P001. Abstract number: 12

New Fluorine Containing Amino Acids As Labels To Study Antimicrobial Peptides By Solid State 19f Nmr

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Solid state 19F-NMR is a powerful method to study membrane-active peptides, as it can reveal their conformation, orientation and dynamics when embedded in a lipid bilayer.1 For this purpose the peptide of interest has to be selectively labeled with a suitable 19F-containing amino acid at several different positions. The resulting battery of singly 19F-labeled analogues then has to be tested for full functional integrity, before they are reconstituted into lipid bilayer samples for the solid state 19F-NMR analysis.

The main limitation of this approach, however, lies in the poor repertoire of suitable 19F-labels, as only very few of the known 19F-containing amino acid can serve as proper 19F-labels. Therefore, we have rationally designed and synthesized compounds 1 and 2, to serve as proper 19F-labels for the substitution of Leu, Ile, Val, Ala and Pro, respectively, in peptides.2-9These novel amino acids have been successfully used to study the structure and dynamic behaviour of the antimicrobial peptides GS, PGLa and cell penetrating peptide SAP in model membranes by 19F-NMR.

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P002. Abstract number: 56

Synthesis of biaryl cyclic peptides through solid-phase borylation and cyclization by a Suzuki-Miyaura cross-coupling

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Cyclic peptides containing a biaryl bridge are widespread in nature. They possess diverse structural features and range from monocyclic to bicyclic to the structurally complex polycyclic glycopeptide antibiotics. These macrocycles have attracted considerable interest due to the significant biological activities that most of them exhibit, including proteasome inhibition, and antimicrobial or cytotoxic activities [1].

Within our current research on the synthesis of biaryl peptides, we focused our attention on the solid-phase synthesis of cyclic peptides containing a biaryl bond between the side chains of two aromatic amino acids. A method of choice for the preparation of biaryl systems is the Suzuki-Miyaura cross-coupling of an aryl halide with an arylboronic acid [2]. Recently, we have reported the synthesis of biaryl peptides involving the borylation on solid support of a phenylalanine followed by the coupling of a variety of aryl halides [3]. This is the first example of the solid-phase borylation of a phenylalanine peptide, being a successful approach for the preparation of modified peptides in a highly flexible manner.

Here we report an extension of this methodology to the synthesis of biaryl cyclic peptides. First, we prepared the linear precursor containing the corresponding amino acid boronate and the haloaryl residue. The boronate was synthesized using our solid-phase borylation protocol. Cyclization via a Suzuki-Miyaura cross-coupling was then assayed. Microwaves significantly enhanced this reaction leading to biaryl cyclic peptides in moderate to good purities. Then, we explored the scope of this methodology using various peptide sequences.

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P003. Abstract number: 71

Synthesis and characterization of FlAib, a completely rigidified benzophenone-containing a-amino acid

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Photoreactive amino acids with benzophenone side chains, such as the widely used Bpa 3-(4-benzoyl-phenyl)alanine, have been used as photoprobes for the covalent modification of enzymes and receptors in protein-mapping studies. However, results of photocross-linking experiments need to take into account the flexibility of the Bpa side chain, which allows reaction with amino acid residues up to 10 Å distant. Since incorporation of a cyclic structure into the amino acid side chain reduces flexibility, we have designed and synthesized an extremely highly constrained fluorenone-containing amino acid, FlAib, belonging to the sub-class of the C_i^{α} <-> C_i^{α} cyclized, C^{α}-tetrasubstituted α-amino acids, which are known to induce β-turn and helix formation in peptides. The 3,4-dimethyl-fluorenone structure required for the synthesis of FlAib was obtained by diazotization of the corresponding dimethyl-aminobenzophenone (prepared from the known 2-amino-3,4-dimethyl benzoic acid). Bromination of the side chains of this dimethyl-fluorenone gave 3,4-(bis)bromomethyl-fluorenone, which was used in the bis(alkylation) of ethyl-isocyanoacetate under phase-transfer conditions to give the cyclic, racemic amino acid H-FlAib-OEt after acid hydrolysis. N^{α}-Benzoyl protection, saponification of the ester function, and coupling with H-(S)-Phe-NHChx gave dipeptide diastereomers which could be resolved by crystallization and chromatography. A crystal of one of these diastereomers, Bz-(R)-FlAib-(S)-Phe-NHChx, subjected to X-ray diffraction analysis, allowed the assignment of the absolute configuration of the FlAib residue. As expected, the (R)-FlAib residue is able to induce a (type-I) β-turn in the molecule, thus behaving as an (S)-protein amino acid.

P004. Abstract number: 72

A practical large scale synthesis of cyclic RGDfK peptide suitable for further decoration of various biomaterials through 'click' chemistry

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Cyclic pentapeptides containing RGD (Arg-Gly-Asp) motif were developed as highly active and selective antagonists for the $\alpha_v\beta_3$ integrin receptor. A class of this heterodimeric transmembrane protein shows an important role in cell-cell and cell-matrix interactions and recognitions. A number of cyclic RGD pentapeptides[1] were synthesized (solid-phase method) and frequently tested for they crucial location in tumor angiogenesis and metastatis as well as for the stimulation of cell adhesion.

The *ε*-amino lysine (K) moiety of the *c*RGDfK peptide can be readily modified to the *ε*-azide[1c,d] and used for a further functionalization with alkynes using a 1,3-dipolar cycloadition (”click”-chemistry)[2] to afford the corresponding 1,4-disubstituated 1,2,3-triazoles. In many examples the method was applied in the synthesis of glycoconjugates and oligosaccharides.

We will present our most recent results on a new efficient large scale solution-phase synthesis of the *N*-*ε*-azido derivative of *cyclo*[Arg-Gly-Asp-D-Phe-Lys] peptide and its further functionalization through ”click”chemistry with various biomaterials in the field of tissue engineering, e.g. decoration of polysialic acid, hyaluronic acid, alginate or modification of polymethylpentane foils which are currently developed in our group.

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P005. Abstract number: 81

Straighforward syntheses of deuterated precursors to be used as powerful tracers under fermentative conditions

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Some varietal thiols as 4-methyl-4-mercaptopentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and its acetate (3MH-A) are responsible for fruity notes in young white wines. These odoriferous compounds are released during the alcoholic fermentation from non-aromatic precursors identified as cysteine conjugates1, 2 under the action of the yeast trough its β-lyase enzymatic activity2.

Recent investigations reported the occurrence of glutathione conjugates3-5 in grapes and could be good candidates as novel precursors. To prove the direct relationship between glutathionylated precursors and the corresponding thiols, straightforward syntheses of deuterated analogues were developed. The S-3-(hexan-1-ol)-glutathione (G3MH) and S-3-(4-methylpentan-2-one)-glutathione (G4MMP) were synthesized through a Michael reaction addition of free glutathione on (E)-2-hexenal and mesityl oxide, however in different yields, 10 and 80 % respectively. The G3MH-d2 and G4MMP-d10 were obtained under similar conditions using deuterated (E)-2-hexenal5 and mesityl oxide.

These labeled precursors were then used as tracers in Sauvignon blanc fermentation experiments. The release of deuterated 3MH and 4MMP in resulting wines demonstrated the ability of the yeast to metabolize glutathionlyted precursors under enological conditions. The conversion yield of such transformation was estimated closed to 4.5 %5 and 0.3 %6 for G3MH and G4MMP respectively, opening avenue for additional studies on varietal thiols biogenesis.

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P006. Abstract number: 82

Tert-BuNH2 as an Efficient Reagent for the Deprotection of Fmoc Protected Amino Acids

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Fmoc group is one of the most important protecting groups for the protection of N-terminal amino acids and also solid phase peptide synthesis. The removal of the temporary Fmoc protecting group from the N-terminus of the peptidyl-resin is normally achieved by short treatment with 20% piperidine in DMF. Due to the hazardous parameter, piperidine has some problems such as potential symptons. Finding another efficient reagent with the same activity is still required demand. In this research work, we have demonstrated t-BuNH_2 as an efficient reagent for Fmoc deprotection in solid phase peptide synthesis instead of piperidine. This method was examined with different Fmoc protected amino acids and also for the synthesis of Leu- and Met-enkephalins. In all cases, the reaction conditions were examined with different percentages of t-BuNH_2. The best results were obtained with 20% t-BuNH_2 in DMF. Fmoc removal was monitored by UV-spectroscopy. The results were indicating that t-BuNH_2 is essential for deprotection and its contribution determines the rate of reaction. The amount of t-BuNH_2 was optimized and the best condition was obtained with 20% t-BuNH_2. The details of the results will discussed.

P007. Abstract number: 86

Comparison of Peptide Coupling Reagents COMU and HCTU

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For many years, HATU has been viewed as the most efficient activator available for peptide synthesis. However, it is also extremely expensive, and is often reserved for only the most difficult couplings. Hood, et al. found HCTU to be a highly efficient, low-cost alternative to HATU [1].

With it, they were able to produce peptides with coupling times of 5 minutes or less. COMU is a new, highly efficient coupling reagent that initial reports have found has similar reactivity to HATU [2]. In this study, we compare the activator COMU with HCTU for the Fmoc solid-phase synthesis of a variety of peptides.

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P008. Abstract number: 88

Peptide diketopiperazine thioester formation at the Cys-Pro-Cys position

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A peptide thioester is one of the key intermediates in protein synthesis by the ligation strategies, such as the thioester method [1] and native chemical ligation [2]. We have focused on an N to S acyl shift reaction for producing peptide thioesters. In previous studies, we reported on the development of a CPE autoactivating unit, which is spontaneously transformed into a diketopiperazine (DKP) thioester via an intramolecular N to S acyl shift reaction [3]. The ester group in the CPE group played a crucial role as a leaving group at the DKP formation step. To find a general sequence that leads to thioester production without an ester moiety, we initiated a search for a CPE-like autoactivating unit.

We prepared a peptide library containing a peptide-Cys-Pro-Xaa-Xbb-Xcc-Arg-NH_2 (**1**) (peptide: Ala-Lys-Leu-Arg-Phe-Gly) structure, in which the Cys-Pro sequence was fixed and the Xaa-Xbb-Xcc contained random sequences of 3 amino acids consisting of Asn, Asp, Cys, Gln, Gly, His, Leu, or Ser residues. The library was treated with H-Cys-Tyr-NH_2 (**2**) in neutral buffer solutions to examine the peptide thioester formation, resulting in the production of a ligated product, H-Ala-Lys-Leu-Arg-Phe-Gly-Cys-Tyr-NH_2 (**3**). The ligated product was observed as a small but distinct peak by RP-HPLC and mass spectroscopy, when a Ser or Cys residue was located at the Xaa position, although the ligation efficiency was low. In a subsequent experiment, some of the selected peptides, such as H-Ala-Lys-Leu-Arg-Phe-Gly-Cys-Pro-Cys-NH_2 (**4),** were incubated in a 0.1 M hydrochloric acid or heptafluorobutyric acid at 110 °C in an evacuated sealed tube. In the reaction of peptide 4, the mass number corresponding to peptide DKP thioester **5**, cyclo(-Cys(R)-Pro-) (R = H-Ala-Lys-Leu-Arg-Phe-Gly-), was observed [4]. Details of the thioester formation will be discussed.

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P009. Abstract number: 100

Fmoc deprotection in the synthesis of cyclic part of oxytocin like peptides; some biological effects and physical properties

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The cyclic part of oxytocin (OT) is known to have some central pharmacological effects,
in which the cycle size plays the most important role. This part of OT molecule can be interesting as a model for examination of molecular geometry and dynamics, followed in ECD, VCD and ROA.
Methodological reason was also for the preparation of models I and II and III.
c(C-Y-I-Q-N-C)-NH2 (I), c(C-A-G-A-G-C)-NH2 (II), and c(C-Y-I-Q-N-C)-OMe (III). It consists of the exploitation of tert-butylamine as a Fmoc deprotecting agent not only and with advantage in LPPS (liquid phase) but also in SPPS (solid phase).
Piperidine, commonly used as Fmoc deprotecting agent in SPPS can unfortunately only hardly be used in LPPS due to the problems with peptides isolation. Removal of piperidine is usually difficult. The exploitation of Fmoc strategy in LPPS can be made possible by
tert-butylamine, which was used in various solvents in reaction mixture containing some dibenzofulvene scavengers - preferably resin bound. HBTU as preferred SPPS coupling agent was used also in LPPS.
Comparison of results of Fmoc release test made by both tert-butylamine and piperidine will illustrate the deprotection efficiency. Effects of I, II and III in the open field pharmacological test and spectral properties of the compounds will be mentioned.
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P010. Abstract number: 109

Backbone- Cyclic Octapeptide-Ligands with N-functionalized Phosphotyrosine for the N-terminal SH2-Domain of Phosphatase SHP-1

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Backbone cyclization can help to stabilize peptides against proteolytic degradation and to preform their bioactive conformation. We started different approaches to form building units for the assembly of backbone cyclic peptides, optimized the synthetic strategies and studied structure activity relationships. We developed peptide ligands for SH2- domains. These phosphotyrosine containing peptides are of great interest to influence the activity of kinases, phosphatases and other functional proteins.
Till now backbone cyclization was never performed with phosphotyrosine. The assembly of such peptides with backbone cyclization requires special building units. We used N-functionalized dipeptides with unprotected phenolic group because all our different attempts failed to form in solution or on SASRIN-resin dipeptide building units with already phosphorylated tyrosine. Thus, phosphorylation of resin bound peptides was performed after assembly of the protected resin bound octapeptides and after cyclization. The advantage is that this offers the possibility to synthesize simultaneously peptides as well as with phosphorylated and unphosporylated tyrosine [1].
We tested our strategy on the synthesis of octapeptide-ligands for the N-terminal SH2-domain of the protein tyrosine phosphatase SHP-1.
The obtained backbone cyclic ligands were tested for their influence on the phosphatase activity of full length SHP-1. All linear and cyclic octapeptides stimulated the

phosphatase activity of SHP-1. No reduction of the basal activity could be detected The cycles force the peptides to form a folded conformation and bind to the deep and hydrophobic pocket of the SH2-domain, probably additionally by the lactam bridge. The found activities agree well with the molecular modelling. Thus the modelled conformation seems to be verified.
The backbone cylic octapeptide ligands are stable against proteolytic degradation by chymotrypsine, proteinase K and cell homogenate. For functional characterization the octapeptides were internalized into NIH 3T3 cells using cell penetrating peptides.
The project was financially supported by the ”DFG”(Re 853/10-1).
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P011. Abstract number: 111
Radical Scavenging Activity of Hydroxycinnamoylamides of Amino Acids - Precursors of Biogennic Amines
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A number of hydroxycinnamic acid amides were prepared from the ferulic, sinapic, caffeic acids and amino acid residues of tryptophan and phenylalanine by EDC/HOBt method. Their structures were elucidated by UV, 1H-NMR and mass spectroscopy.
The ability of hydroxycinnmaoyl amino acid amides to act as radical scavengers was compared with the amides, consisting of the corresponding biogennic aromatic amines using DPPH test.

P012. Abstract number: 113
Repetitive Cleavage of Aib-Peptides by Trifluoroacetic Acid
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Crystalline homopeptides of the general structure Z-(Aib)n-OtBu (n = 3-11) form consecutive β-turns of type III resulting in full turns of 310-helices for n = 5, 8, and 11 (Aib, α-aminoisobutyric acid, 2-methylalanine) (1-4). We have treated Z-(Aib)10-OtBu 1 (2) and Z-(Aib)7-OtBu 2 (3) at 37 °C with anhydrous trifluoroacetic acid (1 mg peptide in 1 ml TFA) for 0.5 - 24 h and analyzed the resulting products by RP-HPLC and on-line LC-ESI-MS. Besides the expected and fast removal of the tert. butyl group we observed an unexpected peptide bond cleavage even though peptide bonds of protein amino acids are usually stable towards TFA. From 1 formation of a regular series of Z-(Aib)10-5-OH were detected with 0.5 h. Furthermore, concomitant release of the series of H-(Aib)10-3-OH occurred, indicating also the fast cleavage of the benzyloxycarbonyl (Z) group. From 2, after 3 h formation of the regular series Z-(Aib)3-7-OH were observed. From these data the common and fast cleavage of the tert.butyl group is deduced followed by a repetitive cleavage of the peptide bonds via intermediate formation of C-terminal oxazolones (5,6). Internal nucleophilic attack of the oxygen of the carboxamide group of the second to last Aib on the carboxy group of the C-terminal Aib residue is probably favored by the so-called geminal dimethyl group or THORPE-INGOLD effect. Notably, ion source collision-induced dissoziation (CID) mass spectrometry of 1 and 2 produced also regular b-series of positive acylium/oxazolonium ions. From the data the generation of intermediate oxazolonium ions in both gas and liquid phase is concluded and related fragmentation/cleavage mechanisms are proposed. The selective acidolytic cleavage can be extended and used for the sequencing of native peptides containing Aib residues (peptaibiotics) (6) but has also to be considered in synthetic peptide chemistry.
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P013. Abstract number: 115
Antioxidant Potential Of Phenolic Acid Amides Of Aromatic Amines
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The lipid peroxidation is one of the important primary events in the free radical-mediated oxidative damage of biological membranes and tissues. Antioxidants are organic molecules which can avoid or delay the progress of lipid oxidation. Their ability to do this is based mainly on their phenol-derived structure.
The aim of the present investigation was to elucidate the antioxidative activity of eight synthesized cinnamoyl- and hydroxycinnamoyl amides of biogenic amines by oxidation of pure triacylglycerols of sunflower oil at 100°C. The results obtained were compared with the inhibiting effects of the main hydroxycinnamic acids. It was established that all compounds possessed excellent antioxidant effectiveness, comparable with this of the caffeic acid. The antioxidant activity increases in the following order: caffeoyldopamine ≈ cinnamoyldopamine > caffeoylphenylethylamine > caffeoyltyramine ≈ caffeic acid ≈ caffeoyltryptamine > p-coumaroyldopamine > feruloyldopamine > sinapoyldopamine. The results showed that in contrast to the hydroxycinnamic acids, the presence of methoxy groups in the molecules of feruloyldopamine and sinapoyldopamine decrease antioxidant effectiveness.

P014. Abstract number: 122
Solid phase synthesis and characterisation of a platelet-derived growth factor receptor (PDGFR) specific Affibody molecule
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A 66-amino acid PDGFR specific Affibody peptide was produced by microwave assisted automated solid-phase synthesis. Fmoc-deprotection steps were carried out using piperazine solution in order to suppress aspartimide formation. Pseudo-proline was incorporated in the peptide

backbone, which was assembled on a 100% PEG resin, affording good purity and acceptable yield of crude Affibody. The identity of the synthetic Affibody was confirmed by time-of-flight mass spectrometry (TOF-MS). The secondary structure content was compared with its recombinant counterpart by circular dichroism (CD) and the biological activity assessed by fluorescence polarisation (FP) and surface plasmon resonance (SPR).

P015. Abstract number: 128

Racemization of Amino Acids on Heating with Sugars or Aldehydes

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The reaction of reducing sugars with amino components such as amino acids (AAs) is commonly denoted as non-enzymatic browning or the MAILLARD reaction (MR). Relatively stable conjugates formed at the early stages of the MR from AAs and the aldose glucose (Glc) or the ketoaldose fructose (Fru) are named AMADORI and HEYNS products, respectively. Notably, the MR proceeds, albeit slowly, also at room temperature and under physiological conditions. Glycosylation of amino groups in proteins generates so-called advanced glycation end products (AGEs), sometimes referred to as glycotoxins.

We had shown (1) that heating of reducing sugars (278 mM) and a mixture of twelve protein L-AAs (2.5 mM) in AcOH (1 M) at 100 °C for 24-96 h lead to the fast formation of free D-AAs, a process that is commonly referred to as racemization or enantiomerization.

Here, we report on new experiments on heating L-Ala at 130 °C/48 h under the above conditions with various D-sugars and aldehydes. Relative quantities of D-AAs (in brackets; not corrected for blanks) were determined by enantioselective GC-MS on Chirasil-Val©: Fru (39.5%), Glc (10.4%), galactose (18.9%), lactose (4.6%), xylose (34.3%), ribose (40.8%), 2-desoxy-ribose (11.3%), DL-glyceraldehyde (30.0%), DL-methylglyoxal (31.6%), pyridoxal (41.8%). Furthermore, heating of dry, synthetic AMADORI products such as Fru-L-Phe and Fru-L-Ala generated free D-Phe and D-Ala, respectively (2). From the data a refined enantiomerization mechanism (3,4) *via* formation of sp^2-hybridized carbanions as the key intermediates in in the aldehyde/amino acid conjugates is presented. The findings are of relevance in the fields of AGE research, glycopeptide synthesis, use of sugars as antifreeze agents or preservatives, and non-enzymatic racemization of AAs in biosystems including foodstuffs and plant products (5,6).

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P016. Abstract number: 153

Nicotianamine and Thermonicotianamine: supported synthesis and chelation ability

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Nicotianamine (NA), which was first isolated from the leaves of Nicotiana Tabacum L, is an important biosynthetic precursor of phytosiderophores. Various studies have proved that nicotianamine plays a significant role in plants as an iron and other heavy metals transporter. Our study aims to synthesize unnatural analogues of NA to investigate the mechanisms of metal transport and accumulation within the plant.

Several chemical syntheses of NA have been described, but they didn't give satisfaction in terms of yield and versatility. We report here a new methodology of synthesis based on a SPOC (solid phase organic chemistry) strategy. The use of supported chemistry allowed us to access easily to NA and new related analogues with high yields and degrees of purity.

Thermonicotianamine (tNA) is a compound related to NA that was discovered very recently into the active site of a NAS like enzyme named MtNAS from an archaeal bacteria (Methanothermobacterthermautrophicus). We applied our strategy on solid phase to the synthesis of the tNA and we compared NA and tNA chelating abilities by MS analysis of metal-ligand complexes.

P017. Abstract number: 158

Protection of Hydroxyl Group via tert-butylation using MTBE

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Traditionally in peptide synthesis, the side chain hydroxyl functions in Ser and Thr are protected as Bzl or t-Bu ether. In order to prepare Ser(tbu) temporary protection of the hydroxyl functional group in side chain is necessary.

Tert-Butylated amino acids could be prepared by reaction with isobutene in organic solvent which is protonated by concentrated sulfuric acid. The existence of strong acid is necessary to generated the tert-butyl cation by protonation of isobutene. In another approach , t-butanol was used as a starting material for the t-butylation of hydroxyl group in the presence of Lewis acid.

Here, we describe a practical synthesis of t-butyl side chain protected serine and threonine derivatives which are useful in Boc and Fmoc chemistry, respectively. Reaction were carried out using methyl tert-butyl ether (MTBE) instead of isobutene in the presence of strong acids and Lewis acids. The details will be discussed. The best yields were obtained using strong sulfuric acid.

P018. Abstract number: 168

Synthesis Of Human Exon 1 Huntingtin

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Huntington's disease (HD) is a genetically derived neurodegenerative disease caused by a CAG-triplet repeat expansion in the huntingtin gene. The protein huntingtin is 3,144-amino-acid long and is encoded over 67 exons with the CAG triple repeat located within the first exon. This CAG sequence codes glutamine. If the CAG repeat number in the gene exceeds 35, the resulting polyglutamine stretch makes the protein pathogenic. Shorter repeat numbers do not result in pathogenicity of the protein. Disease symptoms include depression, personality changes, weight loss, dementia and severe motor disturbances. The length of the poly-Q stretch inversely correlates with the patients' age of first symptoms. A repeat length of 40-55 glutamines typically triggers the first symptoms at an age of 35-50 years.

The expression of exon 1 huntingtin, a 67 amino acid long polypeptide including variable poly-Q stretches was

sufficient to cause full HD like pathology in different animal models. Overexpression of exon 1 huntingtin or longer parts of the protein failed due to cell-toxicity of the pathological form and its high aggregation potential. Here, we describe the synthesis of a 109 residue long exon 1 huntingtin peptide including a poly-Q stretch of 42 glutamines by microwave assisted solid phase peptide synthesis. The yield of the peptide was in the mg range with a very high purity. We also synthesized the non-pathogenic version of exon 1 huntingtin (90 amino acids long including a poly-Q stretch of 23 glutamines) using the same strategy. In CD-spectroscopy, both polypeptides showed alpha-helical properties with the longer peptide showing more pronounced alpha-helices. The pathogenic and non-pathogenic exon 1 huntingtin polypeptides have great potential for further biomedical analysis and could be used e.g. for large-scale pre-screenings for aggregation inhibitors, further structural analyses, and protein-protein interaction studies.

P019. Abstract number: 172

Synthesis Of New Polymer Matrices Including Amino Acids

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Amino acids, carbohydrates and nucleic acids are common biomolecules involved in the diagnostic and treatment (drug delivery) of wide number of diseases. Efforts of many scientific groups concern their encapsulation in organic or/and inorganic matrices during polymerization [1]. During this process some secondary reactions between the biomolecules and host network could occur. These interactions induce a modification of the native polymer structure. Moreover, a change of the initial functionality of these biomolecules could arise. A solution of both problems consists in optimizing the incorporation process. Indeed the choice of this process strongly determines the capability of further amide bond formation.

Herein we present several strategies in order to incorporate amino acids in organic and/or inorganic polymers. The sol-gel (SG) process is a suitable way to obtain hybrid polymer matrices. It is based on the hydrolysis of starting materials and further condensation. Different type of amino acids (either with different pKa or spatial obstructed group) is used and the further amide bond formation is investigated. Either free or protected amino acids are also studied. Additionally, a novel on-line technique to monitor the dynamic rheological properties at microscopic scale is used for polymerization study. This technique gives us information on the final material structure [2]. Using the complex shear moduli G' and G' evolution (respectively linked to elasticity and viscosity) an optimization of the process is proposed in order to design new biosensors and bioreactors.

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P020. Abstract number: 179

Convenient synthesis of Tfm-dipeptides from unprotected enantiopure a-Tfm-Proline and a-Tfm-Alanine

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The major disadvantages of peptides as therapeutic agents are their rapid degradation by peptidase and their low lipophilicity. In this context, α-trifluoromethyl α-amino acids (α-Tfm-AAs) are very attractive compounds for the design of biologically active molecules. Indeed, the incorporation of trifluoromethyl group containing amino acids into peptides increases chemical and thermal stability, resistance to protease degradation and enhances hydrophobicity giving a better affinity for lipid membranes. Moreover, their incorporation into peptides induces particular conformational stabilisations and better auto-assembly. In addition, labeled ^{19}F peptides should be of a big interest for structure elucidation and for the investigations of biochemical processes.

Despite these promising properties, two major drawbacks have to be overcome for their use in medicinal chemistry: 1) the synthesis of α-Tfm AAs in enantiopure form in large scale remains a challenge, 2) their incorporation into peptide sequences is very limited due to both the low nucleophilicity of the amino group and the steric hindrance imparts by the trifluoromethyl group.

We are already interested in the development of efficient synthetic routes for the preparation of enantiopure α-Tfm AAs starting from chiral CF_3-oxazolidines (Fox) or imines. We are now particularly focused on the development of efficient peptide coupling reactions of our α-Tfm-AAs in order to incorporate them into peptide chains. Here, we report the convenient synthesis of *N*-terminal dipeptides from unprotected enantiopure α-Tfm-Alanine and α-Tfm-Proline. Moreover, the coupling reaction at the *N*-terminal position deactivated by the trifluoromethylated group is also described. The reaction requires the use of a Fmoc-amino acid chloride.

P021. Abstract number: 180

Concise access to (S)- and (R)-a-Tfm-Serine and a-Tfm-Aspartic acid from chiral trifluoromethyoxazolidines (Fox)

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α-Trifluoromethylated amino acids (α-Tfm AAs) are current synthetic targets due to the unique physical and biological properties imparted by the fluorinated group. However, their preparation in enantiopure form remains a challenge.

Our group is involved in the development of efficient synthetic routes for the preparation of enantiopure trifluoromethylated amino acids starting from chiral CF_3-oxazolidines (Fox) or imines and their incorporation into a peptide chain.

We will report here the straightforward preparation of both enantiomers of α-Tfm-Serine and α-Tfm-Aspartic acid in enantiopure form starting from oxazolidines derived respectively from ethyl trifluoropyruvate and ethyl trifluoroacetoacetate. The key step of both synthesis involves a Strecker-type reaction.

P022. Abstract number: 181

CF3-pseudoprolines : Synthesis and conformational study of hydrolytically stable proline surrogate containing dipeptides.

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The incorporation of proline derivatives is known to restrict the peptide bond *cis*/*trans* isomerization, to control the protein folding and consequently to modulate the biological activity of peptides. Based on these observations, Mutter's group introduced pseudoproline building blocks (ΨPro) into peptide sequences as reversible protecting groups for Ser, Thr and Cys. ΨPro residues proved to be versatile tools for overcoming the aggregation encountered during solid phase peptide synthesis (SPPS). They also turned out to be inducers of β-turns containing predominantly *cis*-amide bond and useful tools in peptide cyclization.
Our group is interested in the development of efficient routes for the preparation of enantiopure trifluoromethylated amino acids (Tfm AAs), particularly pyrrolidine-type α-Tfm AAs and their incorporation into a peptide chain. As a complementary strategy, we are now focused on the use of trifluoromethylated pseudoprolines (CF_3-ΨPro). 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 acid surrogates into peptide chain. In addition, a high degree of stability toward acidic media is expected since the chemical stability of ΨPro systems strongly depends on the electronic effects of the C2 substituents. This property could make them hydrolytically stable authentic proline surrogates compatible with SPPS strategy.
We will report the preparation of various CF_3-ΨPro as well as the methodological study developed to optimize the synthesis of various *C*-terminal and *N*-terminal CF_3-ΨPro containing dipeptides. Their conformation and their stability toward hydrolysis have been studied and will also be reported.

P023. Abstract number: 198
Solid Phase Synthesis Substituted Peptide Amides On Aryl Hydrazine Resin
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Solid phase synthesis in classical approach starts with attachments of the first amino acid to resin for free acid or an amide peptide. Such approach could not be applied when C-terminal residue does not possess carboxyl group. Y. Kwan et al. [1] presented an approach to synthesize p-nitoanilides and other anilides analogues using aryl hydrazine resin. We used [2] very similar approach to synthesize Pyr-Arg-Thr-Lys-Arg-AMC with slightly modified procedure. This procedure is based on solid-phase peptide synthesis on aryl hydrazine resin a whole sequence less C-terminus amine residue. When the synthesis is completed, the fully protected peptide aryl hydrazide resin is oxidized with NBS in presence of pyridine. The resulting acyl diazene resin is then cleaved by peptide displacement with C-terminus amine. The protected peptide amide is then deprotected and purified. Finally peptide amides were identified by mass spectrometry and their purity determined by analytical HPLC. Cleavage/displacement reaction efficiency depends on amine nucleophilicity which in case of aromatic amines is not significant. Aliphatic amines should have much more nucleophilisity then aromatic amines therefore react faster and more efficient in described procedure. In our approach we used aliphatic amines as decarboxyl amino acid derivatives, which could mimic peptide C-terminus residue. Several peptide substituted amides were synthesized on solid-phase this way very efficiently. Those peptides were tested for their expected biological activity.
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P024. Abstract number: 196
Solid-Phase Synthesis C-Terminus Chloromethyl Ketone Peptides
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Halomethyl ketones and in particular chloromethyl ketones are principal tools in enzyme inhibitory examination. Cholromethyl ketones of amino acids, their analogues and peptides are the most commonly used. There is numerous ways to make halomethyl ketons of amino acids or peptides in solution. For the small molecules like amino acids transformation to chloromethyl ketons in classical organic synthesis seems to be not problematic. Peptides chloromethyl ketons preparations give few problems such as peptide solubility, racemization process when amino acid chloromethyl ketone couple from C-side of the peptide or peptide transformation to chloromethyl ketone. Solid-phase synthesis of peptide chloromethyl ketone would eliminate most problems. Classical solid phase synthesis of these peptide derivatives in a standard form could not be applied due to missing C-terminal carboxyl group. A new strategy to build a peptide on special resin was used by Kwon et al [1]. Aryl hydrazine resin we used to assemble peptide sequence until last C-terminal chloromethyl ketone residue. Continuous flow synthesizer with Fmoc strategy was used to synthesize peptide. The protected peptide-resin was then mildly oxidized to diazene and displaced from the resin with amino acid chloromethyl ketone derivative. Peptide was then deprotected and purified. The final product was identified by mass spectrometry (MALDI) and its purity tested on HPLC and TLC. Prepared peptide chloromethyl ketones were tested for their expected biochemical activity.
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P025. Abstract number: 206
Photo-uncaging of neurotransmitter amino acids from fluorescent 5,6-benzocoumarinyl precursors
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The spatiotemporal controlled release at target sites in or around cells and switching on of biological processes can be achieved by the photochemical stimulation (uncaging) of bioactive molecules from their inactive photolabile precursors (caged compounds) [1].
A diversity of structures, such as 2-nitrobenzyl [2], 1-(4,5-dimethoxy-2-nitrophenyl)-ethyl [3], 4,5-dimethoxy-2-nitrobenzyl [4], 6-bromo-7-hydroxy-coumarin-4-yl-methyl [5], 7-[(diethyl-amino)-coumarin-4-yl]-methyl [6] and 7-dinitro-indolinyl [7] have been evaluated as caging groups for the photo-regulation of calcium ions, neurotransmitters, carboxylic acids, proteins, nucleotides, peptides, RNAs and DNAs [8].
Considering these facts, and following our recent research interests, which are also connected with the investigation of alternative photoreleasable protecting groups [9], we now report the uncaging of several neurotransmitter amino acids, namely β-alanine, tyrosine, 3,4-dihydroxyphenylalanine (DOPA) and glutamic acid from

the corresponding 5,6-benzocoumarinyl fluorescent conjugates. Photocleavage studies were carried out in a photochemical reactor equipped with lamps of 254, 300, 350 and 419 nm and cleavage kinetic data obtained by HPLC/UV monitoring will be discussed.
Thanks are due to Fundação para a Ciência e Tecnologia (Portugal) for financial support through project PTDC/QUI/69607/2006 and a Ph.D. grant to M.J.G.F. (SFRH/BD/36695/2007). The NMR spectrometer Bruker Avance III 400 is part of the National NMR Network and was purchased in the framework of the National Program for Scientific Re-equipment, contract REDE/1517/RMN/2005 with funds from POCI 2010 (FEDER) and FCT.
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P026. Abstract number: 209

Photoswitchable Intercalating Peptides: Synthesis and Characterization

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Novel highly active and specific DNA binding molecules have considerable potential particularly with regard to applications in medical science. The major or minor grooves of the DNA serve as recognition areas. Controlling DNA-binding abilities of synthetic molecules is of high interest for Chemical Biology. In this context, light is an additional possible modulator when DNA-binding molecules are equipped with photoswitchable moieties such as azobenzenes.

Triostin A belongs to a family of peptide antibiotics produced by marine bacteria. The cyclic depsipeptidic structure is composed of two identical subunits each containing alanine, D-serine, *N*-methyl-cysteine and *N*-methyl-valine. The cysteine sidechains bridge the cycle by forming a disulfide bond and quinoxaline-2-carboxylic acid (Qxc) is attached to the amino group of each D-serine.

Triostin A shows antibacterial and cytotoxic activity like other members of this family including echinomycin, BE-22179 and thiocoraline.[1] Upon binding to the DNA minor groove, triostin A exhibits a sequence preference for GpC and intercalates the Qxc chromophores in such a way that triostin A bridges a two base pair site. The synthetic analogue des-*N*-tetramethyltriostin A, (TANDEM), which lacks the four *N*-methyl groups, displays a change of selectivity to TpA sequences due to the different hydrogen bonding pattern.[2]

The replacement of the cystine bridge of TANDEM by an azobenzene unit[3] leads to photoswitchable triostin A analogues. Key step of the synthesis is the double macrolactamization which is accomplished under pseudo high dilution conditions. In order to differentiate between *cis*- and *trans*-configurations of the azobenzene moieties, NMR-spectroscopy was employed. The photoswitchability of different analogues was investigated by irradiation and RP-HPLC analysis.

P027. Abstract number: 217

What could be the role of quinacrine in Creutzfeldt-Jakob disease treatment?

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A conversion from normal cellular form of prion protein (PrPC) to a toxic one (PrPSc) is suspected to cause prion diseases.[1] This transformation can be prevented, at least in vitro, by the reduction of the disulfide bond of the PrPC and subsequent alkylation of formed thiol groups.[2]

To better understand and potentially manipulate the prion sulfide residues, we used quinacrine as a labeling agent.

The acridine moiety of quinacrine can undergo nucleophilic substitution with primary amines and thiols to afford quinacrine analogs, where the original aminoalkyl chain was replaced by another aminoalkyl group[3] or thioalkyl group[4], respectively. This fact was utilized for alkylation of free thiol groups of prion protein.

We investigated quinacrine reactivity towards the thiol groups of prion-protein derived peptides and mouse prion protein: firstly as a model, we prepared short prion fragments with acridine moiety on cysteine sulphur atom. Secondly, we tried acridinylation of the mouse prion protein on both cysteines. All acridinylations were carried out at physiological condition (phosphate buffer, pH=7.4, 37 °C) by mixing together quinacrine and corresponding peptide/protein.

To rationalize the labeling, we also modeled acridinylation theoretically on simpler systems, such as 4-chloropyridine and SH^- ion. The B3LYP functional with the 6-31+G** basis set provided realistic activation energies. Predicted substituent and *N*-protonation influence on activation energy of the reaction of 9-chloroacridines with SH^- were consistent with experimental observations.

Probable mechanism of the acridinylation is discussed on a model reaction of *N*'methyl-4-aminopyridine with SH^-, and extended to *N*-methyl-9-amino acridine.

Since the quinacrine is potential drug for the Creutzfeldt-Jakob disease, which is believed to be caused by prion, we suggest that covalent bonding of acridine moiety to the prion protein free thiol groups can be crucial for quinacrine action in the disease treatment. Moreover, the understanding of the acridinylation mechanism may play a very important role in the design of new acridine drugs.

This work was supported by the Czech Science Foundation (GA CR) grant no. 203/07/1517 and Research Project Z40550506.

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P028. Abstract number: 219

Synthetic Antifreeze Glycopeptide Analogs

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Efficient biological antifreeze agents are crucial for survival in arctic and antarctic waters, where the temperature declines below the colligative freezing point of physiological fluids. One of these agents are classified as antifreeze glycoproteins (AFGP), which have been barely investigated due to problematic purification from natural sources and challenging synthesis of heavily glycosylated

peptides.AFGPs usually consist of a varying number of $[AAT]_n$-repeating units (n=4-50), where every threonine side chain is glycosidically linked to *β*-D-galactosyl-(1-3)-*α*-N-acetyl-D-galactosamine. Although this pattern is highly conserved among different species, minor sequence mutations were found, e. g. substitution of alanine by proline. The antifreeze activity is usually proven by suppression of the recrystallisation and ice nucleation, thermal hysteresis and change of the crystal habitus. This antifreeze activity is influenced by certain properties, e. g. the N-acetyl group at the C2 position of the galactosamine, the *α*-configured glycosidic bond between the carbohydrate and the threonine residue as well as the *γ*-methyl group of threonine, what should be considered in synthesis. Furthermore it has been postulated that AFGPs adopt a threefold left-handed helix similar to a polyproline type II helix. The structure seems to be highly flexible and may changes significantly upon contact to the ice surface.Such glycosylated peptides with varying sequences and lengths can be successfully obtained by solid phase peptide synthesis. The difficult synthesis of peptides, because of the heavy glycosylation, is efficiently performed upon HATU activation and microwave-assistance during the cycles.By introduction of fluorophores at the termini, hydroxyproline or proline residues, which occur especially in the smaller natural AFGP 6-8, further important information regarding conformation and rigidity could be gained.Synthetic AFGP analogs were examined by CD and NMR studies in water and DMSO at different temperatures. Furthermore the peptides were analysed microphysically according to their effect on ice recrystallisation, inhibitory activity and influence on the crystal habitus.

P029. Abstract number: 220

Peptide Based Artificial Receptors for Carbohydrate Recognition

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Protein-mediated natural chiral carbohydrate recognition is one of the most sophisticated recognition processes in biological systems. This process mediates many important aspects of cell-cell recognition, such as bacterial adhesion to human tissue, inflammation, cell differentiation, tumor cell colonization and metastasis. However, due to its complexity and weak binding affinities, carbohydrate recognition by natural systems is still poorly understood. Studies on synthetic carbohydrate receptors could make significant contributions to a better understanding of this process and lead to the development of new analytical agents or therapeutics.

Peptide based macrocyclic structures such as bicyclic peptide, are particularly interesting for the design of artificial carbohydrate molecules because of their facile assembly using standard SPPS, and the three dimensional structure that would permit carbohydrate substrates to be encapsulated to afford polyvalent binding, enhancing therefore receptor's selectivity and affinity. In addition, incorporated bipyridine aromatic units exhibit strong absorption and fluorescence emission bands, and complexation induced spectral changes can be exploited for the discrimination among variety of monosaccharide structures. To demonstrate suitability of bicyclic peptide structures for design of artificial carbohydrate receptors we have successfully prepared bicyclic peptide modeled on the cyclic cationic decapeptide antibiotic polymyxin B, which is known to bind the lipid A moiety of bacterial lipopolysaccharides with the high affinity. Combinatorial chemistry approach is applied to improve receptors binding properties and selectivity.

P030. Abstract number: 226

Synthesis of glycopeptides potential inhibitors of human rhinovirus 3C Protease

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Recently, due to rapidly and dramatically changing climatic conditions, our environment is also varying quickly. Simultaneously, we are witnessing the emergence of several new and more resistant viruses such as HIV virus, AH1N1 influenza virus and an ever increasing resistance of viruses to applied drugs. In this global context, synthesis of completely new or modified antiviral agents and vaccines is of a great interest for medical practice.

Human rhinovirus (HRV) is a small picornavirus responsible for the common cold. Some enzymatic and structural proteins required for viral replication are generated by the viral encoded 3C Protease (3CP). The HRV 3CP is specific cysteine protease able to cleave P1-P1' Gln-Gly amide bond. Structurally, it closely resembles trypsin-like serine protease [1]. A tripeptide aldehyde Z-Leu-Phe-Gln-H is a well know inhibitor of human rhinovirus 3CP [2]. Several literature data reveal that P3 Leu residue is not important for antiviral activity and many groups could be tolerated at this place [3]. Herein, we report a series of mimetics of this tripeptide by replacement of P3 Leu residue in order to make possible further glycopeptides synthesis. An original strategy for solid phase synthesis of such type of Gln containing peptide by means of Glu residue attached to Ring amide resin will be presented. Additionally, some experiments to introduce COOH function in several carbohydrates molecules in order to realize the aim compounds design were done. Several synthetic schemes and strategies will be described and discussed.

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P031. Abstract number: 232

Stereoselective Synthesis of Tetrahydro-β-carboline using typtophane and Pictet-Spengler Reaction

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The Pictet-Spengler reaction is a key step in the total synthesis of a large number of indole alkaloids. In recent years, stereoselective synthesis of this skeleton continue to attract considerable interest due to the broad range of medicinal activities they possess. In this research, we would like to report the cis-selectivity of Pictet-Spengler reaction of tryptopha propargyl ester with aromatic and heteroaromatic aldehydes to generate cis-tetrahydro- β-carbolines. In all cases, reactions were carried out under kinetic control. Fmoc-Trp(BoC)-OH was used as starting materials and the propargyl ester function was formed using TBTU as coupling reagent. Meanwhile, trifluoroacetic acid was used for cyclization and formation of β-carboline scaffold. These products could be used as starting materials for Click chemistry. The details will be be discussed in conference.

P032. Abstract number: 236

Synthesis of aza-β3-Homoserine, incorporation of this new monomer into 26RFa(20-26) and microwave-assisted deprotection of its side chain

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Aza-β^3-peptides, mixing α- and aza-β^3-amino acids (the aza analogs of β^3-amino acids), represent an exciting type of peptidomimetics.[1] In particular, we have shown that an aza-β^3-amino acid induces a N-N turn 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 different conformations and in particular 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 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 to mobilize $[Ca^{2+}]i$ in GPR103-transfected cells. 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-NH_2). As the γ-turn is centered on the Ser23 of 26RFa we focused our modifications on position 23. During the synthesis of a new monomer, the aza-β3-homo serine (aza-β^3-Hse), some problems occured as well as during its side chain deprotection. We will present (i) the synthesis of Fmoc-aza-β^3-Hse(R)-OH, (ii) the incorporation of this surrogate into the heptapeptide, (iii) the side chain deprotection attempts, in particular the microwave-assisted deprotection and (iv) the in vitro effect of this pseudopeptide on GPR103-transfected cells.

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P033. Abstract number: 240

Dimerization strategies of the immunosuppressory decapeptide ubiquitin fragment.

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Our previous studies demonstrated that fragments located in the β-loop of HLA-class II molecules suppress the humoral and cellular immune responses [1, 2]. It may be expected that properly designed dimeric analogs of biologically active bioligands are more selective in their affinity towards dimeric receptors. Basing on the three-dimensional structure of the HLA-DR superdimer, we designed bivalent analogs able to mimic the dimeric nature of the immunosuppressive fragments of the HLA molecules [3]. The synthesized analogs possess enhanced immunosuppressory potencies as compared with their monomeric counterparts [4].

Some topological correspondence between the ß164-172 loop of HLA-DQ and the external 50-59 loop of ubiquitin suggests that their biological roles could also be similar. We found that a decapeptide 50-59 fragment of ubiquitin with LEDGRTLSDY sequence exhibits strong immunosuppressive effects on the cellular and humoral immune responses, comparable to that of cyclosporine [5].

Herein, we designed and synthesized new dimeric analogs of the ubiquitin fragment, to probe whether the ubiquitin receptors may form oligomeric structures. Three dimerization strategies: head to tail, C-terminus to C-terminus, and N-terminus to N-terminus, were used to synthesize the dimeric peptides on solid support. We selected polyethylene glycol derivatives as linking agents because of their flexibility, solubility and bioavailability. In the course of our research, we developed a new and straightforward procedure for the direct dimerization of the C-terminal peptide side-chains on solid support by a polyethylene glycol spacer.

Our results indicate that particularly the head to tail dimerization results in enhanced immunosuppressive activity of the conjugated decapeptide fragments of ubiquitin. This may suggest a parallel orientation of the molecules of the hypothetic ubiquitin receptors responsible for the immunomodulatory activity.

