapidly increasing. Two types of HIV are known to infect humans; HIV-1 and HIV-2. HIV-1 is the more common of the two and the one that causes the larger number of fatalities. An essential step in the replication cycle of all retroviruses is the integration of the viral DNA (after reverse transcription) into the host genome. The research set forth here deals with the molecular mechanisms of the integrase (IN) activity and its regulation.

The HIV-1 IN mediates the integration of the viral genome into the host cell DNA. In HIV-1 infected cells there are only one or two integrated viral genomes (proviruses) per cell while in other retroviruses the number of proviruses per cell is much higher. The mechanism which prevents multiple integrations is specific to HIV-1 and is yet unknown. Thus elucidation of the detailed mechanism of the regulation of IN activity may lead not only to a better understanding of the viral replication cycle but also to the development of new anti-HIV-1 drugs. We suggest that one of the HIV-1 early translated proteins, namely the Rev protein, may be responsible for this regulation.

The viral Rev protein, mediates nuclear export of viral un-spliced and partially spliced RNA. We have observed that Rev binds to IN and furthermore it inhibits the IN activity in-vitro. These results may indicate that the Rev protein may regulate the enzymatic activity of the IN and consequently the viral DNA integration process. Support of this view obtained from results showing that Rev derived peptides are able to inhibit the IN activity in-vitro and in-vivo while peptides derived from the IN, which binds to the Rev, abrogate those inhibitions and increase the IN activity in cell cultures.

P72311-030

The Role of the Non-Helical Tailpiece in Myosin II Assembly

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Self-assembly of macromolecules into large complexes is often mediated by folding of disordered regions. We used peptides to investigate the role of the non-helical tailpiece from non-muscle myosin IIC (NM-IIC) in its self-assembly process. NM-IIC is a motor protein composed of a globular motor domain in the N-terminal region and a coiled-coil rod domain in the C-terminal region, which terminates with a non-helical 47-residue tailpiece (residues 1954-2000). NM-IIC molecules undergo self-assembly into filaments that are necessary for its contractile activity. The tailpiece has an unfolded character and participates in the assembly process of NM-IIC. The N-terminal region of the tailpiece (residues 1954-1968) has a net positive charge of +3 while its C-terminal region (residues 1969-2000) has a net negative charge of -10. We designed peptides that correspond to the entire tailpiece and to its negative and positive regions. These peptides were used to study interactions with the rod, their structures in the free and bound states, and structural changes they undergo upon binding to residues 1296-1854 of NM-IIC rod. Fluorescence anisotropy binding studies showed that a peptide corresponding to the positive region of the tailpiece (residues 1947-1968) bound the NM-IIC rod (residues 1296-1854) with an affinity of 13μM. Circular dichroism studies showed that the positively charged peptide became folded upon binding the rod, indicated by a shift of the spectral minimum from 200 nm to 220 nm upon binding. A peptide corresponding to the negative region of the tailpiece (residues 1969-2000) did not bind NM-IIC (residues 1296-1854). Based on our results, we suggest that the positive region of the tailpiece is required for ordering and aligning neighboring molecules by electrostatic attraction, leading to filament formation. Our results provide molecular insight into the role of the structurally disordered tailpiece of NM-IIC in the self-assembly process.

P72321-032

Targeting leukemia cells with synthetic peptides and small-molecules

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Acute myelogenous leukemias (AML) are characterized with non-random patterns of medullary and extramedullary invasion. We hypothesized that a supramolecular complex, the leukemia cell invadosome, which contains certain integrins, matrix metalloproteinases (MMPs) and other as yet unidentified proteins, is essential for tissue invasion and may be central to the phenotypic diversity observed in the clinic. Here we show that the specific binding of MMP-9 to leukocyte surface áMβ2 integrin is required for pericellular proteolysis and migration of AML-derived cells. An efficient antileukemia effect was obtained by peptide inhibitors that prevented proMMP-9 cell surface binding, transmigration through an endothelial cell layer, and extracellular matrix degradation. Notably, the functional protein anchorage between áMβ2 integrin and proMMP-9 described in this study does not involve the enzymatic active sites targeted by any of the existing MMP inhibitors. Taken together, our results provide a biochemical working definition for the human leukemia invadosome. Disruption of specific protein complexes within this supramolecular target complex may yield a new class of anti-AML drugs with anti-invasion (rather than cytotoxic) attributes.