Supported by grant No. N N401 222734 from the MSHE (Poland).

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P034. Abstract number: 257

The application of the new tin(IV) chloride deprotection for the preparation of glycosylated peptides

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Glycopeptides are a rapidly growing family of biologically important molecules. The carbohydrate moieties are attached through the oxygen in the side-chain of serine/threonine in O-linked glycoproteins or through the carboxamide nitrogen of asparagine in case of N-linked glycoproteins. The rational preparation of the glycosylated peptides is still one of the most challenging tasks of peptide chemistry especially of those having oligosaccharide moieties.

There are two main strategies for the synthesis of glycopeptides: the synthon and global (convergent) method. Based on our earlier results, the glycosylated aspartic acid and serine derivatives could be suitable building blocks for the solid-phase peptide synthesis. In some cases the preparation of Boc-protected glycosylated amino acid building blocks is more convenient than the Fmoc protected ones.

We describe here the solid-phase synthesis strategy for the preparation of glycopeptides by using a new, mild and selective Boc deprotecting agent. For the incorporation of the Boc-protected glycosylated amino acid derivatives in

the model peptides as selective Boc deprotecting agent tin(IV) chloride was used. The glycopeptides were synthesised using Fmoc chemistry. Many resins commonly used in Fmoc chemistry were tried: Rink amide, 2-chlorotrityl and Wang resin, but most of them showed removal of peptide during cleavage with tin(IV) chloride. The TFA cleavable Rink-amide MBHA resin proved to be sufficient for the synthesis of glycopeptides because of the acid sensitive O-glycosidic linkage and also the peptide-resin linkage was left intact, permitting the incorporation of di and trisaccharide moieties. As model peptides we used a fragment of the Trp-cage mini protein (Leu-Lys-Asn*-Gly-Gly-Pro) and an aggrecan fragment, from the most glycosylated region of the protein (Gly-Val-Glu-Asp-Ile-Ser*-Gly-Leu-Pro-Ser-Gly), where * is site of glycosylation. As synthons N^{α}-Boc-protected [GlcNAc(β1-N)]Asn, [GlcNAc(β1-4)GlcNAc(β1-N)]Asn, [Man(β1-4)GlcNAc(β1-4)GlcNAc(β1-N)]Asn and [Xil(Ac)3(β1-O)]Ser were used.

P035. Abstract number: 264

Efficient Microwave-Assisted Synthesis Of Mitochondrial Signal Peptide Using Cltr-Cl And Wang Resin

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Mitochondrial targeting peptide plays an important role in transporting all the necessary information of proteins into mitochondria. In this study we demonstrate the synthesis of 18-mer signal peptide (H_2N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-COOH) using the CEM Liberty automated microwave peptide synthesizer. Microwave assisted peptide synthesis is an attractive alternative for traditional SPPS. This synthesis is 10 to 20 times faster compared to traditional methods and produces cleaner peptides with higher yields. In this report we demonstrate an efficient microwave assisted synthesis using Fmoc/*t*-Bu strategy. The effect of two different resins, CLTR-Cl and Wang resin was compared. The first amino acid was loaded to the solid support and was transferred to Liberty. The two synthetic procedures were performed in the same conditions, on 0.25 mmol scale, using HOBt/DIC as coupling reagents. The total synthesis was completed in 11 hours and the crude peptide was purified by Prep-HPLC. The final product was identified by UPLC/MS and the purity was determined by RP-HPLC. The present comparison of the two synthetic protocols showed that the results were quite similar, while CLTR-Cl resin gave higher yield compared to Wang.

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P036. Abstract number: 271

A New Practical, Scalable, Enantioselective Synthesis Of Substituted Cyclohexyl Glycine Amino Acids.

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The hydro (Boc or Z) enamine derivatives (I) were prepared from commercially available Boc/Z-phosphonoglycine trimethylester and protected cetone using Schmidt's protocol. An asymmetric hydrogenation of (I) in the presence of Rh-chiral biphosphine catalysts generated protected glycine derivatives (II) with excellent enantiomeric excesses. Starting from (II), a variety of side chains can be incorporated.

We report here a scalable, high-yielding enantioselective synthesis of enantiopure substituted-cyclohexyl-glycine amino acids (III) which are considered as protected non-natural amino-acids and can be used for extensive structure activity studies.

P037. Abstract number: 282

Electrochemical reduction of beta-aryldehydroamino acid derivatives

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In our research group we have been interested in the synthesis of dehydroamino acids and in their use as substrates in several types of reactions, namely addition and substitution reactions and palladium catalyzed cross-couplings [1]. The latter allowed the synthesis of several beta-aryldehydroamino acids by reaction of aryl boronic esters with beta-bromo dehydroaminobutyric acid and dehydrophenylalanine derivatives or with a beta,beta-dibromodehydroalanine derivative [2].

Following our previous studies on the electrochemical behaviour of dehydroamino acid and beta-halodehydroamino acid derivatives [1,3], we decided to investigate the electrochemical properties of beta-aryldehydroamino acids. Cyclic voltammetry studies gave for all compounds an irreversible reduction peak at potentials ranging from -1.74 to -2.01 V vs SCE. When beta,beta-diaryldehydroamino acid derivatives were subject to controlled potential electrolysis at the potential of their cathodic peak, reduction of the double bond took place to give the respective beta,beta-diarylamino acid derivative. In general, the double bond is not reducible under electrochemical conditions except, when it is linked to strong electron withdrawing elements, or in case of conjugation which lowers the energy level of the lowest pi*-orbital [4]. Thus, in the case of beta,beta-diaryldehydroamino acids, the further conjugation created by the two aryl substituents allows reduction of the double bond to occur.

In this communication the results obtained in electrochemical reduction of beta-aryldehydroamino acid derivatives will be presented, as well as those obtained by using chemical reduction methods, namely, reduction with mercury activated aluminum or magnesium in methanol.

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P038. Abstract number: 295

New Building Blocks for Solid-Phase Synthesis of Peptide Analogues: Nβ-Fmoc-Nβ-Methyl- aza-β3-amino acides

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N-Methylation is a precious tool to modify lipophilicity, proteolytic stability, and bioavailability and to induce conformational rigidity to the peptide backbone. However,

multiple N-methylation has been seldom employed, probably owing to the availability of N-methylated amino acids, as few *N*-methyl amino acids are commercially available, and their synthesis is tedious.
Unnatural aza-β^3-peptides are synthetic compounds designed to mimic peptides and increased their bioavailability. They can have chemically diverse side chains, but they need to have increased unproteolytically labile amide bonds, resistance to proteolysis. Yet, additional modifications are required to generate peptides with enhanced enzymatic stability and improved oral bioavailability.
We have reported previously a method to prepare N^{β}-Fmoc-aza-β^3-amino acids via reductive amination of glyoxylic acid and Fmoc-hydrazine.[1] Taking account of this previous method we try to reproduce the same reactions starting from methyl hydrazine instead of hydrazine.
We present the general method for the synthesis of N^{β}-Fmoc-N^{β}-Methyl-aza-β^3-aminoacid monomers. We described also the integration of these new monomers during the solid phase synthesis of peptide analogs of RRASVA, known as the ”minimal substrate”of the catalytic subunit of the cAMP-dependent protein kinase (PKA).
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P039. Abstract number: 305
Synthesis and biological studies of 4-nitrophenylalanine modification of Cyclolinopeptide A
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Immune response suppressors are used in the medical praxis to prevent graft rejection after organ transplantation and in the therapy of some autoimmune diseases including dermatology. Cyclolinopeptide A (CLA) (**1**), a cyclic, hydrophobic nonapeptide isolated from linseed, possesses strong immunosuppressive and antimalarial activity[1]. It is suggested that both the Pro-Pro cis-amide bond[2] and an 'edge-to-face' interaction between the aromatic rings of two adjacent Phe residues[3] in tetrapeptide unit are important for biological activity.
CLA(**1**): c(Pro1-Pro2-Phe3-Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
This edge-to-face interaction can be influenced by substitutents in the aromatic rings of phenylalanine residues[4].
In this communicate new analogues of CLA modified by three different substituted phenylalanines: 4-nitrophenylalanine (p-NO2)Phe (2), 4-aminophenylalanine (p-NH2)Phe (3) and 4-acetamidophenylalanine (p-AcNH)Phe (4) in positions 3 or 4 or both 3 and 4 (**5**-**13**) will be presented. CLA analogues **5**-**13** were prepared by cyclization of linear precursors by use of the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide/1-hydroxy-benzotriazole (EDC/HOBt) as a coupling reagent. Linear peptides were synthesized by the manual solid-phase peptide synthesis (Merrifield resin) strategy. The synthetic strategy and biological activity as well as conformational analysis will be evaluated.
(**5**) c(Pro1-Pro2-(p-NO2)Phe3-Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**6**) c(Pro1-Pro2-Phe3-(p-NO2)Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**7**) c(Pro1-Pro2-(p-NO2)Phe3-(p-NO2)Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**8**) c(Pro1-Pro2-(p-NH2)Phe3-Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**9**) c(Pro1-Pro2-Phe3-(p-NH2)Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**10**) c(Pro1-Pro2-(p-NH2)Phe3-(p-NH2)Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**11**) c(Pro1-Pro2-(p-AcNH)Phe3-Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**12**) c(Pro1-Pro2-Phe3-(p-AcNH)Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
(**13**) c(Pro1-Pro2-(p-AcNH)Phe3-(p-AcNH)Phe4-Leu5-Ile6-Ile7-Leu8-Val9-)
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P040. Abstract number: 316
Synthesis and Folding of Kv1.3 Ion-Channel Blocking Peptides
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T cell-mediated autoimmune diseases, such as multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis and others, afflict millions of people worldwide. Disease modifying immunotherapies have improved the management of autoimmune diseases, but these therapies induce a variety of unwanted side effects. Consequently, there remains an enormous unmet medical need for novel immunomodulators with different mechanisms of action and/or adverse-effect profiles from existing drugs. Preclinical and clinical evidence suggests that antagonizing Kv1.3 channels is effective in alleviating MS and RA syndromes by specifically affecting disease causing TEM cells while leaving the remaining components of the immune system unaffected.
An example of such antagonists is the naturally-occurring toxin peptides produced by a variety of organisms, such as snakes, scorpions, spiders, bees, snails and sea anemones. The venom that they produce can serve as a source of small bioactive toxin peptides that potently and selectively target ion-channels and receptors. In most cases, these toxin peptides have evolved as potent antagonists or inhibitors of ion-channels by binding to the channel pore and physically blocking the ion conduction pathway. Unfortunately due to their length and complexity (most have multiple disulfide bridges), they are often overlooked at the research stage.
Here we describe the strategy, synthetic details and folding of a peptide found in the venom of the Asian scorpion Orthochirus scrobiculosus and reported to be a potent antagonist of the Kv1.3 ion-channel. We also briefly describe the conjugation of this peptide to an antibody to greatly enhance its pharmacokinetic properties.

P041. Abstract number: 317
O-Acyl isopeptide method: an efficient preparation of amyloidogenic peptide by use of racemization-free segment condensation
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The synthesis of ”difficult sequence”-containing peptides such as amyloidogenic peptides is one of the problematic areas in peptide chemistry. These peptides are often obtained with low yields and purity, due to their hydrophobic and aggregative properties. We discovered that the presence of an *O*-acyl instead of *N*-acyl residue within the peptide backbone significantly changed the secondary structure of the native peptide. In addition, the target peptide was subsequently generated by an *O*-to-*N* intramolecular acyl migration reaction. These findings led to the development of a novel method, called ”*O*-acyl isopeptide method”, for the peptide synthesis [1, 2].
Along the line, we have developed a novel convergent method of peptide synthesis, racemization-free segment condensation methodology based on the *O*-acyl isopeptide method [3, 4]. The idea was that an *N*-segment with a C-terminal *O*-acyl isopeptide structure at Ser or Thr residue could be coupled to an amino group of *C*-segment without epimerization, since an amino group of the C-terminal isopeptide part is protected as a urethane-type protective group. We have successfully synthesized the *O*-acyl isopeptides of the bioactive peptides such as humanin, orexin-B and amyloidogenic peptides by use of the convergent method. Peptide segments containing C-terminal *O*-acyl Ser/Thr residues were successfully synthesized using a lower nucleophilic base cocktail for Fmoc removal, and then coupled to an amino group of a peptide-resin without side reactions or epimerization. Final deprotected peptides were effectively purified by HPLC, because the introduced *O*-acyl structure remarkably changes the physicochemical properties of the native peptides. Finally, the synthesized isopeptides released their native peptides via the *O*-to-*N* acyl migration reaction. The *O*-acyl isopeptide method-based segment condensation methodology would contribute to the field of chemical synthesis of long peptides and proteins.
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P042. Abstract number: 319

The synthesis of (glyco)protein by the ligation methods

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The ligation methods, the native chemical ligation (NCL) method [1] and the thioester method [2], are routinely used for the synthesis of (glyco)proteins. However, through the synthesis of several (glyco)proteins, we realized that both methods still require improvements to overcome their intrinsic drawbacks.
In the NCL method, the ligation site is limited to Xaa-Cys site. As the abundance of cysteine in natural proteins is quite low, this problem severely limits the selection of the ligation site. We recently developed an extended ligation reaction at the Xaa-Ser/Thr site, which is far more abundant in natural proteins than Xaa-Cys site. In this method, a mercaptomethyl group on the hydroxyl group of Ser and Thr was used as a thiol auxiliary group. The advantage of this method is that this group is a labile hemithioacetal and thus, an additional deprotection step for this auxiliary group is not required, as it is spontaneously hydrolyzed after the ligation. The efficiency of the method was demonstrated by the synthesis of glycopeptide, contulakin-G, and human calcitonin.
The thioester method has the advantage that there is no restriction on the selection of the ligation site. In stead, the side chain amino and thiol groups have to be protected. To realize this, the Boc groups have to be reintroduced to the side chain amino groups, which were made free during the deprotection after SPPS. We examined the direct synthesis of the side chain-N-protected peptides by introducing azido-protected Fmoc-Lys during SPPS to overcome the inconvenience. In the second part, the synthesis of N-, and O-glycosylated POMC (1-74) will be described by the thioester method with above optimized protocol [3].
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P043. Abstract number: 349

Replacement of disulfide bonds with ditellurides: Synthesis and biological activity of ditelluro-oxytocin

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The correct arrangement of disulfide bonds is an essential requirement for maintaining the structures and biological activities of many peptides and proteins. However disulfide bonds are susceptible to scrambling and reduction in vivo, and their regioselective synthesis can be challenging. While the use of selenocysteine to direct the folding as well as to improve the redox stability of peptides is now becoming routine, the substitution of cysteine with tellurocysteine remains largely unexplored. In addition to the potential as a novel structural isosteric disulfide bond mimic, the applications of tellurium incorporation into peptides may include radiolabels, NMR probes or heavy atom labels for structure determination by X-ray crystallography.
In this study, the single disulfide bond containing peptide hormone oxytocin was used as a model to evaluate the feasibility of the synthetic incorporation of tellurocysteine into peptides. The linear precursor was assembled using Boc solid-phase chemistry, substituting cysteine residues with serines that were subsequently converted to β-chloroalanines. Reaction of the resulting dichloropeptide with Na2Te2 produced ditelluro-oxytocin, which to our knowledge is the first chemically synthesised ditelluride analogue of a biologically active peptide. Activity at the oxytocin receptor of the ditelluride analogue was comparable to that of wild-type oxytocin. It is envisaged that using this method, it would be possible to insert ditelluride bonds into other sequences in combinations with disulfides and diselenides to assess their utility in controlling peptide folding.

P044. Abstract number: 352

Understanding the role of the unusual constrained eight-membered disulfide ring of spider toxins

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The κ-hexatoxin-Hv1c is a cysteine-rich peptide found in the venom of Australian funnel-web spider. It is the prototypic member of a unique family of excitatory peptide

toxins that contains a rare vicinal disulfide bridge. These toxins are lethal to a wide range of insects, but are harmless to many vertebrates, making them promising candidates for biopesticide development.
Two adjacent cysteine residues found in the κ-hexatoxin Hv1c structure form an unusual vicinal disulfide bond that is constrained in an eight-membered ring formation. This motif occurs only very rarely in protein structures but it is functionally important to those few proteins that posses it. In many cases, the vicinal disulfide bridge is thought to act as a redox conformational switch, where changes in peptide backbone conformation are observed on going from the reduced to the oxidised (constrained) state. In other cases, the ring is believed to facilitate trans/cis isomerization, allowing the protein to adopt a different conformation upon ligand binding.
For the case of the spider toxin κ-hexatoxin Hv1c, the role of the vicinal disulfide bridge is unclear. Mutation of this motif to a vicinal serine pair resulted in no change in tertiary structure; however, the mutant peptide displayed no insecticidal activity. Thus, the ring is not architecturally important.
In order to test the redox conformational switch hypothesis, we prepared a series of κ-hexatoxin Hv1c analogs in which the native vicinal disulfide S-S bond was mimicked by redox-stable linkages of Se-Se, C-C and C-S bonds. Considering that the mimetics assume the native fold, a critical loss of insecticidal activity for these analogs would suggest that redox changes are important for biological function. The maintenance of activity would rule this theory out.
All peptides were successfully synthetised by solid-phase peptide synthesis, where the two vicinal cysteine residues were selectively replaced by selenocysteine residues or by a building block containing the C-C or the C-S eight-membered ring mimetic. The structure of two toxin analogs was determined by NMR spectroscopy and compared to the native protein. The peptide backbone accommodates well the mimetic scaffold and no significant change in the overall 3D structure was observed.
The insecticidal activity of the new mimetics is currently been tested in blowflies as well as their ability to block calcium activated potassium channels currents in cockroach neurons.

P045. Abstract number: 353

S-Acyl isopeptide method: preparation of thioester-containing isopeptides by Fmoc-based SPPS with Aloc group

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We have discovered that replacing the native amide by an ester within the peptide backbone at Ser or Thr significantly changed the secondary structure of the native peptide, and the target peptide can be easily generated from the formed isopeptide via an *O*-to-*N* intramolecular acyl migration reaction. These findings led us to the development of an effective strategy, the *O*-acyl isopeptide method, for the preparation of peptides containing difficult sequences [1,2].
Based on these results, we developed a novel *S*-acyl isopeptide method [3] as an extension of the isopeptide strategy with a successful synthesis of model pentapeptide Ac-Val-Val-Cys-Val-Val-NH_2 (**1**). Using the Aloc protective group, the preparation of thioester-containing *S*-acyl isopeptide H-Cys(Ac-Val-Val)-Val-Val-NH_2 (**2**) by Fmoc-based SPPS was effectively achieved in 44% yield. Catalytic amount of $Pd(PPh_3)_4$ in the presence of scavengers such as $PhSiH_3$ and dimedone selectively removed the Aloc group with neither decomposition of the thioester structure nor epimerization at the thioesterified residue. Finally, the isolated *S*-acyl isopeptide **2**, possessing high water-solubility (8.5 mg mL^{-1}), was quantitatively converted to the desired peptide **1** via the *S*-to-*N* intramolecular acyl migration reaction in phosphate buffer (pH 7.4) at room temperature. These results indicated that the *S*-acyl isopeptide method would provide a useful tool in peptide chemistry [4].

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P046. Abstract number: 364

Computational Study on Helical Structure of a,a-Disubstituted Oligopeptides Containing Chiral a-Amino Acids

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Prediction of the conformation of oligopeptides using computational simulation presents an interesting challenge to design functionalized and bioactive peptides. We have shown that the MCMM conformational search method using MacroModel is useful to predict 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, etc). 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.

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P047. Abstract number: 365

En route to bicyclic biaryl peptides

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A rich variety of cyclic peptides containing an aryl-aryl bond can be found in Nature [1]. This heterogeneous group of natural products includes compounds showing proteasome inhibition, antimicrobial or cytotoxic activity. The presence of the biaryl bridge confers all of them conformational rigidity. Additionally, aromatic-aromatic or π-cation interactions can be established between the biaryl system

and the residues present on protein surfaces. This last structural feature has so far not been exploited in the design of peptide ligands for protein surface recognition. With this in mind, the scope of our project is to prepare cyclic peptide ligands which are further constrained by means of a biaryl bond. We envision this type of biaryl-containing bicyclic peptides as constrained scaffolds, which can be customized according to the corresponding target.
For the synthesis of the biaryl bridge we have chosen the Suzuki-Miyaura reaction, which in recent years has been applied also to peptide modification [2,3]. Our initial work has been devoted to setting-up reaction conditions for the individual steps leading to the bicyclic systems. The different synthetic approaches which are being explored regarding cyclization and cross-coupling procedures, as well as reaction order and protection schemes, will be discussed.
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P048. Abstract number: 384
Synthesis of C-11 linked active ester derivatives of vitamin D3 and their conjugations to 42-residue helix-loop-helix peptides
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The chemical synthesis of two analogs of Vitamin D3 carrying a linker arm protruding from C-11, and their activation and conjugations to the lysyl side-chain amino group of a 42-helix-loop-helix peptide is described. The Vitamin D3 analogs were designed based on the known structure of Vitamin D-binding protein (VDBP) - ligand (Vitamin D3) complex where C-11 is outside of the binding pocket, and attachment of a C-11 linker arm to vitamin D should therefore not interfere with VDBP binding. The synthesis of the C-11 modified derivatives started with natural Vitamin D3 which was cleaved in two fragments. After further modification (including attachment of a linker arm at C-11 of the ”upper”fragment), the two fragments were joined together again by a Wittig-Horner reaction. The resulting molecules were activated and coupled to lysyl side-chain amino groups of 42-residue peptides. The obtained Vitamin D3 -polypeptide conjugates are intended for use in measurement of VDBP levels in plasma and cerebrospinal fluid. Changes in VDBP levels has been correlated to diseases such as Alzheimers and Parkinsons diseases.

P049. Abstract number: 388
Synthesis and Biophysical Characterization of a Backbone-Cyclized Minimized Z Domain
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Antibodies are to date the most widely studied and employed class of affinity molecules. However, other protein-based affinity scaffolds have several properties that make them attractive as alternatives, both for technical applications and for therapeutic use. Their small size, robustness, and the amenability to production by means of solid phase peptide synthesis are features that are appealing for many purposes.
We have produced a backbone-cyclized affinity protein based on the S. aureus-derived IgG-binding Z domain, which is a three-helix bundle of 58 aa. In the Z domain, only helices 1 and 2 are directly involved in the immunoglobulin binding. By leaving out the third stabilizing helix from the protein and generating a native peptide bond between the N- and C-termini, we have assembled a new, minimized Z scaffold denoted Zmin.
The amino acid residues in helices 1 and 2 that in the three-helix bundle protein are situated in the stabilizing hydrophobic core, have been substituted to more hydrophilic amino acids according to an earlier published study on a minimized Z domain protein [1]. The N- and C-termini of the protein were linked by native chemical ligation, producing a circular, backbone-cyclized, two-helix protein with a unique cysteine residue at the ligation site, which could be used for directed immobilization or site-specific labeling.
Biophysical characterization shows that Zmin has retained high affinity to human polyclonal IgG. The smaller size (35 aa) facilitates solid phase synthesis and might provide advantages over the native scaffold for specific applications such as tumor imaging and targeting (as a result of better tissue penetration and faster clearance). Furthermore, the lack of N- and C- termini is expected to make the scaffold more robust and insensitive to exoprotease degradation. By immobilizing Zmin onto solid support the protein can be used for affinity purification of IgG, thus showing a potential technical application for the new scaffold. The cyclized two-helix structure of Zmin has comparable binding affinity to the three-helix Z domain, but with the aforementioned advantages that the smaller size and lack of termini provides.
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P050. Abstract number: 390, *withdrawn*

P051. Abstract number: 397
Structural studies of host-quest complexation between with 9-aminoacridine and amino acids
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9-Aminocridine and its derivatives present a very interesting object of research. This group of compounds exhibits a wide spectrum of biological activities as well as antibacterial, antiamoebic, antitumor, antiprion, anti-implantation, anti-inflammatory, hypertensive and mutagenic. The potency of acridines as agents is due to their ability to bind DNA through intercalation. 9-Aminocridine is also used as a ΔpH-probe in a variety of biological systems. X-ray crystallographic investigations have been carried out on the salts of 9-aminoacridine derivatives with different kind of ”guests”to understand their specific properties. In this communication we report the synthesis and X-ray characterization of a series of salts with the 9-aminoacridine as ”host”and the some amino acids as ”guests”.

P052. Abstract number: 416
Racemization-free synthesis of cyclic peptides by use of the O-acyl isopeptide method
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Cyclic peptides generally possess higher biological activity and metabolic stability than their corresponding linear peptides due to conformational constraints. Despite the promises of the cyclic peptides in drug development, a head-to-tail cyclization reaction of the corresponding linear peptide is often accompanied by incomplete coupling and/or epimerization.[1] The epimerization occurs because peptides, in contrast to urethane-protected amino acids, easily form chirally labile oxazolones upon C-terminal carboxyl activation.

We previously reported a novel convergent method of peptide synthesis: racemization-free segment condensation methodology based on the *O*-acyl isopeptide method.[2-5] The idea was that an *N*-segment with a C-terminal *O*-acyl isopeptide structure at a Ser or Thr residue could be coupled to an amino group of the *C*-segment without epimerization, because the amino group of the C-terminal isopeptide is protected by a urethane-type protective group. Thus, formation of the racemization-inducible oxazolone was suppressed on the carboxyl group activation. Finally, the target peptide was generated from the *O*-acyl isopeptide via an *O*-to-*N* intramolecular acyl migration reaction.

We herein demonstrate that the cyclic peptide can also be synthesized in a racemization-free fashion by use of the *O*-acyl isopeptide method. A head-to-tail cyclization of the protected linear peptide with the C-terminal *O*-acyl isopeptide proceeded by HATU-based coupling to give the cyclic *O*-acyl isopeptide without epimerization. Interestingly, the cyclic *O*-acyl isopeptide possessed different secondary structure compared to the native cyclic peptide, suggesting that a well-known secondary structure disrupting effect by the introduced *O*-acyl isopeptide, observed in many linear peptides, was exerted in the cyclic peptides. Finally, the isopeptide was efficiently converted to the desired cyclic peptide via the *O*-to-*N* acyl migration with a silica gel-anchored base.

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P053. Abstract number: 418

New Synthetic Strategy for N-Alkylation Reaction: Validation of the Method by Synthesis of N-Alkylated Fmoc-Amino Acids.

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Therapeutically relevant peptides themselves are seldom used in the clinic because of their poor biostability, unfavorable absorption properties, as well as poor receptor subtype selectivity. Nevertheless, they can be used as a template in order to synthesize modified peptides, called peptidomimetics, which are endowed with increased pharmacological activities. One possible strategy to favor the formation of bioactive conformations is modifying the side chain of amino acids.[1] In fact, the field of amino acids modifications has gained a big relevance in recent years, particularly with the emergence of new building blocks that allow introducing chemical and functional diversity into molecules with therapeutic potential.

Herein we describe a facile and general procedure for N-alkylated Fmoc-amino acids, as building blocks for peptide synthesis. The reported synthetic strategy is straightforward and affords desired products in very high yield. One of the building blocks obtained was proven to be efficiently introduced into peptide sequence, thus allowing the preparation of site-specific alkylated peptide molecules.

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P054. Abstract number: 428

Synthesis and activity studies of cyclic analogues of KLK3-stimulating peptide B-2

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Human prostate produces kallikrein-related peptidase 3 (KLK3, also known as prostate specific antigen), which is widely used as a prostate cancer marker. Moreover, proteolytically active KLK3 has been shown to inhibit angiogenesis and its expression decreases in poorly differentiated tumors. Thus, with agents that stimulate the proteolytic activity of KLK3, it may be possible to control prostate cancer growth. We have earlier developed synthetic peptides, which bind specifically to KLK3 and promote its proteolytic activity (Wu et al. 2000). These peptides are cyclic containing a disulfide bridge between the N- and C-terminal cysteines. We have previously showed that head-to-tail cyclization of KLK2 inhibiting peptide increased the proteolytic stability of the peptide in vitro (Pakkala et al. 2007). To increase the in vivo stability of the KLK3-stimulating peptide B-2, we designed and synthesized a set of differently cyclized analogues of peptide B-2 (Pakkala et al. 2010). We replaced both terminal cysteines and the disulfide bridge between them using variable cyclization strategies. Stimulation of activity of KLK3 were studied with cyclic analogues and results were compared to original B-2 peptide. One of peptides where disulphide bridge was replaced with mimetic consisting of gamma-amino butyric acid and aspartic acid, was found to be more active than the original B-2 peptide at high concentrations. Furthermore, we studied in vitro stability of the most potent peptide analogue in human plasma and recombinant KLK3. The most potent of the analogues was found to be more stable than original B-2 peptide in both studies.

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P055. Abstract number: 439

Traceless chiral triazine based coupling reagent. A new concept for synthesis of optically peptides products from racemic carboxylic acids

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The rapid development of the methods of combinatorial chemistry in the systematic exploration of molecular diversity and in the search for improved activities and properties of a broad range of synthetic materials have resulted in a vigorously growing demand for numerous new chiral substrates with diversified structural features. In most

cases, enantiomerically active substrates are needed only in tiny amounts. Therefore, an approach based on the enantiodifferentiating transformation of usually easily available racemic substrates would be valued as more advantageous than the classic procedure involving racemate resolution or asymmetric synthesis. Such an approach would be considered the most convenient in the case of coupling chiral building blocks like amino acids used for the construction of more complex molecules. In order to facilitate these studies in case of syntheses involving condensation of chiral carboxylic acid we developed the concept of a traceless chiral coupling reagent useful for enantioselective synthesis of optically active peptides from racemic carboxylic acids (amino acids). According to the concept, coupling reagents consists of two fragments with chiral auxiliary responsible for enantioselectivity excluded just during activation of carboxylic group. Thus, each achiral TBCR could be (potentially) an ancestor of the whole family of traceless chiral coupling reagents.

Chiral N-(4,6-disubstituted-1,3,5-triazin-2-yl)ammonium tetrafluoroborates 3 were prepared in high yield by the treatment of chiral ammonium tetrafluoroborate (1) with 2-chloro-4,6-disubstituted-1,3,5-triazine (2) in the presence of sodium bicarbonate [1]. 3 were found stable at room temperature and as a solution in broad range of solvents yielding acylated products (amides, esters and peptides) with fully predictable configuration and ee (dr) ranging from 95/5 to 60/40 under reaction conditions and yield identical as optimized for its achiral ancestor [2].

This work was supported by Ministry of Science and Higher Education, Grant 6/PMPP/U/30-09.08/E-370/2009

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P056. Abstract number: 467

A critical comparison of sequence independent solid phase methods for the site-specific synthesis of multiple sulfated peptides.

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Although much less known than protein phosphorylation, the significance of protein sulfation is rapidly gaining momentum. Based upon the analysis of tyrosine sulfation in Drosophila Melanogaster, it is estimated that as much as 1% of all tyrosine residues occurring in proteins can be sulfated. Together with phosphorylation, it is the most frequently occurring post-translational modification of tyrosine.

To study the role and the importance of sulfation, the availability of reliable and convenient methods for the preparation of crucial sulfated peptides is required. Until recently no reliable general sequence independent method was available for the site-specific incorporation of several sulfated tyrosine residues. Clearly, the limited number of methods reflected the relative instability of sulfated tyrosine residues as compared to phosphorylated tyrosine residues in peptides and proteins.

Recent methods for the synthesis of sulfated peptides include the approach of Ali and Taylor [1] using sulfated tyrosine building block protected with a dichlorovinyl group in conventional Fmoc/tBu SPPS. In addition, Simpson et al. [2] described the use of a neopentyl protected sulfated tyrosine building block. We have described [3] a general sequence independent solid phase method by which a peptide is synthesized by Fmoc/tBu SPPS, followed by selective deprotection of the tyrosine residues to be sulfated and introduction of a 2,2,2-trichloroethyl protected sulfate moiety. Upon completion of the synthesis of the sulfated peptide, it is cleaved from the resin by acidolysis and protective groups are removed with the exception of the sulfate protective group thereby preventing undesired acid induced removal of the sulfate group(s) during this step. Finally, after purification by HPLC (TFA-buffers) the sulfate protective groups were removed in a slightly acidic (pH 6.4) reductive step, leaving the sulfate groups untouched. In this communication we will present a critical comparison of these methods and discuss new developments for the synthesis of multiple sulfated peptides.

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P057. Abstract number: 470

Microwave-assisted reaction of building blocks for glycopeptides synthesis

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Solid Phase GlycoPeptide Synthesis (SPGPS) is an efficient method to obtain glycosylated peptides bearing different sugar moieties on the side chains of Asn, but it requires an excess of building block units to achieve high yields.[1] As a consequence, it is important to set up convenient reactions, in terms of yield and time-consuming, leading to glycosyl asparagine residues orthogonally protected for SPPS by Fmoc/tBu strategy. Therefore, we focused our attention on optimization of the key synthetic steps by large-scale microwave-assisted synthesis of N-β-linked glycans linked to side chains of Asn and Gln, via glycosylamines.[2] In fact, microwave technology is widely and advantageously used in different fields of organic synthesis, because reactions are usually clean, efficient and require short times.

Herein we report the effects of microwave irradiation on the coupling reaction of β-D-glucopyranosylamine with the carboxylic side-chain of aspartic acid.[3] The synthesis of N-protected glycosylamino acids from amines has been investigated and it was found that, under strong microwave conditions, glycosylamines could be hydrolyzed leading to new derivatives containing a glycosyl ester linkage. The efficiency of the microwave-induced glycosylation of aspartic acid was studied comparing the microwave-activity between amide and ester bond formation. Different sugar moieties have been employed to demonstrate the simple and reproducible coupling methodology.[3]

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P058. Abstract number: 475

Sulphonates Of N-Triazinylphosphonium Salts As Coupling Reagents For Peptide Synthesis In Solution

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N-triazinylammonium tetrafluoroborates [1] and sulphonates [2] were found highly efficient coupling reagents useful in the peptide synthesis in solution and solid phase (SPPS). Herein, we attempt to expand the family of triazine based coupling reagents by including a new generation of coupling reagents based on N-triazinylphosphonium salts **5**. Sulphonates of N-triazinylphosphonium salts **5** were obtained by treatment 2-chloro-4,6-dimethoxy-1,3,5-triazine **1** with sulphonates of tertiary phosphine **4** in the presence of sodium bicarbonate.

Broad range of sulphonates of N-triazinylphosphonium salts were found useful for activation of carboxylic components, with the activation rate depended mostly on the structure of tertiary phosphine constituent **2a-b**. The participation of triazine ”superactive ester”as intermediate in the condensation has been proved in the model experiments. Utility of reagents **5** was confirmed by peptide synthesis in solution. All of them were found efficient as condensing reagents and gave Z-, Fmoc- and Boc-protected peptides in 70-93% yield.

This work was supported by Ministry of Science and Higher Education, under the Research Project: N N204 228734.

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P059. Abstract number: 477

Strategies for macrocyclic cyclotide synthesis

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Cyclotides are a recently discovered class of proteins that have a characteristic head-to-tail cyclized backbone stabilized by a knotted arrangement of three disulfide bonds (1, 2). Cyclotides display a number of bioactivities including, uterotonic, anti-bacterial, anti-HIV and anti-tumour activities, but their natural physiological role is thought to be in plant defence (3). They are resistant to chemical, thermal and enzymatic treatments and as such their high stability has motivated their development as scaffolds to stabilize peptidic drugs (4, 5). Currently, cyclotides are mainly extracted from plants of Rubiaceae and Violaceae families (1, 6). To facilitate their structure-activity studies and to exploit their development into pharmaceutical and agricultural agents, a convenient route for the synthesis of cyclotides is essential. Until now a thioester mediated solid phase synthesis strategy based on Boc chemistry has been the most robust approach for cyclotide synthesis (7). However, the acid based deprotection step during amino acid coupling and the potentially harmful strong acid based step in the cleavage of the peptide from the resin at the end of synthesis have raised questions regarding the safety of this protocol in routine cyclotide synthesis. A solid phase peptide synthesis protocol based on Fmoc chemistry, assisted by the solvent dimethyl formamide and microwave irradiation was recently optimized by us for the production of cyclotides. This is an advancement in cyclotide synthesis as currently this remains the most safe, cost-effective and time-efficient strategy to synthesize cyclotides by chemical intervention. In this research paper all existing chemical, recombinant and biosynthetic routes to the synthesis of cyclotides will be discussed.

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P060. Abstract number: 511

Triazine condensing agents for synthesis of peptide bond in aqueous media

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Successful synthesis of the peptides bond in aqueous media still remains the challenge although several attempts made to resolve the problems. To perform peptide synthesis in water, the coupling reagent must be water-soluble, maintain solubility and reactivity of intermediate products, preserve enantiomeric homogeneity of products in strongly polar environment and form conveniently removable waste (if any) in order to facilitate isolation the main product. The pursuit for aqueous-compatible coupling reagent is promoted both by environmental problem of the safe disposal of large amounts of organic solvents required for SPPS, as well as by demand for efficient method useful for modification of complex, incompatible for organic solvents, substrates such as proteins and carbohydrates.

Taking advantage of modular structure of triazine coupling reagents we tested the efficiency of the entire family of N-(4,6-(disubstituted-1,3,5-triazinyl-1)ammonium salts in aqueous media. It has been found that under advantageous conditions, yield of isolated peptides was increased to 90-95% after optimization of coupling procedure and structure of reagents. The procedure has been found suitable for synthesis of small peptide and modification of lysozyme by attachment of chromogenic marker.

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P061. Abstract number: 512

The Synthesis Of The Pb1 Protein Fragment Of Rna-Polymerase Of Influenza A Virus

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RNA-polymerase of influenza A virus consists of three subunits namely PB1, PB2 and PA. The tertiary structure of this trimer was determined what allowed to localize domains of intermolecular interaction [1]. The fragment 1-25 of subunit PB1 was demonstrated to effectively connect the subunit PA. Said fragment competes with PB1 and thereby prevents the organization of active RNA-polymerase complex [2].

The objective of the present work was to synthesize the fragment 1-25 in order to check literature data ant to use it in our further investigations. The amino acid sequence of the fragment 1-25 is represented below:

1 2 3 4 5 6 7 8 9 10 11 12 13 14
H-Met-Asp-Val-Asn-Pro-||-Thr-Leu-Leu-Phe-Leu-Lys-Val-Pro-||-Ala-
15 16 17 18 19 20 21 22 23 24 25
Gln-Asn-Ala-Ile-Ser-Thr-Thr-Phe-Pro-Tyr-Thr-OH

The target compound was synthesized via solid-phase synthesis using the Fmoc-t-Bu strategy by sequential elongation of the peptide chain on the 2-Cl-Trt resin. DIC and HOBt were used as condensing agents and DMF was used as a solvent for reactions, 2-3 excess of each amino acid were used. Purifying of the compound obtained was performed with the aid of the preparative reversed-phase HPLC and the resulting purity was higher than 95%. The molecular ion in the mass-spectrum (MALDI) corresponded to the calculated value.

The peptide 1-25 was also synthesized in a convergent manner to optimize the synthesis and to obtain fragments of the sequence under discussion for biological tests.

The convergent manner of synthesis of the peptide 1-25 of subunit PB1 is preferred since it allows to simplify final product purification in the conditions of reversed-phase HPLC and to use fragments being obtained during the synthesis of the peptide 1-25 for biological tests.

P062. Abstract number: 523

Cationic Peptides that Increase the Thermal Stability of 2'-O-MeRNA/RNA Duplexes, but that do not affect DNA/DNA hybridization

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Several different cationic nonapeptides have been synthesized and investigated with respect to how these can influence the thermal melting of 2'-O-methylRNA/RNA and DNA/DNA duplexes. The peptides have a C-terminal L-phenylalanine and are otherwise uniformly composed of a sequence of a specific basic D-amino acid that in most cases will be largely charged at neutral pH. These N-terminal 8-mer stretches are composed of either of the amino acids D-lysine, D-diaminobutyric acic (D-Dab), D-diaminopropionic acid (D-Dap) or D-histidine. None of the peptides affected the thermal melting of the DNA/DNA duplexes substantially which was in strong contrast to the effect on the 2'-O-methylRNA/RNA duplexes. In particular, the diaminopropionic and diaminobutyric acid containing peptides had a strong positive effect on the melting points of the 2'-O-MeRNA duplexes (up to 16 °C higher with 1 equivalent of peptide compared to RNA alone) at pH 7, and at pH 6 the effect was even more drastic (ΔTm up to + 25 °C). The shorter R-groups of the Dap and Dab groups appear to be of more optimal length than lysine for enhancement of the thermal melting of the 2'-O-MeRNA/RNA duplex, an effect that is more pronounced at lower pH but substantial even at pH 7, where the Dap derivative is not likely to be fully protonated. The dramatic difference between the influence, or lack thereof, on the 2'-O-MeRNA/RNA and the DNA/DNA thermal meltings suggest that, although electrostatic interaction probably plays a role, there is another major and structurally related component influencing the properties of the duplexes. This is also seen in that the oligo- Dap and oligo- Dab peptides give a greater melting point enhancement than both the lysine peptide (with a longer side chain) and a β-linked Dap peptide with a shorter side chain and longer backbone.

P063. Abstract number: 546

Is oxidation the trigger of the Amyloid Cascade?: A synthesis of 2-oxo-histidine for incorporation into the Amyloid β sequence.

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Amyloid beta (Aβ) peptide aggregations form the amyloid plaques found in the brains of Alzheimer's sufferers. Currently the Amyloid Cascade Hypothesis is the best understood theory for the downstream events that occur once the neurotoxic, oligomeric form of Aβ is produced in this aggregation pathway. However, the trigger for the initial misfolding of Aβ is less well defined. One possible trigger is the oxidation of Aβ to the oxidised form occurring in amyloid plaques. In addition, the Aβ peptide co-ordinates metal ions, which have roles in metal catalysed oxidation (MCO). We hypothesise that this MCO is directed towards the histidines which co-ordinate the metal, producing 2-oxo-histidine. This may be involved in the subsequent mis-folding of Aβ, altering the rate of its fibrillisation.

Histidine cannot be selectively oxidised in the Aβ sequence, so we devised a synthesis of 2-oxo-histidine for incorporation into the Aβ sequence via solid-phase synthesis. Initially synthesis was attempted from L-histidine using three methods: MCO with a Cu(II)/ascorbate system; Bamberger cleavage of the imidazole ring with subsequent ring closure; and dimethyldioxirane-mediated oxidation. Many derivatives of oxidised histidine were successfully produced, including 2-oxo-histidine in low yields using MCO. Furthermore, novel oxidised derivatives with addition of oxygen at C4(5) or to nitrogen in the imidazole ring were produced using dimethyldioxirane mediated oxidation.

A more successful synthesis of 2-oxo-histidine was developed starting from urea and tartaric acid. These were condensed together to form the known imidazolin-2-one. Esterification and protection followed by reduction converted this into the imidazoline alcohol. This alcohol was oxidised to an imidazoline aldehyde which was coupled to a phosphonate with amino acid functionality in a Horner-Wadsworth-Emmons reaction to form the dehydro-derivative of 2-oxo-histidine. Hydrogenation of this led to a protected form of racemic 2-oxo-histidine. Current work focuses on obtaining 2-oxo-histidine by asymmetric hydrogenation of the dehydro-derivative of 2-oxo-histidine. Once we have an enantiomerically pure form of 2-oxo-histidine we will incorporate this into the Aβ sequence to study the fibrillisation rate of oxidised Aβ using a Thioflavin-T biochemical assay. We hope that these fundamental studies may lead to better understanding of the etiology of Alzheimer's pathology and thus aid rational drug design.

P064. Abstract number: 549

Total Chemical Synthesis of a Glycoprotein and Structural Analysis by Quasi-Racemate X-ray crystallography.

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We report the total chemical synthesis of the chemokine I309, a small glycoprotein having an N-linked oligosaccharide, for X-ray structural analysis by the racemic crystallization method. Glycosylation is one of the most frequent post-translational modification of proteins in a biological system and impacts several aspects of protein function such as immunogenicity, protein folding, or clearance of the protein from blood fluid. In spite of the

significance of three-dimensional structure of a protein for its biological function, there are well known difficulties in X-ray diffraction structural analysis of glycoproteins, which can be hard to crystallize because of the heterogeneity of the oligosaccharide moiety. In order to access a single glycoprotein crystal, a homogeneous glycoprotein is needed, as well as powerful crystallization methods. Convergent chemical synthesis of I309 was performed by use of two peptide and one glycopeptide segments. Coupling these segments by native chemical ligation followed by conventional folding reaction afforded three distinct protein molecules: desired glycosyl-L-I309; non-glycosyl L-I309; and, non-glycosyl D-I309 made from D-amino acids. Crystallization of the homogeneous glycocyl-L-protein was still difficult. However, in contrast, we could get a single crystal by means of quasi-racemic crystallization, from a mixture of glycosyl-L-I309 and non-glycosy D-I309. Diffraction data has been acquired to 2.5 Angstroms, and structural analysis is now ongoing. Quasi-racemate crystallization may be a powerful method for the structural analysis of glycoproteins.

P065. Abstract number: 559

Organic chemistry on synthetic peptide surfaces

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The design and application of foldamers is expanding and maturing rapidly. In particular the ability of β-peptides (i.e. peptides consisting entirely of cyclic and/or acyclic β-amino acids) to form stable, helical structures in solution has led to applications in peptidomimetics as well as new materials. The 14-helix is particularly important because of its almost perfect pitch (i.e. almost exactly 3 residues per turn).

We have recently developed an efficient, metathesis-based, approach to ”stapling”β-peptides. In this presentation recent we will describe the physical and chemical properites of these new molecules with an emphasis on better understanding factors which stabilize their helical structure. Results from our laboratories on the chemical functionalisation of the surface of such structured materials, employing a variety of synthetic protocols (including, inter alia, metathesis, hydrogenaton, dihydroxylation and Pauson-Khand reactions) will be also be discussed. The presentation will conclude with a description of our recent discovery that these molecules show a remarkable propensity to self-assemble as well as crystallize.

P066. Abstract number: 567

Aza-β3-amino acid containing peptidomimetics as cAMP-dependent protein kinase substrates

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Peptidomimetic analogs of RRASVA, known as the ”minimal substrate”for the catalytic subunit of cAMP dependent protein kinase (PKA), were synthesized by consecutive replacement of natural amino acids by their aza-β^3-analogs. These pseudopeptides were tested as PKA substrates and kinetic parameters of phosphorylation reaction were determined. It was found that binding of these peptidomimetics with the enzyme active center was sensitive to location of the backbone modification, while the maximal rate of the reaction was practically not affected by the position of aza-β^3-analog residue in pseudopeptide. The kinetic parameters of phosphorylation reaction were in correlation with calculated data of structure modeling and computational studies of peptide docking in enzyme active center. It was concluded that certain structural fragments, modification of which influences binding kinetics can be distinguished for consensus sequence aza-β^3-analogs. Defining these structural features might open new perspectives for pharmacophore design of peptide and peptide-like PKA ligands.

P067. Abstract number: 573

Structure-activity studies of Angiotensin IV analogues containing the conformationally constrained Aia residue.

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Angiotensin IV: H-Val-Tyr-Ile-His-Pro-Phe-OH (Ang IV) is a bioactive metabolite of Ang II which mediates a wide range of physiological actions such as the ability to improve learning and memory, the inhibition of induced seizures and which has vascular and renal actions. It was proposed that Ang IV exerts its effects by binding to AT4 receptors. Those were recently identified as cystinyl aminopeptidase (CAP, also denoted as IRAP). It is still not clear how Ang IV exerts its biological effects. This could be through inhibition of the activity of IRAP or through activating its receptor function. Ang IV is also capable to inhibit the activity of aminopeptidase N (AP-N), which might represent an alternative target for Ang IV [1,2]. We have reported earlier that the □-homo amino acid containing analog H-□2hVal-Tyr-Ile-His-Pro-□3hPhe-OH (AL-11) is a potent, selective and stable Ang IV antagonist, in which the □2hVal is responsible for stability and the □3hPhe for selectivity [3]. The substitution of the His4-Pro5 dipeptide sequence in Ang IV by the constrained Trp analog Aia-Gly, in combination with □2hVal substitution at the N-terminus, provided a new stable analog H-(R)-□2hVal-Tyr-Ile-Aia-Gly-Phe-OH (AL-40) that is a potent ligand for the Ang IV receptor IRAP, and selective versus AP-N and the AT1 receptor.[4]

In this study we have replaced the Gly residue in AL-40 by different substituted amino acids and investigated the influence on the potency to inhibit the rate of Leu-pNA cleavage in membrane homogenates of HEK293 cells transient transfected with human IRAP or AP-N in the presence of different concentrations of compound. [3]

Stability experiments were performed by incubating the peptides in the presence and in the absence of metal chelators, with membrane homogenates of CHO-K1 cells which contain endogenous IRAP, followed by a competition binding assay by adding [3H]Ang IV. Moreover stability in human serum will be reported.

We thank the 'The Fund for Scientific Research - Flanders' (FWO, Belgium) for financial support.

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P068. Abstract number: 9

The Best Stationary Phase for Your Process HPLC Purification

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Though at analytical scale HPLC 'all ODS are the same' as common argument states, the small differences grow to very important proportion upon scaling up your projects.
Silica based stationary phases come in a great variety, and the important aspects are not clear or neglected. That leads often to the bad scenario where the purification process is adjusted, fine tuned for the properties of the stationary phase. Definitely the opposite should be the case: Chose the best fitting kind of stationary phase for each process.
The way of choosing is described here. The physicochemical properties of chemically bonded silica is explained in view of its strong influence of the separation characteristics.
Examples of peptide and peptide based API separation are shown especially on insulin.

P069. Abstract number: 29

A Novel Method to Prepare Cyclic Peptides at Non-cysteine sites using the Auxiliary group, 4,5-dimethoxy-2-mercaptobenzyl (Dmmb)

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Many cyclic peptides are of therapeutic interest but the preparation of them is a challenge even though several methodologies using cyclization on-resin or in solution is well described in the literature [1]. This work presents a new general cyclization method which apply the auxiliary group, 4,5-dimethoxy-2-mercaptobenzyl (Dmmb) to form end-to-end cyclic peptides in aqueous solution solution without protection of the side-chains. For native ligation [2] several examples are known [3,4] using N□-(4,5-dimethoxy-2-mercaptobenzyl) (Dmmb) peptide fragments to form longer peptides segments at X-Gly or X-Ala sites with C-terminal thioester peptides in three step (1) transthioesterification (2) S to N acyl shift and (3) removal of the acyl transfer auxiliary group. This strategy was applied for synthesis of cyclic peptides and peptide (1) was prepared using standard Fmoc/tBu chemistry followed by the cyclization reaction. The overall synthetic strategy for preparing cyclic peptides using the auxiliary group Dmmb as well as other cyclization methods for comparison will be discussed.

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P070. Abstract number: 35

Do you need small particle HPLC media for peptide purification?

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Recent developments in synthesis resins have resulted in improvements in the quality of synthetic peptides being produced by solid phase synthesis strategies. However, in many instances there is still a requirement to improve peptide purity. Reverse phase HPLC has been the method of choice for synthetic peptide purification but the capital outlay for the gradient HPLC instrument needed to run small particle preparative columns can contribute a significant cost to the peptide production process.
Our work investigates the media and instrumentation requirement for purification and the format and type of liquid chromatography column needed to achieve the desired purity. Data will also be presented to show how media selectivity can be exploited to improve throughput and minimize the column size needed.

P071. Abstract number: 43

Synthesis, Cyclization and Oxidative folding of backbone engineered Cyclotides

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Cyclotides are a cyclic peptide with a cystine knot. Due to their exceptional stability, there has been significant interest in developing these molecules as a drug design scaffold. For this potential to be realised efficient methods for the synthesis and oxidative folding of cyclotides need to be developed. Herein we present a successful strategy for the synthesis, cyclization and oxidative folding of engineered cyclotides.

P072. Abstract number: 48

A Novels Polymer Encapsulated Silica Stationary Phases for Reversed-Phase HPLC of Peptides

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With ”Pathfinder® BIO”, we introduce a new generation of RP-HPLC packing materials for biochromatography which is essentially different form the known conventional RP stationary phases. In comparison to other new novel hybrid materials, these new hybrid silica have organic groups only at the surface. This preserves the characteristics of the silica with only minimize influence of residual silanol groups and share the chemical inertness, temperature and pH stability of organic polymers. We will show in comparison to other RP-silica that his material can be used across the pH range from 1 to 12, while providing extended level of performance and lifetime. It shows a powerful separation of Insulin (hRI). As a further special advantage, we wil show that these new Pathfinder® phases have extremely high hydrophobic capacity and strong enhanced sterical selectivity at entire pH ranges that is nearly analogous to polymeric phases. Providing these properties in the range of 1.5 - 200 micron silica, Pathfinder® BIO can be used for high resolution peptide analytics (UPLC) and analytical HPLC and can be also linear upscaled to large scale preparative HPLC and Flash-LC peptide purification.

P073. Abstract number: 58

Mouse insulin-like peptide 5: chemical synthesis and structure-function relationship

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Insulin-like peptide 5 (INSL5) is a member of insulin/relaxin superfamily of peptides that includes insulin, insulin-like growth factor I and II (IGFI and IGFII), insulin-like peptide 3, 4, 5 and 6 (INSL3, 4, 5 and 6), relaxin-1 (H1 relaxin), -2 (H2 relaxin) and -3 (H3 relaxin)1. Although it is expressed in relatively high levels in the gut, its biological function remains to be clarified. However, a recent patent application points to a significant orexigenic action and a role in appetite regulation which suggests that both agonists and antagonists of the peptide may have significant therapeutic applications. INSL5 has also

recently been identified as the cognate ligand for the G-protein-coupled receptor, RXFP42.

We have previously prepared human INSL5 via a combination of solid phase peptide synthesis and regioselective disulfide bond formation3 and determined its solution structure4. However its chemical synthesis was unexpectedly difficult due to the intractability of the A-chain and the overall poor solubility of the peptide. For this reason, we have focused on the preparation and use of the more hydrophilic mouse INSL5 and its point-mutated analogues for the study of its structure-function relationship in order to identify key receptor binding and activation residues.
Synthetic mouse INSL5 was assembled in improved overall yield relative to the human peptide and shown to inhibit cAMP activity in CHO cells that expressed the human RXFP4 receptor with a similar activity to human INSL5. In contrast, it had no activity on the human relaxin-3 receptor, RXFP3. Synthetic mouse INSL5 in which the two constituent chains were assembled as their C-terminal amides has also been prepared. Chain truncation studies are also underway which will identify the minimum sequence required for RXFP4 binding activity.
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P074. Abstract number: 59

Temporary Solubilization Of Synthetic Peptides By C-Terminal-Linked Poly-Lys Tail: Application To The SPS Of Insulin Glargine

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The post-synthesis handling of poorly soluble peptides often remains a significant hindrance to their purification and further use. Use of strong solvents, acids or chaotropes for their solubilization do not allow convenient purification nor their chemical characterization. Alternative approaches to improving the solubility of synthetic peptides have involved temporary chemical modification of the peptide itself with, for example, O-acyl isoacylpeptides1. However, their use is limited by their high cost as well as the requirement of a convenient amino acid sequence for their introduction. Polycationic peptides have long been known to enhance the solubility of peptides and proteins. A recent study showed that pronounced solubilization was afforded by five Lys or Arg residues particularly if these were linked at the C-terminus rather than the N-terminus of the target protein2. Given that lysine derivatives are cheaper and generally easier to couple in SPPS , we decided to exploit these findings and we describe here a simple approach based on the use of penta-lysine 'tags' that are linked to the C-terminus of the peptide of interest via a base-labile linker. After ready purification of the now freely solubilized peptide, the 'tag' is removed by simple, brief base treatment giving the native sequence in much higher overall yield. The applicability of the method was demonstrated by the first ever preparation of insulin glargine via solid phase synthesis of each of the two chains - including the notoriously poorly soluble A-chain - followed by their combination in solution via regioselective disulfide bond formation. At the conclusion of the chain combination, the solubilizing peptide tag was removed from the A-chain to provide synthetic human glargine in nearly 10% overall yield3. This approach should facilitate the development of new insulin analogues as well as be widely applicable to the improved purification and acquisition of otherwise poorly soluble synthetic peptides.
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P075. Abstract number: 62

Design And Synthesis Of Cyclodextrin/Gnrh Analogue Conjugates For The Treatment Of Hormone Depended Cancer

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Cyclodextrins are cyclic oligosaccharides consisting of a hydrophobic cavity that is capable of including a variety of hydrophobic compounds via host-guest complexation.[1] This property has been extensively exploited in the past to change the physicopharmaceutical properties of lipophilic drugs such as water-solubility, bioavailability, improved stability, and effectiveness. Covalent linkage of bioactive peptides to cyclodextrins has also been proposed[2,3] to possibly take advantage of this complexation in terms of solubility and reduced enzymatic degradation. Gonadotropin Releasing Hormone (GnRH) is a linear decapeptide which is produced in the hypothalamus under the control of neurotransmitter type compounds[4] and it is the central regulator of the reproductive system[5]. GnRH agonist analogues, such as Leuprolide, Buserelin are widely used for the treatment of hormone depended cancer and they have found to be more potent than the natural hormone. The goal of this study was the design and synthesis of β-cyclodextrin/GnRH analogues conjugates with improved biophysical properties, compared to wild hormone, in order to be studied for antitumor activity[6].
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P076. Abstract number: 85

Synthesis of Peptide Libraries on the Overture□

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Peptide libraries are groups of peptides with systematic combinations of amino acids that are an extremely useful tool for drug discovery. Peptide libraries can be used to identify target ligands and then optimize them for potency, selectivity, and stability [1]. Automation plays a pivotal role in increasing the speed and efficiency of this process. The Overture® Robotic Peptide Library Synthesizer was designed to generate and synthesize peptide libraries in an easy to use, high-throughput format. The Overture's® flexible reaction vessel configurations and software allow libraries from 4 to 96 compounds to be made, including overlapping peptides for epitope mapping, and alanine-scanning libraries, positional scanning libraries, random libraries, truncation peptide libraries and scrambled peptide libraries for sequence optimization.

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P077. Abstract number: 92

The use of a cleavable 'Tag' moeity to dramatically increase peptide purification yield.

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Modern methods in peptide synthesis have resulted in the ability to synthesis peptides of ever increasing length and complexity. Whilst resins, solvents, and coupling agents have all been the focus of much attention in offering improvements and alternatives to synthetic methodology, purification techniques have received relatively little attention, and preparative HPLC remains the workhorse method. Traditional HPLC methods have difficulty in separating related impurities from the desired peptide product. We describe a method of purification of peptides synthesised by Fmoc chemistry, which provides product of equivalent or superior quality, when compared with HPLC, but with a dramatic two to three-fold increase in yield.

The improved method involves the use of a cleavable Tag, and takes advantage of metal-binding properties, which are used in the Immobilised Metal Affinity Chromatography (IMAC) purification of recombinant proteins. The Tag incorporates 8-hydroxyquinoline which acts as a metal binding moiety, and is coupled to the peptides via succinimidyl carbonate functionality. The Tag is cleavable under basic conditions through the presence of a beta-sulfone group.

Full length, protected, resin-bound peptides are labelled on the N-terminus with the Tag using standard coupling conditions. The peptide is then cleaved from the resin under standard conditions, with the only the full length peptide of choice labelled with the Tag. The crude peptide is then subjected to IMAC which selectively removes the Tagged peptide from the crude peptide mix. The Tag is removed by treatment at pH 11, and the cleaved Tag removed by a further IMAC pass.

Direct comparisons between Tagging methods and classical HPLC purification methods were carried out using several peptides of different lengths (12-76 amino acids). The study indicated that the Tagging approach resulted in products of at least the same purity as HPLC purification, but that yield was increased by two to three times. Furthermore, the IMAC purification and Tag cleavage process causes no detrimental effect on the peptide.

P078. Abstract number: 103

Novel synthesis of benzophenone units for photo-affinity labeling

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Peptides are a common tool for the study of protein-protein interactions. Benzophenone (BP) is an attractive photo-affinity label which covalently binds to the inactive C-H bonds of the protein, upon exposure to UV irradiation. In order to use BP for the study of protein interactions, a peptide-BP conjugate must be synthesized. The addition of BP to the target molecule has often proven to be a difficult synthetic step. Here we describe a one pot, two step synthesis of BP units that are compatible with standard solid phase peptide synthesis procedures. The proximity between the BP moiety and the peptide pharmacophores can affect both binding and activity, thus we synthesized BP units using spacers of varying lengths. In addition, the spacer has a dominant effect on the site at which the BP binds to the protein, and the use of various lengths enables more complete mapping of the binding site. The incorporation of the BP units into the peptide sequence on solid support was performed using the "sub-monomer"procedure for peptoid synthesis of Nuss et al.1 The formation of "peptoid like"BP units could also increase the metabolic stability of the peptide. We used the BP units to study the interaction between Protein Kinase B (PKB/Akt) and it's substrate based inhibitor, PTR61542. We also synthesized a yeast permeable peptomer containing BP unit, Biotin and Fluorescein, to study protein-protein interactions in a native cellular environment of yeast cells.

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P079. Abstract number: 121

New L-amino acid ligase from Pseudomonas syringae NBRC14081 producing tabtoxin

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L-Amino acid ligase catalyzes ATP-dependent peptide synthesis using unprotected L-amino acids as the substrates. Our research is about peptide synthesis using L-amino acid ligases. We found a new L-amino acid ligase from *Pseudomonas syringae* NBRC14081 producing a tabtoxin peptide-phytotoxin. Tabtoxin is the dipeptide toxin containing tabtoxinine-β-lactam (TβL) linked by a peptide bond to L-threonine, and TβL induces the chlorosis through inhibition of glutamine synthetase in plant cells. The biosynthetic gene cluster of *Pseudomonas syringae* BR2 was identified, but the biosynthetic pathway has not been clarified. We assumed that tabtoxin is combined by an L-amino acid ligase, and searched for the gene possessing ATP-grasp motif, a signature of the ATP-dependent carboxylate-amine/thiol ligase superfamily, in the gene cluster. The result showed that *tblF* had the ATP-grasp motif and therefore may encode L-amino acid ligase. The corresponding gene was then cloned from *P. syringae* NBRC14081, and was named *tabS*. TabS has the same amino acid sequence as TblF from BR2 strain, and whose amino acid sequence shared about 20% homology with those of known L-amino acid ligases. TabS was prepared by the *Escherichia coli* gene expression system as an N-terminal His-tagged protein and was assayed for L-amino acid ligase activity. The substrate specificity was examined using 20 proteogenic amino acids and β-alanine (β-Ala). The LC-MS analysis showed that TabS has broad substrate specificity, and synthesized 136 kinds of

dipeptides. When 20 mM Ala and 100 mM Pro was used as the substrates, 6.3 mM Pro-Ala, 0.6 mM Ala-Ala, and 0.4 mM Pro-Pro were synthesized. Synthesis of dipeptide containing Pro at the C-terminus by an L-amino acid ligase is the first report, although the yield was very low. In addition, TabS synthesized the peptides containing β-Ala, *e.g.*, Phe-β-Ala, a hair-growth inhibitor. Furthermore, our result suggests tabtoxin may be synthesized by L-amino acid ligase, because *tabS* (= *tblF*) is the only gene encoding peptide synthesizing enzyme in the tabtoxin biosynthetic gene cluster.

P080. Abstract number: 149

The chains of insulin-like peptides reveal properties of oxidoreductases

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The synthesis of insulin-like peptides (INSL) and their chimeric derivatives by the folding of their A- and B-chains proceeds fast and selectively. In the key step of the chain combination, a bisoxidized A-chain is formed and folds then fast with the corresponding linear B-chain. The A- and B-chains of the INSL reveal a high recognition ability to each other. So, even the regions of the IGF peptides, which correspond to the A- and B-chains, combine fast and selectively with the corresponding chains of other INSL. These results indicate that the chains of the INSL follow the same basic principles during their recognition and folding, which are independent of their sequence. This is similar to the properties of natural oxidoreductases, which are able to recognize and fold with a variety of different proteins.

P081. Abstract number: 150

Synthesis of the thiolactone derivative of Enterococcus faecalis gelatinase-biosynthesis activating pheromone using the GyrA mini-intein

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The expression of pathogenicity-related extracellular proteases in *Enterococcus faecalis* is positively regulated by the quorum sensing system mediated by an autoinducing peptide termed gelatinase biosynthesis-activating pheromone (GBAP). GBAP is an 11-residue cyclic peptide containing a lactone linkage formed by the side-chain hydroxyl group of Ser^{3} and the main-chain carboxyl group of Met^{11} in the following amino-acid sequence, Gln-Asn-Ser-Pro-Asn-Ile-Phe-Gly-Gln-Trp-Met. Secreted GBAP binds to its membrane-bound receptor FsrC and triggers a two-component signal transduction cascade of FsrC and FsrA. We already succeeded in chemical synthesis and solution structure determination of GBAP. In order to analyze the intermolecular interaction of GBAP and FsrC by heteronuclear NMR, we need tp establish a preparation method of isotopically labeled GBAP. Here, we describe a new method to synthesize the thiolactone derivative of GBAP, a GBAP agonist, from the fusion protein of [Cys^{3}]GBAP and *Mycobacterium xenopi* GyrA mini-intein. With this method, we obtained 0.25 mg of ^{15}N-labeled GBAP thiolactone from 1 L of *Escherichia coli* culture, and measured its ^{1}H-^{15}N 2D HSQC and ^{1}H-^{1}H-^{15}N 3D NOESY-HSQC spectra to analyze its interaction with FsrC.

P082. Abstract number: 155

Automated Solid-Phase Synthesis of Peptide Thioesters with Self-Purification and Application in the Construction of Immobilized SH3 Domains

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Peptide thioesters are important building blocks in the total synthesis of proteins and protein domains via fragment ligation. However, synthetic access of peptide thioesters still is a bottle-neck of this powerful ligation chemistry. The commonly used methods for the Fmoc-based synthesis of peptide thioesters involve non-automated solution steps that have to be performed after the solid-phase assembly of the peptide. Usually, HPLC purification is required. Herein, a method that enables crude peptides to be used in divergent native chemical ligations reactions is described. [1]

We present an automated Fmoc-based solid-phase synthesis of peptide thioesters with self-purification which facilitates access to these important building blocks, since the often cumbersome HPLC purification can be avoided. Fmoc-protected amino acids are coupled on a safety catch sulfonamide resin. The self-purifying effect is achieved through the combination of a) N-terminal coupling of a cleavable cyclization linker, b) subsequent backbone-to-side chain cyclization and activation of the sulfonamide linkage by alkylation, c) thiolysis for the selective detachment of truncation products and d) TFA cleavage for the liberation of the desired peptide thioester in unprotected form. The potential of the method is demonstrated in the parallel synthesis of 20-40 amino acid long peptide thioesters, which were obtained in excellent purities. The thioesters and cysteinyl peptides were used without purification in the assembly of immobilized SH3 protein domains of SHO1 in yeast. A cysteine scan by native chemical ligation suggested single amino acid to cysteine substitutions that a) confer useful ligation yields, b) support correct folding and c) sustain the function of the folded protein domain.

[1] F. Mende, O. Seitz, Angew. Chem. Int. Ed. 2007, 46, 4577-4580

P083. Abstract number: 163

Critical evaluation of in situ coupling reagents for SPPS

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With the plethora of coupling reagents available for mediating amide bond formation in peptide synthesis, making a rational decision as to the optimal reagent for a given application can be difficult. In this poster, we compare a wide range of novel and commercially available coupling reagents for efficiency, solution stability, solubility and cost, with a view to simplifying the selection of coupling reagents.

P084. Abstract number: 164

Practical aspects of the use of the Dbz linker for making thioesters by Fmoc SPPS

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Dawson's Dbz linker [1] is an important new tool for making peptide thioesters by Fmoc SPPS. The linker consists of 3,4-diaminobenzoic acid which is attached *via* the carboxyl to an amino functionalized resin. Peptide chain extension is performed on one of the anilino groups, before formation of a imidazolidinone (Nbz) with *p*-nitrophenyl chloroformate, and cleavage form the resin with TFA. The peptide Nbz is

used directly in chemical ligation reactions to generate *in situ* the desired peptide thioester.
During our attempts to incorporate this new linker into our routine synthesis of peptide thioesters, we observed formation of branched peptides resulting from acylation of the second anilino group, particularly with glycine. In this poster we present the results from our experiments to overcome this problem.
[1] J. B. Blanco-Canosa & P. E. Dawson (2008) *Angew. Chem. Int. Ed.*, **13**, 6851.

P085. Abstract number: 185
Automated 'X-Y' robot for SPPS with microwave heating: Application to difficult peptide sequences and protein domains
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Precise microwave heating has emerged as a valuable addition to solid-phase peptide synthesis. New methods and reliable protocols, as well as their embodiment in automated instruments, are required to fully utilize this potential. Here we describe a new automated robotic instrument (Biotage Syro WaveTM) for solid-phase peptide synthesis with microwave heating and report protocols for its reliable use. We report the application for the synthesis of difficult peptides as well as long sequences including the □-amyloid (1-42) peptide and a LysM2 protein, which were all successfully assembled in excellent crude purity. The instrument is built around a valve-free robot, originally developed for parallel synthesis, where the robotic arm dispenses reagents instead of pumping reagents via valves. We have demonstrated that a valve-free synthesizer based on an 'X-Y' robot can be optimized for synthesis of long sequences. The synthesis of a series of difficult peptide sequences will be presented using microwave irradiation. These findings show that in general, the synthesis of peptides using microwave irradiation was accomplished with greater speed and purity than with conventional room-temperature protocols.

P086. Abstract number: 194
Solvent-free synthesis of peptides in a ball-mill
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A novel solvent-free methodology for the synthesis of peptides was developed: the coupling of an urethane protected N-carboxy anhydride (UNCA) of an aminoacid with another aminoacid derivative gave a protected dipeptide in very high yield under ball-milling conditions. The reaction is taking place in the solid state. This solvent-free synthesis was applied to the preparation of dipeptides and tripeptides, and exemplified in the preparation of the sweetner aspartame, in three steps, using the UNCA route, without any organic solvent and purification. In contrast to the classical methods (solution or solid-phase), this solid-state method represents a new paradigm for the synthesis of peptides.

P087. Abstract number: 208
A study to assess the cross-reactivity of cellulose membrane bound peptides with detection systems: An analysis at the amino acid level
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The growing demand for binding assays to study protein-protein interaction can be addressed by peptide array-based methods [1]. The SPOT technique is a widespread peptide-array technology, which is able to distinguish semi-quantitatively the binding affinities of peptides to defined protein targets within one array. The quality of an assay system used for probing peptide arrays depends on the well-balanced combination of screening and read-out methods. The former address the steady-state of analyte capture, whereas the latter provide the means of detecting captured analyte. In all cases, however, false positive results can occur when challenging a peptide array with analyte or detecting captured analyte with label conjugates. Little is known about the cross-reactivity of peptides with the detection agents. Here we describe at the amino acid level the potential of 5-(and 6)-carboxytetramethylrhodamine [5(6)-TAMRA], fluoresceinisothiocyanate [FITC], and biotin/streptavidin to cross-react with individual amino acids in a peptide sequence.
Peptides with different amino acid cores were synthesized and tested for interaction with common dyes and detection systems. Our goal was to discover the potential of 5-(and 6)-carboxytetramethylrhodamine [5(6)-TAMRA], fluoresceinisothiocyanate [FITC], biotin and streptavidine to crossreact with individual amino acids.
To this end we designed 20 peptides of the sequence XXX[aa]5XXX, where [aa]5 denotes five repeats of one of the 20 amino acids, and prepared them via SPOT synthesis [2]. Glycine was chosen as the flanking residue X to act as a spacer molecule. As analytes small peptides (gly-gly-gly) were solid phase synthesized and afterwards labelled with the detection compound of interest. The resulting amino acid library was then incubated with the glycine labelled detection system and evaluated via optical and fluorescent methods.
Our approach identified several amino acids interacting with different detection systems. These results will strengthen the reliability of the analysis of SPOT synthesis generated data in the future.
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[2] Frank, R. The spot-synthesis technique. Synthetic peptide arrays on membrane supports - principles and applications. *J Immunol Methods*, **267**(1):13-26, 2002.

P088. Abstract number: 216
TERA-linker: Safety-catch linker for peptide thioesters via Fmoc solid-phase synthesis
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C-terminal peptide thioesters are key intermediates in native chemical ligation (NCL) of peptide fragments. However, peptide thioesters are susceptible to aminolysis by the piperidine used for repetitive removal of Fmoc moieties. Instead, several two-step protocols have been described. We recently reported a new strategy for SPPS of peptide thioesters by activation of a peptide backbone amide bond via first formation and then thiolysis of a pyroGlutamyl (pGlu) imide functionality in the peptide backbone (Tofteng et al, Angew. Chem. 2009, 7411).
Here, we introduce a new safety-catch linker based on 2-(aminomethyl)TERephthalic Acid (TERA) for the Fmoc-based SPPS of peptide thioesters. First the linker is attached to the solid support, then peptide chain elongation proceeds in a straight-forward manner. The safety-catch linker is activated in a two-step protocol by (1) removal of a transient and Fmoc-orthogonal carboxyl protecting group

and (2) subsequent formation of a 5-membered hemi-cyclic imide via an asymmetric anhydride. The increased electrophilicity of this species is then exploited by nucleophilic displacement with a suitable thiol and concomitant release of the peptide thioester.

P089. Abstract number: 222

The Microwave Revolution: Recent Advances in Microwave Assisted Peptide Synthesis

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One of the greatest breakthroughs in solid phase peptide synthesis (SPPS) in the past decade is the use of microwave irradiation to overcome incomplete and slow reactions typical of conventional SPPS. Microwave energy has been applied successfully in a manual and automated approach for enhancing the synthesis of peptides and peptidomimetics. During the course of conventional peptide synthesis, the growing peptide chain can form aggregates with itself or neighboring chains producing low quality peptides. Due to its highly charged resonance structure, the peptide bond will readily absorb microwave energy inducing molecular motion within the peptide. This random motion can overcome chain aggregation within the peptide allowing for free access to the *N*-terminus of the growing peptide chain, and therefore results in a significant increase in the peptide purity. In addition, microwave irradiation can considerably increase the speed at which peptides are synthesized. Traditionally, peptide coupling reactions require from 30 minutes up to two hours to reach completion. Microwave energy allows the amino acid coupling to be completed in just five minutes. The Fmoc deprotection reaction can also be accelerated in the microwave decreasing the reaction time from at least 15 minutes to only three minutes. We have recently demonstrated common side reactions such as racemization and aspartimide formation are easily controllable with optimized methods that can be applied routinely.[1] Our latest research has focused on the microwave assisted synthesis of modified peptides. Such modifications include *N*- and *C*-terminal modifications, cyclizations, and the incorporation of unnatural amino acids. We have also continued to develop methods for the synthesis of difficult peptides. These peptides were synthesized in a fraction of the time compared to conventional peptide synthesis without the need for unusual or expensive reagents and in a fully automated fashion to give peptides in high yield and purity.

[1]Palasek, S. A.; Cox, Z. J.; Collins, J. M. *J. Pept. Sci*. **2007**, *13*, 143-148.

P090. Abstract number: 223

Novel Core-shell Particle Technology Aiding Synthetic Peptide Purification

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Fraction analysis is a time consuming part of the peptide purification process. Traditionally, conventional HPLC columns, typically of 15-25 cm length are used for fraction analysis requiring a significant amount time for the screening of all fractions of interest. This in turn causes delays in deciding if the purification process is effective or it may require further optimization. The advent of highly efficient short HPLC columns allows for a significant reduction in the time required for fraction analysis. While such columns have been made until recently with sub-2 µm particles, requiring the use of high pressure-capable (expensive) analytical HPLC instrumentation, the recently introduced core-shell particle based columns provide superior resolving power and analysis times, while fully compatible with conventional HPLC instruments. Core-shell based HPLC columns are currently the ”state of the art”in modern HPLC analysis and their use is continuously expanded to the various aspects of drug discovery, including peptide analysis.

In this presentation we demonstrate the excellent performance of core-shell based HPLC columns for the analysis of fractions collected in the purification of several generic synthetic peptides. Results to date suggest that such columns provide excellent chromatographic resolution, reproducible quantitative results and fast run times on the order of 1-3 minutes, while operated on conventional HPLC instruments capable of delivering consistent flow rates at back pressures below 400 bar. Hence, the most recent advancements in HPLC column technology can be successfully implemented also to fraction analysis in peptide purification.

P091. Abstract number: 247

MICROWAVE-ASSISTED SOLID-PHASE PEPTIDE SYNTHESIS OF THE 60-110 DOMAIN OF HUMAN PLEIOTROPHIN (hPTN) ON CLTR-Cl RESIN

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Human pleiotrophin (hPTN) is a heparin-binding growth factor with diverse biological activities, the most studied being those related to the nervous system, tumor growth and angiogenesis. It is interesting to determine which regions of hPTN are responsible for its diverse functions, in order to identify the molecular mechanisms involved and to identify possible therapeutic targets or/and agents. The binding of hPTN to heparin is mediated by the two central regions that are homologous to the thrombospondin type I repeat (TSR-1), with the carboxyl terminal TSR-1 domain (60-110) being the main heparin-binding site of PTN [1,2]. Here we report the Microwave Enhanced Solid Phase Peptide Synthesis (MW-SPPS) of the C-terminal 60-110 domain of hPTN composed of 51 amino acids using the Fmoc/tBu methodology. The linear protected peptide was synthesized using the LibertyTM Microwave Peptide Synthesizer (CEM) on 2-chlorotrityl chloride resin (CLTR-Cl) [3]. Fmoc deprotection was achieved with 20% Piperidine in DMF, while for the coupling reactions HOBt/DIC in DMF were used. Moreover, this domain is supposed to contain two disulfide bonds, one between 67-99 residues and one between 77-109 residues [2]. In order to achieve selective formation of these disulfide bonds, the Cys(Trt) at 77, 109 positions and Cys(Acm) at 67, 99 positions were used. After cleavage of the protected peptide from the resin and removal of the side chain protecting groups [except for $Cys(Acm)^{67}$ and $Cys(Acm)^{99}$], the first disulfide bond was formed by dimethyl sulfoxide (DMSO). The second disulfide bridge was formed contemporaneously with the Acm group removal using iodine. In this report, we demonstrated that microwave energy can also be applied in the case of the solid-phase synthesis of large peptides utilizing the acid sensitive CLTR-Cl resin.[1] Papadimitriou, E. et al. (2009) Eur. Cytokine Netw., 20, 180.[2] Mikelis, C. et al. (2007) Recent Patents on Anti-Cancer Drug Discovery., 2, 175.[3] Barlos, K. et al. (1991) Angew. Chem. Int. Ed., 30, 590.

P092. Abstract number: 302

Solid-Phase Synthesis of the Lipopeptide Myr-HBVpreS/2-78, a Hepatitis B virus Entry Inhibitor

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Chronic HBV infection is the leading cause of liver cirrhosis and hepatocellular carcinoma. Synthetic peptides derived from the *N*-terminus of the large HBV envelope protein have been shown to efficiently block HBV entry. Myr-HBVpreS/2-78, the parent compound of these drugs, inhibits human HBV infection *in vitro* and *in vivo*. Constituting a novel class of anti HBV drugs an efficient synthesis of this peptide is required. Consequently, the solid phase synthesis of the *N*-terminal 77 amino acids of the viral L-protein was studied in detail. The sequence was *N*-terminally myristoylated to resemble the natural, postranslationally modified protein. The synthesis was monitored using the Fmoc cleavage pattern of the solid phase synthesis on a standard peptide synthesizer and by HPLC-MS analyses of the arising side products. NMR studies suggest that the peptide belongs to a class of intrinsically unfolded peptides and is therefore not prone to aggregations within the synthesis. Nevertheless, ”difficult sequences”in the positions 42-47 of the peptide sequence complicate the efficient synthesis of the 77-mer peptide HBVpreS/2-78. Attempts were undertaken to optimize the synthesis by heating, double coupling or the use of pseudoproline dipeptides. HPLC-MS analyses showed that the efficiency of the synthesis could be increased best by applying elevated temperature resulting in a higher purity of the crude product after solid phase synthesis. It was possible to minimize the occurrence of side products related to incomplete coupling steps. In conclusion, the peptide is accessible by stepwise SPPS without the necessity of segment coupling.

P093. Abstract number: 310
Beyond 100mer peptide synthesis using CS336 automated synthesizer
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The total chemical synthesis of peptides provides a rapid and effective route for the production of homogenous proteins free of biological contaminants, while at the same time offering flexibility through the incorporation of unnatural amino acids or other chemical modifications that improve protein efficacy. Furthermore, chemical synthesis allows facile introduction of biochemical and biophysical probes that cannot be made by biology methods.
In the synthesis of a peptide of 101 residues, reaction efficiency of 99% per cycle would give 36% of the peptide with the correct amino acid sequence. The remaining 64% would consist of a mixture of peptide contaminants, including 100 polypeptides with a single deletion, 5000 with two deletions, and a lot more remainders with three or four deletions. To obtain a crude peptide with ~80% purity, the reaction efficiency of 98.8-99.9% per reaction cycle has to be reached. Recently, we finished a 102mer peptide synthesis using CS336 automated synthesizer with a very good yield. The 3-RV (reaction vessel) CS336 allowed us to aliquot the resin during AA assembling and customize the coupling strategy whenever needed. For example, for the ‘difficult sequences’ of a growing peptide chain, double/triple coupling could be required, which can be simply done by editing the program on CS336 control panel. Additionally, we alternatively used HBTU/DIPEA and DIC/HOBt for the same sequence when double coupling and triple coupling were performed, which improved the completeness of each cycle before capping. Another 108mer was synthesized using the same strategy on the CS336 automated synthesizer within three weeks. Although long peptides can also be obtained by chemical ligation, a stepwise synthetic approach is still the option as it is easier and faster to handle than other strategies, especially when the automated synthesizer is used.

P094. Abstract number: 312
Automated Microwave-Assisted Peptide Synthesis with a Novel Robotic Synthesizer: Synthesis of Difficult Sequences and N-Methylated Peptides
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New coupling reagents and matrices have improved the yields of peptide bond formation. However, 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 has provided significant improvement in terms of speed, purity, and yields, particularly in the synthesis of long and ”difficult”peptide sequences. Thus, precise microwave heating has emerged as a new parameter to be considered for SPPS, in addition to choice of coupling reagents, resins and solvents. The synthesis of peptidomimetics and N-methylated peptides, often requires incorporation of modified or non-proteinogenic amino acids. These amino acids are often expensive and as a consequence not used in large excess in peptide assembly. Microwave heating has the potential to increase yields in couplings of these amino acids, even when used in low excess, making this type of acylation chemistry affordable. We have previously reported on microwave heating to promote a range of solid-phase reactions in SPPS. Here we present the recent applications of a new, flexible automated instrument, the Syro WaveTM, for the application of precise microwave heating in SPPS. The instrument incorporates a modified Biotage Initiator microwave instrument and the Syro I parallel peptide synthesizer from MultiSynTech, thus the instrument retains it parallel capability while additionally having a single channel microwave reactor. The solvent handling is achieved by a robotic arm and the mixing is performed by vortexing. N-methylated peptides were prepared using commercially available N-methylated Fmoc-amino acids. The N Methylated peptides were prepared using microwave-assisted SPPS giving the desired peptides in high purity.

P095. Abstract number: 330
Tridegin - insights into the synthesis and bioactivity of a factor XIIIa-inhibitor
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A major concern regarding the origin and development of different cardiovascular diseases is the pathophysiological role of factor XIII/factor XIIIa, a transglutaminase from the blood coagulation cascade [1]. Moreover, there are reports indicating that increased levels of factor XIIIa in humans are involved in atherosclerosis or Alzheimer’s disease [2, 3].
For a better understanding of these processes studies with effective enzyme inhibitors are indispensible, while at the same time such compounds could have therapeutic potential. Up to now the most effective inhibitor for factor XIIIa is Tridegin, a 66-mer disulfide-bridged peptide isolated from the Amazon leech Haementeria ghilianii [1]. Due to aspects such as low yields and impurities which

have been described for the isolation from leech glands or the recombinant expression [1, 4], we decided to investigate different methods for chemical synthesis. In addition, another important aspect concerning the therapeutic potential of Tridegin is the elucidation of specific amino acid positions essential for drug development. Both, the preparation of Tridegin as well as the bioactivity of this peptide and a series of analogs will be reported.

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[4] Patent application WO2003054194

P096. Abstract number: 331

Bioactive cysteine-rich peptides are interesting targets for oxidative folding and native chemical ligation in ionic liquids

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Disulfide bridges play an essential role in maintaining biologically active conformations of several natural and synthetic peptides. Disulfide bridge formation in peptides either occurs in solution at high dilution by means of redox active agents or in light basic conditions or on a solid support (on-resin oxidation) [1]. In addition, cysteine residues are involved in the selective formation of a stable covalent peptide bond by native chemical ligation (NCL) [2]. A new promising method of disulfide bridge formation in biocompatible ionic liquids (ILs) was proposed to possess many advantages compared to other existing methods commonly used [3,4]. For selected targets it was found that the reaction outcome of desired product depends on the structure of the IL, its hydrogen bond acceptance properties and the ability to interact with the solute, as well as the chain length, the sequence and the net charge of the peptides [4]. Several peptides differing in their cysteine content, disulfide bridge pattern and structure have been chosen to investigate their synthetic generation and structure-activity-relationships. Completion of oxidative folding and NCL was monitored and characterized using several analytical and biochemical methods. Biological activities of the peptides (e.g. different conotoxins) were investigated by respective assays in order to confirm correct folding or ligation to the desired product.

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P097. Abstract number: 333

Oxidation of methionine occurring in TFA treatment of peptides on Sieber amide resin

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Sieber resin loading the xanthenyl (Xan) linker can be cleaved using very low concentrations of TFA (1-2%) [1]. This allows the Fmoc/*t*Bu peptide synthesis of peptide amides and carboxamides to be produced with their protecting groups intact. These are used for the subsequent fragment condensation reaction performed either in solution or on a solid support to assemble larger peptides. Moreover, the acid-lability with this linker is suitable to produce acid-susceptive derivatives such as *O*- and *N*-glycosides, sulphate ester and phosphorylated ones. However, the TFA treatment of the peptide on Sieber resin, regardless of its concentrations, was found to be accompanied by a significant extent of Met-sulfoxide formation. In particular, the addition of triisopropylsilane (TIS) as a quenching agent to the TFA reaction mixture accelerated Met(O) formation rather than suppressing it. As for Boc/Bzl peptide synthesis mediated by phosphonium or uronium reagents, the Xan group on the Asn residue is routinely employed as a temporary protecting group to suppress dehydration of its side chain amide [2]. After incorporation of the Asn residue, the Xan group can be simultaneously removed by the TFA cleavage conditions necessary for removal of the Boc group. During this step, by the same token, the Met residue located on the peptide resin was also found to be considerably oxidized. To fine out measures to this side reaction associated with the use of the Xan group, a model peptide, Met-enkephalinamide, was subjected to various TFA conditions in the presence of Sieber resin, Asn(Xan) or xanthene.

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P098. Abstract number: 346

The oxime-based family of coupling reagents

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The recent discovery of the explosive nature of benzotriazole-based additives (HOBt, HOAt, 6-Cl-HOBt) and derived onium salts has prompted the search for a new family of reagents. With the aim to fill the gap in the methodology for amide formation, oximes were found to be a promising scaffold for developing new safe and efficient reagents. Thus, Oxyma [ethyl 2-cyano-2-(hydroxyimino)acetate] and its parent uronium salt COMU, which are commercially available, were studied exhaustively.[1,2] These compounds showed high control of optical purity, solubility, stability, and outstanding performance in the assembly of sterically hindered sequences. Both reagents are compatible with microwave irradiation and comply with thermal safety requirements, as shown by DSC and ARC calorimetry assays.[3] In view of these excellent results, we seek to increase the scope of available reagents with novel oxime-based reagents.

Phosphonium salt PyOxP, easily obtained in a one-pot procedure from Oxyma, is presented as a superior reagent for slow couplings such as the assembly of peptide fragments or cyclizations. This reagent shows enhanced solubility, stability and efficacy in linear and cyclic peptide

models, compared with PyAOP, PyBOP and PyClock. Other oxime scaffolds have been designed, like the one derived from Meldrum's acid (HONM). Although the suitability of HONM as additive is compromised by the appearance of side reactions caused by its high reactivity, its parent uronium salts have proved to be useful reagents for the acylation of poor nucleophiles, presumably because of the presence of a neighboring group effect. The dimethylmorpholino-HMMU is a reliable alternative for peptide bond formation, ensuring low racemization and high coupling extentions. Finally, the need for Fmoc/Alloc-introducing reagents also focused our efforts on the development of oxime carbonates. *N*-Hydroxypicolinimidoyl cyanide derivative analogues were found to combine efficient protection of the *N*-terminus with the absence of side-products, such as dipeptides.

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P099. Abstract number: 350

A new peptide-based scaffold of six-helical bundle for bio-mimic studies

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In past twenty years, peptide-based bio-mimic systems were insensitively studied worldwide. From small peptides to the dendrimers, α-Helixes and 4-helical bundles of peptides were tested as scaffolds [1-3]. Construction of framework and positioning the key functional groups on a scaffold as natural biomacromolecules are the key points for the design of artificial enzymes. Therefore, a robust and alterable scaffold is required for bio-mimic studies and applications. Recently we used C34/N36 six-helical bundle (6HB), which is part of intermediate core structure formed during the cell fusion of HIV-1 gp41 with its target, as scaffold to mimic function of biomacromolecules. In the beginning of our study, we built up a multiple-His 6HB as an artificial hydrolase to test its stability and activity. The sequences of the peptides were designed by combining the X-ray crystal structure of C34/N36[4] and molecular modeling. Histidine residues can function as nucleophiles or general base in their unprotonated form, and as general acid and hydrogen-bond donors in their protonated form. The well formed 3-D structure and the stability of 6HBs with multiple His mutations were confirmed by N-PAGE, SE-HPLC, CD spectrum, Tm measurement and micro-DSC. The new 6HB system can catalyzed the hydrolysis of p-nitrophenyl acetate (PNPA) which was wildly used as substrate for artificial enzyme assay. One of 6HBs, $C34_{H13H20}/N36_{H15H22}$ showed enzyme-like saturation kinetics, and gave a K_M of 598.9μM, k_{cat} of 2.19 -10^{-3} sec^{-1}, k_{cat}/k_{uncat} of 607 and k_{cat}/K_M of 3.66$M^{-1}sec^{-1}$ by the data analysis. The data showed obviously catalytic efficiency of the 6HB formed with $C34_{H13H20}/N36_{H15H22}$. K_M is referred to affinity of substrate to enzyme. The K_M value of the system is clearly smaller and means higher affinity to the substrate than most other artificial catalysts.

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P100. Abstract number: 358

Improved synthesis of backbone and macrocyclic peptides: application to PKB, gp120 and integrase inhibitors

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Cycloscan is a method that enables the fast discovery of peptido- and proteinomimetic molecules with desired pharmacological properties (e.g. metabolic stability, selectivity and intestinal permeability) [1]. Cycloscan is based on the synthesis of backbone cyclic focused libraries with conformational diversity, namely all the members of the library have the same sequence of the parent peptide or the active region of the protein. Screening of these libraries leads to the discovery of the desired peptido- or proteinomimetic. Some of the drawbacks of backbone cyclization, that limited its use, are synthetic problems derived from the cumbersome synthesis of protected building units, their incorporation into peptides and the cyclization step. We have developed (i) a facile, large scale synthesis of Allyl protected Gly building units (AGBU) (ii) the incorporation of AGBUs into backbone cyclic peptide using automatic SPPS (iii) facile synthesis of backbone to backbone cyclic peptides with on resin urea cyclization [2] (iii) microwave assisted solid phase synthesis of focused macrocyclic libraries with regio, stereo and conformational diversity. The synthetic improvements led to the discovery of potent substrate inhibitors of PKB, potent cell permeable inhibitors of integrase and an orally available macrocyclic entry inhibitor of HIV-1 replication. We hope that the synthetic improvements will facilitate the synthesis of backbone- and macrocyclic peptide- and proteinomimetics.