P72400-033

Influence of the lysine dendrimers on functional activity of serpentine type receptors and G_i protein

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The molecular mechanisms of action of the polycationic lysine homo- and heterodendrimers on functional activity of adenylyl cyclase signaling system (AC system) in the myocardium and the brain of rats were studied. The lysine homodendrimers of the third (I), the fourth (II) and the fifth (III) generations, as well as the lysine heterodendrimers of the fifth generation – $[(NH_2)_{64}(Lys\text{-}Glu)_{32}(Lys\text{-}Glu)_{16}(Lys\text{-}Glu)_8(Lys\text{-}Glu)_4(Lys\text{-}Glu)_2Lys\text{-}Ala\text{-}Ala\text{-}Lys(ClAc)\text{-}Ala\text{-}NH_2]$ (IV), $[(NH_2)_{64}(Lys\text{-}Ala)_{32}(Lys\text{-}Ala)_{16}(Lys\text{-}Ala)_8(Lys\text{-}Ala)_4(Lys\text{-}Ala)_2Lys\text{-}Ala\text{-}Lys(ClAc)\text{-}Ala\text{-}Ala\text{-}NH_2]$ (V) and $[(NH_2)_{64}(Lys\text{-}Gly\text{-}Gly)_{32}(Lys\text{-}Gly\text{-}Gly)_{16}(Lys\text{-}Gly\text{-}Gly)_8(Lys\text{-}Gly\text{-}Gly)_4(Lys\text{-}Gly\text{-}Gly)_2Lys\text{-}Gly\text{-}Gly\text{-}Lys(ClAc)\text{-}Ala\text{-}Ala\text{-}NH_2]$ (VI) stimulated by receptor-independent mechanism the activity of heterotrimeric G proteins, preferably of inhibitory type, and interacted with C-terminal regions of their α-subunits. The homodendrimers II and III and heterodendrimer V were more effective G protein activators. The treatment of the membranes with pertussis toxin (inactivating G_i protein), but not with cholera toxin, led to a decrease of G protein activation effect of the dendrimers. The lysine dendrimers disturbed the functional coupling of the receptors of biogenic amines and peptides hormones with G_i proteins and, to a smaller extent, with G_s proteins. It was illustrated by the decrease of regulatory effects of the hormones on AC activity and G protein GTP binding and by the decrease of receptor affinity to agonists in the presence of the lysine dendrimers, as result of receptor–G protein complex dissociation. It was shown that the molecular mechanisms of the action and the G protein selectivity of the polylysine dendrimers are similar to those of mastoparan and melittin, natural toxins of insect venom, which also preferably activate $G_{i/o}$ proteins and inhibit $G_{i/o}$-coupled signaling.

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P72505-034

DNA-Peptide Interaction Forces On The Single Molecule Level

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DNA-protein interactions are a key element in the regulation of cellular processes. Transcription factors are able to recognize their cognate DNA sequences and regulate the expression of proteins. As a model system, the specific DNA binding of the transcription factor PhoB from *E. coli* is investigated in single molecule experiments.

Structurally, PhoB belongs to the family of winged helix-turn-helix proteins. It is composed of a transactivation domain (amino acids 1-127) and a DNA binding domain (amino acids 123-229). After phosphorylation of the transactivation domain, the protein binds to specific DNA sequences containing a TGTCA consensus sequence. Different C-terminally modified protein epitopes representing parts of the DNA binding domain of PhoB were chemically synthesized using microwave assisted solid phase peptide synthesis. The binding contributions of these molecules are compared to the complete DNA binding domain (127-229). This protein was purified using intein mediated protein splicing, an additional cysteine was ligated to the protein by intein mediated ligation.

Performing single molecule force spectroscopy experiments, kinetic *off*-rates were obtained, and sequence specific DNA-binding of both peptide and protein was proven in competition experiments. Alanine scans of strategic residues revealed the contributions of single amino acid residues for peptides and proteins.

Structural investigations of the peptides, the proteins and DNA/protein complexes were performed using circular dichroism measurements. This method revealed structural differences of the peptides, proteins and DNA upon complex formation. Furthermore, the protein/DNA or peptide/DNA interaction was determined by surface plasmon resonance experiments and electrophoretic mobility shift assays.