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P101. Abstract number: 379

Fmoc SPPS of peptide thioesters: Towards new pyroglutamyl-like linkers

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The use of C-terminal peptide thioesters in synthetic protein chemistry has inspired the search for optimal solid-phase peptide synthesis (SPPS) strategies for their assembly. Thioesters are not directly available by standard Fmoc SPPS due to the sensitivity of the thioester linkers to the piperidine used for repeated Fmoc removals. Instead, several two-step protocols have been described. We recently reported a new strategy for SPPS of peptide thioesters by activation of a peptide backbone amide bond via first formation and then thiolysis of a pyroGlutamyl (pGlu) imide functionality in the peptide backbone (Tofteng et al, Angew. Chem. 2009, 7411). This linker strategy is based on the proteinogenic amino acid Glu, however, to enable more facile ring-closure and thiolysis we have

studied new potential linkers for the formation of a pGlu-like structure.
Here we report studies on o-carboxy-phenylglycine and 4-fluoro-glutamic acid, which are either sterically restricted, thus pursuing easier pGlu-like ring closure in-chain, and/or structures which are more acidic at their amide function than pGlu, thus pursuing better leaving group abilities.

P102. Abstract number: 386
Engineering of Amyloid-β-Binding Affibody Molecules for Improved Chemical Synthesis and Higher Binding Affinity
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The oligomerization and aggregation of Amyloid-β (Aβ) peptide is generally considered to be one of the most important contributors to the onset and progression of Alzheimer's disease (AD). Selective and high affinity binding proteins directed towards Aβ peptide are important tools to better understand the aggregation process and could possibly be used for diagnosis and treatment of AD.
Affibody molecules, based on the engineered domain Z derived from the B domain of staphylococcal protein A, are a class of affinity proteins with several advantageous properties, such as small size (58 aa), permitting the use of peptide synthesis for the production, and rapid and reversible folding.
An Affibody molecule ($Z_{Aβ3}$) previously selected by phage display has been shown to bind Aβ peptide with nanomolar affinity and to inhibit Aβ-peptide aggregation.[1, 2, 3] By using chemical synthesis for the production of this protein, various chemical modifications of the molecule such as introduction of unnatural amino acids or site-specific labeling with reporter groups are possible. We here describe the design, synthesis and evaluation of six N-terminally truncated variants, together with a full-length version of the $Z_{Aβ3}$ Affibody molecule.
The $Z_{Aβ3}$ Affibody molecules were prepared by Fmoc solid phase peptide synthesis (SPPS) and their affinities to Aβ peptide (1-40) were determined by surface plasmon resonance (SPR)-based biosensor analysis. Our results show that three of the truncated variants can be produced in considerably higher yield than the full-length protein. The synthetic yields of $Z_{Aβ3}$(18-58), $Z_{Aβ3}$(15-58) and $Z_{Aβ3}$(12-58) were 35%, 30% and 29%, respectively, while the yield of the full-length protein, $Z_{Aβ3}$(1-58), was only 8% using the same reaction conditions.
When analyzed by SPR, the shortest variant, $Z_{Aβ3}$(18-58), showed a complete loss of binding to Aβ(1-40), whereas both $Z_{Aβ3}$(12-58) and $Z_{Aβ3}$(15-58) displayed approximately a ten-fold increased affinity for Aβ(1-40) compared to the full-length protein.
In conclusion, our work shows that the N-terminally truncated Affibody variants $Z_{Aβ3}$(12-58) and $Z_{Aβ3}$(15-58) are improved versions of $Z_{Aβ3}$, both in terms of synthetic yield and binding affinity. We suggest that they are promising molecules for further engineering and use for the studies of Aβ peptides.
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P103. Abstract number: 413
Chemical Synthesis of Fluorescent-Labeled Affibody Molecules for Use in Cancer Diagnostics
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Affibody molecules are a class of affinity proteins, which is based on the non-immunoglobulin Z domain, derived from staphylococcal protein A. The Z domain is a three-helix bundle protein, where 13 residues in helices 1 and 2 have been randomized to generate a combinatorial Affibody library, from which binders to a variety of different target proteins have been selected by phage display. The protein is only 58 amino acid residues, making it suitable for production by solid phase peptide synthesis.
In the present study, two Affibody molecules targeting known cancer biomarkers, EGFR [1] and HER2 [2], have been synthesized. Both targets belong to the epidermal growth factor receptor (ErbB) family, which is involved in signal pathways regulating processes such as cell proliferation and differentiation. High expression of HER2 has been associated with different types of cancer, such as breast, ovary and bladder cancer. Similar findings have been reported for EGFR, which is up-regulated in many different human cancers.
The Affibody molecules were synthesized in high yield on solid phase by Fmoc/tBu chemistry with a C-terminal Cys-Gly dipeptide extension to enable site-specific labeling with fluorophores in solution by maleimide chemistry. The Affibody molecules were conjugated to three fluorophores, which have high fluorescence quantum yield and high photo-stability; ATTO-488, ATTO-594 and ATTO-647N (Atto-Tec Gmbh, Siegen). The fluorescent-labeled Affibody molecules have subsequently been used in high resolution fluorescence microscopy and fluorescence correlation spectroscopy to study the relative expression, spatial distribution and dynamics of the EGFR and HER2 proteins in human breast cancer cell lines, with the aim of identifying differences that can be of diagnostic or prognostic value in the analysis of patient samples.
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P104. Abstract number: 422
Oxyfold : a new solid supported reagent for the simple and effective formation of disulfide bond in peptides
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The importance of disulfide bridges in peptide conformation and activity make their formation a fundamental step of peptide synthesis. Formation of disulfide bond is probably one of the most challenging steps to achieve regarding the formation of unwanted by-products and oligomerization. In order to minimize the latter phenomenon, disulfide bridge cyclizations are performed under high diluted conditions which require time consuming removal of solvent at the end of the reaction. Dimethyl sulfoxide (DMSO) is one of the oxidizing reactants most commonly used for this. One of its major drawbacks is its elimination from the reaction medium, which requires evaporation under strong vacuum or repeated lyophilizations. Furthermore, the dimethyl sulfide generated during the reaction is volatile and toxic.On the other hand, supported reactants are

particularly advantageous for promoting intramolecular reactions. In fact, they are known to cause a phenomenon of ”pseudodilution”which makes it possible to minimize oligomerization and to use much smaller amounts of solvents [2].Here we present the synthesis and the use of novel oxidation reactants on solid support for disulfide bond formation. This family of supported reagents consists in a series of oxidized methionines grafted by step-wise SPPS or methionine N-carboxyanhydride oligomerization on solid support. The formation of disulfide bond in peptides is carried out in aqueous solutions and the desired peptide is recovered by filtration from the resin. We demonstrate the efficiency and easiness of these supported reagents for the formation of disulfide bridges in peptide.Their characteristics make the use of our supported reagents a fast, cheap and green procedure for disulfide bridge formation.References1. J.P. Tam, C-R Wu, W. Liu, J-W Zhang, J. Am. Chem. Soc. (1991) 113, 6657.2. R. Shi, F. Wang and B. Yan J. Int. J. Pept. Res. Ther. (2006), 13, 213.

P105. Abstract number: 461

A study on the microwaves synthesis of somatostatin dicarba-analogues

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We previously synthesised several octreotide dicarba-analogues lacking the disulfide bridge via Ring Closing Metathesis (RCM) on linear sequences containing allylglycines in strategic positions [1;2]. The RCM reaction was firstly performed in an oil bath, with second generation Grubb’s catalyst and under severe experimental conditions i.e. anhydrous argon atmosphere and long reaction times [3;4]. Subsequently, we assayed the microwaves assisted technique (MW) that emerged as the more effective for the cyclopeptides yields as well as for shortening the reaction times. Moreover, we found that MW works well also on specific sequences containing alkenyl glycines where the classical oil heating failed. In particular, we evaluated the efficacy of the MW for obtaining cyclic octa-mer-peptide ring of somatostatin dicarba-analogues. Surprisingly, we noticed that the classical oil-bath RCM did not work well on our octa-peptide linear chain. Moreover, it clearly resulted that the yields in cyclopeptides where in any case strictly related to the aminoacid sequences. We, therefore, investigated the different MW methods in order to comprehend which variables played an important role in the RCM reaction. In fact, not all the methods give the same results and it’s important to comprehend which is the decisive variable among MW power, time and temperature. Once we found an appropriate MW method, we focused our attention on improving the reaction conditions, in order to increase yields and reduce reaction times.

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P106. Abstract number: 464

Microwave-assisted solid phase synthesis of [Asn641(Glc)]FAN(635-655): a new case study for optimisation of glycopeptide synthesis

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One of the best known PTMs affecting proteins before their synthesis is complete is represented by glycosylation. It is clear that defects in glycosylation or aberrant glycosylation of proteins from their native state are found to be extremely important for the identification of neoantigen recognized as non-self, and thus triggering an autoimmune response.

Factor associated with neutral sphingomyelinase activation (FAN) represents a member of the WD-repeat family of proteins that includes mainly regulatory proteins.[1] Even if FAN is expressed in different human tissues, a bioinformatic study revealed high sequence homology of the fragment 635-655 of this protein with CSF114(Glc), the synthetic glycopeptide developed to detect antibodies in Multiple Sclerosis (MS) patients’ sera.[2] As FAN(635-655) is characterized by the minimum consensus pattern for N-glycosylation of asparagine residues (Asn-Xaa-Ser/Thr, where Xaa is any amino acid except Proline), we investigated a possible involvement of this peptide as linear natural epitope of antibodies in MS.

The bottleneck of this study was to obtain by Solid-Phase Peptide Synthesis (SPPS) [Asn^{641}(Glc)]FAN(635-655) in high purity and good yield to test in immunoenzymatic assays on sera. In fact conventional SPPS protocol failed during the coupling of the sterically hindered building block Fmoc-Asn(GlcOAc4)-OH and of other bulky amino acid residues. Therefore, we optimized the synthesis of this glycopeptide using microwave irradiations.[3] Moreover considering the formation of sequence-specific secondary structures reducing reagents penetration and decreasing reaction rates in both coupling and deprotection steps, thermal disruption of peptide aggregation induced by microwaves, was demonstrated to be favourable for obtaining this particularly difficult sequence.

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P107. Abstract number: 479

Conventional and microwave-assisted SPPS approach: a comparative study of PTHrP(1-34) synthesis

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Parathyroid hormone (PTH) is an 84-amino acid polypeptide playing a significant role in calcium homeostasis. Parathyroid hormone-related peptide (PTHrP) is a distant homologue of PTH and is not a true hormone. It is synthesized in cartilage and in many more tissues than parathyroid hormone, and its secretion is not regulated by serum calcium. It mimics the actions of PTH as a result of its structural homology and its ability to bind

and signal via the PTH/PTHrP receptor in bone and kidney.[1]
Based on the amino acid sequence, it was deduced that the first 34 amino-terminal amino acids should be sufficient for biological activity. Availability of highly purified parathyroid polypeptide and active synthetic fragments made it possible to develop radioimmunoassay.
Considering the presence of sterically hindered amino acid residues in the sequences, such as Arginine and Phenylalanine between position 19-23, and the length of the peptide, we optimised the synthesis of PTHrP(1-34), comparing conventional Solid-Phase Peptide Synthesis (SPPS) protocol with a microwave-assisted one.
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P108. Abstract number: 503
Microwave-assisted total synthesis of macrocyclic cystine knot miniproteins
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Cyclotides are mini-proteins of approximately 30 amino acids residues that have a unique structure consisting of a head-to-tail cyclic backbone with a knotted arrangement of three disulfide bonds [1]. This unique structure provides exceptional stability to chemical, enzymatic and thermal treatments [2,3]. Cyclotides display various bioactivities, such as anti-HIV, uterotonic, cytotoxic, and insecticidal activity [4]. Due to the unique structural stability, cyclotides have been implicated as ideal drug scaffolds and for development into agricultural and biotechnological agents [2]. In the current work, we represent the first method for total synthesis of cyclotides based on Fmoc-SPPS assisted by microwave. This protocol adopts a strategy that combines the optimized microwave assisted chemical reactions for Fmoc-SPPS of peptide backbone synthesis, thioesterification of the C-terminal carboxylic acid of the peptide and a one pot reaction that promotes cyclisation through native chemical ligation and oxidative folding. The application of this protocol was exemplified for the synthesis of two prototypic cyclotides; kalata B1 and MCOTI-II.
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P109. Abstract number: 514
Racemization in Automated Solid Phase Peptide Synthesis
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Automation of solid phase peptide synthesis has become an essential tool in laboratories synthesizing large numbers of peptides. The most widely applied methodologies utilize Fmoc-protection in combination with uronium/phosphonium activating agents for synthesis of peptides on a solid support. Racemization occurring during the activation process is an important side reaction which has been extensively studied by others. In our recently presented studies we tested levels of racemization occurring during automated peptide synthesis with two of the most sensitive amino acids, cysteine and histidine. We will extend our work to other common amino acids. The model peptides having a general formula Z-Ile-Xxx-Pro-OH will be used in these studies. We will reevaluate the degree of racemization of several amino acids (Arg, Asp, Leu, Phe, Ser, Tyr) using a selection of currently available activating agents applied to a typical automated peptide synthesis with an in-situ activation method. HPLC analysis will be used to evaluate the extent of racemization during their incorporation in peptide synthesis.

P110. Abstract number: 517
Microwave-assisted solid phase synthesis of collagen peptides
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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints. The primary protein in cartilage, type II collagen is crucial to joint health and function. Involvement of this homotrimeric collagen in the process of joint inflammation has proven difficult to substantiate. There are strong indications that citrullination, catalyzed by peptylarginine deiminase, is involved in autoantibody response in RA converting a nonimmunogenic protein into an autoimmunogenic one [1]. A diagnostic assay based on citrullinated peptides is considered a reliable marker of RA [2]. R and E residues in collagens are known to significantly contribute to the triple helix stability by electrostatic interactions [3] and are involved in sequential and spatial RGD motifs for cell adhesion processes [4]. To study the effect of a potential citrullination of R residues on the thermal stability of the collagen triple helix and on cell adhesion processes we selected collagen model peptides containing the cell adhesion epitope of collagen type I (GFOGER), flanked N- and C-terminally by two and three GPO triplets, respectively, to induce and stabilize triple helix formation. The synthesis of collagen model peptides containing larger numbers of the GPO triplets has been performed both in stepwise manner and with suitably protected GPO synthons to prevent with more or less success microheterogeneity deriving mainly from diketopiperazine formation. In the present work the efficiency of a MW-assisted strategy for the stepwise synthesis of Arg and citrulline containing collagen model peptides was studied. Indeed upon the due optimization, the peptides were obtained by this procedure as crude products at a quality superior to that of standard protocols thus allowing for their purification in satisfactory yields. These results clearly indicate this strategy as an efficient alternative for collagen peptides. Thermal stability as monitored by CD served to analyze the effect of citrullination of a single Arg residue on the triple helix fold of the related self-associated homotrimers. ELISA assays performed with RA patients' sera could be useful to be compared with the commercially available assay based on citrullinated peptide antigens
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P111. Abstract number: 538

O-Glycopeptide Discovery Platforms for Seromic Profiling

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We have developed high-throughput O-glycopeptides screening platforms for profiling of glycan binding proteins, monoclonal antibodies and disease-associated autoantibodies.

Our O-glycopeptide microarray library is fabricated by a robotic solid-phase parallel peptide synthesizer followed by direct immobilization of crude glycopeptide mixtures on NHS-activated microarray glass surface. Since capping of deletion products is undertaken at every elongation cycle, only full-length structures will preserve the N-terminal amine, thus ensuring on-slide enrichment of desired glycopeptide products. This approach allows complete control of site(s) of GalNAc O-glycan occupancy and combined with on-slide enzymatic glycosylation with different polypeptide GalNAc-transferases and other elongating glycosyltransferases, an extended diversity in O-glycan density and O-glycan structures can be generated.

Another high-throughput (HTP) methodology for seromic profiling is a random combinatorial O-glycopeptide library synthesized on PEGA beads. The glycopeptides are attached to the beads with a safety-catch cleavable linker easily released under mild conditions. Thus, beads showing positive reactivity with patient sera in an immunochemical assay can be selected out, released from the bead and sequenced by ESI-MSn methods. After identification, the glycopeptides are re-synthesized and validated using our microarray technology.

We have also developed an antibody capture microarray for lectin glycoprofiling of captured serum proteins. This antibody array can also be charged with tumor specific glycopeptides and glycoproteins for seromic profiling of aoutoantibodies.

Utility of these platforms in different screening applications will be discussed.

P112. Abstract number: 545

Proline editing: control of peptide and miniprotein structure and function via synthesis of diversely functionalized peptides

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Protein structure and protein function are tightly linked. In proline residues, proline ring pucker, and consequently protein main chain conformation (phi, psi, omega), is controllable by the stereochemistry, electronics, and sterics of substitution on the 4-position. We have developed a practical solid-phase approach to incorporate diverse functional groups with defined stereochemistry at the 4-position of proline residues in peptides, allowing control of protein structure via stereoelectronic or steric effects. This approach has been applied to tune the stability of peptides and miniproteins, leading to tunability of miniprotein stability (Tm) of greater than 50 ˚C. This approach has been broadly applied to allow incorporation of a wide range of functional groups, including fluorines, ketones, sulfonates, azides, amines, thiols, selenides, guanidiniums, alkynes, alkenes, alkyl halides, aryl halides, and dienophiles, with defined stereochemistry into peptides, utilizing no solution phase chemistry. This approach is generalizable to allow incorporation of multiple bioorthogonal functionalities into peptides, resulting in polyfunctional peptides that permit the ready synthesis of multidomain proteins. This approach has been applied to the synthesis of novel multidomain proteins for application to the development of novel smart biomaterials.

P113. Abstract number: 548

Efficient Direct Solid-Phase Synthesis of β-Amyloid (1-42) Peptide Using Controlled Microwave Irradiation

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Alzheimer's disease is a neurodegenerative disorder characterized by extracellular accumulation of amyloid senile plaques within the brain tissue. The major proteinaceous component of amyloid deposit consists of aggregates of sparingly soluble β-amyloid peptides, which are derived from proteolytic cleavage of the amyloid protein precursor. Aβ (1-42) plays a crucial role in the pathogenesis of Alzheimer's disease, forms β-sheet structures and amyloid fibrils which induces neurotoxicity both *in vitro* and *in vivo* experiments.

The Aβ (1-42) peptide has received a considerable amount of attention in the peptide community during the last decades, and as one of the most prominent examples of a so-called "difficult peptide"is well-known for its complexity of preparation. Therefore, the development of efficient direct and linear methods for the solid-phase synthesis of the Aβ (1-42) peptide are still of great importance.

Herein, we report a rapid and simple protocol for the preparation of the β-amyloid (1-42) peptide employing a microwave-assisted Fmoc/*t*Bu solid-phase method that utilizes standard N,N´-diisopropylcarbodiimide/1-hydroxybenzotriazole (DIC/HOBt) coupling reagents. While standard solid-phase protocols typically result in peptides of poor quality, the application of controlled microwave heating provides the Aβ (1-42) peptide in high purity in only 15 h of total processing time. Our best conditions utilized 5 equiv of activated Fmoc-amino acid at a temperature of 86 °C for 10 min coupling and 3 min deprotection time. The coupling of His residues was performed at room temperature to eliminate the effect of racemization for this sensitive amino acid. The comparison of cytotoxic effect of β-amyloid (1-42) peptide synthesized under microwave and conventionally heated conditions will be presented.

P114. Abstract number: 553

Rebuilding Protein Surfaces using Peptide Fixation Technologies

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Small mimics of proteins with intact bioactive surfaces, but with improved pharmacodynamic and kinetic properties, could be of great value in developing new drugs or therapies.

We present three different examples of protein mimics, exploring the use of CLIPS technology for chemical fixation (Chemical LInkage of Peptides onto Scaffolds).[1] This proprietary technology quantitatively cyclizes linear peptides in solution, without the need for orthogonal side-chain protection or the use of expensive catalysts. The technology is applicable to peptides in solution as well as on microarrays used e.g. in epitope mapping studies (PEPSCAN method).

Our first case shows a protein mimic of the fertility hormone Follicle Stimulating Hormone (FSH). We designed CLIPS-based protein mimics covering the major part of the discontinuous b1-b3 epitope on hFSH-beta.

Immunization with these mimics successfully generated neutralizing antibodies against FSH, in case where the linear peptides failed to do so.[2]
The second example involves a protein mimic (~25% of entire protein) of Vascular Endothelial Growth Factor (VEGF), that is involved in the growth of blood vessels and strongly upregulated in most tumor tissues. Data from a recent study in Swiss nu/nu mice clearly demonstrated the strong anti-tumor activity of antibodies generated by immunization with this VEGF-mimic.[3]
Our final example illustrates the use of CLIPS technology in the mimicry of membrane-integrated proteins. One class in particular, i.e. the G-protein coupled receptors (GPCRs), receives enormous attention because ~60% of the currently used drugs is targeted against these. However, there is currently a lack of methods that allow antibody generation against intact or membrane-bound GPCRs. We have found that CLIPS technology can be used to address this problem and we will present in this lecture some of our results in this area.[4]

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P115. Abstract number: 2

Total solid-phase synthesis of N-acylated macrocycle depsipeptides for antibiotic screening

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In past decades, more and more cyclic peptides including acylated cyclodepsipeptides were separated and identified from terrestrial microorganisms and marine organisms. Generally, naturally occurring acylated cyclodepsipeptides contain unusual amino acids and building blocks. Most of them will offer a new frontier in both synthetic organic chemistry and biological activities. In particular, unique unusual structures will offer the challenge of exploitation of novel synthetic methods, reactions, reagents, catalysts, etc. However, practically, it is rather difficult to isolate a larger amount of naturally occurring acylated cyclodepsipeptides because of minute constituents in organisms, difficulty of collection of organisms, and resistance of laboratory culturing. Thus, the limits of the quantity preclude the precise structure determination as well as clarification of detailed biological activities. In fact, the proposed structures of many cyclic peptides have been revised by synthetic works. Thus, the total synthesis is still playing a final means for the structure determination of naturally occurring acylated cyclodepsipeptides just like several decades ago. Furthermore, the efficient large-scale production of naturally occurring acylated cyclodepsipeptides by synthesis will offer an opportunity to investigate their biological activities in detail. One of the characteristic features of cyclic peptides will be their conformational rigidity and stability in vivo, in contrast to their linear counterparts. In addition, unusual amino acid and non-amino acid moieties of naturally occurring acylated cyclodepsipeptides will offer the lead structures of new biologically useful compounds. On the basis of our previous work on total solid-phase synthesis of cyclic peptides, we establish and develop a totally novel chemical approach to synthesize daptomycin analogues by on-resin head-to-tail cyclization. This powerful and universal total solid-phase synthesis strategy will be applicable in the parallel preparation or one-bead one-peptide (OBOP) library of naturally occurring acylated cyclodepsipeptides and the artificial mimic derivatives for systematic research on the structure-activity relation (SAR) of potent lead antibiotic compounds.

P116. Abstract number: 8

Octapeptide ligands derived from the screening of combinatorial libraries with affinity for recombinant erythropoietin

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Recombinant erythropoietin (rhEPO) is used for therapeutics of anemia associated with chronic renal failure and for AZT-induced anemia of AIDS. Monoclonal antibody (mAb) affinity chromatography and dye affinity chromatography are alternative techniques nowadays in use for its purification. MAb are expensive, thus increasing the cost of the final product. Affinity chromatography with Cibacron Blue as the ligand is widely used, but the selectivity is not high. The use of short peptides as affinity chromatography ligands would result in a more economic and selective purification process. Divide-couple-recombine (DCR) method allows obtaining a library with all possible combinations of the amino acids in the form of 'one bead-one peptide'. Peptide ligands can be selected from the library screening. We have developed a rapid and non-expensive strategy for the identification of peptides contained on positive beads, by using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS), and 4-hydroxymethylbenzoic acid (HMBA). In this work the method designed was applied for identification of peptide ligands for rhEPO purification.
In previous works we have obtained low-affinity tetrapeptide ligands for rhEPO. Taking into account those results, a combinatorial library containing the octapeptides XXXFXXAG where X=A, D, E, F, H, L, N, P, S or T was synthesised on HMBA-ChemMatrix resin by the DAR method using the Fmoc chemistry. Side-chain deprotection was carried out with TFA. For the library screening the rhEPO was coupled with either Texas Red or biotin. Fluorescent beads or beads showing a positive reaction with streptavidin-peroxidase were isolated. Fifty beads showed positive reactions. After peptides cleavage with NH4OH, they were sequenced by MALDI-TOF-MS. and those sequences showing more consensus were synthesised and their affinity to rhEPO evaluated using a plasma resonance biosensor. Kd values between 10-5-10-6 M were obtained. Peptides with the highest affinity were immobilised on agarose. All peptide-agarose matrices showed affinity for rhEPO. Also, the affinity of the peptide-agarose matrices for bovine seroalbumin (BSA) - usually present in the culture supernatants - was assessed. Those peptides with the highest selectivity between rhEPO and BSA were selected for future development of a rhEPO purification chromatographic matrix.

P117. Abstract number: 126

Post-synthesis modifications of p-iodo-phenylalanine-containing peptides on solid phase via the palladium-catalyzed Sonogashira reaction

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As recently described, ”Peptides are active regulators and information brokers of key biological functions”. In comparison to small molecules, they have many advantages that make them particularly interesting for drug discovery. As a matter of fact, their high biological activity is often associated with low toxicity and high specificity for their targets. However, due to inherent limitations such as low stability, low oral bioavailability, difficult delivery, as well as costly synthesis, the potential of using peptides as drugs remains relatively low. Hence, to improve key pharmacological properties, structure-activity relationship studies generating libraries of peptide analogs proved to be very helpful to identify new therapeutic peptides. Thus, several modifications including lactamization, ”stapling”of peptides, incorporation of non natural amino acids, pegylation, etc. were developed and showed frequently a significant improvement of the pharmacological properties of peptides, including bioavailability, selectivity and stability. In this regards, we developed an effective method based on the palladium-catalyzed Sonogashira reaction to modify on solid phase p-iodo-phenylalanine-containing peptides. In this study, we used [Ala^{1,2,3}, Leu^{8}]Enkephalin as a template in order to optimize the conditions of the reaction. Following the replacement of the Tyr residue with p-iodo-phenylalanine, the influence of several parameters including the type of base, catalysts, solvent systems, as well as the reaction time or the temperature were

investigated in the Pd-catalyzed cross-coupling reaction. Moreover, the stability of the Fmoc and Boc protecting groups, as well as of different linkers (Wang and Rink amide linkers) were evaluated under these conditions in order to optimize the methodology. Results showed that the best conditions for the reaction were including TEA (base), DMF (solvent), and $PdCl_2(PPhe_3)_2$ (catalyst), at 40 °C during 20 h. No side-reactions involving sensitive and/or reactive amino acids such as methionine, cysteine, histidine and tryptophan have been detected. No cleavage of the Boc protecting group was observed, even after several days, whereas the Fmoc protecting was not stable over an extended period of time. Similar observations were made with the Rink amide linker and Wang linker, respectively. Following the optimization of the procedures, we investigated the usefulness of this reaction in the preparation of a new potent PACAP agonist.

P118. Abstract number: 200

Specific substrates and inhibitors of human furin

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Proprotein convertases (PCs) are calcium-dependent serine proteases of the subtilisin family which are known to generate the important biologically active peptides by the specific cleave of peptide bond at the C-terminal fragments R-X-(K/R)-R of their precursors. Such group of enzymes are initially synthesized as inactive proenzyme forms that undergoes activation under physiological condition. Furin, one of the seven endoproteases that belong to the PC family, is ubiquitously expressed in mammalian cells processing and activating a wide variety of proprotein substrates. The endogenous substrates of furin are receptors such as insulin proreceptor, plasma proteins, growth factors and hormones as well as proteases such as matrix metalloproteinases - like membrane type 1-matrix metalloproteinase (MT1-MMP). Increased activity of furin was identified in head and breast tumors, neck tumors also in lung cancer. Recent reports have indicated that furin is a target of hypoxia-inducible factor 1 (HIF-1) and that hypoxia induces elevated levels of furin, leading to more aggressive tumors. Therefore PC inhibitors may constitute new promising antitumor agents. The aim of this study is to select, using combinatorial chemistry methods, specific furin substrates that will display FRET and chromogenic properties. The general formula of the peptide library synthesized is as follows:

ABZ-X4-X3-X2-X1-ANB-NH2,

where ABZ = 2-amino benzoic acid (donor of fluorescence); ANB-NH2 = amide of 5-amino 2-nitro benzoic acid (acceptor of fluorescence); in positions X1 - X4 non-proteinogenic basic amino acids and peptoid monomers (Har, Orn, Dab, Gnf, Amf, Nlys) were introduced.

Iterative method was applied for selection process. Selected the most effective substrates were converted into aldehyde and/or phosphonic peptide derivates and tested as potential inhibitors of human furin.

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P119. Abstract number: 218

Novel peptide conjugates. Heterocyclic modifications of the immunomodulatory ubiquitin fragment.

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Ubiquitin is one of the pivotal proteins in cellular physiology. The immunomodulatory properties of ubiquitin were discussed by us recently [1]. We discovered that the peptide DGRTL is the shortest active fragment of the immunosuppressory loop of ubiquitin and we analyzed the impact of the specific amino acid side chains on the activity [2].

To investigate the influence of heterocyclic modifications on properties of DGRTL peptide, we synthesized a series of peptide-heterocycle conjugates, in which the aspartate residue was replaced by 2-substituted 1*H*-benzimidazol-5-yl alanine [3] or 2,3-disubstituted quinoxalin-6-yl alanine [4]. The peptide conjugates were obtained using the solid phase strategy developed in our research group, based on the on-resin synthesis of the heterocyclic moiety after the peptide assembly [3, 4]. Such procedure is especially suitable for synthesis of a series of conjugates for screening purposes.

We examined the heterocyclic modifications containing 2,2`- bipyridyl motif, known for high metal ion affinity. The coordination properties of the DGRTL conjugates in respect to Cu(II) and Pt(II) were investigated using HR-ESI-MS. The fragmentation pathways of conjugates and their complexes provided information on localization of metal ion in the molecule and on the relative stability of the coordination compounds. The NMR spectra were used to confirm the location of Cu ion in the benzimidazole conjugate.

The immunomodulatory activity of the conjugates and their complexes was examined in vitro using antibody forming cells test. The effect of the heterocyclic motif was established after comparison with the DGRTL peptide, whereas the sequence involvement was examined in relation to a respective modified dipeptide (Xaa-Gly).

The results show the significant influence of the heterocyclic modification on the biological and complexing abilities of peptides. Such conjugates could be of use as starting structures for design of novel bioactive compounds of pharmacological interest as well as catalysts, indicators and nanomaterials.

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P120. Abstract number: 332

Determination of heme binding motifs by a combinatorial peptide library approach

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The binding of heme and heme degradation products (BOXes) to distinct motifs in proteins, e.g. ion channel proteins, was shown to affect protein function [1]. In order to explore and better understand the role of heme and BOXes in physiological and pathophysiological conditions, the molecular basis of heme-protein interactions should be investigated in more detail. Therefore, in a first approach a combinatorial peptide library was used to evaluate the binding capacity of heme to specific peptide motifs in order to find out whether there are consensus sequences for this

association [2]. For a selection of peptides proposed by screening of the library X4[C/H/Y]X4 (X = any amino acids, except Met, Cys, including Nle) binding constants were determined by UV-Vis spectrometry. The consensus sequences derived from this approach were subjected to a database search that aimed at the determination of known motifs as well as the discovery of yet unknown proteins those function might be influenced by heme and its degradation products.

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P121. Abstract number: 367

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

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

ABZ-X4-X3-X2-X1-ANB-NH2 where:

ANB-NH2 - amid of 5-amino-2-nitrobenzoic acid (acceptor of fluorescence);

ABZ - 2-aminobenzic acid (donor of fluorescence);

X1; X2, X3, X4 were 19 proteinogenic amino acid residues excluding Cys.

Partial results of the substrate mapping of those three enzymes indicate that StpA and StpB display very similar substrate specificity. StpC reveal quite different substrate preferences. The obtained substrate sequence(s) of all three proteases will be characterized biochemically. C-terminal moiety of potent substrates were converted into aldehydes. Inhibitory activity of such synthesized compounds was tested for all enzymes studied.

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P122. Abstract number: 410

Discovery of Esterolytic Histidine Oligomers Using Peptide Arrays

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Solid-phase synthesis of peptide constructs such as linear, cyclic or dendritic peptides represents one of the most practical entries into synthetic enzyme models. The structure-activity relationships in such peptide constructs are generally complex and their study requires combinatorial chemistry approaches involving the synthesis and screening of large libraries, typically using one-bead-one-compound libraries (N. Maillard et al., *J. Comb. Chem.* **2009**, *11*, 667-675). Herein we report a simple and practical catalysis screening protocol that allows one to assay peptide arrays prepared by the SPOT-technique on cellulose membranes (R. Frank, *Tetrahedron* **1992**, *48*, 9217-9232). The SPOT format delivers spatially encoded libraries which circumvents the problem of bead decoding. In addition assays in the SPOT format are known to be predictive of activities of the peptides in solution for the case of binding to biomolecules.

To test the compatibility of SPOT-libraries for catalysis screening we prepared a 192-member library of dendritic and linear analogs of the catalytic peptide dendrimer **A3C** (Ac-His-Thr)$_8$(*Dap*-His-Thr)$_4$(*Dap*-His-Thr)$_2$Dap-His-Thr-NH_2 (Dap = L-2,3-diaminopropanoic acid branching unit) previously identified in a focused structure-activity relationship study of esterase peptide dendrimers and which catalyzes the hydrolysis of fluorogenic 8-acyloxypyrene 1,3,6-trisulfonates with high catalytic efficiency (k_{cat}/k_{uncat} = 90,000, E. Delort et al., *J. Org. Chem.* **2006**, *71*, 4468-4480). Catalysis screening of the SPOT library confirmed the high activity of peptide dendrimer **A3C** over other analogs, providing the first evidence that SPOT library display is favourable for catalysis screening. The screening also pointed to the remarkable esterolytic properties of a simple linear histidine undecapeptide for the reaction. A closer investigation of histidine oligomers from one to fifteen residues shows that these simple peptides indeed efficiently hydrolyze acyloxypyrene trisulfonates with rate accelerations up to k_{cat}/k_{uncat} = 5,000.

P123. Abstract number: 423

Preparation of Novel Polymer Support and Its Application for Peptide Engineering

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In recent days, solid-phase peptide synthesis was widely used with its strength such as high purity and easy separation in the peptide engineering field. To increase efficiency of solid-phase peptide synthesis, many research groups have tried to improve solid-phase peptide synthesis technique by modifying the polymer supports. The ideal polymer supports should have some important characters; i) stability in harsh chemical and physical condition, ii) compatibility with various solvents including water, iii) preventing the non-specific binding when used for bioassays, iv) advanced kinetics for chemical reaction, v) low background signal, such as fluorescence or Raman spectroscopy.

In this presentation, we will present the preparation and application of polymer support, which have the important characters as mentioned above. The polymer support was prepared from aminomethyl polystyrene resin by grafting of surface with poly(ethylene glycol) groups. Because of the cross-linking reagents were not used while preparation, the polymer support was well-swollen in the various solvents and had less auto-fluorescence. On this polymer support, one-bead one-compound peptide library was synthesized by using ”split-and-mix”method, and the peptide substrates of Ser/Thr kinase were successfully screened by using this peptide library. In addition, several compounds containing amide groups were released in aqueous media by using

pyrazolone active ester linker which bounded on the polymer supports as well.

P124. Abstract number: 456

QSAR modeling of calcitonin gene-related peptide antagonists

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Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide generated by alternative tissue- specific splicing of the primary transcript of the calcitonin gene.CGRP is involved in the regulation of blood flow to the brain and pain-sensitive meninges. A clinical study has shown that inhibition of CGRP binding to its receptor may be useful for therapeutic purposes. Despite improvements in receptor potency, CGRP peptide antagonists suffer from poor metabolic stability. Both the native human CGRP and a derivative of CGRP (8-37) have demonstrated short plasma half-lives of approximately 10 min [1]. Quantitative structure activity relationship (QSAR) studies can be employed to identify CGRP peptide antagonists with prolonged in vivo circulating time and improve metabolic stability, selectivity, and receptor affinity.

In the present work, a library containing 97 CGRP peptides were used, derived from two structure activity experimental studies through which systematic modification of the C-terminal segments CGRP Y0-28-37 and CGRP 27-37 have been done [1,2]. Conventional, inductive [3,4] and Dragon descriptors were calculated and different linear activity prediction models were constructed. The poor values of the statistical parameters derived from these models indicated the probability of a nonlinear relationship between the descriptors and the peptide activities. However, applying an artificial neural network as a nonlinear modeling method could not improve the statistics of the models significantly due to using descriptors selected by linear methods. The score plot obtained from principal component analysis (PCA) of the dataset, demonstrated grouping of the peptides into subpopulations, indicating their different behavior in modeling resulted from different ranges of activities. In an attempt to overcome the problems of modeling these peptide activities we employed the shuffeling-ANFIS method [5]. This powerful nonlinear feature selection technique was used to select four descriptors amongst the 195 cross-validated descriptors (conventional, inductive and Dragon). The artificial neural network model constructed using the four Shuffling-ANFIS descriptors could successfully predict 88% of the activities of the peptides in the test set. The descriptors applied in the final model reveals the importance of peptide properties such as surface area, 3D structure and atomic partial charges as the main factors affecting the antagonist activity of these peptides.

P125. Abstract number: 21

Chemical synthesis vs. expression of the HIV-1 Rev protein

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The HIV-1 Rev protein (116 amino acids, 13kDa) is responsible for shuttling partially spliced and unspliced viral RNA out of the nucleus. This is a crucial step in the HIV-1 life cycle, and thus Rev is an attractive target for the design of anti HIV drugs. Despite its importance, there is lack of structural, biophysical and quantitative information about Rev as well as about the role of the post translational modifications on the protein. This is mainly due to the inability to obtain the protein in sufficient quantities that are required for biophysical and structural characteristics. The production of recombinant Rev was described only very recently (Marenchino M. et al, Protein Expression and Purification, 2008). Here we present two new highly reproducible methods to produce Rev in large quantities: (i) Chemical synthesis: To achieve our synthetic goal, we have synthesized the wild type protein from three fragments combining native chemical ligation coupled with desulfurization; (ii) A new method for expression and purification: Expressing Rev from a vector containing the HLT tag followed by purification with a Ni column and a cation exchange column. The Rev protein was obtained in excellent yields and quantities using both methods. The chemically synthesized protein was subjected to structural and functional analysis, which revealed a similar behavior to the expressed recombinant protein. Our synthetic strategy should enable straightforward access to homogenously posttranslationally modified HIV-1 Rev in order to reveal the exact role of these modifications on the protein structure and function.

P126. Abstract number: 38

A DNA and Metal Binding Hybrid Based on Triostin A

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Delivering reactive metalated species to targeted DNA utilizing the DNA affinity of small peptides is a promising approach in drug development. Peptide scaffolds can be derived from a number of natural products such as histones, zinc finger proteins or small cyclopeptides like the quinoxaline antibiotics. One of the most prominent members of the latter family is Triostin A.[1]

Triostin A binds DNA via bisintercalation in the minor groove, spanning two base pairs. The rigid bicyclic backbone preorganizes two quinoxaline moieties in a distance of 10.5 Å which facilitates bisintercalation and is likewise the distance found for every i+4 base pair in B DNA.[2] Substituting the intercalating moieties with nucleobases should result in a modified interaction with DNA. Such derivatives are supposed to bind DNA via hydrogen bonding to the exposed Hoogsteen flanks within the major groove. This specific interaction enables potential sequence specificity for these triostin analogues.[3]

The synthesis of a nucleobase functionalized triostin analogue with a metal binding site was realized in a modular approach using Cu(I) mediated ”click”chemistry as key step. The synthesis as well as metal binding studies (mass spectrometry) and DNA binding (UV-melting curves) of the hybrid are presented.

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P127. Abstract number: 70

Convergent syntheses of HuPrP106-126 (difficult sequence) using native chemical ligation and desulfurization/deselenization

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Prion proteins are causative agents of neurodegenerative diseases such as scrapie, CJD, mad-cow disease (nvCJD), GSS, etc.[1] Prion-derived peptide HuPrP106-126 was found as a difficult sequence for the Fmoc approach.[2] We have described its synthesis using divergent approach in 9% yield.[3] In order to improve yield of this peptide by Fmoc approach, we employed 2- and 3-segment convergent approaches using a native chemical ligation with subsequent desulfurization/deselenization[4] of peptide precursors. In 2-segment approaches, we studied ligation of segments such as KTNMKHMAG* with CAAAGAVVGGLG or UAAAGAVVGGLG; and KTNMKHMAGAAAAG* with CVVGGLG or UVVGGLG (asterisk indicates activation by ester, thioester[5] or cyclic-urea[6]). Since the syntheses of the longer peptides are also limited with yields, we moved to 3-segment convergent approaches. We studied possibility of kinetically controlled ligation[7] of KTNMKHMAG*, CAAAG* and UVVGGLG using chemoselectivity between sulfanyl and selanyl groups. We observed that CAAAG-*SEt* stored as TFA salt in a freezer is stable for several months. Another 3-segment convergent approach was based on consecutive modular ligations.[8] The first ligation was between Thz-AAAG* and UVVGGLG. After thiazolidine ring opening with NH_2OH, the second ligation with KTNMKHMAG* was carried out. The products were converted to HuPrP106-126 by radical desulfurization/deselenization using Danishefsky’s procedure.[4] The 3-segment convergent approach led to significant improvement of the yield.

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P128. Abstract number: 147

Functionalized PNA Backone Building Blocks eligible for Diels Alder Click Chemistry in Molecular Imaging

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Therapies of cancer via ”old fashioned”chemotherapeutics normally lead to undesired side effects followed by abortion of the therapy due to the physical and chemical attributes of the drug. Modern chemotherapeutics are marked by their property of cell specific interaction with DNA on a molecular basis without harming the surrounding healthy tissue. This rapid progress in chemotherapeutic development led to molecular therapies which demand molecular diagnostics : Hereby, radiation free methods of imaging metabolic processes become increasingly topic of current scientific research. Open questions concerning the molecular imaging techniques remain to be answered: 1) The transport of imaging components in blood circulation, 2) the differentiation between tumor and healthy tissue and 3) the local enrichment within target tissue and target cells or in subcellular components. Development of needed molecules led to complex, modularly built, functional molecules. This results in extreme requirements not only of synthesis and quality insurance but of attributes like fast, specific, quantitative and irreversible ligation of single modules. A series of for improving approaches for improving the ligation of components finally led to the development of the ”click chemistry”.

P129. Abstract number: 159

Native chemical ligation as a versatile tool to connect peptides with internal modified oligonucleotides

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Native chemical ligation (NCL) is a favoured technique to link short peptide sequences to other peptides and even whole protein domains. Mild reaction conditions and high yields make this method attractive not only for peptide-peptide couplings but also for couplings with other biomolecules such as oligonucleotides. NCL has been used to attach peptides to the terminal ends of cysteine modified oligonucleotides. This attachment mode is limited to end-labeling of oligonucleotides. We sought for a method that allows the attachment of peptides at any desired position within DNA. We herein report the first ligation of peptides to sequence internal sites using cysteine-containing phosphoramidite building blocks that can be used in automated DNA synthesis. For this purpose, Fmoc/StBu-protected cysteine was attached via an aminopropargyl linker to the C5-position of uridine. The rigid triple bond conferred a high reactivity in native chemical ligation reactions which involved 5mer peptide thioesters and up to 15 nucleotides long oligonucleotides. The desired peptide-oligonucleotide conjugates were obtained in high yields after purification. Internal peptide-modified oligonucleotides offer a better control of the spatial arrangement of peptides presented on self-assembled nucleic acid complexes. Because modifications can have an enormous effect on hybridization efficiency we studied the thermal stability of the achieved peptide-DNA hybrids. UV melt experiments revealed that the peptide modification does not hamper nucleic acid hybridization. This finding marked an important step in our research program devoted to studies of multivalent presentation of peptides via modular assembly of nucleic acid complexes.

P130. Abstract number: 174

Expression and characterization of human interleukin-8 analogues

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Despite modern techniques, implant rejection and wound healing are still significant problems in regenerative medicine. Improved biomaterials have been reported to be advantageous to conservative materials. However, it is necessary to control and limit the inflammatory potential of implants coated with biomaterial layers.
The chemokine interleukin-8 (IL-8, also named CXCL8) has been reported to mediate inflammatory processes and hence, the investigation of IL-8 interaction is a powerful tool to follow and estimate the inflammatory potential of biomaterials [1]. IL-8 belongs to the family of CXC chemokines and selectively binds to the seven transmembrane G protein-coupled receptors CXCR1 and CXCR2 with nanomolar affinity [2].
In previous work it could be shown that the C-terminal helix of IL-8 is not directly involved in the binding of the chemokine to hyaluronic acid [3]. Whether this holds true for different biomaterials or extracellular matrix components have been investigated. Proteins with truncated C-termini were synthesized, as well as N terminally reduced IL-8 segments. The hIL-8 N-terminus was expressed in E. coli and purified by affinity chromatography. C-termini were synthesized by solid phase synthesis (SPPS). Both fragments were fused by Expressed Protein Ligation (EPL) and characterization of these analogues was performed by analytical and biological methods.

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P131. Abstract number: 176

Fluorescent Modification of the Regenerative Chemokine SDF1 a to Study Receptor Internalisation

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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 embryogenesis and later in adults 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 SDF-1α could serve as a therapeutic target. For studies on this 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-terminal segmet SDF-1α (1-49) has been cloned and expressed recombinantly in E. coli ER 2566, while the C-terminal segment SDF-1α (50-68) has been synthesized via solid phase peptide synthesis. Modifications are thereby introduced in the C-terminal segment at Lys^{56}, e. g.. Up to now carboxyfluorescein has been coupled to the ε-amino group of the lysine residue. The two fragments then have been ligated by Expressed Protein Ligation (EPL), a subform of the Native Chemical Ligation (NCL).[2] The activity of the conjugate has been tested in an inositol 3-phosphate turnover assay as well as in chemotaxis assays. The

potential of this compound to bind specifically to CXCR4-transfected HEK293 cells as well as to induce internalisation has been proven by fluorescence microscopy.[3]

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P132. Abstract number: 183

Do intramolecular hydrogen bonds play a role in native chemical ligation?