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P72600-035

Fast Separations of Peptides using Small and Large Pore, Sub-Two Micron Columns

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Reversed-phase chromatography is important in the development of well-characterized peptide pharmaceuticals. Several factors influence sensitivity, resolution, and retention for LC and LC-MS applications: bonding chemistry, pore size, particle size, column configuration, and solvent composition. By decreasing the particle size of HPLC packings, column efficiency is increased. We demonstrate the use of short 10-mm length HPLC columns packed with 1.5 µm, 500 Å C18 silica for ultra-fast, one-minute peptide analysis with moderate backpressures (< 3000 psi) using a conventional HPLC system. For more complex peptide samples, such as protein digests, on conventional HPLC, a novel column configuration packed with 1.5 µm, 100 Å C18 is described for 10-to-20-min. separations that traditionally take 30 to 80 minutes. We also discuss fast two-minute separations on an ultra-high pressure system using a variety of 1.5 µm, small pore columns with unique selectivity.

P72627-036

"Unknown-genome" proteomics- based identification of a new NADP-epimerase/dehydratase from Desulf. phosphitoxidans by inverted-PCR, Edman-sequencing and high resolution mass spectrometry

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Protein identification in proteomics is generally based on the availability of genomic data. Using amenable databases, identification in "bottom–up" proteomics is often straightforward but is highly complex in the absence of genome data, which typically requires "de novo"- identification approaches. We present here a new approach for identification of proteins from a bacterial strain, Desulfotignum phosphitoxidans, with unknown genomic background, using a combination of (i), inverted PCR of degenerate primers derived from N-terminal Edman sequencing, and (ii) high resolution MALDI-FTICR mass spectrometric peptide mass fingerprint-proteomics of expressed proteins. Desulfotignum phosphitoxidans is an anaerobic sulfate-reducing bacterium from marine sediment in which phosphite oxidation was found crucial for energy metabolism. Culturing under different growth conditions provided 4 specifically expressed proteins with molecular masses of ca. 40 kDa in the presence of phosphate, which were subjected to 2D-gel electrophoretical separation for soluble and membrane fractions using PDQuest comparative analysis. N-Terminal sequences of the proteins, determined by Edman analysis were used

for inverted PCR of degenerate primers, and provided a series of ORF candidates, one of which coded for a putative NAD-dependent dehydratase. In a complementary approach, protein spots from the 2D-gels were excised, digested with Lys-C protease, and digestion mixtures analysed by MALDI-FTICR-MS. The accurate peptide masses unequivocally matched to identify a new NAD-dependant epimerase/dehydratase. The detailed functional characterization of the new protein, and further development and application of this approach to proteome analysis with unknown genomic background, are currently in progress.

P72629-037

Vydac MS RP-HPLC Columns Provide Unique Selectivity and High Recovery for Peptide and Protein Separations

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Based on specially treated large pore silica and enhanced with a proprietary bonding process, Vydac MS reversed-phase (RP) HPLC columns offer superior performance for peptides and proteins. The deamidation of human growth hormone (HGH) has been monitored for many years by RP using Vydac columns. The Vydac MS C4 column provides the best overall performance characteristics (recovery, resolution, and peak symmetry) for the common important assay of HGH and desamido HGH. Although hydrophobic membrane proteins are particularly difficult to separate, the Vydac MS C4 column provides better separation and recovery (up to 86% higher vs. other leading columns) for a reptilian reovirus p14 protein and myristolyated form, a component of a potentially new vaccine delivery system. Separation of the trypsin digest of fetuin, a glycoprotein, exhibits improved selectivity for peptide mapping on a Vydac MS C18 column compared to other C18 columns, revealing some peaks otherwise not seen. The improved selectivity for peptides on the Vydac MS columns results in better primary structure definition and easier identification of degradation products and other protein characteristics.

P72710-038

Hemoglobin peptides in mammalian tissues: facts or artifacts?

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Comparative RP-HPLC and MS analysis of peptide composition of rat brain, heart, lung and spleen acidic extracts was performed. The extracts were prepared from snap-frozen organs both in the presence and in absence of protease inhibitors (10^{-6} M pepstatin A, 10^{-4} M PMSF, 2 mM EDTA) which stabilize the peptide composition of tissue acidic homogenates. The absence of inhibitors led to appearance of novel peptides and did not affect the concentration of predominant peptide components detected in samples prepared with protease inhibitors. Therefore, the latter were considered as present in the tissues prior to extraction, i.e. as endogenous. The majority of predominant peptide components were found common in the studied tissues. The structures of 105 peptides (both endogenous and extraction artifacts) were established by MS/MS analysis. The predominance of hemoglobin fragments in the tissue peptidome was confirmed. The results were compared with those obtained by other authors [Skold K. et al., Proteomics 2 (2002) 447-454; Svensson M. et al., J. Proteome Res. 2 (2003) 213-219; Che F.Y. et al., Mol. Cell. Proteomics 4 (2005) 1391-1405] and with our earlier findings [Karelin A.A. et al., J. Pept. Sci. 6 (2000) 345-354]. The endogeneity of hemoglobin-derived peptides and other tissue protein fragments was discussed in the context of *in vivo* and *ex vivo* stability of peptides and proteins, and of sample preparation approaches in peptidomic studies. We believe that the presence of predominant (> 100 pmol/g) peptide components in the protease-inactivated tissue extracts can be reliably extrapolated to their in vivo presence in the tissues. The endogeneity of minor (< 10 pmol/g) components requires a separate consideration. Generally, the results of peptidomic studies strongly depend on sample preparation procedures involved, making difficult the straightforward comparison of the results obtained by different research groups.

P72715-039

Urine Peptidomics with LC-MS

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Urine is a promising source of endogenous peptide biomarkers, because the protein concentration is 1000-fold lower than in plasma but the peptide concentration is equal. Urine contains small metabolites that interfere with peptide analysis and urine flow varies between subjects and within a day. These variations pose a challenge to urine sample preparation, which, in case of serum, has been shown to be critical for reproducibility. We aim to optimize the enrichment method for urine peptides, to characterize these and to find potential biomarkers. All methods resulted in good peptide recovery if the peptide concentration was normal and metabolite content low. Slightly different peptides were obtained with different methods. The DF preferentially recovered hydrophilic peptides, while SEC-SPE recovered high molecular weight peptides better than the DF-method. SEC-SPE provided the best peptide enrichment especially for samples with high metabolite concentration. Normalization was critical especially with very dilute samples. Without normalization the number of peptides detected was highly dependent on urine concentration. Normalization against specific gravity proved the most reproducible results. We have identified several peptides found in all samples and some differences between cancer and control samples. Validation of these differences is currently under way.

P72726-040

Novel Reversible Biotinylated Probe for the Selective Enrichment of Phosphorylated Peptides from Complex Mixtures

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In order to improve the detection of phosphorylated peptides/proteins, we developed a novel protocol that involves the chemical derivatization of phosphate groups with a chemically engineered biotinylated-tag (biotin-tag), possessing three functional domains; a biotin group for binding to avidin, a base-labile 4-carboxy fluorenyl methoxy-carbonyl (4-carboxy Fmoc) group, and a nucleophilic sulfhydryl moiety on the side-chain of cysteine. Using this approach, the derivatized, enzymatically digested peptides were selectively separated from unrelated sequences and impurities on immobilized avidin. Unlike previously published phosphopeptide enrichment procedures, this approach upon treatment with mild base, liberates a covalently bound Gly-Cys analog of the peptide(s) of interest, exhibiting improved RP-HPLC retention and MS ionization properties compared to the precursor phosphopeptide sequence. The results obtained for a model peptide Akt-1 and ovalbumin protein digest, demonstrated that the method is highly specific and allows selective enrichment of phosphorylated peptides at low concentrations of femtomoles/µl.

P72729-041

In search of physiological substrates of brain prolyl oligopeptidase.

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Prolyl oligopeptidase (POP) is a serine protease, which cleaves small peptides at the carboxyl side of an internal proline residue. Substance P, arginine-vasopressin, thyroliberin and gonadoliberin are proposed physiological substrates of this protease. POP has been implicated in a variety of brain processes including learning, memory, and mood regulation, as well as in pathologies such as neurodegeneration, hypertension and mood disorders. However, there is no definite information about the physiological substrates of POP in brain. We have determined previously that some POP inhibitors, when administrated orally or intraperitoneal, are able to cross the blood-brain barrier and inhibit POP ex vivo. In this work also inhibition duration according to the doses were determined. Using this system we studied the natural occurring peptide profile in brain tissues from inhibited animals and compare it with the profile in control tissue. We were able to design a method in which the background peptide level was importantly decreased which allowed us to identify, with high degree of confidence, the peptides which were changed in rat hypothalamus upon POP inhibitor treatment. Conclusions about the physiological substrates of POP are discussed.