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Native chemical ligation enables the chemoselective coupling of two unprotected protein segments. This reaction proceeds via a two-step mechanism. First, the side chain of N-terminal cysteine residues reacts with the peptide thioester under formation of a peptide thioester ligation intermediate. After this thiol exchange, the N-terminus of the cysteinyl peptide is involved in a subsequent intramolecular aminolysis reaction, which leads to the ”native”peptide bond. The rate of this reaction sequence usually depends on the first step. The C-terminal amino acid of the thioester and the thiol leaving group play a critical role. Ligation of β-branched or proline thioesters proceed slowly and often did not result in a quantitative ligation. Proline is the only amino acid comprising a cyclic side chain and a secondary amino group. We hypothesized that intramolecular hydrogen bonds may participate in stabilizing the transition state of the thiol exchange reaction. To address this issue we investigated native chemical ligation reactions of proline, alanine and N-methylated alanine thioesters. We also assessed the influence of hydrogen bond donors at the thiol leaving group (3-mercaptopropionic acid, 3-mercaptopropioamide and N-alkylated 3-mercaptopropioamide). The results from the kinetic studies indicate that hydrogen has an activating effect on the thioester reactivity and therefore increases the ligation rate with the thiol side chain of the cysteinyl-peptide.

P133. Abstract number: 230

Convergent Synthesis of Peptide Dendrimers by the CLAC (Chloroacetyl) Ligation

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Herein we report the convergent synthesis of large, protein-sized 4th and 5th generation peptide dendrimers by the convergent multiple (up to 8x) CLAC (chloroacetyl) ligation of 2nd and 3rd generation peptide dendrimers. In the CLAC ligation, dendrimers with a C-terminal cysteine residue at their core couple in multiple copies by substitution of the chlorine atom at the multiple chloroacetylated N-termini of another peptide dendrimer. This ligation does not require special building blocks or catalysts and provides products in high yields and purities. The method opens the way to the investigation of large peptide dendrimers as protein and enzyme models.

P134. Abstract number: 304

Fmoc solid phase synthesis of peptide thioesters and thioacids for native chemical ligations employing a tert-butyl thiol linker

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Herein we describe the first direct synthesis of peptide thioesters and thioacids using Fmoc SPPS with a base-stable thioester linker.[1] Thioesters are generally known to be base labile, which has previously precluded their use as linkers in Fmoc-based SPPS. Tert-butyl thioesters, however, are special and distinct from from other thioesters: While being stable under under alkaline conditions[2] they are easily cleaved under only slightly basic conditions by primary thiolates. In stability experiments, we have shown that Boc-Phe-S-t-butyl has a half life of 6.5 days when treated with 20 % piperidine in DMF being 5.5 times more stable than Boc-Phe-S-ethyl (half life of 5.5 hours). Accordingly, a tert-butyl linker attached to an acid sensitive resin might allow a direct synthesis access to peptide thioesters.

We developed 4-mercapto 4-methylpentanol (MMP) as a tert-butyl thiol linker which was coupled to a standard 2-Cl-tritylchloride resin via its hydroxyl function. Acylation of the tert-butyl thiol with the first amino acid was accomplished using EDC/DMAP with yields ranging from 70-90 %.[3] For most dipeptides tested, no diketopiperazine (DKP) formation was observed after coupling of the 2nd amino acid. DKP formation could be fully avoided by quantitative coupling of dipeptides to the first resin bound amino acid using HATU/HOAt. After standard Fmoc-SPPS synthesis, the bound peptide thioesters could be cleaved either with acid to yield fully deprotected or by nucleophilic displacement with thiolate to furnish fully protected peptide thioesters. By displacing the peptide from the resin with ß-eliminable 2-cyanoethylmercaptane followed by base treatment, the method was extended to the preparation of peptide thioacids. An in-situ generated pentapeptide thioacid could be transformed via addition of tosyl azide to the corresponding sulfonamide.[4]

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P135. Abstract number: 308

Assembly of the Zinc Finger Domain Zif268

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Zinc finger domains are known as specific DNA binding motifs found in many transcription factors. The first and best investigated zinc finger protein Zif268 specifically recognizes a DNA sequence of nine base pairs. Its zinc finger domain consists of three zinc finger motifs composed of 30 amino acids each. For sequence specificity a tandem repeat of at least three zinc finger motifs is necessary. The zinc atom is, thereby, tetrahedrally coordinated by a Cys2His2 motif and known for being exclusively of structural importance.[1]

The Zif268 domain sequence was chosen for incorporating an artificial metal chelating building block. The introduction of a second metal binding site into the system might result in a cooperative effect with the structural one. The assembly of the entire sequence of the zinc finger motif is to be achieved by means of expressed ligation. Therefore, the desired peptide-thioester is achieved by protein

expression, whereas the cysteine containing peptides are synthesized by solid phase peptide synthesis (SPPS).
Protein expression was the method used for the implementation of zinc fingers one and two (Zf12). The respective coding region of these zinc fingers was cloned into an intein vector. This vector includes an intein- as well as a chitin-binding-domain (CBD)-coding region and while expressed in E. Coli these protein domains were attached to the C-terminus of the target peptide. The intein-tagged peptide was loaded on chitin beads and cleaved via thioesteri-fication with 2-mercaptoethanesulfonate (mesna). Through cleavage a C-terminal thioester, needed for expressed ligation, was generated. The peptide was verified by ESI-HR mass spectrometry.
The peptide syntheses of the third zinc finger (Zf3) and its variants were carried out by automated microwave assisted SPPS. Besides the sequence of zinc finger three with an incorporated unnatural metal chelating amino acid at position His85, also the natural occurring sequence was synthesized for comparison reasons, as well as a modification of the sequence known for possessing DNA cleavage ability by lacking one of the histidine residues at its active center.[2,3] Furthermore, a fluorophore is to be attached to the peptides to alleviate the observation of the following ligation step.

P136. Abstract number: 359
Synthesis of Neuropeptide Y analogues as PET imaging agents
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Radiolabelled peptides are an important class of radiopharmaceuticals for imaging and therapy of tumours. They can be designed upon endogenous peptide hormones making them an attractive target.
Neuropeptide Y (NPY) is a 36 amino acid peptide that exhibits a wide range of central and peripheral activities. These actions are mediated by at least six G-protein coupled receptor subtypes. The Y1 receptor subtype is over expressed in a number of human tumours and can potentially be exploited for detection of tumours and also for the delivery of radiotherapy.
This project aims to develop highly selective Y1-receptor peptide ligands suitable for imaging and/or delivery of radiotherapy with improved in vivo properties over native NPY and other pancreatic polypeptide family members.
Y1-selective cyclic and linear peptide antagonists previously described in the literature[1] have been further derivatised by substituting amino acids with alkyne or azide terminating side chains into the sequence. These functional groups allow for ”click chemistry”modification and introduction of radiolabels under mild conditions.
The peptide may contain either of the required functional groups (azide or alkyne) and the radiolabel the corresponding partner (alkyne or azide). The click reaction is chemoselective, lacking reactivity towards other functional groups on the peptide. The reaction can be carried out in an aqueous environment at room temperature and it generates a stable triazole moiety bridge between the peptide and radiolabel.
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P137. Abstract number: 377
Control Function - Towards Activatable SDF-1a Analogues
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SDF-1 (CXCL12) is a representive of the CXC chemokine family, but its distinct chromosomal localization and low homology to other group members makes this protein unique along the CXC chemokines. It is constitutively expressed in many tissues, signals through CXCR4 and at least binds to CXCR7, however the role of this recently dicovered receptor is not fully understood, yet.
SDF-1 attracts stem cells in a concentration dependend manner, in particular guide hematopoietic progenitor cells (HPCs) to the bone marrow and modulates neuronal progenitors. Furthermore, it participates in embyonic heart and intestine development as well as in the metastasis of CXCR4/7 positive cancer cells toward SDF-1 expressing tissues.
This broad range of biological functions make this protein interesting as a potential drug in regenerative medicine to support repair of damaged tissues through stem cell recruitment. Different labelled and activatable analogues of SDF-1 are desirable, in order to study protein action in more detail.
Herein we present a convenient synthesis strategy, which allows the modification of SDF-1 site-specific all in its C-terminal region by applying the expressed protein ligation approach. The N-terminal segment was prepared as C-terminal peptide thioester by using the IMPACT® system and the C-terminal segments, which carry a N-terminal cysteine residue, were synthesized by SPPS. After ligation and refolding of the proteins, the biological activity of the compounds was tested in cell-based assays.

P138. Abstract number: 378
3TS : Thiazolidine-Triggered Thioester Synthesis
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The chemical synthesis of proteins is a great challenge for organic chemists. Chemoselective ligation methods such as native chemical ligation (NCL)[1] or Staudinger ligation[2] allow the assembly of proteins of moderate size (150 amino acids) and provide ready access to natural as well as modified proteins. Native chemical ligation is based on the reaction of a peptide bearing a C-terminal thioester group with a N-terminal cysteinyl peptide. Transthioesterification is followed by an intramolecular *S,N*-acyl shift that leads to the formation of a peptide bond at a X-Cys junction. Application of this chemistry to N-terminal selenocysteine peptides was also described. Methods based on the use of N-linked thiol-containing cleavable auxiliaries or thiol-modified proteinogenic amino acids were used to extend the principle of native chemical ligation to sites other than Cys residues.
The key starting materials for native chemical ligation are unprotected C-terminal peptide thioesters. Consequently, Fmoc/*t*Bu SPPS methods for peptide thioesters are of high interest. For example, Kenner sulfonamide safety-catch linker and an intramolecular *N,S*-acyl shift permitted thioester formation after peptide assembly (Ollivier N., Behr J.B., El-Mahdi O., Blanpain A., Melnyk O. Org. Lett., 2005, 7(13), 2647-2650).
Recent studies in the lab led to the discovery of an innovative and efficient method for thioester synthesis based on the Fmoc/*t*Bu SPPS of a highly stable peptide amide derivative, featuring a beta-amino thiol arm at the C-terminus. Thiazolidine ligation in solution between the beta-amino thiol moiety and glyoxylic acid triggers the formation of the thioester.
Importantly, thioester formation is performed in solution (i.e. can be easily monitored) using a simple chemical reaction. The synthesis of the peptide amide precursor is

straighforward. This peptide derivative is highly stable and can be converted to the thioester just before the NCL. This method, which is called 3TS for thiazolidine-triggered thioester synthesis, complements favorably the existing methods for thioester synthesis.

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P139. Abstract number: 450

Application of an Omonasteine Ligation Strategy for the Total Chemical Synthesis of a 108-AA Bromodomain.

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Bromodomain containing protein 7 (BRD7, also known as Celtix-1) is a protein that is associated with nasopharyngeal carcinoma and recently, BRD7 has been shown to suppress tumorigenesis by serving as a cofactor of p53.

To enable detailed biochemical and cellular studies with the BRD7 bromodomain, we aimed at total chemical synthesis of this domain by solid phase peptide synthesis and native chemical ligation between a C-terminal mercaptopropionic acid-Leu (mpaL) thioester and an N-terminal cysteine. However, the bromodomain is relatively long (108 aa) and contains only a single cysteine residue located at position 76, making this a challenging target. Since synthesis of this domain by classical native chemical ligation would involve synthesis of a 75 aa long peptide-thioester, which we thought unfavorable, we chose to use a alternative approach.

We extended the previously developed homocysteine/methionine ligation strategies to allow for 2 sequential ligations of three peptide fragments. The key innovative step in our work was the on-resin S-/N-deprotection of the N-terminal homocysteine of the middle fragment, followed by on-resin reaction with formaldehyde and cleavage from the resin to give an N-terminally protected omonasteine-peptide-mpaL thioester. Omonasteine or 1,3-thiazinane-4-carboxylic acid can be readily reconverted to homocysteine by reaction with N-methyl-hydroxylamine.HCl, analogous to the conversion of thiaproline to cysteine, which is routinely used in sequential NCL strategies. After the first homocysteine ligation, the omonasteine was reconverted to homocysteine and the second ligation could be peformed. Methylation of homocysteines to native methionines in the completed amino acid chain was done with an excess of iodomethane in a chaotropic buffer and, following removal of the S-Acm protecting group for the single cysteine, we obtained the BRD7 bromodomain with a single free cysteine in adequate quantities for biological examination. The same strategy was used to prepare a fluorescently labeled derivative of BRD7 bromodomain. Biological activities of the BRD7 bromodomains are assessed.

P140. Abstract number: 452

Maintaining bioactivity with triazoles as disulfide bond mimetics

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The scope of triazoles as disulfide bond mimetics was investigated. In contrast to disulfides, triazoles are not affected by disulfide isomerases or reducing agents. Moreover, triazoles can be formed in a two component approach, comparable that of two cysteins forming a disulfide bond. A Huisgen-type cycloaddition of alkyne and azide generates the triazole very readily[1]. Since the introduction of Cu(I)-catalysis[2], this reaction has been applied in many cases[3]. The two-component approach allows the substitution of cysteines in peptides by alkyno- and azido-funtionalized amino acids. A straight forward peptide synthesis is followed by a cyclization reaction leading to triazoles as disulfide bond mimetics.

The ”click”strategy was applied to substitute the two disulfide bonds in tachyplesin-I. This antimicrobial active peptide consists of 17 amino acids forming a β-hairpin-fold[4]. The two disulfides create a ribbon like structure and are essential to stabilise the active conformation of the peptide[5].

Two triazole-analogues of tachyplesin-I has been produced and studied with respect to their chemical and biological properties. Propargylglycin and azido-ornithin/ azido-amino-butyric acid were used to replace the cysteines upon peptide synthesis. The following click reaction generates bicyclic peptides, which contain triazoles instead of disulfides. Successful cyclization was proved by HPLC retention time shifts, MSMS-fragmentation and NMR-studies.

Additionally, the biological activity of the cyclic and linear species was investigated in an antimicrobial assay. Only the correctly folded peptide was able to inhibit the growth of different bacterial strains. More importantly, the minimum inhibitory concentration was similar to that of the wild type tachyplesin-I.

Therefore the introduction of triazoles in place of disulfide bonds in tachyplesin-I did neither change the structure, nor the biological activity. Hence, triazols can be regarded as true disulfide mimics.

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P141. Abstract number: 471

Ne-(Thiaprolyl)-lysine as a handle for site-specific protein conjugation

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Covalent bioconjugation of proteins is an attractive approach for the selective modification of proteins for structure-function analysis and biomolecular imaging. A number of strategies exist for the functionalization of proteins, and most of these rely on the use of either amines or thiols as attachment points for derivatization of native proteins. However, these strategies come with significant limitations and an alternative strategy involves the introduction of orthogonally reactive or protected reactive groups into (semisynthetic) proteins.

Here, we introduce the use of a thiaproline modified lysine side-chain (Lys(Thz)), as an unlockable handle that enables late-stage, site-selective modification of chemically synthesized proteins. The Lys(Thz) residue was incorporated into the murine chemokine RANTES to demonstrate its compatibility with Boc/Bzl solid phase peptide synthesis, native chemical ligation, and disulfide bond formation. After oxidative folding of the protein, the thiol was liberated under mild reaction conditions (0.2 M hydroxylamine (NH_2OH) or O-methylhydroxylamine ($MeONH_2$), pH 4) and was subsequently reacted with thiol-selective tags. This side chain protection strategy enables the use of readily available thiol-reactive probes for the modification of internally disulfide bonded proteins not interfering with their native fold.

P142. Abstract number: 539

Convenient synthesis of C-terminal glycopeptide conjugates via Click chemistry

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Glycoproteins are involved in many biological processes including among other things, immune defense, cell growth or inflammation. Access to natural glycoproteins and glycopeptides are usually achieved by recombinant techniques or, by total or semisynthetic chemical ligation strategies. Another approach relies on the conception of glycopeptides mimetics containing unnatural linkages between the peptide and oligosaccharide moieties using chemical ligation. To that purpose, the regioselective Cu-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) developed independently by Meldal[1] and Sharpless[2] proved to be a very efficient and versatile tool. Indeed the chimioselective 1,3-dipolar cycloaddition between an organic azide and a terminal alkyne lead to the linkage 1,4-disubstituted triazole. Previous works[3,4] have reported the CuAAC ligation of unprotected azide carbohydrates and alkyne side chain peptides.

Recently our research focused on the design of an innovative synthesis of unprotected C-terminal azide linker peptides and their application to copper catalyzed cycloaddition with oligosaccharide-based acetylenes. First the method encompasses a reliable, safe and efficient synthesis of amine-azide linker salts. Next aminolysis cleavage from solid support of side-chain deprotected peptides is achieved using the amine-azide linker. The released C-terminal azide peptides undergo Copper (I)-catalyzed [3+2] cycloaddition in mild reaction condition with various glycosides such as quinic and shikimic acids derivatives.[5]

The C-terminal azide linker peptides approach represents an alternative to the commonly used side chain modified peptides and an original strategy to access C-terminal glycopeptides. This method could also be applied to other carbohydrate scaffolds or alkyne peptide conjugates.

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P143. Abstract number: 551

Preparation of Thioacid-containing Amino Acids and Peptides and their Application in Ligation Reactions

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Ligation reactions have emerged as an important tool for the chemoselective conjugation of large and complex molecules. One recent example is the reaction of thiocarboxylic acids (thioacids) with electron-deficient organic azides, such as sulfonyl azides [1]. This reaction proceeds at room temperature in different solvents and leads to *N*-acylsulfonamides in excellent yields. In the peptide field, this ligation reaction has been applied for the synthesis of peptide mimetics [2] and C-terminal labeling of peptide thioacids [3].

Here we present the preparation of amino acid and peptide derivatives containing aspartic acid (Asp) and glutamic acid (Glu) residues with a suitably protected thioacid in their side chain. Several protecting groups such as trityl thioesters and other acid-labile groups have been evaluated for their stability under peptide synthesis conditions and their ease of deprotection. These compounds then served as precursors for amino acids and peptides with a side-chain thioacid. Whereas the more traditional approach to thioacids, i.e. the hydrothiolysis of side chain-activated Asp and Glu derivatives, was accompanied by diacyl disulfide byproducts due to thioacid oxidation, our procedure gave the thioacids in high purity. Subsequent ligation of sulfonyl azide-modified carbohydrates and fluorescent dyes gave access to the corresponding peptide conjugates (glycopeptides, fluorescently labeled peptides).

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P144. Abstract number: 552

Molecular dynamics calculation and NMR conformational studies of heterodetic triazolyl cyclo-nonapeptides: a comparative study

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A solid phase assembly of model peptides derived from human parathyroid hormone-related protein (11-19) containing omega-azido- and omega-yl-alfa-amino acid residues in positions i and i+4 was cyclised in solution by an intramolecular Cu(I)-catalyzed azide-alkyne 1,3-dipolar Huisgen's cycloaddition. These series of heterodetic cyclo-nonapeptides varied in the size of the disubstituted-[1,2,3]triazolyl-containing bridge, the location and the orientation of the [1,2,3]triazolyl moiety within the bridge. The [1,2,3]triazolyl moiety, presented at either 1,4- or 4,1-orientation, is flanked by side chains containing 1-4 methylenes that result in bridges comprised from 4-7 methylenes connecting residues 13 and 17. We have recently reported a comprehensive conformational analysis employing CD, NMR and molecular dynamics in water-hexafluoroacetone (1:1, v/v).(Scrima et al. Euro JOC 2009) Our data showed that all heterodetic cyclo-nonapeptides in

which the [1,2,3]triazolyl is flanked by a total of 5 or 6 methylenes nicely accommodate alfa-helical structures.Here we present an extensive unconstrained molecular dynamic calculation on the mentioned series of heterodetic cyclo-nonapeptides. The results of MD studies were compared to the experimental NMR data obtained in DMSO/water and in HFA/water solvents. MD trajectories confirm NMR structural data. MD data allow to assess the role of the triazolyl moiety, the size of the methylen bridge, the side chain specificity on the stabilization of the cyclo-peptide regular secondary structures

P145. Abstract number: 565

Reverse ligation approach to dendrimeric peptide platforms: solution and solid phase studies

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Multimerization is recognized as one of the most effective tools for enhancing peptide immunogenicity. MAPs (multiple antigenic peptides; Tam, 1988) are the most popular type of multimeric immunogen, based on a core of Lys dendrites onto which a number of peptide epitopes are attached. MAPs were first prepared by standard SPPS methods and later through various forms of solution ligation that allegedly improved on the all-SPPS approach. The most frequent solution approach uses a dendrimeric Lys platform functionalized with α- and ε-chloroacetyl (ClAc) groups to which peptide epitopes (with a C-terminal Cys and N-terminally acylated) are then linked via thioether bonds. We have explored the reverse approach, namely a thiol-functionalized dendrimeric platform to which ClAc-modified peptide epitopes are linked. This approach is advantageous in that (i) in situ TCEP reduction avoids disulfide formation and continuously maintains the thiol platform reactive; (ii) TCEP amounts are low enough to pose minimal damage to ClAc groups in the peptide epitope; (iii) byproduct formation is significantly reduced and the end product is therefore more homogeneous and easy to purify; (iv) last but not least, ligations can be run to quantitative replacement at all thiol sites, vs. the partial replacement levels inevitably encountered in the original procedure. Examples of application of this reverse ligation approach in both solution and solid phase contexts will be given.

P146. Abstract number: 6

Artificial Supersecondary Structure Composed of Triple Helix-Triple Helix Dimer from Three-Armed Collagen-Like Peptides

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Exploring the self-assembling properties of artificial peptides has been useful for designing functional nanomaterials as well as structural model of native proteins. As part of our research into peptide nanoarchitectures[1], we descibe herein a collagen-like foldamer. Collagen is defined by a triple helix motif, where three left-handed polyproline II-like chains supercoil together to form a right-handed super helix. Collage is composed primarily of Gly-Xaa-Yaa repeating trimeric units, where Xaa and Yaa residues are predominantly Pro and Hyp, respectively. In synthetic collagen structures, primarily sequence of peptides can affect significantly folding stability, and however the incorporation of a scaffold into the design of peptidomimetics can reinforce intramolecular folding of peptides. In the present study, a novel collagen-like triple helix foldamer, in which three $(Gly\text{-}Pro\text{-}Hyp)_5$ chains were connected by Lys through a flexible and hydrophilic linker, were newly prepared by standard solid phase synthesis. This peptide was found to form a new class of artificial supersecondary structure composed of triple helix-triple helix dimmer in MeOH. In addition, effects of temperature and molecular structures on stabilization of such triple helix-based supersecondary structure were investigated in detail.

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P147. Abstract number: 10

Dimerization of tertiary amides leads to improved affinity for melanocortin receptors

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Melanocortin receptors are G-protein coupled, seven coil transmembrane entities, playing an important role in many living processes in animals and humans. They are known five subtypes ($MC_{1\text{-}5}R$). Endogenous ligands of melanocortin receptors are peptides, called melanocortins. Melanocortin receptors are potential targets for therapeutic drugs. Working on design of such drugs, we have previously created a simple solid phase process for preparation of highly diverse tertiary amides imitating active core of melanocortins [F. Mutulis et al, Bioorganic & Medicinal Chemistry 15 (2007) 5787-5810]. Now, we report on extension of our method leading to dimers of tertiary amides. To achieve dimerization, we employed capability of 2,3-unsubstituted indole derivatives to form oligomers in presence of acids. For example, when attached to polymer *N*-(2-aminoethyl)-*N*-benzyl-1*H*-indole-5-carboxamide (**1**) was treated with trifluoroacetic acid in presence of 1,2-ethanedithiol, the tertiary amide (**1**), its dimer - N^5,$N^{5'}$-bis(2-aminoethyl)-N^5,$N^{5'}$-dibenzyl-2,3-dihydro-1*H*,1'*H*-2,3'-biindole-5,5'-dicarboxamide (**2**), and the dimer with incorporation of 1,2-ethanedithiol - 3-(2-{2-amino-5-[(2-aminoethyl)(benzyl)carbamoyl]phenyl}-1-(2-mercaptoethylthio)ethyl)-*N*-(2-aminoethyl)-*N*-benzyl-1*H*-indole-5-carboxamide (**3**) were simultaneously yielded . A series of 17 compounds was prepared. Tested on recombinant melanocortin receptors $MC_{1,2\text{-}5}R$, they showed binding affinity to them reaching submicromolar K_i range. Typically, dimers were more active than corresponding monomers. So, affinities of **1-3** to MC_5R were 87, 0.39 and 0.66 μM, correspondingly. According to our interpretation, dimers fill receptor binding pockets more efficiently than corresponding monomers, finding more positive interactions and, as a consequence, displaying an increased affinity.

P148 Abstract number: 15

Peptoid-Peptide Hybrides as Targeting the Polo-box domain of Polo-like Kinase1

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Poly-N-substituted glycines or peptoids are excellently suitable for transformation of peptides consisting of several amino acid residues into mimetics retaining the biological activity of the original peptides while reducing of some of their defects such as enzymatic degradation and membrane permeability.

Polo-like kinase 1(plk1) has a pivotal role in cell proliferation and is considered a potential target for anti cancer therapy. Bang et al.1 have described the structural and functional analyses of minimal phosphopeptides targeting the polo-box domain of polo-like kinases 1. According to this result, minimal phospho-pentapeptide Ac-PLHSpT-NH2 specifically binds to the PBD of Plk1, but not those of the closely related Plk2 and Plk3.

To better understand the structural requirements for this interaction, we generated peptoid-peptide hybrids. Here, we provide, in detail, the systematic transformation of the former pentapeptide ligand to all possible combination of the corresponding peptoid-peptide hybrids with the appropriate side chains. A peptoid-peptide hybrid library containing 34 ligands based on Ac-PLHSpT-NH2 was synthesized using ”submonomer”approach of Zuckerman method

P149. Abstract number: 31

Regiospesific derivatization of hydroxy and amino benzoic acids by SPOC

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Substituted benzoic acids, that possess several hydroxy and amino groups, provide a useful template for combinatorial chemistry. Specifically, a protocol has been developed that employs amidation, alkylation, urea formation, Mitsunobu reaction1 and other reactions used in Solid Phase Organic Chemistry (SPOC), for regiospecific derivatization of benzoic acids. In this work, starting hydroxy or aminobenzoic acid scaffolds are loaded directly on the chlorotrityl resin through the more nucleophilic carboxylate anion, leaving the hydroxy and amino groups untouched and ready for the following derivatization .

The protecting groups of the tethers used in this work were matched to the orthogonal or semiorthogonal combinations namely Alloc/Allyl, o-Nosyl, Fmoc, Boc and Cbz. The synthetic routes reported in this work are short, general and applicable for the preparation of libraries, controlling protection and positioning of peripheral moieties. This method is operationally simple and provides the products with high purity and in good yields.

P150. Abstract number: 33

Peptidomimetic PKB/Akt inhibitors as anti-cancer drug leads

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Protein Kinase B (PKB/Akt), a member of the Ser/Thr kinase family, is involved in cell proliferation and suppresses cell death (apoptosis). Persistently activated PKB/Akt is associated with many types of human cancer, such as breast, colon, ovary, pancreas, head and neck, and prostate cancer. Inhibition of PKB/Akt is therefore an attractive strategy for targeted cancer therapy. Screening of small molecules as enzyme inhibitors is very common. However, in the case of ATP-mimetic kinase inhibitors, the resulting small molecular inhibitors usually exhibit low selectivity towards the desired kinase, because kinase ATP-binding sites are strongly conserved. Substrate-based inhibitors, especially those based on the peptidic nature of the substrate, are more likely to be selective, because the substrate-binding site on each kinase is specific.
Recently, a series of peptides derived from a PKB/Akt substrate - the protein Glycogen Synthase Kinase 3 (GSK-3) - was developed and their interactions with PKB/Akt were studied. The peptide Arg-Pro-Arg-Nva-Tyr-Dap-Hol (PTR6154), based on the GSK-3 substrate peptide Arg-Pro-Arg-Thr-Ser-Ser-Phe, was found to be a selective PKB/Akt inhibitor.1 In order to improve the potency and pharmacological properties of PTR6154 and of its cholesteryl analog PTR6164,1 we generated various peptidomimetics based on PTR6154. Modifications to the parent peptide included backbone cyclization2 and the following mono scans: N□-methylation,3 peptoid3 and aza-amino acid. We found few analogs that are up to 5-fold more potent than PTR6164/PTR6154 in vitro. We are now evaluating their potency as inhibitors of cell proliferation, utilizing cell lines derived from cancers in which the PKB/Akt pathway is over-activated. The most successful inhibitors will be evaluated as anti-tumor agents, using xenograft animal models based on the same cell lines.
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P151. Abstract number: 42
Application of Fragment Based Drug Design for the discovery of peptidomimetic as inhibitors of cyclophilin's.
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Hepatitis C virus (HCV) is a major causative agent of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Approximately 200 million individuals are infected worldwide and HCV infection causes approximately 280,000 deaths per year. The current standard treatment for chronic hepatitis C is based on the use of pegylated interferon (IFN-□) in combination with ribavirin for up to one year. However, only up to 50% of patient with HCV genotype 1 infection can eradicate infection upon therapy. Moreover, both IFN-□ and ribavirin are associated with adverse effects. Therefore, more efficient and better tolerated therapies are needed for hepatitis C. Current HCV ”Drug Discovery”efforts focus primarily on developing molecules that specifically inhibit the function of two viral enzymes: the NS3-4A serine protease and the NS5B RNA-dependant polymerase, both of which are essential for viral replication. However, due to the high genetic variability of the virus, amino acid substitutions that confer drug resistance are likely to emerge during treatment with specific inhibitors of the HCV protease or polymerase. Since viruses depend on host-derived factors that are required for viral replication and may be less prone to the development of drug resistance. Cyclophilin’s are cellular factor that were initially identified as having high affinity for cyclosporine A (CsA), an immonusuppressive agent. Cyclophilins form a family of peptidyl-prolyl isomerases that catalyze the cis-trans interconversion of amino-terminal peptide bonds to praline residues, facilitating changes in protein conformation. Some authors have recently reported the involvement of CypA and Bin HCV genome replication and proposed a model for the molecular mechanism, where Cyp’s would interact with NS5B and promote its RNA binding affinity. The Cyp’s represents an interesting target for the development of new antiviral strategies targeting HCV replication without targeting viral enzymes. The oral presentation or poster will shown the application of Fragment Based Drug Design to Cyclophilin’s for the design and synthesis of peptidomimetic as inhibitors for these enzymes. In particular, we will demonstrate the use of this technique for the rational design of peptidomimetic based on cyclosporine scaffold.

P152. Abstract number: 52
SPOS route to Novel 9-Anilinoacridine Derivatives: Biological Evaluation
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The 9-aminoacridine (9-AA) core fragment is a structure of interest to medicinal chemists and appears in many biologically active compounds, mostly with anticancer and anti-malaria applications. In the field of antitumor DNA-intercalating agents, 9-anilinoacridines derivatives play an important role due to their antiproliferative properties1. Several cancer chemotherapeutics based on the 9-aminoacridine scaffold, such as Amascrine and Ledakrin, have been developed. So far, 9-AA analogs have been prepared through several step synthesis involving harsh conditions and laborious purification of intermediates and final compounds. Thus, finding short and efficient methods for the rapid generation of new 9-AA core based compounds will greatly enhance their availability for examination in biological systems. We have previously demonstrated a new, highly-efficient, one-pot derivatization in solution of 9-AA at the 9-amine position by simple reductive amination, addition-elimination or SNAr reactions yielding series of novel substituted N(9)-benzylaminoacridines, N(9)- quinoneaminoacridines and N(9)-anilinoacridines correspondingly2. This unique method allows formation of aniline, benzyl and quinone tether with electron withdrawing (EW) groups in 9-AAs. This is a difficult task to accomplish using traditional nucleophilic substitution of the deactivated amines on 9-chloroacridines. Recently, we also developed a solid phase organic synthesis (SPOS) approach to novel 9-AA derivatives and various mono- and bis-9-anilinoacridine peptidyl conjugates. Such synthetic strategy rapidly generates 9-AAs with variable spacer lengths and charged, polar or hydrophobic residues at desired positions, which can increase binding affinity, conformation stability and/or biological activity.
This observation can be explained in terms of SAR centered on the substitution pattern on the aniline moiety.

We focus our current studies on the optimization of the recently discovered submicromolar leads against MDM-MD-A31 (renal cancer), OVCAR8 (ovarian cancer) and MCF7mito (mitoxantrone selected and associated with MDR phenomena) breast cancer. Our latest most promising results will be presented.

1. Sebestík et al., "A role of the 9-aminoacridines and their conjugates in a life science". Curr. Pro. Pep. Sci. 2007, 8: 471-83.
2. Gellerman et al., 'One-pot derivatization of medicinally important 9-aminoacridines by reductive amination and SNAr reaction' Tet. Lett., 2010, 51, 836-839.

P153. Abstract number: 83

Hepatitis C Virus NS3 Protease Inhibitors based on 2(1H)-Pyrazinones

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Protease inhibitors based on substituted pyrazinones have proved to function as non-peptidic β-strand conformation inducers. Consequently, these heterocyclic systems are interesting from a medicinal chemistry point of view. Molecular modelling of HCV NS3 protease inhibitors suggested that the space occupied by the P2 side chain could be reached by the substituent in position six on the P3-pyrazinone. We have previously reported a rapid microwave method for synthesis of N-1, C-6-disubstituted 3,5-dichloro-2(1*H*)-pyrazinones[1] which enables the introduction of a variety of substituents in crucial positions. We herein present our further optimization of these pyrazinone based inhibitors.

(1) Gising, J., et al. Org. Biomol. Chem., 2009, 7, 2809-2815.

P154. Abstract number: 102

Design, synthesis and evaluation of GPCRs ligands based on 1,2,4-triazole scaffold.

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A large part of the hormonal communication is mediated by peptide - GPCR (G-protein coupled receptor) interactions. At the central nervous system (CNS) level, the physical and pharmacokinetic properties of such peptides are responsible for their poor bioavailability when administrated per os. To overcome the problems linked to the nature of the peptide bound, a strategy of replacement of the amide bond by heterocycles led us to develop a new family of peptidomimetic compounds based on the 1,2,4-triazole scaffold trisubstituted in positions 3, 4 and 5. We described a new synthesis for these compounds using silver benzoate as a key reagent.

Starting from small active peptidic sequences with high affinity for the receptor we tried to rigidify each amide bond, in a kind of "triazole scan". Two biological targets have been chosen to study the validity of this approach: CCK2-R and opioids receptors. For each of these compounds we measured the affinity for the targeted receptor. This studies results in an exploration of the binding pocket with these constrained analogs. With the obtained affinity data we could have a better comprehension of the spatial side chains arrangement during the interactions between the native peptide and its biological target. The first results and interpretations will be discussed.

P155. Abstract number: 105

Novel Peptidyl 9-Anilinoacridines and Their Biological Evaluation: from Solution to SPOS

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9-Anilinoacridines (9-AA) and their conjugates play an important role in anticancer and anti-malaria applications as drugs and biomolecular probes. The mode of binding of acridine derivatives involves intercalation of the acridine core in the DNA duplex interfering the normal DNA function by blocking the polymerases to synthesize RNA and DNA leading to inhibition of protein synthesis.

We previously have shown a new one-pot synthesis in solution of 9-aminoacridine by simple reductive amination, addition-elimination or SNAr reactions, yielding series of novel substituted N(9)-benzylaminoacridines, N(9)-quinonoaminoacridines and N(9)-anilinoacridines correspondingly.1

Recently, we developed a convenient and short synthesis of novel mono- and bis-9-anilinoacridine peptidyle conjugates on solid support using these reactions. Such a synthetic strategy rapidly generates 9-AAs with variable amino acid tethers, spacer length and charged residues at desired positions avoiding tedious purification and can increase binding affinity, conformation stability and extensive SAR evaluation.

Solid-phase synthesis and chemistry of such compounds suitable for library construction and biological screening will be presented.

P156. Abstract number: 108

Peptide Nucleic Acids With Chiral Backbone - Synthesis, Properties And Some Applications

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Peptide nucleic acids (PNAs) are new completely artificial DNA/RNA analogs in which the backbone is replaced by pseudopeptide. Originally conceived as agents for double-stranded DNA binding, the unique properties of PNAs as DNA mimics were first exploited for gene therapy drug design. Now PNAs are used in different fields as antigene and antisense agents, as delivery agents, in PCR and Q-PNA PCR, Nuclear acid capture, Solid-phase hybridization techniques. Many types of PNAs were synthesized with the aim to increase their binding affinity to specific DNA/RNA, their bioavailability and stability *in vivo*.

We present here synthesis and some physico-chemical properties of new PNAs with chiral backbone, containing unnatural amino acids. Amino acid analogs were based on the natural amino acids Arg, Orn and Lys.

We are grateful to the European Social Fund and Ministry of Labor and Social Policy (Contract BG051PO001-3.3.04/58 - 2009) and NFSR of Bulgaria (Contract MY-FS-13-07).

P157. Abstract number: 110

The Synthesis of antibacterial Dihydropyridine based peptide -Drug Hybrids

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The antibacterial pentapeptide KAAAK motive present in Dermaseptin 1 was modified to a hybridic constract where the central AAA were replaced by a 4-(4-methoxyphenyl)-1,2,6-trimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid

moiety in solution . The synthesis and biological activity of the resulting KAAK peptide surrogates will be presented.
The phenomenon of fault tolerance bacteria resistant, is a global problem and hard to treat, that is getting worse.
Therefore, it is necessary to discover new antibiotics.
These needs surface the necessary effort and pressure to investigate new derivatives of various known and novel Scaffolds, among them are the 1,4-Dihydropyridines. The latter are abundant source of molecules with proven biological activity.
The present research refers to the phenomenon of pharmacokinetics as well as pharmaco-dynamics in pharmacologic activity. Namely, the modification that extends use of existing medicines. In particular, the final changes of the medications which are known and usable. For medicines known, this change is achieved by combining them with peptides units sequences (based on Dermaseptin fragments), in order to produce hybrid drug like molecules with better desired potential therapeutic features.

P158. Abstract number: 116
The role of disulfide bridge in SFTI-1 trypsin inhibitor in the inhibition of serine proteinases.
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Series of 14 monocyclic analogues of trypsin inhibitor SFTI-1 isolated from sunflower seeds were synthesized by the solid phase method. The intention of this work was to establish the role of disulfide bridge involved side chains of Cys3 and Cys11 present in the inhibitor on association with serine proteinase. This cyclic element was replaced by disulfide bridges formed by Pen (L-pencillamine), homo-L-cysteine (Hcy), N-sulfanylethylglycine (Nhcy) or combitation of these residues with Cys. In substrate specificity P1 position of the analogues synthesized Lys, Nlys (N-(4-aminobutyl)glycine), Phe or Nphe (N-benzylglycine) were present and therefore they were checked for trypsin and chymotrypsin inhibitory activity. The results obtained clearly shown that Pen and Nhcy are not accepted in position 3, yielding inactive analogues whereas second Cys residue can be substituted without significant impact on affinity towards proteinase. On the other hand, lengthening of Cys3 side chain (introduction of Hcy) does not affect inhibitory activity, and analogue with Hcy - Hcy disulfide bridge was more that twice as effective as reference compound ([Phe5] SFTI-1) in inhibition of bovine α-chympotrypsin.

P159. Abstract number: 136
Structure, Biophysical And Biological Characterization Of Somatostatin-Derived Peptidomimetics Inhibiting 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's Phase II clinical trials are completed) 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 [DPhe-c(Cys-Tyr-DTrp-Lys-Cys)-Thr-NH2] verified that the side chains of Tyr, DTrp and Lys are important pharmacophoric groups for somatostatin-like biological activity. Linear, cyclic and branching derivatives were designed and synthesized using different amino acids to selectively inhibit inflammatory actions. A series of simple, branching peptidomimetics were developed by our group comprising a flexible core [tris(2-aminoethyl)amine] and three protected or unprotected amino acids situated in equal positions, representing basic, aromatic and heteroaromatic groups in the molecule. Synthesis of the molecules was carried out in solution phase. The final products were characterized by HPLC, MS and NMR.
Biophysical characterization of the compounds based on their lipophilic character measured by HPLC retention time, calculated logP values, as well as by their ability to cross artificial biological membrane (PAMPA assay). Substance P release from sensory nerve terminals of the isolated rat trachea was used to screen their biological effect in vitro. The effective molecules were further tested in vivo for their actions on cutaneous plasma protein extravasation and plantar incision-induced mechanical hyperalgesia in the rat hindpaw.
The structure of the novel compounds and the relationship among their structure, lipophilicity and biological activity will be discussed.

P160. Abstract number: 148
Efficient access to orthogonally protected disulfide bridge mimetics
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Many naturally occurring and engineered biologically active peptides are cyclized via disulfide bridges. However, the disulfide bond is not as strong as a carbon-carbon bond, it can also be easily reduced *in vivo* to the open chain peptide. Interest in peptidomimetics and *in vivo* stabilization of peptides has led us to examine disulfide bridge analogues where the disulfide bridge is replaced by different carbon chain linkers. Our primary interest is the bicyclic peptide C-4 that stimulates the activity of human tissue kallikrein 3 (KLK3, also known as prostate specific antigen, PSA). The KLK3 stimulating peptide has the potential to provide a new non-invasive therapy for prostate cancer. The peptide consists of 13 amino acids, with one terminal disulfide bridge between cysteines 1 and 13 and one internal between cysteines 5 and 10.
We have successfully developed efficient, high-yielding, enantiopure synthetic procedures of four disulfide bridge mimetics, possessing an alkyne, a *Z*-alkene, a *E*-alkene and an fully saturated linker instead of the disulfide bridge. These linkers have different geometries and should be able to mimic any conformation of the disulfide bridge. The mimetics are protected by completely orthogonal protecting groups (Fmoc and Boc as *N*-terminal protecting groups and *tert*-butyl and methyl esters as *C*-terminal protecting groups). We have utilised alkyl copper-zinc/haloalkyne

chemistry (previously developed by Knochel) to synthesise a disulfide bridge mimetic containing an alkyne moiety. Selective and full reduction of the triple-bond using $Pd/BaSO_4$ and Pd/C respectively, enabled access to the *Z*-alkene and the fully saturated mimetics. For the enantiopure synthesis of the corresponding mimetic possessing an *E*-alkene, we successfully employed a Grubbs cross-metathesis reaction.

P161. Abstract number: 165

Design And Synthesis Of Protein-Protein Interaction Mimics As Plasmodium Falciparum Cysteine Protease Falcipain-2 Inhibitors

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Cysteine proteases play a significant role in the growth and the development of several human parasites such as Plasmodium, Leishmania, and Trypanosoma which affect more than 3.5 billion people worldwide. Falcipain-2 (FP-2) is an important cysteine protease of the human malaria parasite, P. falciparum (Pf), and is one of the most promising target for the development of new Pf inhibitors.1 FP-2 plays roles in haemoglobin degradation and merozoites egression at the asexual blood stages of Plasmodium development. Endogenous cysteine protease inhibitors have been described in a number of eukaryotic systems and they represent an important platform for new drug design. Cystatin and its homologues are natural inhibitors of Papain and others cysteine proteases, but the high molecular weight and the peptidic structure raise difficulties in their exploitation in the drug design process. In the present study, we designed a series of small peptides that mimic the protein-protein interaction between falcipain-2 and Chicken Egg White Cystatin and analyzed their effects on falcipain-2 activity.

These peptide mimics were designed keeping in mind the distance of about 4-5 Å between the carbonyl of Pro11 or Val12 of cystatin. Three different linkers, γ-aminobutyrric acid (GABA), cis-4-aminocyclohexane carboxylic acid and a macrocycle formed by GABA and two cysteines joined by a disulfide bridge were used to link peptides. Some of these compounds showed considerable inhibition (≥50%) at 5 μM (Ki 2-7.5 μM). When these peptide mimics were tested against the mature parasite, they also produced morphological abnormalities especially in the food vacuole, similar to what have been observed with others cysteine protease inhibitors. We believe this approach could be an excellent starting point for the development of new cysteine protease inhibitors and in general for the development of new drugs against P. falciparum.

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P162. Abstract number: 170

Derivatization of Selective Integrin Ligands via Click-Chemistry for Surface Coating

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Chemoselective ligation is a powerful technique for bioconjugation because of its application in low concentrations and its compatibility with unprotected functional groups.[1] In here, we describe the modification of highly active and selective α5β1 and αvβ3 integrin ligands with an azide functionality for CuI-catalyzed azide-alkyne 1,3-dipolar Huisgen's cycloaddition (CuAAC). In contrast to peptides these highly active ligands are able to bind selectively to specific integrin subtypes. Based on docking experiments and SAR-studies it was found that in derivatives of such ligands the isopropoxy group points out of the binding pocket and offers the best position for functionalization with the azide. In this way the synthesis of modified integrin ligands was achieved representing building blocks for click-chemistry with arbitrarily functionalized alkines for different applications like e.g. surface coating.

Coating of artificial surfaces with cell-adhesive molecules provides a strong mechanical contact between cells and the surface. Especially α5β1 and αvβ3 are known to additionally bind to osteoblasts and osteoclasts as well as to activated endothelial cells.[2] So, there is a high demand for such coated materials in the field of medicinal chemistry, especially for bony ingrowth of implantation surfaces like e.g. in orthopedics or in dentistry.[3]

For anchoring on titanium, which is often used in implant surgery, phosphonic acids can be used. In this manner, the modified integrin ligands bearing the azide functionality undergo a CuI-mediated azide-alkyne cycloaddition with alkyne-functionalized protected phosphonate anchors under formation of a triazole ring system, followed by deprotection of the phosphonate groups. First assays on surfaces show a significant improvement of integrin adhesion due to the coating with these phosphonic acids anchored selective integrin ligands.

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P163. Abstract number: 173

Highly Active and Selective Integrin Ligands and Their Application for Surface Coating via Thiol Anchoring

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The advantage of peptidomimetics is their specifity to adress distinct integrin subtypes in contrast to peptides. So up to now it was not possible to investigate the different roles of the integrins because there was a lack of highly active and selective nonpeptidic integrin ligands.

Starting from a recently published αvβ3 nonpeptidic integrin ligand,[1] we describe the rational design of highly active αvβ3 integrin ligands and the optimization of their selectivity against α5β1 by means of extensive SAR-studies and docking experiments.[2] Based on a ligand with a tyrosine scaffold we succeeded in obtaining compounds with affinities in the low nanomolar range.

Based on previous experiences of the sterical demand of the ligands by extensive docking experiments, it was

shown that the aromatic moiety which is attached to the aspartic acid mimic points out of the binding pocket. At this position, derivatization of the aromatic system in the para-position with a spacer (aminohexanoic acid) and a thiol anchor group can be performed without changing the activity or selectivity profile of the parent ligand. To prove this hypothesis, the modified compounds were again tested which showed that activity as well as selectivity could be conserved.
This thiol anchor functionality enables coating of titanium or gold surfaces by αvβ3 as well as α5β1 selective ligands to compare and further investigate the single roles of the different integrin subtypes for integrin mediated cell adhesion. First tests of the functionalized ligands immobilized on surfaces show enhanced integrin adhesion. In the future the use of subtype specific functionalized integrin ligands will yield new opportunities in biological and biophysical investigations with high potential for future applications in medicine.
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P164. Abstract number: 202
Stuctural features of antimicrobial aza-β3-peptides
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Designing antimicrobial molecules based on pseudopeptides to increase the activity, selectivity and bioavailability of natural peptides is now widespread. Recently, numerous peptidomimetics have been developed for biological applications including azapeptides, β-peptides, peptoids, oligoureas. In this context, aza-β^3-aminoacids were used as new blocks to compose antimicrobial peptide sequences. These monomers are analogs of β^3-aminoacids in which the CHα is replaced by a nitrogen stereocenter conferring a better flexibility to the pseudopeptide due to the chiral nitrogen (with a non-fixed configuration) bearing the side chain. The nonnatural oligomers have an extended conformational space and are supposed to adopt non-canonical secondary structures.
From a natural antimicrobial peptide, depending on the aza-β^3 residue insertions, the modifications can result either in inactive pseudopeptides or in a drastic enhancement of the antimicrobial activity without cytotoxicity. To understand how the incorporation of aza-β^3-aa modulates the antimicrobial activity, we studied the structure of aza-β3-peptides by CD and NMR spectroscopy. To date, no solution structures of peptides containing aza-β^3-aa have been solved. Crystalline structures of linear aza-β3-peptides and aza-β^3-cyclopeptides have been determined and exhibit an internal hydrogen-bond network leading to bifidic eight-membered ring pseudocycles, called N-N turn or hydrazino turn. Recently, it was demonstrated that the nitrogen configuration inversion could be fixed, by incorporating chiral monomers among these heteromacrocycles.
We have determined the first three-dimensional structures of naturally related linear pseudopeptides containing aza-β^3-aa in SDS micelles. Insertion of aza-β^3-aa systematically breaks the natural antimicrobial peptide amphipatic helices and induces either stable hydrazinoturn conformation or flexible unordered structures. When one modified residue is incorporated in a linear sequence, the hydrazinoturn seems to be classically surrounded by β-turns stabilizing the overall peptide structure over 5 residues. This typical fold was not observable if several aza-β^3 residues are inserted in closer positions destabilizing this motif. We studied the propagation of the hydrazino/β-turns succession on model peptides with sequences based on aza-β^3 residues introduced every 3 residues: αα(aza-β^3)αα(aza-β^3)αα[3DOTS]Structure and activities will be presented depending on the modifications.

P165. Abstract number: 237
Screening and optimization of constrained peptides mimicking the binding epitope of the CCR5 antibody 2D7 for structure determination.
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The creation of peptides as potential protein surface mimics has always presented a great interest for the development of new therapeutics (mABs, inhibitors, vaccines[3DOTS]), especially in difficult cases such as the HIV, GPCRs. The chemokine receptor CCR5 belongs to the family of Gprotein-coupled receptors and is known to be the main coreceptor used for HIV entry (1). However little is known about the CCR5 structure, which is generally based on rhodopsin crystal structure models (2).
We present here a screening of constrained peptide mimicking the ECL2a loop of CCR5, which is the epitope of the neutralizing CCR5 antibody 2D7, in order to know which structure is recognized by this antibody and determine the conformation of the ECL2a loop. The constrained peptides were cyclic peptides and the cyclization was performed using the proprietary CLIPSTM technology (Chemical LInkage of Peptides onto Scaffolds) (3) since this technology was shown very suitable for this kind of application (4).
Those peptides were screened in a competition assay using 2D7 in order to optimize their binding to 2D7. This optimization touches upon the peptide length, choice of the amino acids to be included, and the position of the scaffold. With the best peptide, changes were made using different scaffolds. The scaffold variation causes dramatic changes of the peptide recognition by 2D7. In one hand, scaffolds decreased 2D7 recognition of the sequence down to the level of the linear peptide, in the other hand, other scaffolds increased the sequence binding potency to 2D7 by a factor 10 compare to the original scaffold.
From the literature it is know that constraining the peptides further increases their structure (5). Therefore we used combinations of constraining motifs (scaffold/scaffold, scaffold/backbone cyclization, scaffold/disulfide). Those doubly constrained peptide showed up to a 100 fold better binding than the original single constrained peptide.
Finally we associated the increase in binding potency of the peptides with the increase of b-turn and anti-parallel b-sheet content in the peptide by using Circular Dicroism. The capacity of the best peptide to adopt a b-hairpin conformation suggests that the ECL2a loop of CCR5 adopt this conformation.
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P166. Abstract number: 251
The detailed studies of the Pictet-Spengler reaction used as a method of cyclisation of tryptophan moiety
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The Pictet-Spengler (PS) reaction is widely applied in the synthesis of tetrahydroisoquinoline and tetrahydro-beta-carboline skeletons, which are found in many alkaloids. Such moieties are also useful in peptide chemistry as constrained analogs of aromatic amino acids.
In order to obtain cyclic analogs of tryptophan we have utilized P-S reaction with the use of L-TrpOMe as an amino component and α-amino aldehydes as chiral carbonyl components. Depending on the chirality of α-amino aldehydes diastereomeric mixture of tetrahydro-beta-carbolines is formed [1].
Recently, the influence of conditions such as solvents, temperature and amount of acid on the final result of the reaction have been examined. Our studies have shown that differently structured compounds (tetrahydro-beta-carboline and octahydrobipirolloindole moieties) may be obtained depending on conditions. In apolar and aprotic solvents normal, 6-membered compounds were formed as main products, whereas in polar and protic solvents 5-membered products were obtained. It was also found that the rate of formation 5 and 6-membered compounds in apolar solvents was probably similar, but octahydrobipirolloindole moieties were transformed into tetrahydro-beta-carbolines. The temperature and amount of acid were also crucial for the results of the PS reaction, especially with α-amino aldehydes, because those compounds could easily racemize.
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P167. Abstract number: 255
Synthesis of peptidomimetics by the Pictet-Spengler reaction
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Tryptophan is often a key pharmacophore which determines the affinity of peptide ligands for their receptors. Various constrained tryptophan analogues or tryptophan containing motifs have been utilized to generate highly potent and selective ligands to biological target receptors.
Cyclic analogues of tryptophan which introduce local constraints reduce the flexibillity of the indol moiety are very valuable tools to probe the bioactive conformation of the peptide ligands. The Pictet-Spengler reaction [1] has been one of the possibilities to prepare such analogues, with freezed the indol moiety in tryptophan, containing 1,2,3,4-tetrahydro-β-carboline skeleton. The heterocyclic skeleton of 1,2,3,4-tetrahydro-β-carbolines possesses multiple sites for functionization, therefore they are an ideal choice for the design of pharmacophore-based libraries in drug discovery, through generation of a large member of structurally diverse compounds.
We report the synthesis of β^3- and β^2-*homo*-tryptophan and their derivatives as components to Pictet-Spengler reaction. β-Amino acids represent an important class of biologically relevant molecules. Oligomers composed of β-amino acids can form predictable secondary structures stable to metabolic transformations and they can mimic α-peptides in peptide-protein interactions. For these reasons β-amino acids are very usefull for peptidomimetics design. Pictet-Spengler condensation of L- and D-α-aminoaldehydes as carbonyl components with dipeptides with N-terminal α-Trp or β^3-hTrp as arylethylamine substrates lead to 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines. The reactons with β^2-*h*Trp derivatives is still under investigation. The reaction was studied in terms of double stereodifferentiation. The influence of the carboxyl terminus of α-Trp and β^3-*h*Trp on the ratio of *cis/trans* products was also investigated. The conformations of cyclic products were studied by 2NMR ROESY spectra.
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P168. Abstract number: 267
Induction Of A Gamma-Turn In 26rfa And 26rfa(20-26) by Insertion Of Azetidine-Derived Amino Acids
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26RFa is a novel neuropeptide of the RFamide family that exhibits nanomolar affinity for the orphan G protein-coupled receptor GPR103. 26RFa and GPR103 mRNAs are intensely expressed in the hypothalamus of rodents. Icv injection of 26RFa or the strictly conserved C-terminal heptapeptide ($26RFa_{(20-26)}$, GGFSFRF-NH_2) induces a potent orexigenic effect in mice. It has also been found that GPR103-KO mice exhibit an early arrest of osteogenesis and a kiphotic hump, a phenotype that mimics the symptoms of osteoporotic patients. *In vitro*, 26RFa increases $[Ca^{2+}]_i$ in GPR103-transfected cells (IC_{50}=10.2±1.1 nM) while $26RFa_{(20-26)}$ is about 75 times less potent. Molecular modeling under NMR constraints of 26RFa shows that, in DPC micelles, the N-terminal region encompasses an α-helix while the C-terminal region adopts an inverse γ-turn (Phe-Ser-Phe). $26RFa_{(20-26)}$ exhibits a marked distortion of this turn that may be responsible for its weak potency. The aim of the present study was to stabilize an inverse γ-turn in $26RFa_{(20-26)}$ and in 26RFa. For this purpose we have introduced an azetidine-derived residue in position 23 of the peptides since this moiety has been shown to promote the stabilization of a γ-turn in peptide models. Two Fmoc-azetidine-derived amino acids harbouring the native Ser (AzSer) and Nva (AzNva) side chains have been prepared in racemic form starting from H-L-Ser(tBu)-OMe and H-L-Nva-OMe, respectively. [$AzSer^{23}$]$26RFa_{(20-26)}$ and [$AzNva^{23}$]$26RFa_{(20-26)}$ diastereoisomers and their full length 26RFa counterparts have been obtained in pure forms and characterized by Maldi-tof MS. The ability of each analog to displace [^{125}I]-26RFa from its binding site has been studied on membranes from GPR103-expressing CHO cells. Neither the couple of diastereoisomers [$AzSer^{23}$]$26RFa_{(20-26)}$ nor the couple of diastereoisomers [$AzNva^{23}$]$26RFa_{(20-26)}$ could compete with [^{125}I]-26RFa contrary to the diastereoisomers [$AzSer^{23}$]- and [$AzNva^{23}$]26RFa. The results from functional assay showed that the more hydrophilic [$AzNva^{23}$]26RFa diastereoisomer exhibited an EC_{50} between those of 26RFa and $26RFa_{(20-26)}$, while the 3 other compounds were less potent than $26RFa_{(20-26)}$ in mobilizing $[Ca^{2+}]_i$. Structural characterization of these peptidomimetics should help to elucidate why they are less active than the parent molecule. These data constitute a step towards the development of GPR103 analogs that could prove useful for the treatment of feeding disorders and/or osteoporosis.

P169. Abstract number: 283
Probing the Charge Capability of the MIDAS: Phosphinic- and Phosphonic Acid Containing Compounds as New Potent allbβ3 Integrin Antagonists
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Inhibition of integrin function is a major challenge in medicinal chemistry. Tirofiban is an intravenously administered synthetic αIIbβ3 receptor antagonist drug that specifically inhibits fibrinogen-dependent platelet aggregation.[1] The carboxylic acid group in integrin ligands is essential for the coordination of the bivalent metal cation at the metal-ion-dependent adhesion site (MIDAS) of all integrins.[2] It is the most conserved functionality in all integrin ligands. Recently, the first successful bioisosteric replacement of the carboxyl group with the pharmacologically favorable hydroxamic acid group has been described.[3] Based on these results we were interested in the question if the MIDAS tolerates any other bioisosteric group and negative excess charge. Being aware that phosphinic- and phosphonic acids possess very good coordination properties for bivalent metal cations, we assumed that Tirofiban analog structures with the above-mentioned motive are highly valuable compounds.
Herein, we describe the rational design and multistep synthesis of phosphinic- and phosphonic acid analog Tirofiban derivatives to gain insights into the binding modes of ligands to the MIDAS region under physiological conditions. The phosphinic acid derivative, bearing a single negative charge, has similar activity for αIIbβ3 as compared to the known carboxylic acid derivative (4.3 vs. 1.2 nM). However, the phosphonic acid compound, being double negatively charged, gave a 60-fold lower activity than the phosphinic acid. Obviously, the additional negative charge in the phosphonic acid destabilizes ligand binding in the MIDAS region. We used modeling, docking and *ab-initio* calculations of the ligand-receptor-interactions as well as ^{31}P-NMR studies on the protonation state of the phosphorus containing derivatives to support the hypothesis that the unoccupied binding site at the octahedral coordinated bivalent metal cation does not tolerate the bulky and double negatively charged phosphonic acid moiety as compared to the phosphinic acid analog.
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P170. Abstract number: 287
From Peptides To Non Peptide Mimetics: The Examples Of Angiotensin Ii And Myelin
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The discovery of Losartan a non peptide Angiotensin II Receptor antagonist was announced in 1989 during the Gordon Research Conference on Angiotensin and the Renin - Angiotensin - System (RAS). The drug was discovered in the Laboratories of Dupont and the announcement at the Conference was the approval for Clinical trials which led to the first Angiotensin II nonpeptide Receptor antagonist. Previous Angiotensin II peptide antagonists such as Sarilesin and Saralasin failed to become drugs due to its peptide nature rendering them susceptible to proteolytic enzymes which hydrolyze them. The announcement was the result of many years work on Angiotensin and the RAS System, since it was discovered 80 years ago. Breakthroughs in this evolution was the discovery of Captopril by Miguel Ondetti in 1975 and Losartan by Timmermans in 1989. In this lecture the main steps followed in our laboratories in Patras are mentioned which led to our Sartan, named Elsartan. Briefly the main steps are: 1. Peptide (The tool), 2. Peptide Model (The ligand - receptor interaction), 3. Cyclic Peptide (The drug lead), 4. Non-peptide mimetic (The Drug).
Immunodominant Epitopes MBP 83-99, PLP 139-151, MOG35-55 of human proteins MBP, PLP, MOG of myelin sheath are implicated in Multiple Sclerosis. These epitopes have been the tools in our laboratories for the Design Synthesis and Preclinical Evaluation in a large number of rationally designed linear and cyclic analogues conjugated to reduced or oxidized mannan via [Lys-Gly] bridge. Specific Analogues have been found to immune rats rendering them potential therapeutics vaccine drugs in the Immunotherapy of Multiple Sclerosis. Furthermore, our cyclic MBP 83-99 peptides, for the first time to be reported as HLA and MHC binders and more stable compared to linear counterparts, possess a series of important immunomodulatory properties rendering them as putative drugs for treating multiple sclerosis and potentially other Th1 - mediated autoimmune diseases. In the light of the results and findings in our research, the main immunodominant peptides MOG35-55, PLP139-151 and MBP83-99 and their head to tail cyclic counterparts conjugated to reduced mannan have been selected to constitute a mixture cocktail drug for preclinical investigation in preparation of New Drug Application (NDA) for Clinical Phase I and II studies in the Immunotherapy of Multiple Sclerosis.

P171. Abstract number: 289
Efficient Synthesis And Biological Evaluation Of Substituted Imidazole At1 Ang Ii Receptor Antagonists Based On 4(5)-Butylimidazole
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Angiotensin II (Ang II) is the octapeptide produced by the Renin-Angiotensin System (RAS) which plays a central role in blood pressure regulation and electrolyte homeostasis. The discovery of losartan has stimulated extensive research in the development of potent and selective antagonists. Herein, we describe a short, efficient and regioselective synthetic approach of AT1 Ang II receptor antagonists based on 4(5)-butylimidazole in which the hydroxymethyl and butyl groups attached to imidazole ring present different topographical positions in comparison to Losartan. Furthermore, a halogen atom is introduced at the 5-position of imidazole moiety as a lipophilic, electron-withdrawing substituent. The analogue 1 as well as the brominated analogue 3 showed high antihypertensive activity (pA_2= 7.97, 7.58, respectively) similar to Losartan (pA_2 = 8.33), indicating that reorientation of butyl and hydroxymethyl groups on the imidazole template retains high antihypertensive activity.
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P172. Abstract number: 294

Synthesis And Biological Evaluation Of 1-??N?Yl And 1-Biphenylmethyl Substituted Imidazole At1 Ang Ii Receptor Antagonists

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Angiotensin II (ANG II) is the octapeptide produced by the Renin-Angiotensin System (RAS) which plays a central role in blood pressure regulation and electrolyte homeostasis. Inactivation of RAS has stimulated many researchers to design drugs either by inhibiting Renin or the ACE or by blocking the ANG II receptors. The DuPont group was the first to develop Losartan (DuP 753), an orally effective Angiotensin receptor blocker, which is metabolized in vivo to the more potent full antagonist EXP 3174. This has encouraged the development of a large number of similar compounds, among them Eprosartan, Irbesartan, Candesartan, Valsartan, Telmisartan, Tasosartan and Olmesartan.

Herein, we report the preparation of *E*-urocanic acid based analogues, focusing our attention on the structural modifications on the imidazole ring which would possibly enhance potency. Consequently, we have designed and synthesized a series of urocanic acid derivatives bearing a benzyl or biphenylmethyl tetrazole moiety at the 1-position and a bulky lipophilic and electron-withdrawing group such as a halogen atom, at the 5-position of the imidazole ring. Additionally, the rigid acrylic or saturated acid side chain was lengthened by esterification, resulting in the methyl ester or the bulky ester group (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl of Olmesartan, which in vivo is metabolized to the carboxyl moiety and may proved an effective structural element, emerging to compounds with improved activity.

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P173. Abstract number: 313

Design, Synthesis and Evaluation of 2,3,6,8-Tetrasubstituted Chromone and Chroman-4-one Derivatives as Somatostatin Mimetics

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The physicochemical properties and rapid enzymatic degradation limit the use of peptides as drugs [1]. To overcome these problems, peptidomimetics are designed to be more stable and at the same time retain the effect of the parent peptide. One strategy to develop these kinds of compounds is to use a scaffold approach. Chromone and chroman-4-one derivatives are frequently found in nature and in this project these heterocyclic ring systems are used as scaffolds for peptidomimetics. Incorporation of amino acid side chain moieties in the 2-, 3-, 6-, and 8-positions of the chromone/chroman-4-one structures makes it possible to mimic a β-turn of a peptide chain. The peptide hormone somatostatin is known to adopt a β-turn conformation when interacting with its receptor(s). Computer based modelling has been used to design the chromone/chroman-4-one based mimetics of somatostatin. Appropriate substituents have been introduced in the ring systems using efficient reactions such as Pd-mediated couplings [2] and a SmI2 mediated Reformatsky reaction [3]. For the incorporation of the 2-substituent in the chroman-4-ones a base promoted reaction using DIPA in EtOH under microwave conditions was developed. Factors such as sterical hindrance, use of different bases, catalysts, solvents, reaction times and temperatures have been evaluated regarding this reaction [4]. The synthesized chromone/chromane-4-one based somatostatin mimetics have been tested for their agonist properties at the somatostatin receptors, some compounds showed EC50-values in the low μM range.

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P174. Abstract number: 341

Galanthamine based hybrid molecules with potential acetylcholinesterase, butyrylcholinesterase and β-secretase inhibition activity.

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Alzheimer's disease (AD) is well known neurodegenerative illness which is affected around 20 million of people worldwide. Different causes are proposed to be responsible for AD occurrence and three hypotheses are arisen. Once of them has not been clearly proven. However, it is well established that some enzymes from cholinesterase family play a key role in the progression of AD and that their levels vary significantly during different stages. Recently, a lot of scientific groups work on the design of new medical drugs which could affect some of AD causes. The design of new substances with combined pharmacological activities is a promising alternative for prevention and treatment of AD.

Herein, we report the synthesis of galanthamine amides comprising peptide moiety at position 11. They were designed to be both β-secretase inhibitors, due to peptide moiety, and acetylcholinesterase/butyrylcholinesterase inhibitors owing to galanthamine molecule. The newly synthesized hybrid molecules contained the following six peptides: Z-Asp-Val-Asn-Leu-Ala(or β-Ala)-Val-NHBzl; Z-Asp-Asn-Leu-Ala(or β-Ala)-Val-NHBzl and Z-Asp-Asp-Leu-Ala(or β-Ala)-Val-NHBzl. These compounds are analogues of b secretase inhibitor OM 99-2 modified in P3-position. They include C-terminal benzylamin and N-terminal Z-Asp with free γ-carboxyl function. Both needed peptides and final hybrid molecules were synthesized in solution. Several different schemes were realized and will be discussed according to yields and purity of products. The condensation of peptide mimetics mentioned above with norgalanthamine using modified carbodiimid method by means of N-cyclohexl-N-(β-N-methyl-morpholino-ethyl) carbodiimid was revealed to be the most successful approach. 1D 1H, 13C and 2D NMR as well as ESI/MS were used to characterize final hybrid molecules. In vitro biological trials and some kinetic investigation according to inhibition activity of newly synthesized molecules toward AChE and BuChE are in progress and will be reported. Additionally, some structure-activity conclusions will be done.

P175. Abstract number: 342

Three Disulfide Bridged μ-Conopeptoids and their Minimized Disulfide-Depleted Selenopeptide Derivatives

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Peptide neurotoxins from cone snails continue to provide compounds with a therapeutic potential. Analgesic μ-conotoxin KIIIA is a three disulfide bridged peptide inhibitor of mammalian sodium channels, but more SAR studies are needed to optimize its subtype selectivity. Recent studies suggested that Lys^7 in KIIIA might be an attractive target for engineering selectivity in μ-conotoxins toward neuronal subtypes [1,2]. Furthermore, converting this three disulfide bridged peptide into a one-disulfide-containing disulfide-depleted selenopeptide analogue did not significantly affect its bioactivity, but dramatically simplified oxidative folding [2]. Here, we report design and synthesis of two series of μ-conopeptoid analogues in which we replaced Lys^7 with peptoid monomers of increasing side-chain size: N-methylglycine, N-butylglycine and N-octylglycine. Each of μ-conopeptoid analogues from the first series contained three disulfide bridges, whereas the second series of peptoid analogues was introduced in the context of disulfide-depleted selenoconopeptides. Chemical synthesis, oxidative folding and the bioactivities of the μ-conopeptoid analogues will be presented.

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P176. Abstract number: 348

Development of a new strategy for the synthesis of 4-amino-tetrahydro-2-benzazepin-3-one containing peptides

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One strategy to overcome the limitations of peptides, such as metabolic stability and lack of selectivity is to introduce conformational constraints in the peptide. These peptidomimetics may possess an improved receptor selectivity and potentially an improved potency by locking the biologically active conformational state.

In this work the rotational freedom around the C_α-C_β of the phenylalanine side chain was reduced by introducing a methylene bridge between the aromatic ring and the amine of the next residue. This 4-amino-tetrahydro-2-benzazepin-3-one (Aba) skeleton only allows the gauche(+) and trans conformation.[1]

A general pathway towards Aba containing peptides, which is applicable in solution and in solid phase peptide synthesis, uses a protected o-formylphenylalanine. We will discuss the synthesis of a *N,O*-protected o-formyl-Phe building block. The asymmetric synthesis of this building block will be presented as well as its tendency to undergo 'reductive lactamisation' (reductive amination/spontaneous intramolecular lactamisation).[2] Applications in opioid peptides will demonstrate the advantages of the constraint.

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P177. Abstract number: 371

Screening of cell-specific adhesion peptides for medical device coating

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Recently, cardiovascular diseases have become major causes of death in the developed countries. Many types of medical devices have been developed to treat ischemic heart diseases, peripheral vascular diseases, and strokes. However, long-term implanting of such devices commonly induces critical complications. Stents that are used for coronary arterial diseases, peripheral vascular diseases and strokes are known to cause thrombosis combined with stenosis derived from the lack of endothelium. Small-caliber vascular prostheses also take risks of obstruction for thrombosis. Patients whom implanted cardiovascular implants are commonly forced to take costly anticoagulant agents for their lifetime to escape from life-threatening risks.

To provide better quality of life to such patients, effective inner endothelialization is an essential criterion for such medical devices. If the early and sufficient endothelialization is accomplished on the surface of medical devices, both the risk of thrombosis and the cost for anticoagulant agent could be reduced. Therefore, safe and effective material that enhances endothelialization on the medical devices is strongly desired. In this aspect, peptides are one of most promised bio-compatible materials that could be chosen for surface modification, since it could eliminate both risks of animal-derived infections and interspecific immune response found in other large proteins and antibodies.

There had been many researches that reports universal cell adhesion peptides, such as RGD peptides, found from extracellular matrixes (ECM) [1]. However, for the medical device surface modification concept for effective endothelialization, such strong cell-adhesion peptides hold the risk to capture unwanted cells. Therefore we designed a peptide array-based screening combined with in silico ECM analysis to obtain cell adhesion peptide that specifically capture endothelial cells than other anchorage dependant cells. From our cell-assay method on SPOT peptide array [2, 3, 4], we succeeded to find more than twenty objective peptide candidates, and also found the common peptide property that could selectively control the cell specific adhesion. We here report the effects of screened peptides.

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P178. Abstract number: 385

Microwave Assisted Synthesis of Backbone Cyclic Peptides

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The use of backbone cyclization to improve the pharmacokinetic properties of peptide drug leads have shown to be a successful strategy [1, 2]. One of the reasons that hamper the use of backbone cyclization is the lack of compatibility with automatic Solid Phase Peptide

Synthesis (SPPS) and Microwave Assisted Peptide Synthesis (MAPS). We described the synthesis of backbone to backbone cyclic peptide libraries, utilizing manual microwave technology.
Two backbone to backbone cyclic peptide libraries based on the parent dermorphin analog TAPS (Tyr-D-Arg-Phe-Sar) [3], with different ring size and bridge chemistry, were synthesized using MAPS.
The first library was prepared by incorporating Fmoc-Nα[(Alloc)□-aminoalkyl] glycine (called herein Alloc Glycine Buiding Units, AGBU), on-resin reductive alkylation of amino functionalized alkyl chains and subsequent urea cyclization [4].
The second library was synthesized by a novel method of backbone cyclization, utilizing AGBU [5], which after Alloc deprotection, were coupled to widely available □-bromo carboxylic acids with varying alkyl chain length. Cyclization was achieved via intra-molecular variation of the Nuss et. al. [6] "sub-monomer"procedure for peptoid synthesis, creating a bond between the alkyl bromide linker and nitrogen of the peptide backbone, producing a backbone cyclic peptide with amide bond bridge chemistry.
Peptides from both libraries were purified and tested for biological activity.
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P179. Abstract number: 447

Design, Synthesis and Evaluation of Inhibitors Targeting the SHP-1 Catalytic Domain.

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Protein tyrosine phosphorylation is a ubiquitous signaling mechanism that is regulated by an intricate balance between the action of kinases and phosphatases. Protein tyrosine kinases (PTKs) phosphorylate cellular substrates and protein tyrosine phosphatases (PTPs) act by removing these phosphate groups through hydrolysis. SHP-1 is a PTP that is primarily expressed in hematopoietic cells and has been recognized as a negative regulator of various signal transduction processes, including erythropoietin (EPO) receptor signaling[1]. Since EPO enhances erythropoiesis, SHP-1 inhibitors are predicted to potentiate this pathway.
EPO is a drug used to treat anemia resulting from chemotherapy or radiotherapy. This study aims to develop an inhibitor of SHP-1 to be used in combination therapy with EPO to efficiently treat anemia by maintaining high levels of haematopoiesis. A substrate-based approach was used to identify these SHP-1 inhibitors, beginning with a collection of novel compounds that have been designed and selected as phosphotyrosine mimetics. Molecular modeling studies have been used to confirm the feasibility of these compounds binding to the active site of SHP-1. A series of these mimetics have been synthetically incorporated into a physiological peptide substrate and biologically evaluated using the in vitro Malachite Green assay. An alanine scan was then conducted to explore substrate specificity. Key groups of residues at the N- and C-terminus important for binding were virtually screened against the ZINC database[2] for organic-based fragment replacements. Finally, these compounds can be converted to inhibitors by replacing the phosphate group with a non-hydrolyzable sulfate moiety.
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P180. Abstract number: 457

Comparison of synthesis and antibacterial activity of a- and β-peptoids

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In most organisms natural antimicrobial peptides (AMPs) are the front line defense strategy against a wide range of pathogens.[1,2] Cationic host-defense peptides constitute the most interesting subclass of AMPs due to their role in the innate immune response to microbial infections. The characteristic features of cationic AMPs are a positive charge together with a high content of hydrophobic residues.
Homomeric β-peptides and α-peptoids as well as heteromers composed of two different types of residues are able to imitate the antibiotic activity of natural AMPs.[3-6] Recently, we reported on alternating α-peptide/β-peptoid chimeras that exhibit promising antibiotic properties.[7-9] Submicromolar activities have been found for S. aureus (methicillin-resistant), S. epidermidis and E. coli while micromolar activities were found for many other bacteria.
Although feasible, preparation of these α-peptide/β-peptoid chimera compounds by using a dimer-based protocol it requires tedious solution-phase syntheses of several different building blocks. Thus, we wish to develop a monomer-based methodology involving microwave-assisted solid phase synthesis of the corresponding homomeric α- and β-peptoids. Here, we describe the preparation of the required α- and β-peptoid building blocks and preliminary testing of antimicrobial activity.
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P181. Abstract number: 462
Conformational Analysis of Aliskiren, a Potent Renin Inhibitor, in Solution using NMR and Molecular Dynamics
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Aliskiren is a non-peptidic molecule that specifically and potently inhibits human renin. It has been newly introduced to the pharmaceutical industry as a standalone antihypertensive drug with various effects, especially blockade of the renin receptor. In previous studies, 1D NMR experiments have been performed on aliskiren, but no structural data is available in terms of its structural characteristics in solution. In this report, the conformational behavior of aliskiren is studied in water (H2O) and *N,N-dimethylformamide* (DMF) solutions, through dynamic 600MHz NMR spectroscopy, by means of 2D-NMR spectroscopy and molecular modeling techniques. These results are used to compare the conformation of aliskiren in solution with the aliskiren-renin co-crystallized structure, in order to examine the structural characteristics of this antihypertensive agent. Data extracted on the interatomic distances between distant protons of the molecule's functional groups are combined with molecular dynamics simulations for inspection and evaluation of the drug's molecular properties.

P182. Abstract number: 474
Development Of A Protease-Resistant Bicyclic Peptide Targeting Human Plasma Kallikrein
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Cyclic peptides have been studied for many years to present a better conformation to their target but also for their ability to create protease resistance[1] . Among them, peptides forced into two loops combine properties of both small molecule drugs and proteins. One way of constraining peptides is to react the cysteine residues with a halogenated core. After synthesis on solid phase, the peptides are purified by HPLC and cyclized with tris(bromomethyl)benzene *via* the cysteines to give bicyclic peptides [2,3] .
The peptidic sequence ACSDRFRNCPADEALCG (PK15) was selected for its high binding affinity (IC_{50} = 4 nM) and specificity to human plasma kallikrein[3], an enzyme involved in angioedema and blood pressure regulation[4] . Despite its bicyclic structure, PK15 is still sensitive to trypsin cleavage, albeit at a much lower level than the linear peptide. In the present study, we conducted an alanine scan of the sequence to reveal the most important residues for binding to kallikrein and we designed variants to make the peptide resistant to proteolysis.
We present here the binding affinities and protease resistance of our modified sequences. These may represent leads for drugs to treat hereditary angioedema.
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P183. Abstract number: 494
A NOVEL AND HIGHLY SELECTIVE IgE-BINDING PEPTIDE WITH ANTI-ALLERGIC PROPERTIES
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Binding of IgE to the high affinity receptor FcεRI on the surface of mast cells and basophils is the key step of allergic reactions. The receptor contains a β-chain, a dimer of γ-chains and an extracellular α-chain that binds with high affinity to the Fc of IgE (KD ≈ 10-9 M). Receptor cross-linking through allergen/antibody interactions activates an intracellular signalling in mast cells that leads to degranulation and to release of histamine or other mediators of the allergic response [1] and it has been shown that preventing the IgE-FcεRI binding is an effective way to block the early events of the allergic response [2,3]. The crystallographic structure of the complex FceRI(αchain)-IgE(Fc) has cleared the molecular details of this interaction and has opened the way to the design of novel modulators of allergic responses [4,5]. We have recently reported a set of peptide-based receptor mimetics containing key residues from the D2 domain (interaction site 2) and from the D1-D2 junction of FcεRIα chain (interaction site 2) joined by a linker. Peptides have a μM affinity for IgE and show a distinct 2-site mechanism of recognition for the immunoglobulin, as it occurs in the native receptor. We report here a new IgE-binding peptide - named Pep-E - where the linker has been optimized in terms of length. Pep-E shows a largely improved affinity (> 30-fold) compared to the first generation peptides [5], the same specificity and a 2-site mechanism of binding for IgE Binding to IgE has been performed using the SPR technique on a BIAcore instrument (Pharmacia Biosensor) and on a SensiQ (Nomadics). For this purpose, biochips were prepared by coupling IgE, IgG and IgA to a CM5 sensor (Biacore), whereas only IgE were bound on a COOH1 SensiQ chip. Notably, assays with the two instruments provided a comparable 600 nM affinity even when the peptide was conjugated to a fluorescent dyes. IgE-binding and the potential anti-allergic properties of Pep-E were further assessed on RBL2H3 cells, confirming disruption of IgE interaction with the cell-bound native receptor and blocking of histamine release following IgE-antigen stimulation. The IC50 for the histamine release assay was about 100 μM.

P184. Abstract number: 495
Structure-activity relationships study of interleukin-1 receptor antagonist by alpha-amino gamma-lactam scanning
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Agl (α-amino γ-lactam) residues in peptides have stabilized β-turn structures and enhanced potency and receptor selectivity [1], underlining their importance as synthetic tools in conformational analysis and drug discovery. The single and multiple insertion of Agl residues into peptide sequences has been performed on solid support using a six-member cyclic sulfamidate derived from homoserine to annulate the amino lactam residue onto the peptide chain [2,3]. Moreover, the introduction into peptides of beta-hydroxy-alpha-amino gamma-lactams, as constrained ser

and thr mimics, was achieved by employing N-(Fmoc)oxiranylglycine as a bis-electrophile in TFE to sequentially alkylate and acylate the growing sequence on solid support [4]. These methods have provided access to several analogs of the allosteric modulator of interleukin-1 receptor APG-101.10 (D-Arg-D-Tyr-D-Thr-D-Val-D-Glu-D-Leu-D-Ala: rytvela) [5]. With the goal to better understand the conformational preferences responsible for the biological activity of APG-101.10, a library of (R)- and (S)-Agl analogs was produced, including analogs possessing two Agl residues, and those in which the Thr residue was replaced by a beta-hydroxy-alpha-amino gamma-lactam. The efficacy of the lactam analogs was ascertained by measuring their influence on IL-1-induced human thymocyte TF-1 proliferation relative to that of the parent peptide. The conformational preferences of the lactam analogs and rytvela were assessed by circular dichroism spectroscopy. Compared to the parent peptide, which was characterized by a CD curve characteristic of a disordered structure, the analogs exhibited curve shapes indicative of turn-like conformations. Our presentation will demonstrate the influence of the lactam constraint on rytvela conformation and biology.

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P185. Abstract number: 501

Design, Synthesis, and Evaluation of a-Helix Mimetics Targeting PCNA-p53 Interaction

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PCNA, well known as the DNA sliding clamp, plays an essential role in DNA replication and different types of DNA repair, including nucleotide excision repair, mismatch repair, and base excision repair.[1,2] We have originally found that PCNA interacts with the CDK inhibitor $p21^{Cip1}$.[3] PCNA functions through its direct interactions with DNA processing enzymes, p21, and other binding partners, which share similar PCNA binding motif, PIP-box.[4] The PIP-box consensus sequence, Qxx(M/L/I)xx(F/Y)(Y/F), is highly conserved in Archae and Eukarya. The PIP-box peptides include three distinct regions, an extended N-terminal region, a central short hydrophobic 3_{10} helical region, and an extended C-terminal region. Short PIP-box peptides, such as the C-terminal region (a.a. 141-160) of p21 has been shown to bind with PCNA and inhibit PCNA-dependent DNA replication and repair.[5] Therefore, such peptides can be used as a template for the design of peptidomimetics with helix mimetics as key scaffolds.

According to the crystal structures of PCNA and PIP-box peptide, we applied individual or combined strategies to design peptidomimetics of PIP-box peptides. As mentioned above, PIP-box motif has the central 3_{10} helical region that positions the hydrophobic residues plugging into the hydrophobic cleft of PCNA. We employed covalent linkage to constrain the 3_{10} helical structure, which include two alternative strategies for the synthesis. The binding mode of PIP-peptidemimetic is similar to that of the p21 peptide in the crystal structure. The hydrophobic side chains pack with the hydrophobic cleft of PCNA tightly. Detailed synthesis will be reported.

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P186. Abstract number: 504

The Synthesis of Peptide DualAG

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Recently, it was reported that peptide DualAG, H-s-QGTFTSDYSKYLDSRRAQDFVQWLMNTKR-NRNNIAC(CH2CO-PEG4-Cholesterol), exhibits superior weight loss, lipid lowering activity, and antihyperglycemic efficacy[1]. The preparation and conjugation of cholesterol with PEG4 linker to the C-terminus of native oxyntomodulin via a Cys sidechain improve pharmacokinetics and is also a key step for synthesis of peptide DualAG. Here we report the synthesis of DualAG with Fmoc solid phase synthesis to obtain peptide precursors and solution phase work to make and react cholest-5-en-3-yl 1-iodo-2-oxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-oate with the thiol-containing peptide to obtain peptide DualAG.

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P187. Abstract number: 507

Structure-activity relationship studies for Cystapep 1 and its analogues

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Cystapep 1 is a peptidomimetic compound structurally based upon the inhibitory centre of human cystatin C, which was found to inhibit growth of Streptococcus pyogenes. Because of its origin it was thought that it inhibits cysteine protease, but subsequent analysis of a number of similar compounds showed that their antimicrobial effect does not include protease inhibition. [1] Despite number of analogues which we synthesized the modus operandi of Cystapep 1 is not known. Isothermal titration calorimetry (ITC) is a great tool to study interactions between various lipid bilayer model membranes and peptides active against pathogens that is why we were using ITC to investigate interaction between Cystapep 1 analogs and model of procariotic or eucariotic cell membrane.

In ITC research we have studied binding for both active and inactive analogs of Cystapep 1. Large unilamellar vesicles composed of zwitterionic (in the case of Eucaryota) and anionic (in the case of Procaryota) phospholipids were used as model membrane system. Our research indicate that Cystapep 1 analogues interact only with model of bacterial cell membrane. None of investigated compounds interact with model of eucariotic

plasmalemma. Interaction between procariotic cell membrane model and Cystapep 1 analogues is not strongly depend on antibacterial activity of studied peptidomimetics. We have found that some of inactive compounds binding with liposomes as well as active peptidomimetics. This fact may indicate that mechanism of action of Cystapep 1 analogues is probably not related with interruption of bacterial cell membrane system.
Structural studies comprising NMR and molecular dynamic calculations demonstrate that only Cystapep 1 is a stabile molecule with one major and very tight conformation, whereas rest of the studied compounds are flexible. We suggest that the characteristic side-chain orientation and the length of the amino-acid side chains of the compounds are the reason for distinct antibacterial properties.
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The work was supported by Ministry of Science and Higher Education, Grant No. N N204 023535 and European Social Fund. The calculations were carried out in the TASK in Gda'sk, Poland.

P188. Abstract number: 516
Supported Synthesis of Pyrrolo[3,2-e][1,4]diazepin-2-one gamma-Turn Mimics.
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1,4-Aryldiazepin-2-ones are privileged structures which display a wide range of biological activities, including antagonism at G-protein coupled receptors, enzyme inhibiton and antibiotic activity. Moreover, the seven-member ring of 1,4-aryldiazepin-2-ones can mimic an inverse γ-turn peptide conformation [1]. We have developed a modular solution-phase synthetic method for accessing the novel pyrrolo[3,2-*e*][1,4]diazepin-2-one scaffold, starting from *N*-(PhF)-4-hydroxyproline employing an asymmetric *Pictet-Spengler* reaction [1]. Towards the diversity-oriented synthesis of a library of pyrrolo[3,2-*e*][1,4]diazepin-2-ones, we have investigated a series of supports (Wang resin, Merrifield resin, PVA-PEG based resin [2] and tetraaryl phosphonium (TAP) methylbiphenyl soluble support [3]). Our presentation will discuss the influence of the support on the heterocycle synthesis and modification as well as its compatibility in a split-and-mix sequence for library synthesis.
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P189. Abstract number: 564
Cellular internalisation of water-soluble helical aromatic amide foldamers
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Intracellular transport of drugs and therapeutics represents one of the most exciting and challenging areas at the interface of chemistry, biology and medicine. Most of the effort in this field has so far been devoted to the development of peptide-based delivery systems that can translocate therapeutic agents into their intracellular targets. More recently, the use of bio-inspired non-natural foldamers has resulted in the successful delivery of cargo molecules possessing a wide range of sizes and physico-chemical properties across the cell membrane. We present here the synthesis of aromatic amide foldamers and their biological evaluation as cell-penetrating agents. Using a well-established synthetic route, a series of fluorescein-labeled cationic aryl amide conjugates has been constructed and their cellular uptake into various human cell lines analyzed by flow cytometry and fluorescence microscopy. The assays have revealed that longer oligomers achieve greater cellular translocation, with octamer Q8 proving to be a remarkable vehicle for all cell lines. Biological studies have also indicated that these helices are biocompatible, showing promise in their application as cell penetrating agents and as vehicles to deliver biologically active molecules into cells.

P190. Abstract number: 101

Detection of the Apo-B,E-binding site of low density lipoprotein receptor

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Apolipoproteins Apo-E and Apo-B100 play an important role in the development of the cardiovascular diseases. Their selective elimination from patients serum is one of the methods of the treatment and prophylaxis such cardiovascular disease as a myocardial infarction, stroke and atherosclerosis. These proteins bind with one receptor - low density lipoprotein receptor (LDLR). Detection of the Apo-B,E-binding site of LDLR and designing its low-molecular analogs can results in a making of the new medical adsorbents and therapeutic agents. The aim of our study was to detect the Apo-B,E-binding site of LDLR.

Low density lipoprotein receptor is a protein with known 3D structure and consists of several independent domains. Ligand-binding domain of LDLR is placed in N-terminal of molecule and consists of 7 repeats with 50% homology. Apo-E binds with repeat 5 of LDLR. Apo-B100 has two binding regions: one of them binds with repeat 4 of LDLR and another binds with repeat 5 of LDLR and has high homology with receptor-binding region of Apo-E. Repeat 5 of LDLR presumably has one Apo-B,E-binding site and both Apo-E and Apo-B100 competitive bind with it.

The binding sites of proteins usually are placed in hydrophobic or hydrophilic cavities on protein surfaces. Therefore for detection of the binding site of proteins we designed the program complex which allows to build the molecular protein surfaces, to detect cavities on the protein surfaces and to carry out the analysis of distribution hydrophobic and hydrophilic areas on protein surfaces. Detection of the Apo-B,E-binding site of LDLR we carried out with the help of this program complex.

At the first step we imported from ProteinDataBank (PDB; http://www.rcsb.org/pdb/) the 3D structures of LDLR (PDB ID 1N7D), repeat 5 of LDLR (PDB ID 1AJJ) and Apo-E (PDB ID 1GS9). Then using our program complex we built surfaces and defined in the repeat 5 of LDLR one cavity with rank one which form highly charged region. This cavity is formed by residues Asp224, Lys225, Ser226, Asp227, Glu228 and Glu229. With the help of the program HEX (http://www.loria.fr/~ritchied/hex/) we carried out the docking of this cavity with the critical for binding region of Apo-E (residues 140-160). It turned out, that these regions are high complementarity. We think that the region of residue 224-229 form Apo-B,E-binding site of LDLR. Now we are synthesizing its peptide's analogues and investigating their ability to Apo-E and Apo-B100 binding.

P191. Abstract number: 106

Silver nanoparticle influence on Staphylococcus aureus peptidoglycan cell wall

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In this study the spherical silver nanoparticles (SNPs) were synthesized and their influences on *Staphylococcus aureus* peptidoglycan cell wall, as gram positive model bacteria, were investigated. For the primary cell wall study, while the gas chromatography tandem mass spectrometry (GC-MS/MS) was performed for the glycan strands variations, the liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for any alterations in peptide branches. In order to study the peptide secondary structure mutation the circular dichroisms (CD) was performed. With respect to the results, the SNPs not only changes the secondary structure (*α-helix*) of bacterial cell wall, but also destroyed its primary structure. Also the transmission electron microscopy (TEM) images prove the condition of distortion of PNGs in the *S. aureus* cell wall in the presence of SNPs.

P192. Abstract number: 146

Peptidomic analysis of human blood serum for specific disease markers

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Peptidomic studies, i. e. total screening of biological samples for peptides is a rapidly growing field of biomolecular research. This approach has been successfully applied to a variety of biological fluids, cells and tissues. Hundreds of new, often bioactive peptides were found, the composition of respective peptide pools being sensitive to the physiological state of the experimental animal - pathology, impact of stress, genetic modification, immune state or action of pharmacological agents.

Considerable effort has been directed at exploiting blood serum and plasma peptidomes for development of early tumor diagnosis based on discovery of new peptide biomarkers [1]. However, these attempts have not yet produced definitive results due to extreme complexity of serum composition and formidable technical difficulties, such as extensive ex vivo peptide formation and tight binding of peptides by huge excesses of albumin and other blood proteins. In this work we present our recent results in that area.

Comparative MALDI-TOF MS profiling of blood serum samples from patients with verified ovarian cancer, colorectal cancer and syphilis as well as from a control group of healthy women has been carried out. Optimal conditions selected for sample preparation implied preliminary fractionation of serum on weak cation exchange magnetic beads and protein-peptide complexes thermal dissociation. Classification models generated on the basis of respective MALDI-TOF MS profiles demonstrated sensitivity and specificity close to 100% for the detection of all studied diseases. The same samples were subjected to nanoelectrospray-Q-TOF HPLC-Chip-MS/MS analysis which resulted in identification of ~1000 amino acid sequences. A number of peptide markers specific for concrete pathology have been revealed. Further steps to verification of the results obtained will be discussed.