P72729-042

Cellular peptidomics: peptide sets produced by rat hepatocytes in vitro

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Earlier we have shown that several cell cultures generate specific sets of peptides [1,2]. In the present work, we further studied the dependence of peptide production by the cells on their functional state. Using rat hepatocytes as a model, we compared peptide sets generated by: (1) primary hepatocytes in a differentiated state; (2) dedifferentiated primary hepatocytes with fibroblast-like phenotype; (3) rat hepatoma cells. Peptides were extracted from the cellular lysates and supernatants by solid-phase extraction and then separated by RP-HPLC. Peaks were analyzed with MALDI-TOF and/or MALDI-TOF/TOF.

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P72825-043

Photoswitchable DNA Bis-Intercalators - Structure and Activity

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Triostin A is a naturally occurring quinoxaline depsipeptide antibiotic originally isolated from *Streptomyces* S-2-210. With its two quinoxaline moieties it bis-intercalates into DNA in a GC selective manner. Triostin A contains N-methylated L-valine and L-cystein. The unmethylated analog TANDEM binds AT selectively due to a different hydrogen bonding pattern. Substitution of the disulfide bridge of TANDEM by an azobenzene moiety leads to photoswitchable analogs. Their photoswitchability was investigated and quantified using UV, CD and NMR spectroscopy as well as HPLC.

In order to determine the three-dimensional structure, conformational analysis by NMR spectroscopy in combination with molecular dynamics calculations was carried out. In this process, distance restraints were obtained from NMR spectra measured in DMSO-D_6 and applied in distance geometry/simulated annealing followed by molecular dynamics calculations. As it is possible to distinguish between *cis*- and *trans*-isomer in NMR spectra due to the difference in the chemical shifts, structures of both isomers can be investigated.

DNA binding studies were carried out using an optical tweezers setup with λ-DNA. Furthermore, UV melting curves were recorded for the TANDEM derivatives in complex with DNA. CD spectroscopy was applied to observe changes in DNA structure upon formation of the complex.

In addition to structural investigations, the antibiotic activity of the TANDEM derivatives was investigated in minimal inhibition concentration assays against gram-positive *Bacillus subtilis*.

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P72900-044

Self-assembling Cyclic Peptides for Peptide Nanotube Formation

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Cyclic peptide nanotubes are formed by self-assembly of cyclic peptides with alternating L- and D-amino acid. However, peptide nanotube self-assembly phenomenon had not yet been made cleared. Intermolecular electrostatic interaction is one of the important driving forces to assemble cyclic peptides to peptide nanotubes. Here in, a series of cycilc hexapeptides, cyclo(-L-Lys-Gly-)$_3$, cyclo(-L-Glu-Gly-)$_3$, cyclo(-L-Lys-D-Ala-)$_3$, cyclo(-D-Glu-L-Ala-)$_3$, cyclo(-L-Trp-D-Lys-)$_3$, and cyclo(-L-Trp-D-Glu-)$_3$, were designed and synthesized by solid-phase peptide synthesis using Fmoc strategy. Turbidity study revealed that the abilities of self-assembly formations of the 1:1 mixtures of cyclo(-L-Trp-D-Lys-)$_3$ and cyclo(-L-Trp-D-Glu-)$_3$ in aqueous solution are extremely higher than the abilities of each individual positively or negatively charged cyclic peptides. Transmission electron microscope (TEM) experiments showed that mixture of cyclo(-Lys-Xxx-)$_3$ and cyclo(-Glu-Xxx-)$_3$ formed fibrous structure. TEM images indicated that, the diameters of the nanotubes were approximately 2-5 nm. Diameter of similar cyclic peptides was found to be 1 nm approx. These results also supported that the bundle formation of peptide nanotube. Conformations of monomer cyclic hexapeptides were calculated based on NMR observation. Estimated formation of peptide nanotube is consisted with the bundle structure.