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P193. Abstract number: 192

Synthesis and Evaluation of Activity-based Sulfatase Probes

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Sulfatases attract considerable attention due to their involvement in numerous pathological conditions. They comprise a family of bacterial as well as eukaryotic enzymes all activated by a unique oxidation event of an active site residue: the post-translational transformation of either a serine or cysteine side chain to a formylglycine

moiety. As for many other enzymes, sulfatase abundance is a poor measure for effective activity, and this demands for the development of activity-based tools for functional proteomic studies.
Activity-based proteomics utilizes small molecule probes comprising a targeting group, a linker, and a reporter tag to selectively label and detect active enzymes. Recent synthetic approaches rely on solid and solution phase peptide chemistry as well as ”click chemistry”for probe assembly.
We have synthesized and evaluated probes including quinone methide (QM) type traps against a panel of both bacterial and human sulfatases. Although QM precursor probes were turned over, and thus activated, their biochemical profiles are not completely consistent with mechanism-based sulfatase inactivation. In addition, fluorescence labeling studies of complex protein samples indicate that a considerable amount of QM intermediate is able to diffuse out of the sulfatase catalytic site. Our findings question the applicability of sulfatase QM probes, and, more generally, suggest a closer evaluation of the numerous previously published results for QM probes targeting other enzyme classes.

P194. Abstract number: 214
Complex networks govern coiled coil oligomerization: A multi-method approach
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Coiled coils are usually described as consisting of two up to seven α-helices that are wrapped around each other. They can associate as either homomeric or heteromeric structures and bind in parallel or antiparallel topologies. Another characteristic of all coiled coils is the periodic recurrence of a sequence $[abcdefg]_n$ called heptad repeat, where n denotes the heptad number. In these repeats *a* and *d* are hydrophobic amino acids at core positions crucial for the tertiary structure. In contrast, the polar positions *b*, *c*, and *f* are hydrophilic and *e* and *g* are charged residues [1].
Since structure and occurrence are well known, it might stand to reason that we have a clearly drawn picture of coiled coils. Yet, the rules for oligomeric formation and thus the key to biological function are poorly understood.
Based on comprehensive peptide libraries of GCN4 mutants, the influence of amino acid substitutions on the association was tested and the stoichiometry was examined by biophysical methods. Our results indicated that the formation of oligomeric structures strongly depends on various specific positions inside a heptad [2].
These amino acid dependencies were investigated using support vector machines. In order to reveal the rules of formation, pairwise feature selection was used to determine several amino acid pairings within and between heptads that are significant for oligomerisation. To this end we expanded the range of analyzed structures to all structurally resolved coiled coils contained in the PDB.
The bioinformatics results indicate that the two-amino acid dependencies found in our biochemical experiments are parts of the equation that describes coiled coil formation, but they are by far not sufficient to completely explain the complex phenomenon. We discovered that a complex network of amino acid dependencies and sequence positions previously thought irrelevant to direct coiled coil interaction have an undeniable impact on stoichiometry. To uncover these dependencies, we developed the online-tool *PrOCoil*, which visualizes the contribution of each individual amino acid to the overall oligomeric tendency of a given coiled coil sequence.
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P195. Abstract number: 277
Propeptide-derived irreversible inhibitors of cathepsin L: a SAR-study
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Currently, eleven human cysteine cathepsins are known that belong to the clan CA (family C1) of cysteine proteases. Most of these enzymes among them cathepsin L show endopeptidase activity preferring hydrophobic P2 residues. Beside their role in the lysosomal protein breakdown, these enzymes are involved in the regulation of important physiological processes like bone resorption, proenzyme activation and hormone maturation. Moreover, it is well accepted that these proteases play a crucial role in a variety of diseases among them osteoporosis and cancer [1].
To better dissect partially overlapping functions of these proteases or even to identify new functions, affinity probes are powerful tools. In this context the thiol-reactive group (2*S*,3*S*)-oxirane-2,3-dicarboxylic acid represent a privileged platform for the development of such affinity probes [2]. With attached peptide portions addressing only interactions along the active-site cleft, however, it is difficult to gain reliable selectivity for most of the cysteine cathepsins due to their structural similarity. Therefore, accessing additional peptide-protein interactions beyond the active-site cleft on the surface of theses proteases would represent an interesting new approach. Without a structural guide, however, it is difficult to identify suitable regions on the surface of these enzymes. In this context it is important that the activity of cysteine cathepsins is generally regulated by their propeptides that address both interactions within and beyond the active-site. Using procathepsin L/cathepsin L as representative model system for the family of cysteine cathepsins, we have shown that indeed structural information provided by the propeptide can be exploited for the development of irreversible cathepsin L inhibitors [3]. The synthesis and detailed SAR-data of these novel inhibitors will be presented.
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P196. Abstract number: 420
Epitope determination of anti-Aβ antibodies by online combination of SAW- bioaffinity and electrospray mass spectrometry
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Biosensors are devices able to detect a biochemical interaction between two molecular species and produce a physical signal, generally optical or electrical. The K5 S-sens® is a recently developed biosensor, capable of detecting small variations of mass by monitoring the changes of amplitude and frequency of the surface acoustic waves (SAW) through electric signals [1]. Nonetheless, biosensors do not enable a chemical structure determination of ligands, which can be achieved

by mass spectrometry [2]. We report here the epitope determination of two antibodies against amyloid beta: 6E10 (anti Aß 1-16) and 4G8 (anti Aß 17-28). The antibodies were covalently immobilised (via a thiol linker) on the surface of a gold chip inside the biosensor. The interaction of the antibodies and a solution containing the enzymatic digestion mixture of Aß 3-40 with GluC or trypsin was detected in the output signal of the biosensor. In addition, the extracted epitope peptides (affinity bound to the antibody) were eluted with a newly developed online interface under acidic conditions from the gold chip and analysed by electrospray ionisation mass spectrometry (ESI- MS), to identify the primary structure. Since the investigation of the affinity interactions is performed in PBS buffer at pH 7.5, representing physiological like conditions, an intermediate desalting step of buffer salts is necessary. This was carried out on a guard column, which also concentrated the eluted sample, rendering it detectable by mass spectrometry with high sensitivity. The combination of SAW biosensor and ESI-MS was found to be an excellent tool for the epitope determination. The results compare well with those reported in the literature.

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P197. Abstract number: 425

The top-down analysis of chemical modification of ubiquitin by ECD method

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The electron capture dissociation (ECD) is a method of choice for top-down analysis of proteins and large polypeptides. In contrast to CID fragmentation method, ECD preserves the labile posttranslational modifications. We investigated the nonspecific chemical modification of ubiquitin by ECD method. The protein was subjected to glycation [1] and N-phosphorylation [2]. Although these modifications are unstable in gas phase during CID experiments, the ECD fragmentation of glycated or N-phosphorylated compounds provided high sequence coverage.

The reactivity of ubiquitin towards glucose [3] and phosphoramidate [2] was investigated. The glycation level of lysine moieties as well as the N-phosphorylation of histidine residues in ubiquitin was investigated on the intact protein using a hybrid FT-MS instrument, equipped with ECD. The ions of the chemically modified ubiquitin were isolated by quadrupole and fragmented in ICR cell by ECD method. The fragmentation spectrum was dominated by c_n and $(z+1)_n$ type ions.

The ECD method was found useful for the localization of chemical modification sites in glycated and phosphorylated ubiquitin as well as for monitoring hydrogen - deuterium exchange with one residue resolution [3]. Its main advantage is a significant reduction of the neutral losses related to aminofructose or phosphoramidate moiety.

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P198. Abstract number: 438

Proteolytic stability of KLK3-stimulating peptide B2 and its most potent peptide analogue.

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Kallikrein-related peptidase 3 (KLK3, also known as prostate specific antigen) is produced by human prostate. Proteolytically active form of KLK3 has been shown to inhibit angiogenesis and its expression decreases in poorly differentiated tumors. We have earlier developed KLK 3 specific synthetic peptides, which promote the proteolytic activity of KLK3. All of these peptides contain disulfide bridge between the N- and C-terminal cysteines (Wu et al., 2000, Pakkala et al., 2004). We modified KLK3-stimulating peptide B-2 with alternatively cyclized analogues by replacing both of the terminal cysteines (Pakkala et al. 2010). Our earlier study showed that head-to-tail cyclization of KLK2 inhibiting peptide increased the proteolytic stability of the peptide (Pakkala et al. 2007) in vitro. Because KLK3 cleaves substrates mainly at the C-terminal side of tyrosine and glutamine (Coombs et al. 1998; Malm et al. 2000) it is feasible that the B-2 peptide also acts as a substrate for KLK3. Two possible initial cleavage sites were characterized for both of the peptides, orginal B2 and the analogue. The initial cleavage site by KLK3 was shown to be between tyrosine-7 and aspartic acid-8 in both peptides with mass spectrometric analysis. The in vitro stability of the most potent peptide analogue was studied in human plasma and recombinant KLK3. The most potent of the analogues was found to be more stable than original B-2 peptide not only in human plasma but also recombinant KLK3 showed decreased cleavage rate of this peptide, respectively.

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P199. Abstract number: 451

Plant cyclotides from an extreme habitat: Characterization of six cyclic peptides from a Violet growing at 3400 m.

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Cyclotides comprise a family of bioactive circular mini-proteins isolated from plants with a unique structure of cyclic backbone and six conserved cysteine residues designated as the cyclic cystine knot (CCK). Besides being a characteristic structural feature of the cyclotide family, the CCK motif makes these mini-proteins exceptionally resistant to chemical, enzymatic or thermal degradation (1). Cyclotides have pharmaceutical/medicinal significance due to their exceptionally stable framework, which makes them potential targets as scaffolds in drug design, and also due to the wide range of biological and pharmacological activities the native peptides exhibit by their own right (2,3). To date more than 140 cyclotides have been characterized, however, this is just the tip of an iceberg since the number of cyclotides even a single cyclotide-producing family i.e. Rubiaceae is estimated to be tens of thousands (4). In our efforts to explore cyclotide structural diversity, we have now turned to plant species from diverse habitats. In the current work we have studied the cyclotide content of the single Violet species reported to be native to East African highlands, Viola abyssinica, which grows at an altitude of 3400 m. It was possible to isolate and characterize six cyclotides by employing HPLC and MS techniques with AAA. Five of them are novel sequences of which three contain an alanine moiety in one of their inter-cysteine loops that has not been reported before for the position. A Fluorometric Microculture Cytotoxicity Assay carried out on two of the novel cyclotides revealed cytotoxic property of these cyclotides. These findings demonstrate that investigations of the cyclotide content of Violets growing in different environments is a promising route for assessing both structural and biological diversity for cyclotides.

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P200. Abstract number: 455

Heat Inactivation Of Tissue Samples To Stabilize Proteins, Peptides And Their Modifications

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Removal of a sample from its natural surrounding leads to major disturbance of the tissue homeostasis. The action of proteases and other protein-modifying enzymes rapidly can change the composition of the proteome and post translational modifications. Subsequent analytical results reflect a mix of in vivo proteome and degradation products. In this experiment a novel stabilization system was used to treat fresh and frozen tissue samples to stop degradation and preserve the in vivo proteome by inactivation early in the sample preparation chain.
The system utilizes rapid heat inactivation to eliminate enzymatic activity in tissue and thereby enable detection of endogenous peptides and monitoring of important post-translational modifications, such as phosphorylation of peptides and proteins in a stabilized and preserved proteome. Stabilization was assessed by nano-LC-MS, MALDI, western blotting, and spectrophotometric assays on samples from brain, muscle, and liver.
Inadequate sample handling normally cause an increase in degradation fragments which has been shown in these experiments. However, after immediate sample stabilization, no protein degradation fragments were detectable. The mass spectrometrically identified peptide peaks in the stabilized samples consisted of several known neuropeptides, endogenous peptides, and novel potentially biologically active peptides. Most peaks detected in an untreated group originate from proteins such as hemoglobin, cyclophilin, NADH dehydrogenase, synuclein and other highly expressed proteins. Accordingly, the assayed functions of proteases, phosphatases and cytochrome C oxidase showed clear inactivation after stabilization. The levels of phosphorylated forms of CREB, GSK and MAPK remained unchanged after 2 hours in room temperature after stabilization treatment as the levels of the same proteins in untreated tissue decreased in only 10 minutes.

P201. Abstract number: 458

Effect of point mutation in the hinge region on a structure of an amyloidogenic protein - human cystatin C

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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 fibrilar aggregates which deposit in brain arteries of elderly persons causing cerebral amyloid angiopathy [1]. At physiological conditions wild-type hCC is a monomeric protein, but under crystallization conditions (pH 4.8) forms a domain-swapped dimer [2]. The dimerization process is facilitated by the presence in the hCC structure of a flexible region created by the loop L1 (55-59, QIVAG) connecting protein subdomains undergoing the exchange process. This loop is the only part of hCC which undergoes significant structural changes during the dimerization process and, according to experimental [3,4] and theoretical [5,6] studies, these changes are driven by the conformational constraints attributed to the located near the top of the loop Val residue (Val57 for hCC). With the aim to check implications of grater or decreased stability of this loop on dimerization and aggregation propensity of human cystatin C, we designed and constructed hCC L1 mutants with Val57 residue replaced by Asp, Asn (residues favored in this position of β-turns) or Pro, respectively. By applying this rational mutagenesis approach we were able to obtain hCC variants stable in the monomeric form both in solution and in the crystal (V57N), monomeric in solution but dimeric in the crystal (V57D) and dimeric in solution and

oligomeric in the crystal (V57P). The results of structural studies of hCC L1 mutants in the context of their dimerization and oligomerization propensities will be presented.

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P202. Abstract number: 459

Crystal structure of L68V mutant of human cystatin C, an amyloidogenic protein

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Human cystatin C (wt cystatin C) is a one-chain protein (13,343 Da, 120 amino acids) that reversibly inhibits cysteine proteases of the papain and legumain families [1]. Besides its inhibitory function, hCC plays a causal role in development of one of neuropathological diseases - an amyloid angiopathy. In brain arteries of elderly individuals suffering from this disease cystatin C forms massive amyloid deposits leading to cerebral hemorrhages and finally death of patients [2]. The naturally occurring single point mutant of human cystatin C - Leu68Gln hCC - is implicated in hereditary cystatin C amyloid angiopathy, also known as hereditary cerebral hemorrhage with amyloidosis, Icelandic type. L68Q variant oligomerizes much more easily than its wild-type analog, even at physiological temperature. Deposition of L68Q aggregates in cerebral and spinal arteries and arterioles leads to recurrent hemorrhagic strokes causing serious brain damage and death of young, less than 40 years old, adults [3].

The increased propensity of the L68Q hCC variant for oligomerization can be connected with its decreased conformational stability caused by the introduction of a bulky and polar residue into the hydrophobic interior of the protein [4]. To get deeper insight into the possible mechanism of hCC oligomerization and asses the impact of modifications introduced into position 68 on this process we designed and constructed hCC variants with Leu68 residue replaced isosteric but polar Asn residue and hydrophobic but smaller in the van der Waals radius valine. The first mutation resulted in strong destabilization of the protein comparable with the one caused by glutamine residue. Valine mutant turned out to be more stable and could be expressed in good yield as a monomeric protein, but it shows increased propensity for dimerization in in vitro tests. Since we were able to obtain well diffracting crystals of the hCC L68V variant at two crystallization conditions (pH=4.6 and pH=8.0), the properties of this mutant will be discussed in connection to the obtained structural data.

Acknowledgements: This work was supported by grant of Polish Ministry of Higher Education No 2739/B/H03/2010/38.

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P203. Abstract number: 500

Mutants of an amyloidogenic human cystatin C in pressure-induced denaturation studies using hydrogen exchange mass spectrometry

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Human cystatin C (hCC) is a 120 amino acids containing protein, ubiquitous in all body fluids. Its main physiological function is to regulate activity of cysteine proteases, either released from damaged or dying lysosomes or originated from microbial invasion. Besides its inhibitory function, hCC plays a role in development of a neurodegenerative disease called amyloid angiopathy, caused by pathological aggregation of the wild-type protein or its naturally occurring L68Q variant [1].

The mechanism of cystatin C amyloid formation has not been elucidated till now. It is postulated, however, that aggregation and fibrilization proceed through propagated 3D domain-swapping process, which was evidenced as a mechanism of hCC dimerization [2]. The dimer reconstructs in duplicate the general fold of the monomeric protein with the exception of the hinge region encompassing residues Q55IVAG59, which links the swapped subdomain to the rest of the molecule. With the aim to check implications of greater or decreased stability of this region for dimerization and aggregation propensity of human cystatin C, we designed and constructed hCC mutants with Val57 residue replaced by Asp, Asn or Pro, respectively. These mutants were the subject of our denaturation studies.

Observation of H/D exchange proceeding during induced by growing pressure unfolding and subsequent refolding of the investigated proteins allowed us to detect differences in their stability and folding/unfolding dynamics. Basing on the obtained results we can conclude that proline residue at the hinge region makes cystatin C structure more flexible and dynamic, what probably facilitate the dimerization process of this protein. Polar asparagine did not influence stability of hCC conformation, while charged aspartic acid in 57 position made the protein more prone to unfolding but concomitantly also more eagerly refolding.

The work was supported by grants BW/8000-5-0253-9 and 1264/B/H03/2009/37.

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P204. Abstract number: 502

Characteristics of hCC-mAb complex

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The immune system has a major role in the defense of organisms against infections. Nowadays scientists are working on the use of antibodies in a search of inhibitors for many kinds of amyloidogenic diseases. The great potential of this concept is hidden in monoclonal antibodies (mAb), which recognize only one epitope of the antigen and are highly specific to the particular antigen. Grubb and

coworkers described the influence of monoclonal antibodies on dimerization process of human cystatin C (hCC). It was found that even catalytical amount of monoclonal antibodies visibly diminished the process [1]. This clearly showed that the mAb can be considered as potential therapeutic agent in amyloidosis caused by aggregation of hCC and its L68Q mutant. The physiological role of cystatin C is to regulate extracellular cysteine protease activity during microbial invasion or release of lyzosomal proteinases from dying or diseased cells. Cystatin C is monomeric in its native physioloigical state while in pathological conditions it is present as dimer.
In this work we present the preliminary results of studies on the influence of monoclonal antibodies on dimerization process of human cystatin C and identification of the epitope for those antibodies with the use of epitope extraction and excision mass spectrometry method.
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P205. Abstract number: 527

Characterization of human Cystatin C (hCC) - Serum Amyloid A (SAA) complex

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Cystatin C (hCC) is the the most abundant human extracellular inhibitor of cysteine proteinases. . Cystatin C is the single-chain protein which dimerize throught 3D domain swapping mechanizm. HCC is monomeric in its native physiological states while in pathological conditions it is present as dimer [1,2,3].Serum amyloid A (SAA) play major, but relatively uncharacterized roles in the acute phase response and are important complnentes of the innate immune systems of humans. Analysis of primary structur of human SAA suggests that approximately 80% of the molecules may consist of a helical bundle while the remaining C-terminal part is disordered [4].Cystatin C can create immunocomplex with SAA. The determination of the interacting sites could also be useful in designing new tools for diagnostics in many neurodegenerative disordes. The identification of the binding site in hCC should be very important for oligomeryzation studies of new oligomerisation inhibitors may be designed baded on SAA binding fragmnets. In this work we present a novel affinity metod for protein-peptide interaction studies that enabled identification of the interactions between human cystatin C and serum amyloid A. For the identification of the binding sites were applied extraction/excision mass spectrometry method together with digestion by means different proteolytic enzymes[5]. The binding sites of for SAA-hCC complex are located in C-terminal part of both protein, namely residues (96-102) in hCC and (87-105) in SAA.Acknowledgements: This work was supported by grant of Polish Ministry of Higher Education No 1264/H03/2009/371. Grubb A., Adv Clin Chem, 2000.35:p63-692. Grzonka Z., Jankowska E., Kasprzykowski F., Kasprzykowska R., Łankiewicz L., Wiczk W., Wieczerzak E., Ciarkowski J., Drabik P., Jankowski R., Kozak M., Jaskólski M., Grubb A., Acta Biochem Pol, 2001. 48(1):p. 1-20.3. Janowski R., Kozak M., Jankowska E., Grzonka Z., Grubb A., Abrahamson M., Jaskólski M., Nat. Struct. Biol., 2001, 8,316-320.4. Stevens F.J., Protein Folding Disord., 2004,11,71-80.5. Macht M., Fiedler W., Kurzinger K., Przybylski M., Biochemistry, 1996. 35(49):p. 15633-9.

P206. Abstract number: 533

Identification of cytoskeletal proteins monoclonal antibodies recognized by CSF114(Glc), the synthetic probe of Multiple Sclerosis

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CSF114(Glc) is a simple, reliable, and efficient tool, detecting for the first specific and high affinity autoantibodies in a statistically significant number of Multiple Sclerosis patients.[1, 2, 3] The corresponding native antigen(s) possibly triggering anti-CSF114(Glc) antibodies have not been yet identified and characterised.
In this study, we demonstrated the ability of CSF114(Glc) to specifically recognize monoclonal IgGs antibodies of cytoskeletal proteins.
Frozen rat brain was homogenized and solubilized in tritonX-100. Solubilized fractions were analysed in 12% SDS-PAGE.
Extracted proteins were transferred onto Nitrocellulose membrane and western blots were performed using affinity purified anti-CSF114(Glc) IgGs. We constantly found three positive bands recognized by affinity purified anti-CSF114(Glc) IgGs at the positions of 130kDa, 98kDa, and 46kDa. On the contrary affinity purification of normal blood donors sera using the same CSF114(Glc) column did not recognize those bands.
Bands of interest from SDS-PAGE were excised and trypsin digested. Digested samples were analyzed by MALDI-TOF and MS/MS. We succeeded in identifying 2',3'-cyclic-nucleotide 3' phosphodiesterase, brain specific Alpha actinin, and spectrin alpha chain (Alpha Fodrin) corresponding to positions 46kDa, 98Da, and 130kDa respectively.
To confirm our data, we purchased monoclonal antibodies to Alpha fodrin and CNPase (Abcam), and Alpha actinin (Sigma). All the monoclonal IgGs were used in BiaCore for binding analysis with CSF114(Glc) coated chips. We found that anti-Alpha actinin and anti-Alpha fodrin were able to bind to CSF114(Glc) while anti-CNPase IgGs were not able to bind to CSF114(Glc) at the same concentration.
In conclusion, we found that CSF114(Glc) specifically recognizes monoclonal IgGs anti-Alpha fodrin and anti-Alpha actinin belonging to the same family of cytoskeletal proteins. Are these two proteins possible native antigens involved in triggering antibodies in MS?
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P207. Abstract number: 536

O-glycopeptide microarrays for identification of IgG antibodies to herpex simplex virus 2.

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We have developed a high throughput O-glycopeptides screening platform for profiling of glycan binding proteins, monoclonal antibodies or disease-associated autoantibodies. In our approach, a parallel peptide synthesizer is used to generate 20-mers designed (typically) as 10-mers overlaps covering the targeted protein portion by means of Fmoc-based solid phase peptide synthesis (SPPS). The site of O-glycosylation is carefully controlled by means of glycosylated Fmoc-protected Serine/Threonine building blocks inserted at the desired positions during synthesis while deletion peptides are capped with acetic anhydride at each coupling cycle. After cleavage from the beads, overall amino acid deprotection, desalting and deacetylation of the sugar moieties, the (glyco)peptide libraries are directly immobilized on NHS-activated microarray glass surface resulting in on-slide enrichment of desired glycopeptide products via free N-terminal amines. Further, the on-slide peptides are amenable to enzymatic glycosylation with different polypeptide GalNAc-transferases and other elongating glycosyltransferases, so that extended diversity in O-glycan occupancy and O-glycan structures can be generated.

We have utilized our platform to study the human antibody response, in blood and cerebrospinal fluid, to Herpes Simplex Virus (HSV) 2 infection. HSV-1 and HSV-2 are two closely related viruses to which the antibody response against several of their envelope proteins is largely cross-reactive. We focused on envelope glycoprotein G (gG) as it harbors the only HSV antigens known to induce type-specific antibody responses, currently in use for serological assays to discriminate between HSV-1 and HSV-2 infections. Peptide 20-mers representing full-length HSV-2 gG were prepared as outlined above and immobilized on-slide. The slides were probed with mAbs as well as patient sera and samples of cerebrospinal fluid from individuals carrying none, either or both viral infections. Our preliminary results indicate that it is indeed possible to apply our microarray platform to retrace known epitopes responsible for HSV-2 type-specificity, along with other unaccounted epitopes in the O-glycan rich region of the mature part of the gG protein.

P208. Abstract number: 555

Advances in MS/MS Sequencing of Cyclic Peptides: What's around the Bend?

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In recent years, the discovery of a large family of cyclic peptides, named the cyclotides, has revealed Nature's ingenuity in drug design. The incorporation of a cyclic peptide backbone and a knotted arrangement of disulfide bridges confer extraordinary chemical, thermal and enzymatic stability on this family of biologically active peptides. However, these attributes present challenges in the identification and characterisation of cyclotides. Mass spectrometry (MS) has become the gold standard for the identification of peptides, but relies on ionization by protonation of the peptide, preferentially occurring at basic sites (Lys, Arg, His) and at the N-terminus. The absence of termini and the relatively low number of basic residues in prototypic cyclotides represents the first major challenge in MS analysis. Tandem mass spectrometric (MS/MS) sequencing is enabled by fragmentation of a peptide along the backbone primarily at the amide bonds. A typical peptide will produce a suite of sequence ions allowing the amino acid sequence to be "read off". In the case of the cyclotides, fragmentation is severely hampered by the cyclic nature and presence of disulfide bridges. Standard proteomic approaches such as reduction, alkylation and digestion may be employed to produce linear derivatives amenable to MS/MS sequencing. However, high sequence homology between cyclotides, the unusual gene structure and lack of genomic data provide additional challenges in piecing back together the cyclotide puzzle.

In this presentation, the challenges in the MS identification and sequencing of cyclic peptides will be addressed and novel analytical and bioinformatic approaches that have been developed to overcome these hurdles will be described.

P209. Abstract number: 566

Enhanced detection of sulfo-peptides in matrix-assisted laser desorption / ionization time-of-flight mass spectrometry

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Protein identification and characterization, has become one of the central activities in proteomics. The biological activity of many proteins is regulated by the extent and positions of posttranslational modifications (PTMs). More than 200 different PTMs have been described but only a minor fraction of those has been studied in detail. Indeed, most of these PTMs are lacking appropriate analytical methods that allow the sensitive, residue-resolved detection and localization of the modifications.

It appears that sulfation of tyrosine is a PTM occurs almost exclusively on secreted and trans-membrane spanning proteins. Evidence suggests up to 1% of all tyrosine residues of the total protein content in an organism can be sulfated.Mass spectrometry has been developed as a key technology for protein modification analysis.

Many mass spectrometric studies on sulfopeptides are MALDI based. It has been reported that the sulfo-moiety from sulfopeptides is readily and quantitatively lost in positive ion mode. Alternatively, MALDI in negative mode has proven useful for analysis of sulfopeptides as deprotonated anionic sulfomoieties are worse leaving groups than protonated neutral ones.

Hence, significant amounts of intact tyrosine-sulfonated species are detectable irrespective of linear or reflectron mode although the linear mode shows less fragmentation.

Here we describe a peptide/small molecule non covalent interaction system allowing detection of the entire sulfo-peptides in the reflectron/ linear positive mode by matrix-assisted desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS). We will highlight strengths and limitations of this strategy and phospho-peptides detection approach will be discussed.

P210. Abstract number: 7

Parental and non-parenteral routes for delivery of peptides drugs: Opportunities and challenges

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As one of four therapeutic modalities to address unmeet medical needs and mitigate risk in development, the market for peptides drugs is growing nearly twice as fast as that for all other types of pharmaceuticals. This growth is largely driven by an increased number of therapeutic targets. The market size for peptides drugs in 2009 is estimated at US$ 3.6 billon, with a projected growth rate of >10 % per year. In recent years, there are at least 33 therapeutic peptides on the market; 29 in Phase III, 83 in Phase II, 66 in Phase 1 and over 400 peptides are also reported to be in advanced stages of pre-clinical research. In the past decade, great progress has been made in the synthesis (large scale), selectivity, and duration of action. Delivery, however, remains the main challenge for further development. Progress has been made in parenteral delivery with broad range of technologies available for necessary release profiles. Each non-parenteral delivery system (i.e., oral, nasal, pulmonary, buccal and transdermal) has specific advantages and disadvantage, but advances in each of these techniques are coming about rapidly. These points and the overall progress in therapeutic peptide field will be addressed in this presentation.

P211. Abstract number: 11

Role of triple Hyp?Pro substitution on conformation and bioactivity of integramide A

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AIDS is produced by HIV-induced infections. HIV integrase is an important enzyme as it is critical for the integration of HIV genome into that of the host cell. Because this complex process is exclusively brought about by the virus, the enzyme, not found in the host cell, represents a safe target for the development of a single or a combined anti-HIV therapy. Integramide A is a 16-mer long, effective peptaib inhibitor of HIV-1 integrase. We have recently described a versatile synthetic strategy in solution to afford this natural compound and its diastereomer at positions 14 and 15, and found that both peptides display a significant inhibitory activity (1). Here, we present our data on the synthesis in solution, in-depth conformational analysis, and biological activity against HIV-integrase of the analogs of the two above mentioned peptides in which all of the three (2S,4R)-Hyp residues at positions 2, 9, and 13 are replaced by L-Pro. This study definitely confirms that the mixed α-/3_{10}- helical conformation of natural integramide A plays a key role in its mechanism of inhibition. Moreover, our data provide evidence that the amphipathic character of this helical structure is not required for the activity of integramide A against HIV-1 integrase. These observations will hopefully be useful to further clarify the precise mechanism of inhibition of this interesting peptaib and to identify shorter peptide sequences active against HIV-1 integrase.

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P212. Abstract number: 17

ACE inhibitors and their protein precursors - the strategy of research

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Angiotensin I converting enzyme (ACE) inhibitors are considered as the molecules that contribute to reduction of blood pressure. These peptides are encrypted in many food-derived bioactive proteins and due to their special properties they are found as the valuable food components that can be regarded as health-promoting ingredients [1].
There is vast amount of data about the ACE inhibitors available in the literature and computer databases. Databases along with some bioinformatic tools allow for the understanding some biological processes by application of specially designed mathematical and statistical algorithms [2].
The aim of the work was to elaborate the strategy of identification of ACE inhibitors in major groups of proteins by applying a BIOPEP database. This strategy involves the application of qualitative and quantitative criteria to evaluate protein as the source of ACE inhibitors as well as the prediction of release of them due to the action of digestive enzymes. The strategy of defining the best/worse source of ACE inhibitors in the protein precursors can be useful in the nutraceuticals design. Moreover, the strategy proposed is consistent with the idea of food peptidomics which covers the research concerning both the composition, changes of the pool of peptides and the methods applied in the studying of these group of molecules [3].

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P213. Abstract number: 27

Cortagen protects brain against ischemic damage in experimental models of focal and incomplete global ischemia

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Although numerous drugs have been advocated for stroke therapy, so far there are no drugs whose efficacy in preventing acute brain damage and/or promoting subsequent rehabilitation has been demonstrated unequivocally. In the present study we investigated the effect of the novel synthetic peptide Cortagen® (Ala-Glu-Asp-Pro) on the volume of the cerebral cortex infarct area and on the dynamics of subsequent neurological deficits in rat stroke models.
Two models were employed. Focal ischemia in Rattus Norvegicus males was induced by a modification of the left

middle cerebral artery occlusion (MCAO) method by combined ligation of the efferent vein and the ipsilateral left carotid artery. We also studied incomplete global cerebral ischemia (IGCI) in Rattus Norvegicus males following irreversible bilateral carotid artery occlusion.Operated animals were injected intraperitoneally with either 10 µg/kg or 150 µg/kg of Cortagen at 15 min, 2 h 15 min, 24 h and 48 h following arterial occlusion (both protocols) and additionally at 72 h and 96 h (IGCI protocol). Control animals received saline. Infarct volume 72 h after surgery in MCAO model was determined on brain cross-sections using quantitative planimetry. Neurological deficits following surgery in IGCI model were monitored during the first 10 h post-surgery and were evaluated according to Sarkisova's scale. Death-rates were assessed at 12 h and 10 days post-surgery.

Infarct volume was significantly reduced in animals receiving Cortagen at 10 µg/kg (5.1 ± 3.7 % of total left cortex volume compared to 9.5 ± 5.5 % in the control group; $p < 0.05$). Both doses of Cortagen (10 µg/kg and 150 µg/kg) attenuated the severity of neurological deficits in IGCI rats, and the number of animals with serious neurological disturbances (9, $n = 15$) was significantly lower than in control group (13, $n = 16$; $p < 0.05$). Both doses of Cortagen decreased acute mortality assessed during the first 12 h after IGCI (7 - 10 % vs 40 % in controls; $p < 0.01$) without having any effect on mortality at 10 days post-surgery.

These data indicate that Cortagen is a promising neuroprotective agent with potential to reduce brain ischemic damage and the subsequent manifestation of neurological deficits. Further studies on the use of Cortagen to extend the therapeutic window following acute cerebral stroke are warranted.

P214. Abstract number: 28

A method for screening peptides bound to EGFR by using multiple fluorescent amino acids as fluorescent tags

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A peptide library is generally used for screening peptides bound to a target protein. The phage display method and the one-bead-one-compound method are widely used methods for screening with a peptide library. However, the methods have a drawback in the case of screening for short peptides because of the need to fix the short peptides on large carriers like phages and beads. The carriers often interact with targets nonspecifically. To overcome this drawback, we have developed a new screening method without large carriers. In this method, a peptide library is labeled with multiple fluorescent amino acids. The peptides binding to targets are detected quantitatively by specific fluorescence from the fluorescent amino acids labeling to peptides. In this study, we screened 8-mer peptides bound to epidermal growth factor receptor (EGFR) by this method. EGFR is a receptor tyrosine kinase over-expressed on surface of many human cancers. It is regarded as a significant target of tumor.

Peptide was prepared by solid-phase peptide synthesis. The sequence is Ac-EE-FI-EE-Sp6-XXXO1O2XXX-NH2. O1 and O2 indicate fifteen D-configurated amino acids other than Cys, Gly, Leu, Glu and Gln. X indicates equimolar mixture of these amino acids. FI indicates a fluorescent amino acid. Fifteen fluorescent amino acids were used in this study. Each of fluorescent amino acids was corresponded to O1 and O2. Sp6 is ethylene glycol linker as spacer.

Peptide library was incubated with EGFR in HEPES buffer (pH 7.2) at room temperature for 2 h. The concentrations of peptides were 190 µM (4.5 nmol) and that of EGFR was 8.6 µM (0.36 nmol). And the peptides binding to EGFR were recovered by gel filtration chromatography. The peptides binding to EGFR was quantified by specific fluorescence in the peptides. Fluorescence was measured by three-dimension fluorescence spectroscopy in HEPES buffer containing 50% MeOH. And each of fluorescence was differentiated and determined these quantities by least-squares analysis. As the result, it found that Ac-EE-FL-EE-Sp6-XXXYFXXX-NH2 is most strongly bound to EGFR. Binding amount of the peptide was 41 pmol. This result also indicates that the peptides binding to EGFR were successfully differentiated and quantified by this method.

P215. Abstract number: 30

A second generation peptide activator derived from the C2 domain of delta protein kinase C and regulates its function in the mitochondria

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The C2 domain (conserved domain 2), identified first in protein kinase C (PKC), is found in a large number of proteins and mediates important protein-protein interactions (PPI) [1]. The C2 domain, a 120 amino acid-long domain, forms a two beta sheet sandwich that binds negatively charged phospholipids and calcium. We study the C2 domain of PKC and its role in PPI in PKC-mediated signaling.

PKC is a key player in a variety of signal transduction pathways such as apoptosis and cell proliferation and it plays a critical role in various diseases such as cancer, stroke, and cardiac ischemia. PKC is a large family of serine/threonine protein kinases that translocate from one cell compartment to another following activation. We demonstrated that the C2 domain is critical in PKC translocation, as this domain serves to anchor PKC to other proteins. Using a number of rational approaches, we successfully generated peptides derived from the PKC C2 domain that selectively disrupts PPI between individual PKC isozymes and their anchoring proteins, Receptors for Activated C Kinase (RACKs).

A peptide delta PKC-selective activator, termed delta-RACK, mediates delta PKC activation and translocation to the mitochondria, which increases tissue damaging after cardiac ischemia and reperfusion (I/R) [2]. Using the same model of isolated rat heart subjected to I/R, we showed that a new peptide, mito-delta, that was also derived from delta-PKC C2 domain, also induces delta PKC translocation to the mitochondria and selectivity regulates delta PKC interaction with distinct substrates. Surprisingly, although both peptides induce translocation of delta PKC to the mitochondria, mito-delta reduces I/R injury whereas the delta-RACK increases I/R damage. The mito-delta represents a second generation peptide that affects PPI that are unique for pathological conditions and not other functions. We suggest that the mito-delta promotes the interaction of delta PKC with proteins distinct from delta-RACK. Current studies focus on identifying those mitochondrial binding proteins that regulate cardiac protection during I/R. Because the C2 domain is found in over 60 signaling proteins, this study opens a new approach to identify pharmacological tools and perhaps even drugs that selectively regulate the functions of these C2-contianing proteins in specific pathological signaling events.

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P216. Abstract number: 41

IMPROVING PEPTIDE THERAPEUTICS FOR HIV AND OTHER VIRAL DISEASES

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Fusion of enveloped viruses with the host cell is driven by specialized fusion proteins. The ”class I”fusion proteins harbor two regions, typically two heptad-repeat (HR) regions, which are central to the complex conformational changes leading to fusion: HR1, adjacent to the fusion peptide, and HR2, immediately preceding the transmembrane domain. An obligatory step for membrane fusion is transition of the so-called pre-hairpin intermediate, where HR1 and HR2 are separated, to the post-fusion structure, where they bind to each other. This transition is inhibited by HR2-derived peptides like, for HIV, C34 and T20: the latter is in clinical use with the name enfuvirtide/Fuzeon®.

A number of HR2-derived peptides have been engineered to interact more strongly with HR1, but none is significantly more potent than C34/T20. A possible explanation is that the potency of fusion inhibitors is kinetically rather than thermodynamically-driven, being strongly influenced by parameters like the lifetime of the sensitive state, and the rate of inhibitor association, so that beyond a certain threshold of potency, the equilibrium binding strength plays a diminished role.

We have devised a general method to increase the rate of association of fusion inhibitors with the pre-hairpin intermediate, by pre-concentrating the peptide in the membrane raft microdomains where viral fusion occurs. The method is based on the addition of cholesterol group and for HIV, addition of cholesterol to C34 led to a dramatic increase in antiviral potency [Ingallinella et al., Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 5801]

We report here that cholesterol tagging is also very effective for three paramyxoviruses: the parainfluenza virus type 3 (HPIV3), which is major cause of lower respiratory diseases in infants, and the emerging zoonotic viruses Hendra (HeV) and Nipah (NiV), which cause lethal central nervous system diseases. Similarly to HIV, we observed > 100-fold increase in antiviral potency for a HPIV3 peptide fusion inhibitor, and 20-fold increase for HeV and NiV.

Moreover, we report preliminary data which extend the approach to an additional group of enveloped viruses: those where activation of the class I fusion machinery occurs intracellularly. For these viruses no HR-derived peptide inhibitor has been reported so far. We found that a cholesterol-tagged HR2-derived peptide was an effective inhibitor of the influenza A/H3N2 virus in cell culture, while the untagged peptide was inactive.

P217. Abstract number: 44

Leptin receptor antagonist peptide is highly efficacious in animal models of leptin-overabundance diseases

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Leptin, a cytokine produced mostly in the white adipose tissue, is a satiety factor regulating appetite and energy expenditure by acting on leptin receptors in the hypothalamus. It also controls multiple physiological processes in peripheral organs, including activation of immune responses and inflammation. In addition, leptin has been implicated in the development of obesity-related cancer. Thus, inhibition of leptin signaling would be a radically new and promising therapy option against different diseases linked to peripheral leptin-overabundance.

We have developed a family of leptin receptor antagonist nonapeptides that selectively inhibit leptin-induced proliferation of brain and breast cancer cells in pico- and nanomolar concentrations as well as pro-inflammatory signaling in immune cells. In cancer cells the peptides inhibit ERK1/2 and STAT3 signaling. These leptin receptor antagonist peptides contain 2-3 unnatural amino acid residues; the lead peptide having the sequence H-alloThr-Glu-Nva-Val-Ala-Leu-Ser-Arg-Aca-NH2 is designated as Allo-Aca. Allo-aca did not exhibit any systemic toxicity in normal CD-1 mice up to the 5 mg/kg highest subcutaneous (sc) dose studied, but at 0.1 mg/kg/day added sc for 38 days reduced (approximately by 50%) the growth of MCF-7 breast cancer xenografts in scid mice. Moreover, at 0.01 - 0.1 mg/kg/day sc for 3 weeks, the peptide statistically significantly improved the survival of scid mice carrying metastatic triple negative breast cancer xenografts. The efficacy was comparable to that of cisplatin without the toxic effects of the chemotherapy treatment. In a rat model of adjuvant-induced rheumatoid arthritis (RA), Allo-aca given at 0.1 mg/kg/day dose sc for 3 days fully reversed several RA symptoms (i.e control animals had 20-30% increase in ankle and paw thickness and width that completely returned to normal upon peptide treatment).

Based on these data, Allo-aca may represent the next sought-after target drug in oncology and autoimmune therapy.

P218. Abstract number: 51

Impact of peptide ghrelin antagonists on metabolic syndrome in female obese mice

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Antagonists and inverse agonists of orexigenic hormone ghrelin are promising substances for treatment of obesity. Based on the fact that ghrelin is stimulated both at estrogen deficienty and consumption of high fat diet, we hypothesize that inhibition of ghrelin could be especially beneficial in postmenopausal females with diet induced (DIO) obesity. As a model of the condition mentioned, we used ovariectomized (OVX) C57Bl/6 mice on high fat (HF) diet and treated them either with ghrelin antagonist [DLys3]GHRP-6 or ghrelin inverse agonist [DArg1,DPhe5,DTrp7,9, Leu11]substance P (SPa).

First, both substances were tested for their effect on acute food intake after their subcutaneous administration to lean fasted mice where both peptides lowered food intake in a dose-dependent way.

Then [DLys3]GHRP-6 or SPa were administered subcutaneously twice a day for one-week-long administration to OVX mice on HF diet and to all possible control groups: OVX mice fed with standard diet, and OVX mice fed either with standard or HF diet under E2 supplementation. In OVX mice on HF diet, [DLys3]GHRP-6 long-term treatment lowered significantly food consumption and body weight and significantly attenuated parameters related to metabolic syndrome such as fat/body weight, and leptin, glucose, and insulin levels. This effect was more pronounced in OVX mice on HF diet than in all control groups. On the other hand, the effect of SPa did not reach that of [DLys3]GHRP-6.
In order to find out mechanism of anorexigenic action of ghrelin antagonists, expression of neuropeptide Y in nucleus arcuatus of hypothalamus was determined. Possible shifts in metabolic efficiency were estimated from the expression of uncoupling protein in brown fat.
In our previous study, we found that ovariectomized (OVX) mice on HF diet developed resistance to an effect of intracerebroventricularly administered leptin on food intake and body weight (Horm. Metab. Res. 42:182, 2010). Here we show that for treatment of obesity and metabolic syndrome resulting from central leptin resistance, antagonists and inverse agonists of orexigenic hormone ghrelin are promising substances, especially at estrogen deficiency such as in postmenopausal women with metabolic syndrome.
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P219. Abstract number: 55

De novo designed cyclic decapeptides with anticancer activity

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Antimicrobial peptides are known to exhibit anticancer activity. Particularly those with low hemolytic activity can be considered as good candidates for the development of new anticancer agents [1-3]. We designed and synthesized a library of cyclic decapeptides, with general structure c(X_5-Phe-X_3-Gln) where X is Lys or Leu, that displayed high antibacterial activity and low hemolysis [4]. In the present study, we tested the cytotoxicity of these peptides on five human carcinoma cell lines, and their effects on apoptosis and cell signaling proteins in cultured human cervical carcinoma cells. Peptides exhibiting a promising anticancer effect in all selected cell lines were identified. Best peptides also showed low hemolytic activity, low cytotoxicity to non-malignant fibroblasts and were stable in human serum. Moreover, induction of apoptosis was observed for these peptides as evidenced by caspase activation. For the peptide with the optimal biological profile, its ability to synergize with cisplatin in HeLa cells was evaluated and will be discussed.
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P220. Abstract number: 76

SYNTHESIS OF TACRINE ANALOGUES COMPRISING PEPTIDE MOIETY

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Alzheimer's disease (AD) is a neurodegenerative illness, which affects millions of people worldwide. According to World Health Organization around 26.6 million people worldwide had AD in 2006. Following the dynamic development of the disease the number of patients with AD may quadruple by 2050. Today a most useful approach for the treatment of AD in a medical practice is to restore the level of acetylcholine by inhibiting of the enzyme acetylcholinesterase (AchE) with reversible inhibitors (AChEIs) as well as the inhibition of the enzyme γ-secretase. The latter is responsible for appearing of senile plaques in the brain. Clinical trials have shown that AChEIs like tacrine and physostigmine improve effectively the memory of some patients. The tacrine, although weaker than physostigmine, is more useful in the treatment of AD. Unfortunately, the use of tacrine is limited by poor oral bioavailability, the necessity for four-times daily dosing, and considerable adverse drug reactions (including nausea, diarrhea, urinary incontinence and hepatotoxicity) such that few patients could tolerate therapeutic doses. Herein, we report on the synthesis of a series of tacrine analogues comprising peptide moiety. These compounds were designed with aim to combine two important for AD treatment effects - AChE and γ-secretase inhibition. Additionally, in our opinion the introduction of peptide moiety in the hybrid structures could decrease their toxicity and to preserve or even increase its biological activity.