P72900-045

Fatty Acylation Improves Membrane Transport Properties of Acylated Cystatin

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Therapeutic proteins offer a promising potential as effective drug compounds. However, proteins are generally poorly transported across biological membranes due to hydrophilicity. Fatty acylation with reactive fatty acid derivatives represents one of the basic methods for increasing the proteins' hydrophobicity and improving membrane permeability.

Using the acylation method, fatty acyl chains are covalently linked to the free amino residues forming a stable amide bond.
In this study, a novel method for acylation of proteins was developed using in situ prepared fatty acyl chloride dispersion in aqueous acetonitrile solution, which allows protein modification under very mild conditions. Chicken cystatin, a reversible 13 kDa inhibitor of papain-like cysteine proteases, was selected as a model protein due to its high potential to inhibit intracellular cathepsins. The protein was modified using fatty acyl chlorides with 6, 8, 10, 12, 14, 16, and 18 carbon atoms.
Based on the cell culture assays, we examined the transport properties of fatty acylated cystatin, the effectiveness of its internalization and efficiency to inhibit intracellular enzymes, which was measured indirectly by cathepsin B inhibition. The experiments showed that acylated cystatin quickly internalized into the cells and effectively inhibited cathepsin B. In contrast, non-acylated cystatin didn't cause inhibition as it was unable to enter the cell. The permeability enhancement effect was shown to depend on the length of the attached fatty acyl chain as the strongest inhibition was caused by cystatin acylated with 18 carbon atoms long stearoyl chloride. Additionally, chemical modification did not influence the protein's immunogenicity.
The results of our study provide clear evidence that fatty acylation greatly improves membrane permeabilization properties of proteins.

P72900-046

DAISOGEL WING, Custom Made Process Scale Stationary Phases

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DAISOGEL WING takes your process separation to a higher level. Custom made solution for your process peptide purification problem can be easily delivered using the DAISOGEL WING polymer grafted silica based platform. "Silica or polymer?"- It used to be an evergreen debate when it came to choose the adequate stationary phase for process scale separation or purification. Silica based stationary phases feature much higher mechanical strength and stability, better controlled porosity and as a result higher separation efficiency, while polymers boast wider pH range. The attempts to bring the good aspects of these opposite methods together failed so far, or failed to provide flexible solutions. The new DAISOGEL WING platform for silica surface polymer grafting preceding chemical modification is presented. The revolutionary new technique offers high versatility: Most variations of the base silica (different pore sizes or particle sizes of choice) can be combined with your choice of familiar chemical surface modifications to tailor make the perfect stationary phase for your given chromatography challenge. The polymer graft on the silica surface provides extreme shielding effect, the produced stationary phase displays outstanding pH stability and durability. Any of your preferred and trusted regular silica surface modifications can be done on the top of the grafted polymer layer. You may enjoy the usual high performance separation, excellent efficiency, high plate numbers, mechanical strength of silica based stationary phases with a dramatically extended pH range you expected so far only from polymer resins. You have the full range of choices of particle and pore sizes with the full choice of desired bonding chemistries. Finally DAISOGEL WING is here to deliver you the most versatile polymer grafted silica hybrid platform to provide the best solution for your demanding process scale peptide separations.

P72900-047

Melanocollagen type I – Obtaining and application

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Melanin, a natural compound formed in the tyrosine metabolic process. According to occurrence, melanin is divided into that present in true skin and in internal organs. The melanin synthesis and secretion process takes place in melanocytes under the control of two neurohormons: MSH – Melanocyte Stimulating Hormone and MCH – Melanocyte Concentrating Hormone. Research has shown the significant role of melanin in the process of breeding and storing, in regulating metabolism of fat tissue, and in carbohydrates regulation. Melanin biopolymer finds application in IT technology as neurotransmitters for non cellular intelligence. Biologically active collagen constitutes the architectonics of all tissues and organs. Due to its piezoelectric and dielectric properties it also provides storage and relay functions. For the needs of medical practice, transplants, pharmacy, cosmetology and information technology the key issue is to obtain a compound of melanin with biologically active collagen. This compound we named melanocollagen type I. Melanocollagen type I is formed in result of protonating collagen amino acids with melanin, which yields coloured gel ranging: grey, graphite and black. /Melanin (H+) + collagen → melanocollagen type I/. The therapeutical capacity of biologically active collagen type I with melanin is an ideal formulation inhibiting aging and mitigating related neurovegetative and dementia symptoms, and in vivo transplantology. Freeze dried melanocollagen type I retains the features of both collagen type I and melanin. It can be a formulation on its own or a supplement for in vivo and in vitro tissue engineering, biotechnology, information technology, pharmaceutical industry and cosmetology. A patent application for melanocollagen was placed with the Polish Patent Office on 05.08.2004 as P-369439, entitled: Melanocollagen type I – method of obtaining.

P72912-048

1.4-DHP-lipid forms a tubular micellae

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1,1'-{[3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridin-2,6-diyl]dimethylene} bispyridinium dibromide (1,4-DHP lipid) is a gene transfection agent [1, 2]. 1,4-DHP lipid structure was calculated with ab initio quantum mechanics to obtain the charges for molecular dynamics with AMBER 8.0 force field. DHP-lipid molecules were subjected to molecular dynamics from the initial structure of a periodic lipid bilayer-water box, with a small amount of excessive water on the lipid edges to ensure the mobility of lipid molecules. After 14 ns of MD simulation the lipid molecules with the fatty acid tails started to squeeze from one bilayer layer to another one. After 35 ns few lipid molecules turned with their charged heads to the side of the lipid bilayer and after 100 ns a profound tubular micelle structure began to form. The tubular micellae structure becomes more perfect during the course of simulation of 300 ns. Conclusion is that one of the gene transfection agent 1,4-DHP lipid structures is a tubular micellae, and we could expect that such the micellaes are capable to form lipoplex for the DNA transfection or peptide delivery.
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P72629-049

New BioPro ion exchange columns

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Introduction A newly developed hydrophilic polymer-based ion exchange chromatography column for biomolecules. Ion exchange chromatography (IEC) is a widely used for analysis and purification of biomolecules. We have newly developed polymer-based IEC column, named YMC-BioPro series, specially designed for separation of proteins, peptides and nucleic acids. The completely spherical and monodispersed porous / nonporous beads (5 μm), optimally packed with advanced technology, provide high theoretical plate number and symmetric peak shape. Excellent resolution is achieved from the high column efficiency coupled with the excellent selectivity of QA and SP chemistries. In this paper, we will show features and benefits of YMC-BioPro series.

P70000-050

Mutation-resistant Antiviral

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Viral entry requires fusion of the viral and cellular membranes. In all Class-1 envelope viruses including HIV-1, fusion is mediated by envelope glycoprotein which has a homotrimeric quaternary structure which forms a hairpin-like assembly of six-helix bundle (6HB) during the fusion event. The 6HB employs a 3-on-3 locking mechanism in which 3 HRC (heptad repeating-carboxyl region) chains crosslink 3 HRN (heptad repeating-aminal region) chains to enable membrane fusion. This locking mechanism confers avidity due to multi-chain interactions and a high genetic barrier to mutations in the viral entry strategy despite the high mutation rate of their envelope protein. This mechanism also provides inspiration to our approach in designing mutation-resistant entry inhibitors of HIV-1.

T20/enfuvirtide, a synthetic HRC-peptide monomer designed to interrupt membrane fusion, is the first and only FDA-approved entry inhibitor against HIV-1. However, T20 becomes ineffective in 20% of AIDS patients due to acquired resistance to T-20 resistant during the treatment course. Our inhibitor design is based on a novel quaternary protein mimetic approach. Key elements include a covalent-link 3-parallel-chain construct mimicking the quaternary structure of gp41 in its pre-fusion state and an inhibition mechanism mimicking the multimeric interactions found in the highly conserved 6HB formation. Our hypothesis is that covalent-linked, 3-chain quaternary mimetics (called 3α mimetics) of HRC peptides can confer multi-chain binding to the HRN region in a multi-chain locking mechanism that may lead to mutation-resistant HIV fusion inhibitors. We also extend our design to 2-chain HRC mimetics (called 2α mimetics) which may bind to the HRN as a 2-on-3 locking mechanism. In contrast, T20 and other single-chain peptide inhibitors with a 1-on-3 binding mechanism lacks the advantages offered by the quaternary mimetics and is susceptible to gp41 mutations. This report will describe all three types of inhibitors as a model to further our understanding of gp41 mutations and viral fusion mechanism in developing mutation-resistant entry inhibitors.