P221. Abstract number: 90

Inhibition of amyloid formation in model peptides

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A de novo designed α-helical coiled-coil peptide A has been shown to inhibit the amyloid formation of two related de novo designed amyloidogenic sequences B and C. The design of the 26 residue peptides was based on the naturally occurring coiled-coil folding motif [1],[2].By introducing a few changes into the primary sequence of an ideal coiled-coil peptide we were able to get a set of variants that dramatically differed in their aggregation behaviour. Peptide A formed stable coiled-coil trimers. The incorporation of three valine residues in b, c, and f positions initiated an α' β conformational change. As a result peptide B formed amyloid-like fibrils. Peptide C follows the design of B whereby positively charged Lysine residues were placed in close spatial proximity at positions b, e, and f, which generates an equally charged domain. As a consequence, the α-helical coiled coil structure is destabilized at pH 7.4 and peptide C underwent a conformational change from random coil to a β-sheet at pH 4 and formed amyloid-like fibrils within few days [3]. To characterize the conformational transition, the fibril formation, and the structure of amyloid fibrils of the pure peptides B and C as well as in the mixtures with peptide A different analytical methods were applied, such as CD spectroscopy, ThT and NIAD-4 fluorescence-monitored kinetic experiments, TEM, and WAXS. For the pure amyloidogenic peptides B and C the data showed a nucleation-dependent growth of amyloid-like fibrils. The formation of amyloids was inhibited however when peptide A was present before incubation. Instead, α-helical oligomeric assemblies and long fibers were found for the

B/A and C/A mixtures, which indicated the formation of heteromeric coiled-coil structures.[4] Moreover, peptide A was able to disassemble the amyloids. We could show that amyloidogenic peptides can become engaged in a stable helical arrangement using complementary interactions with an ideal α-helical coiled coil model peptide. Our studies suggest that stabilizing the helical conformation and the formation of stable helical coassemblies is a promising approach for preventing amyloid formation.

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P222. Abstract number: 95

Synthesis and in vitro Characterization of New, Potent and Selective Oxytocin Receptor Agonists

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Oxytocin (OT) exerts its numerous biological functions by interacting with oxytocin receptors (OTR) located in the periphery (uterine contractions, milk ejection) and in the CNS (social and maternal behavior). OT is widely used as a labor-inducing agent and, in a few markets, for lactation support in term mothers. Extensive research has been focused on identifying OTR antagonists suitable for treatment of premature labor, while efforts to design OTR agonists have been relatively sparse. Side effects associated with the intravenous or intranasal administration of OT are well described in the literature and are attributed to lack of selectivity versus related receptors such as the V2R, which may result in hyponatremia. Therefore, the identification of oxytocin agonists that selectively activate the OTR could be therapeutically useful and free of these dose-limiting side effects.

The biological activity of the OT molecule has been shown to be very sensitive to changes in position 7. OT analogs with Gly^7 or Sar^7 have shown improved selectivity profile versus related vasopressin receptors [1] whereas other modifications in position 7 (Tic, D-Tic) resulted in compounds with different degrees of antagonistic activity [2].

Here we report the synthesis and *in vitro* evaluation of a series of OT agonists with N-alkylglycine residues (-NR^7-CH_2-CO-) in position 7 as a key modification. All compounds were prepared as desamino analogs containing either an unaltered disulfide bridge or its monocarba modification.

The peptides were synthesized by standard SPPS methods using either the Fmoc or Boc strategy. The N-alkylglycine residues in position 7 were introduced either by a two step procedure comprising the acylation of the resin-bound C-terminal dipeptide with bromoacetic acid followed by a treatment with an appropriate primary amine or by using Fmoc-N-alkylglycines prepared separately. The compounds were tested in *in vitro* functional assays for their agonistic potency and efficacy at the OT and the related receptors. The analogs where R^7 was a straight alkyl or an arylalkyl group were found to be extremely potent OTR agonists and showed excellent selectivity versus the related receptors. One analog, carba-1-[4-FBzlGly^7]dOT, has been selected for clinical development as lactation support in both term and preterm mothers.

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P223. Abstract number: 125

INFLUENCE OF CONFORMATIONALLY CONSTRAINED AMINO ACIDS AT POSITION 4 OF UROTENSIN II-RELATED PEPTIDE (URP) ON ITS PHARMACOLOGICAL PROPERTIES

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The urotensinergic system, mostly involved in the pathogenesis and progression of hypertension and atherosclerosis, is composed of two ligands, namely, urotensin II (UII) and urotensin II-related peptide (URP) and one specific membrane-bound UT receptor, belonging to the 1A subclass of the GPCR family. Little attention has been paid to the Trp residue within the cyclic core of U-II and URP, apart from Ala-scan and D-scan approaches. However, NMR analysis of URP, together with Ala-scan results have demonstrated the importance of the triad Trp-Lys-Tyr suggesting that the turn formed by this core is intimately associated with the biological activity of URP. NMR studies revealed that Trp, both in UII and URP, was participating in a structure, a β-turn in UII and an inversed γ-turn in URP, which might be a key element for biological activity. The present work aimed at clarifying the influence of structural constraints within the cyclic core of URP on its pharmacological profile. Thus, we have designed and synthesized new URP analogs where Trp4 was replaced with unnatural and constrained residues, in order to limit conformational freedom, thus forcing the peptide backbone and/or side chains to adopt specific orientations. Using different pharmacological models, we have assessed the impact of several modifications on binding affinity and calcium mobilization using UT-transfected cells as well as ex vivo assays such as the aortic ring contraction. Preliminary binding results demonstrated that turn-inducing residues (Tiq, Phg or Tpi) retained good binding affinity and biological activity whereas other modifications such as Deg, Tic or Sar led to analogs completely devoid of any binding affinity. Inversion of configuration of Tiq or Phg led to full agonists whereas replacement of Tpi by its D-counterpart led to an almost completely inactive compound. Altogether, these data give us new insights regarding the biological conformation of URP and they will be used for the rational design of drug candidates, potentially useful for the treatment of cardiovascular disorders.

All authors are also affiliated to the Laboratoire International Associé INSERM - INRS Samuel de Champlain

P224. Abstract number: 132

Amidine neighbouring-group effect on the stability of B9870, a highly potent anti-cancer bradykinin B1/B2 antagonist peptide dimer

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Bradykinin (BK) is a biologically active nonapeptide that plays an important role in many physiological processes, such as allergies, cardiovascular diseases, hypotension, septic shock, pain, chronic and acute inflammation, and cancer. BK has two receptor subtypes, B1 and B2. The first full-chain, highly enzyme-resistant bradykinin antagonist

which showed high affinity at both B1 and B2 receptors, B9430 (DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg, where Hyp is *trans*-4-hydroxyproline and Oic is octahydroindole-2-carboxylic acid), was developed by Dr. Gera in the Stewart laboratory with the introduction of α-(2-indanyl)glycine (Igl) (1). Since BK is an autocrine growth factor for many types of cancer, notably of lung and prostate, it was logical to try the potent BK antagonist B9430 as a growth inhibitor for cancer. Disappointingly, B9430 was ineffective, but the N-terminal suberimidyl-crosslinked B9430, known as B9870 [Suim-(B9430)2, where Suim is suberimidyl; B201; CU201; NSC 710295; Breceptin] was found to be an extremely potent anti-cancer agent. B9870 was designed as a positively charged N-terminal *bis*-amidine peptide dimer [2,3]. Due to the N-terminal amidine structure, this peptide is pH sensitive and additionally shows unexpected structural changes with amidine neighbouring-group participation in physiological solutions. Interestingly, B10346 (CU301), which is the *bis*-amide analog of B9870, is stable in the same physiological conditions. The synthesis of the peptides, mechanisms of the unexpected structural changes, and the anti-cancer activity of the peptide dimers will be discussed. The stability study of B9870 and B10346 was carried out using HPLC and mass spectrometry. An IND application to test B9870 in small cell lung cancer was recently approved by the FDA and therefore the clinical trial can now proceed.

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P225. Abstract number: 151

In vitro characterization of a novel designer peptide to treat systemic infections caused by Gram-negative human pathogens.

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The incidence of serious bacterial and fungal infections is increasing despite remarkable advances in antibiotic therapy. At a time of the rapid emergence of drug-resistant bacterial strains, there is an urgent need to develop new antimicrobial compounds with novel modes of action. Antimicrobial peptides (AMPs) of the innate immune system appear very promising. Starting from the 2 kDa *Oncopeltus* antibacterial peptide 4, which was originally isolated from milkweed bug (*Oncopeltus fasciatus*), we optimized the antibacterial activities against Gram-negative pathogens. The current focus is on three species of Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*) and two non-fermenting species (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*). These are clinically relevant species, causing serious health care problems due to the acquisition of multiple resistance traits. The optimized peptide leads (called oncocin) showed minimal inhibitory concentrations (MIC) between 0.25 and 4 µg/mL against a panel of 32 different strains and clinical isolates. The serum protease resistance was increased up to a half-life of eight hours by mutating two residues to stabilize the major cleavage sites. The tested peptide derivatives showed neither toxic effects on mammalian cell lines (HeLa, HepG2, HEK 193, and SH-SY5Y cells) nor hemolytic effects on human erythrocytes within the tested peptide concentration range of up to 600 µg/mL. Confocal fluorescence microscopy indicated that the peptide derivatives entered the periplasmic space of *E. coli* at room temperature within 20 min before becoming equally distributed throughout the bacterial cell 30 min later. QCM studies (quartz crystal microbalance) indicated that the peptides can freely penetrate a model lipid membrane consisting of DMPC and DMPG (ratio 4:1) without any sign of lytic activity (i.e. loss of lipids) over the peptide concentration range of 1 to 15 µmol/L. In conclusion, the optimized oncocin derivative represents a very promising candidate for subsequent in vivo models and may serve as a novel lead compound for an antibacterial drug class against multiresistant Gram-negative pathogens.

P226. Abstract number: 160

Synthesis and biological evaluation of cytotoxic peptide conjugates containing 5-fluorouracil

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The efficiency of conventional cancer chemotherapy is limited by the lack of drug selectivity and significant toxicity to normal cells. Conjugation of cytotoxic drugs to receptor-binding peptides is one of the promising approaches for their targeted delivery to cancer cells. 5-fluorouracil (5-FU) is widely applied for the treatment of solid tumors, such as breast, colorectal, and gastric cancers. However, apart from well-known side effects, 5-FU undergoes rapid clearance in blood plasma.

In the present study we describe the synthesis of 5-FU conjugates with peptide carriers and their antitumor activity. Considering poor stability of carbamoyl derivatives during peptide synthesis, 1-carboxymethyl-5-FU p-nitrophenyl ester was used as the reagent of choice for the attachment of cytotoxic agent to carrier molecule. The practical utility of suggested approach was investigated using GnRH analogs for the targeted delivery of cytotoxic agent to cancer cells and RGD peptides as model compounds for optimization of reaction conditions.

A set of cytotoxic peptide conjugates containing 5-FU was synthesized using both classical and solid phase technique. Preliminary HPLC experiments shown that 5-FU containing peptides in contrast to parent carrier molecules are stable to enzymatic degradation in human blood plasma during 24 hr. The biological studies both *in vitro* and *in vivo* revealed that attachment of 5-FU moiety can significantly increase efficiency of antitumor action. These results are in good agreement with literature data regarding cytotoxic properties of 1-carboxymethyl-5-FU and its derivatives.

As an alternative approach to 5-FU containing peptides synthesis we consider an application of 1-carboxymethyl-5-FU hydrazide, easily converted to the corresponding acid-labile hydrazone. An optimization of reaction conditions was performed using model RGDF peptides, modified by the attachment of N-terminal levulinic or pyruvic acid. Keto-peptides were synthesized by SPPS using both BOC/Bzl and Fmoc/Bu^t strategy with some precautions at the stage of final cleavage and deprotection to avoid side products formation. Further experiments with 1-carboxymethyl-5-FU hydrazide revealed that in tested

reaction conditions only pyruvil peptides were prone to hydrazones formation in contrast to levuloyl ones.
Thus, suggested approach can be applied for the synthesis of cytotoxic peptide conjugates containing 5-FU moiety attached via stable or acid-labile linker group.

P227. Abstract number: 161
IMMUNOPEPdb: a novel immunomodulatory peptides database
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Immunomodulatory peptides are known for two common main effects: immunostimulants or immunosuppressant. The understanding the mechanisms of action of these peptides, their properties and further adaptation for clinical use are a real challenge, once that literature in this specific field is recent and widely scarce. In order to support and improve the knowledge of immunomodulatory peptides a new and original IMMUNOPEPdb immunomodulatory peptide database was developed. To ensure the accuracy of the data deposits, all sequences were carefully analyzed such as the correct function assigned in databank, the presence of signal peptide in a Signal Peptide Database (SPdb) and also redundancy degree. IMMUNOPEPdb was developed with PHP (Hypertext Preprocessor) and relational database management system (RDBMS), MySQL, based on SQL (Structured Query Language). Database is monthly update by a semi-automatic procedure. At date, host information detailed of 291 entries in which, the most representative are: 45% are from mammalian, 20% from insects, 9% from amphibians and 7.5% from fungi. Information about peptides may be obtained by using keywords such as peptide name and molecular weight or by submission of a sequence previously determined as an immunomodulatory peptide in common databanks. Due to the capability of these peptides in modulating innate immunity, IMMUNOPEPdb can be a powerful tool to development of a new therapeutic strategy combating infectious diseases and autoimmune disorders.

P228. Abstract number: 171
Peptide inhibitors for human trypsins
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Trypsin has a major function in food digestion, but its different isoforms may also have a role in cancer and other pathological states, and, thus, could be useful drug targets. Although all human trypsin-isoenzymes share extensive sequence similarity, their activity and susceptibility to inhibitors are not identical, especially trypsin-3 (mesotrypsin) differing from others. Using phage display technology, we identified seven peptides that bind to and inhibit the activity of trypsin-3 (Biol Chem 2010, 391, 283-93), which is a minor trypsin-isoform in pancreas, but the major one in brain. All of the peptides contained at least two of the amino acids tryptophan, alanine and arginine, and in all but one peptide proline was found close to N-terminus. All peptides, irrespective whether they were from cysteine engineered library or not, contained two or more cysteines, suggesting that they are cyclic. However, synthetic linear variants of these peptides were also bioactive. By alanine replacements we found that IPXXWFR motif is important for the activity. By molecular modeling the same amino acids were found to interact with trypsin-3. The peptides also inhibited trypsin-1, but only weakly, if at all, trypsin-2 and -C. Interestingly, we were able to modify one of peptides so that it inhibited trypsin-1, but not trypsin-3, and weakly, if at all, other isoforms. As trypsins effectively activate protease activated receptors and enzymes that are involved in tumor invasion and metastasis, these peptides might be useful lead molecules for development of drugs for diseases associated with increased trypsin activity.

P229. Abstract number: 175
Controlled self-assembly of perfluoro-insulin analogues: synthesis and biophysical evaluation
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Here we report the synthesis and biophysical evaluation of a series of insulin variants with perfluoroalkyl substituents. Perfluorinated alkyl chains of varying length were attached to desB30 human insulin by acylation of the ε-amino position of the side chain of LysB29. This surface exposed substituent enables intermoleular fluorous interactions leading to self-assembly, as a new and orthogonal approach to direct the self-assembly of proteins. The self-assembly was studied using small angle X-ray scattering (SAXS), dynamic light scattering (DLS) and size exclusion chromatography (SEC). First, we studied the self-assembly at different pH values without using traditional insulin formulation methods based on phenol and zinc(II). The samples were analyzed by DLS and SAXS, which showed the formation of very large aggregates. The proportion of the aggregation decreased at higher pH values. Next, the effect of formulation of the fluorinated insulin variants with zinc(II) in a 6:2 ratio in the presence of phenol at neutral was studied. Inducing the R6 form of insulin afforded a much more controlled self-assembly visualized both by DLS and SAXS. This afforded lower masses characterized by SEC and the radius of gyration observed by SAXS was much lower compared to the very large aggregates formed in the absence of phenol. Here, the short fluorinated chains exclusively gave hexamer formation and the longer chains induced the formation of larger assemblies. This implies that hexamer to hexamer interactions utilizing the fluorous subcore interaction leads to a more cooperative self-assembly of insulin. Our findings suggest that the rational design using fluorous interactions to form high molecular complexes of insulin could potentially lead to more long acting insulin variants. This concept could be applicable to other peptides or proteins.

P230. Abstract number: 177
Synthesis of Ghrelin Receptor Inverse Agonists
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The ghrelin receptor is a GPCR mainly distributed in the brain, but also in peripheral tissues like stomach. It shows a unique constitutive activity representing 50 % of its maximal activity. The signaling of the receptor controlled by its endogenous ligand ghrelin considerably contributes to

the regulation of appetite, food intake and energy homeostasis [1]. Thus, reducing the constitutive activity can be an approach to decrease body weight and to develop an anti-obesity drug.
Inverse agonists are able to reduce basal signaling of a receptor. Holst et. al showed that variants of substance P provide inverse agonistic activity at the ghrelin receptor. Structure activity relationship studies led to the hexapeptide KwFwLL-NH_2 that significantly reduces the constitutive activity [2]. Therefore, we synthesized analogues of this peptide by using solid phase peptide synthesis with Fmoc/tBu-strategy. The activity at the ghrelin receptor was tested by an inositol trisphosphate turnover assay. Some peptides presented inverse agonistic activity with EC_{50} values in the nanomolar range.
Furthermore, modified analogues containing PEG were synthesized. Pegylation should increase bioavailability and biodistribution [3]. PEG2 was introduced at the N-terminus of the peptide either directly or separated by a lysine or a lysine-β-alanine spacer. Interestingly, both, the spacer as well as the pegylation influenced efficacy.
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P231. Abstract number: 178

Synthesis of peptide vaccines against Influenza A viruses

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Influenza A viruses are causing annual flu epidemics and even threatening worldwide pandemics like the so called swine flu. Due to the high mutation rate, viruses are able to overcome species barriers with the risk of high mortality owing to the insertion of antigenically novel viruses into an immunologically naïve human population [Hay].
Commercially available vaccines are based on the two surface proteins haemagglutinin and neuraminidase. Since these proteins are very sensitive to mutations an annual vaccination is necessary. To overcome these drawbacks a universal vaccine effective against most influenza A strains would be needed.
Such an interesting candidate is M2, the third ion channel protein M2 of the influenza A virus. Since 1933, when the first influenza virus was isolated, the N-terminal extracellular domain of the protein (M2e) was highly conserved, therefore representing a potential universal influenza A epitope. Unfortunately M2 lacks natural immunogenicity in humans. For the development of a universal influenza A vaccine, three branched peptide constructs containing four copies of M2e as B cell epitope and two different species specific T cell epitopes for induction and modulation of immune response were synthesized.
The synthesis of the peptide vaccines backbone including both T cell epitopes was done sequentially using selectively cleavable protecting groups. The M2e sequence was coupled at the end either by sequential synthesis or by oxime ligation. Both synthesis strategies yielded the correct vaccine structure. The highest peptide purity was achieved by ligation of M2e as peptide aldehyde.
Vaccination with the constructs induced higher anti-M2e serum Ab titers against either the M2e peptide or native M2e. The best T helper cell epitope combination was that containing fragments from the Plasmodium falciparum CS protein and the hepatitis B virus antigen. The serum of vaccinated mice was cross reactive against the influenza virus subtype A/FM (H1N1). The new peptide vaccine regimen showed significant protection with remarkably reduced viral titers in lungs of mice after challenge with influenza A strains X31 (H3N2), PR/8 (H1N1) and two mutant viruses, termed P10H and P10L. These results confirm the potential ”universal”influenza vaccine with M2e as a target and peptide-based subunit vaccines to reach that goal.

P232. Abstract number: 188

Delta-Sleep Inducing Peptide (DSIP) and Its Analogues: Studies On Their Therapeutic Potency

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One of the therapeutic anti-tumor strategies has relied on cytostatics application. This approach often leads to a number of side effects owing to the high toxicity and lack of specificity. So the search for additive agents capable to mitigate the severity of the cytostatics treatment remains to be an urgent task for cancer therapy.
Previous examination of protective properties of DSIP and series of its analogues already gave first promising result. DSIP was found to increase the anti-tumor resistance of animals after inoculation of leukemia cells under stress and inhibit dissemination of Lewis carcinoma after its surgical removal. DSIP and several analogues were shown to enhance the efficacy of cytostatics application in model experiments on mice with the Lewis carcinoma. The DSIP ability to limit the spontaneous carcinogenesis and mutagenesis was also revealed. The previous studies have found some DSIP related compounds that might be useful for medical purposes, in particular, for possible inhibition of metastasis, decrease of cytostatics toxicity and limitation of metabolic disturbances.
In this connection, we continued the studies of DSIP family peptides to select the most effective derivatives. The novel set of analogues was synthesized, and their antioxidative, detoxifying properties and influence on a growth of experimental tumors were studied. Among tested compounds we have found the peptides with direct antioxidant action and ability to considerably inhibit the mortality of mice under cisplatin application in semi lethal dose. The most active analogues also favored a decrease in toxic action on liver and kidney under cytostatics application. Combine treatment of the tumor-bearing animals with the cytostatics and DSIP or its active analogues did not result in both an elimination and increase in the therapeutic effect of the cytostatics [1].
Thus, our studies demonstrated the expedience of the further investigation of the detoxifying effect of these regulatory peptides and rational way for its putative medical application.
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P233. Abstract number: 189

Total Synthesis of Azide Functionalized Cryptophycins

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Cryptophycins are a family of 16-membered cyclic depsipeptides produced as secondary metabolites by cyanobacteria.[1] The first representative, cryptophycin-1, was isolated in 1990 by Schwartz.[1] The retrosynthetic analysis of cryptophycin-1 leads to the four units A-D, from which unit A with four stereogenic centers and the epoxide is the most challenging part. Cryptophycin-1 and some artificial analoges proved to be extremely cytotoxic and cytostatic in biological screening studies.[2] The bioactivity of cryptophycins is based on their ability to interact with tubulin. They show considerable cytotoxicity both against multidrug-resistant tumor cell lines and solid tumors. Cryptophycin-1 and cryptophycin-52 display an antitumor activity with 100- to 1000-fold increased potency compared to the chemotherapeutics paclitaxel and vinblastine. As a consequence, cryptophycin derivatives are potential antitumor drugs. Cryptophycin-52 entered phase II clinical trials but failed because of its high neurotoxicity.[3]

We focus on the synthesis of modified cryptophycins for structure activity relationship (SAR) studies and biochemical application to circumvent this problem. Recently, we developed a short and efficient synthesis of a cryptophycin unit C building block containing a side chain azide function. The cytotoxic activity of these functionalized cryptophycins was determined in a resazurin assay.

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P234. Abstract number: 193

Dual Acting mu-/delta-Opioid Antagonist, H-Dmt-Tic-Lys-NH-CH2-Ph, Ameliorates Obesity-Related Factors

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Opioid antagonists represent an important family of synthetic drugs affecting neural reward mechanisms intimately associated in regulating homeostatic mechanisms including craving, addiction and compulsive behavior such as obesity. Amelioration of obesity by opioid antagonists is a relevant approach since agonists enhance consumption of excess high caloric foods. Since stimulation of the μ-opioid receptor results in addictive behaviors, μ-antagonists should play a central role in diminishing its activity.

Toward this goal, we prepared a unique dual acting μ-/δ-antagonist MZ-2 (H-Dmt-Tic-Lys-NH-CH_2-Ph) by stepwise solution synthetic methods (Balboni *et al.*, *J. Med. Chem.*, **2006**, *49*, 5610-5617) due to the presence of heterodimeric μ/δ-opioid receptors. MZ-2 also inhibited the development of morphine tolerance (Jinsmaa *et al.*, *Pharmacol. Biochem. Behav.*, **2008**, *90*, 651-657) and was applied at 3-10 mg/kg to suppress the reward pathway in two mouse models: ob/ob mice (daily oral administration) and diet-induced obesity (continuous mini-pump administration). Both groups had free access to food and water, and exercise wheels or kept sedentary for 3-12 weeks. Data were compared to controls: saline-treated obese mice and lean mice with and without the drug housed under identical conditions. Numerous parameters were measured: body weight (BW), tissue composition, glucose tolerance, blood chemistry, bone mineral density (BMD), and action on human osteoblast cells (Marczak *et al.*, *Eur. J. Pharmacol.*, **2009**, *616*, 115-121).

MZ-2 treated obese animals had reduced BW gain, body fat, insulin and glucose levels, improved glucose tolerance, enhanced HDL:LDL ratio and BMD, stimulated voluntary running on wheels in both lean and obese animals. In cell culture, MZ-2 enhanced mineral nodule formation (> 30%) comparable to naltrexone, but decreased with morphine.

Conclusion: MZ-2 portends a potential application in the clinical management of factors leading to obesity and the amelioration of osteoporosis.

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P235. Abstract number: 203

Molecular Modeling of the Interactions between μ-Conotoxin SmIIIA and the Pore of Voltage-Gated Sodium Channel Subtypes Nav1.2 and Nav1.4

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μ-Conotoxin SmIIIA is the only known peptide toxin that binds nearly irreversibly to two subtypes of voltage-gated sodium channels, $Na_v1.2$ and $Na_v1.4$; this unique bioactivity presented an opportunity to analyze molecular interactions between μ-conotoxins and both isoforms of sodium channels. A homology model of a pore of $Na_v1.2$ was build based on sequence similarities to four domains of $Na_v1.4$. The NMR-based model of SmIIIA was subjected to molecular dynamics simulations that resulted in minimized and equilibrated structures after 120 ns runs. These μ-conotoxins models were subsequently used for docking experiments with the pore models of $Na_v1.2$ and $Na_v1.4$. As a control experiment, we also carried out the identical procedures for modeling of molecular interactions between GIIIA and $Na_v1.4$ ion channel that are characterized by a reversible block with a nanomolar potency. Our results showed multiple binding modes of μ-conotoxin SmIIIA docked into $Na_v1.2$ and $Na_v1.4$; the key residues responsible for ionic bonds in both, the conotoxin and the sodium channel models were defined. Strikingly, from the three highest populated clusters of residues showing the strongest ionic interactions, SmIIIA shared one common mode of the interactions between both subtypes of sodium channels. GIIIA also exhibited a variety of orientations within the pore of $Na_v1,4$, but one of three highest populated modes of the interactions matched that of the well-established model, previously verified by a double-mutant cycle analysis. The selected residues of SmIIIA and GIIIA involved in occupying the sodium channel filter (DEKA-motif) are proposed to be mutated to verify the modeling results. Our work suggests that the emerging molecular diversity of μ-conotoxins may be mirrored by multiple modes of their interactions with the pore of the sodium channel subtypes.

P236. Abstract number: 210

Bradykinin Analogues Acylated On Their N-Terminus

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Two receptors, B_1 and B_2, mediate biological activities of BK. These receptors belong to a G-protein-coupled family and their activation stimulates smooth muscle cells, sensory nerve endings, causes vasodilation and microvascular leakage and modulates the response of immunocompetent cells. There is also considerable evidence that BK contributes to the inflammatory responses. Moreover, after injection to the skin, BK produces all of the basic signs of inflammation. Due to its ability to lower blood pressure, BK has been implicated in the pathogenesis of several shock syndromes. The classical B_2 receptors are of high affinity and they seem to require the full bradykinin sequence for effective activation. Accumulated evidence indicates that most of the clinically relevant effects of bradykinin are functions of B_2 receptors this being the reason why research on their antagonists is a topic of great interest.

Our previous studies suggested that acylation of the N-terminus of several known B_2 antagonists with various kinds of bulky acyl groups consistently improved their antagonistic potency in rat blood pressure assay. On the other hand, our earlier observations also seemed to suggest that the effects of acylation on the contractility of isolated rat uterus depended substantially on the chemical character of the acyl group, as we observed that this modification might either change the range of antagonism or even transform it into agonism.

In some recent work we placed two of the previously used groups (9-acridinecarboxylic acid and 9-anthracenecarboxylic acid) in the N-terminus of the BK molecule. Proposed modification transformed activity of analogues from agonistic to weak antagonistic in the rat blood pressure test and depressed its agonistic activity in the interaction with rat uterine receptors. It should be pointed out that there were first reported B_2 blockers without any changes in main peptide chain.

In the present study we continue our previous investigations to find structural requirements which in the case of BK analogues result in high B_2 antagonistic activity. Several new BK analogues acylated on their N-terminus were synthesized using SPPS method. The potencies of the analogues were assessed by their ability to inhibit vasodepressor response of exogenous BK in conscious rats and by their ability to inhibit the contractions of isolated rat uterus evoked by BK.

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P237. Abstract number: 213

Synthesis and Biological Activity of Quercetin Derivatives of Endogenous Opiod Peptides Leu- and Met-enkephalin

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Quercetin, 3,3',4',5,7-pentahydroxyflavon is well-known flavonoid distributed ubiquitously as a glycoside in fruits, vegetables and herbs (apples, onions, Ginkgo biloba) and related products, specially in red wine [1]. In plants, flavonoids are involved in energy production and exhibit strong anti-oxidant properties, possibly protecting plants against harmful ultraviolet rays. Quercetin has become a subject of many investigations because of strong anti-cancer, anti-inflammatory, anti-oxidative and other therapeutic activities of significant potency such as cardioprotective, bacteriostatic and nevertheless its systemic toxicity is quite low.

In this paper quercetin derivatives of endogenous opioid peptides Leu- and Met-enkephalin (H-Tyr-Gly-Gly-Phe-Leu/Met-OH) were prepared starting from differently protected N-terminal amino group (Boc- or Z- protection). Different protecting groups were used to exchange the position and number of bounded peptide. Modifications were performed in intention to increase biological activity of quercetine as well as enkephalins which also examine antitumor activity. Antitumor evaluation and interactions with ct-DNA of prepared compounds were investigated.

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P238. Abstract number: 227

Cryptophycins - Synthesis of potent functionalised new antitumor agents

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Cryptophycins are a class of 16-membered highly cytotoxic macrocyclic depsipeptides isolated for the first time in 1990 from cyanobacteria.[1] The biological activity is based on their ability to interact with tubulin. Strong antiproliferative activities with 100- to 1000-fold increased potency compared to paclitaxel and vinblastine have been observed. Cryptophycins are also remarkably cytotoxic against several multidrug-resistant tumor cell lines and solid tumors.[2] Cryptophycin-52 was subjected to phase II clinical trials, but failed due to its high neurotoxicity.[3] Next-generation members of this compound class are currently under investigation in industry and academia.

We started to investigate this field of research to considerably simplify the synthesis of these promising molecules, taking specific emphasis on the synthetically most challenging unit A of the molecular backbone.[4] In addition, new interesting functionalities have been introduced in different positions giving rise to further possible attachment points for conjugation. A resazurin assay with the KB-3-1 and KB-V1 tumor cell lines was established as a cytotoxicity assay for SAR-studies.[5] All these research efforts have led to significant insight in cryptophycin chemistry and triggered our current interest in innovative cryptophycin bioconjugates.[6]

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P239. Abstract number: 233

Efficient Synthesis of Fertirelin Acetate as a reproductive Control Drug: A GnRH Hormone Analogue

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Fertirelin acetate is a Luteinizing Hormone-Releasing Hormone (LHRH) agonist intended for the treatment of ovarian follicular cysts in mammals and for the improvement of conception rates in this species. It induces ovulation in mammals and it is used as a drug for cows.
In this research, the synthesis of fertirelin acetate was done according to solid-phase peptide synthesis using 2-chlorotrityl chloride resin and using Fmoc-protected amino acids in the presence of TBTU as coupling reagent. Cleavage of the synthesized peptides and the final deprotection were done according to the standard methods. Final N-ethylamidation was done using reaction of synthetic nona-peptide containing free carboxylic acid with ethyl ammonium chloride using TBTU as coupling reagent.
This methodology was expanded for the synthesis of N-alkylated C-terminal peptides. The structure of product was confirmed according to the MALDI-mass data.

P240. Abstract number: 238

Synthesis, characterization and activity of Antamanide and its analogues as inhibitors of the mitochondrial permeability transition pore

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Antamanide, a cyclic decapeptide derived from the mushroom Amanita phalloides, is known for its antitoxic activity against phallotoxins and amatoxins contained in the fungus itself. The mechanism of this action is still unclear. In the present study, we show that this peptide also desensitizes the opening of the mitochondrial permeability transition pore (MPTP), a central effector in the induction of necrotic and apoptotic cell death.
We show that Antamanide inhibits Cyclophilin D (CypD), a protein implicated in the regulation of MPTP, and that its activity is not additive to that of Cyclosporin A (CsA), a well known CypD inhibitor. Like CsA, Antamanide requires the presence of phosphate and is not effective on CypD knockout mitochondria and cells.
Our studies on Antamanide analogues show that two amino acid residues (Phe^6 and Phe^9) are crucial for the preservation of activity toward CypD. Indeed, analogues in which these two Phe residues are replaced by Gly or Tyr did not show any regulation of MPTP. We also demonstrate how the inhibition of toxic effects of phalloidin occurs with a MPTP-independent mechanism. Therefore, our studies identify Antamanide as a CypD ligand and a new MPTP inhibitor.
Inhibition of CypD, preventing the opening of MPTP and, therefore, apoptosis may play an important pharmacological function, for example in neurodegenerative diseases. The recent discovery that lack of CypD improves learning and memory in animal models of Alzheimer's disease supports this hypothesis.

P241. Abstract number: 239

'Clicktophycin-52': A Bioactive Cryptophycin-52 Triazole Analogue

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The cryptophycins are a family of macrocyclic depsipeptides. They are produced as secondary metabolites by cyanobacteria of the genus *Nostoc* [1]. The cryptophycins bind to tubulin which results in disturbance of microtubule dynamics. Hence, they display a very high level of cytotoxicity even against multi drug-resistant tumor cell lines. Cryptophycin-52 was investigated as a cytostatic drug in clinical phase II studies [2]. However, it failed because of dose-limiting neurotoxicity. Cryptophycins consist of two hydroxy acids and two amino acids (unit A-D), which are connected by two ester and two amide bonds. Ester and amide bonds can be cleaved by enzymes, which lowers the in vivo stability and activity. It is known that the physicochemical properties of triazoles are similar to the properties of *trans*-amides [3]. Former studies showed a *cis*-amide bond between unit AB and a *trans*-amide bond between unit BC [4]. We synthesized a cryptophycin with a triazole replacing the *trans*-amide bond which connects unit B and C [4].
The cytotoxicity of the ”clicktophycin-52”and cryptophycin-52 was compared in cell-based cytotoxicity assays [6] using both the non-multidrug-resistant cell line KB-3-1 and the multidrug-resistant cell line KB-V1 expressing P-glycoprotein (P-gp) [7]. P-gp is an important transmembrane transporter which excretes a variety of drugs leading to multi-drug resistance.
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P242. Abstract number: 241

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

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Integrins are cell adhesion molecules consisting of non-covalently associated heterodimers that play a physiological role in cell adhesion and migration, wound healing, cytoskeletal organization and signal transduction across cell membranes and a pathological role in tumor invasion, metastasis and infection by microorganisms.[1] The amino acid sequence RGD is present in many natural ligands as a prominent recognition motif.[2] Thus, small peptides containing the RGD sequence have emerged as an excellent starting point for the identification, synthesis and development of selective integrin ligands.[3][4]
The affinity and selectivity of the peptide ligands towards different integrins depend strongly on the secondary structure and the overall three-dimensional shape. It is known that constrained amino acids, such as D-amino acids and β-amino acids, highly reduce the conformational space by inducing β- or γ-turns, respectively, which results in a higher affinity and selectivity.[5] Therefore, a series of cyclic peptides containing the RGD motif and new constrained amino acids (α-methylated amino and

dehydroamino acids) have been synthesized and tested for their activity in comparison to the known αvβ3-ligand c-(-Arg-Gly-Asp-d-Phe-Val-). In cell adhesion assays it was found that some peptides showed affinity to integrin αvβ3 comparable to that of the reference peptide.
Due to the pronounced relationship between structure and activity, the conformations of these cyclic peptides should also be comparable to that of the reference. A conformational analysis was done by NMR and molecular dynamics calculation. Based on atom distances calculated from NOE spectra, starting structures were obtained by distance geometry calculation with a simulated annealing step. After torsion angle clustering, restrained and free MD calculations with GROMOS resulted in structural proposals for the cyclic peptides.[6]
The main conformations are compared to each other and influencing properties of the new constrained amino acids are evaluated.
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P243. Abstract number: 250
CD and fluorescence screening of a-synuclein-peptide interactions
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α-Synuclein (AS), a natively unfolded protein, is the major components of the intracellular protein-aggregates, the Lewy bodies, found in the dopaminergic neurons of Parkinson's disease patients. The aggregates called 'protofibrils,' an intermediate in the fibrillogenesis process, are more cytotoxic than the amyloid-like fibrils in most of the proteins which generate fibrils. On one hand, aggregation inhibitors are expected to reduce AS cytotoxicity by preventing protofibril formation; on the other, an aggregation accelerator has recently been reported to reduce AS cytotoxicity, likely by causing protofibril precipitation. Therefore, amyloid aggregation modulating ligands are expected to serve as therapeutic medicines.
In the present study, we evaluated the interaction of peptide ligands with AS by CD and fluorescence spectroscopies. For this purpose, we synthesized two peptides, H-RKVFYTW-NH_2 and H-RGAVVTGR-NH_2, named BB1 and BB2, respectively, and their all-D amino acid analogues. In addition, a rotamer-scan of the phenylalanine residue into the BB1 peptide was performed with the aim to evaluate the influence of the topography of this residue in the binding process. To this end, the Phe residue was replaced by L- or D-NMePhe, L- or D-Tic and Ala residues.
Far-UV CD studies showed that AS conformation was strongly influenced by the interaction with these peptides. While the interaction with BB1 and BB2 induced an increase of the negative band at 198 nm, suggesting a corresponding increase of the unordered conformation of AS, other peptides caused a decrease of the same band. Surprisingly, L-NMePhe and L-Tic BB1 analogues did not interact with AS. The binding properties of the BB1 analogues was also confirmed by near-UV CD and fluorescence spectroscopies.
The preliminary results of these studies on AS-peptide interactions and their effects on AS conformation will be reported and discussed.

P244. Abstract number: 258
Antidepressive action of short human urocortin III fragments and analogues
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Depressive disorder is some of the most prevalent psychiatric diseases and there is no good antidepressive drug available uniformly appropriate for treatment.
In the activation of stress mechanism the corticotropin-releasing factor (CRF) is important. The action is elicited via CRF1 receptor, stimulating the ACTH-adrenal system and increasing the corticosteroid secretion of the adrenal cortex, and it is responsible for the anxiety related stress activation. Discovery of the CRF family peptides led to urocortin III, which is present in highest concentration in the hypothalamus and amygdala, and it is a specific ligand of CRF2 receptor. The urocortin III-CRF2 receptor stimulates serotonin release from the basolateral amygdala and strengthens the antidepressive action in depression tests.
Urocortin III, consisting of 38 amino acids, its shorter fragments and their analogues, obtained with deletion or replacement of amino acids from the original human urocortin III sequence, were synthesized and tested for treatment of depression and anxiety. We especially focused on the C-terminal alanyl-glutaminyl-isoleucine tripeptide of urocortin III and its analogues, because of our previous investigations showed that this short fragment conserves the above mentioned biological action of the longer urocortin III, and we have found that many of them show similar or improved antidepressive and anxiolytic action.
For improved pharmacokinetic properties we designed and synthesized several ureas and azapeptides by modification of the peptide backbone via formation of a resin-bound isocyanate or activated carbamate intermediate, followed by nucleophilic attack with an amine or an amino acid acyl hydrazide.

P245. Abstract number: 260
Effect Of A Synthetic Peptide Corresponding To Residues 313-320 Of The Aiib Integrin Subunit N Carotid Artery Thrombosis In Rabbits
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The platelet integrin receptor $\alpha_{IIb}\beta_3$ plays a critical role in thrombosis and haemostasis. The role of platelets in the pathogenesis of atherosclerosis, acute coronary syndromes, ischemic complications after percutaneous interventions, stroke and peripheral vascular disease, has been lucidly established. Of particular importance is the platelet integrin receptor $\alpha_{IIb}\beta_3$, which plays a critical role in thrombosis and haemostasis by mediating interactions between platelets and several ligands, primarily fibrinogen. Furthermore, an important role in the interaction between

$\alpha_{IIb}\beta_3$ and fibrinogen in humans is played by the αIIb subunit of the receptor and the HHLGGAKQAGDV sequence located at the carboxyl terminus of the γ-chain of fibrinogen. Previous results of our research group have demonstrated that the YMESRADRKLAEVGRVYLFL (313-332) sequence of the αIIb subunit may be important not only for fibrinogen binding to $\alpha_{IIb}\beta_3$ and platelet aggregation but also for platelet activation through the $\alpha_{IIb}\beta_3$-dependent outside-in signal transduction pathway (Biris N et al., 2003). Moreover, the octapeptide YMESRADR (sequence 313-320) showed the highest inhibitory activity (Mitsios JV et al., 2004). This peptide inhibits platelet aggregation and secretion as well as fibrinogen binding to the activated $\alpha_{IIb}\beta_3$, possibly by a non-RGD like mechanism (Mitsios JV et al., 2004). The aim of the present study was to investigate the effect of the synthetic peptide analogue of αIIb subunit YMESRADR (sequence 313-320) on experimentally induced arterial thrombosis in rabbits. In summary the present study shows that YMESRADR is a novel antiplatelet agent that can effectively inhibit thrombus formation and carotid artery occlusion without causing hemorrhagic complications in a rabbit model of arterial thrombosis that mimics clinical arterial thrombosis in humans. *References: Biris N. et al. (2003), Eur. J. Biochem. 270, 3760-3767, Mitsios J.V. et al. (2004), Eur. J. Biochem. 271, 855-862. Acknowledgements to the Greek ministry of health and social solidarity [KE.S.Y.] for the financial support.*

P246. Abstract number: 261

A Highly Constrained Cyclic (S,S)-Cdc- Peptide Is A Potent Inhibitor Of Carotid Artery Thrombosis In Rabbits

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Glycoprotein IIb/IIIa receptor (integrin $\alpha_{IIb}\beta_3$) is the most prominent member of adhesion receptors that mediate platelet aggregation mainly by binding to fibrinogen. The $\alpha_{IIb}\beta_3$ human platelet integrin primarily interacts with its ligands through a common tripeptide sequence, the RGD (Arg-Gly-Asp) sequence. Despite the fact that this tripeptide motif was originally identified as the cell binding domain of the integrin on fibronectin, the RGD sequence is present in various adhesive ligands, including fibrinogen, fibronectin and von Willebrand factor (vWf). Our research group has previously reported on the synthesis of constrained cyclic peptides incorporating the (S,S)-CDC- sequence presenting potent in vitro antiplatelet activity in human platelets. One of these highly constrained cyclic peptides, the (S,S) PSRCDCR-NH2, exhibited significant inhibition of human platelet aggregation and secretion as well as fibrinogen binding to the activated $\alpha_{IIb}\beta_3$ in vitro, probably by a non-RGD like mechanism (Kouki A. et al, 2005). In the present study we evaluate the antithrombotic and antiplatelet effect of the (S,S) PSRCDCR-NH2 peptide, on experimental carotid artery thrombosis in rabbits. It was found that this cyclic inhibitor can effectively inhibit thrombus formation and carotid artery occlusion without causing hemorrhagic complications in a rabbit model of arterial thrombosis. References: Kouki A. et al (2005) J. Thrombosis and Haemostasis 3, 2324-2330. Acknowledgements to the Greek ministry of health and social solidarity [KE.S.Y.] for the financial support.

P247. Abstract number: 263

Design, Synthesis And Biological Activity Of Peptides Which Act With A Non-Rgd-Like Inhibitory Activity

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Platelets play a significant role in the pathogenesis of acute coronary syndromes. The Arg-Gly-Asp RGD motif of adhesive proteins is recognized by the activated platelet integrin $a_{IIb}b_3$. Binding of fibrinogen (Fg) to activated $a_{IIb}b_3$ causes platelet aggregation and thrombus formation. Thousands of RGD analogues have been developed, targeting the blockage of the receptor ligand interaction. In previous studies we presented cyclic (S,S)-CDC-containing compounds (IC_{50}~2μM) that exhibit a non-RGD-like inhibitory activity [1,2]. This aspect could be the basis for the design of a new class of anti-platelet agents. Taking into account the previous data and the fact that the peptide (S,S) Pro-Ser-Arg-Cys-Asp-Cys-Arg-NH_2 is one of the best inhibitors, we designed, synthesized and tested for their inhibitory potency the following peptides: i) (S,S) His-Ser-Arg-Cys-Asp-Cys-Arg-NH_2, ii) (S,S) His-Ser-Lys-Cys-Asp-Cys-Arg-NH_2, iii) (S,S) His-Ser-Orn-Cys-Asp-Cys-Arg-NH_2 and iv) (S,S) DPro-Ser-Arg-Cys-Asp-Cys-Arg-NH_2. The synthesis of the peptides was carried out by solid-phase peptide synthesis (SPPS) method on a Rink Amide resin, using low substitution (0.21 mmol/g) and following the Fmoc chemistry. The removal of the Acm group and the disulfide bond formation were performed by one step reaction on solid support, using thallium trifluoroacetate $Tl(tfa)_3$. Purification and identification of the peptides was performed by HPLC and ESI-MS respectively. Assessment of their inhibition was made using ADP-induced platelet aggregation in human platelet rich plasma (PRP). The inhibition assays revealed that the nature of both the amino terminus amino acid and the cationic side chain of the X amino acid in the (S,S)-XCDC- motif affect the biology activity of the inhibitor.*References:[1] Kouki A. et al (2005) J. Thrombosis and Haemostasis 3, 2324-2330. [2] Mitsios J.V. et al. (2004) Eur. J. Biochem. 271, 855-862.*

P248. Abstract number: 270

Cyclotheonamide E4-based beta-tryptase inhibitors

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Human beta-tryptase is a trypsin-like serine protease that is virtually exclusively expressed in mast cells. Upon activation by an IgE-mediated process as a response to allergic stimuli, these mast cells release beta-tryptase along with other inflammatory mediators such as histamine and heparin proteoglycan into the surrounding tissue by exocytosis. An increasing number of studies point to a pivotal role of this secreted protease in modulating bronchial tone, airway inflammation and tissue remodeling, the hallmarks of asthma. Thus, inhibition of beta-tryptase has emerged in the last decade as promising approach for the treatment of allergic asthma [1].

Recently, based on the cyclotheonamide E4 scaffold we have developed a potent and selective beta-tryptase inhibitor [2]. For this the natural product was modified at two key positions: On the one hand the alpha-amino function of (*S*)-2,3-diamino propionic acid was used as anchoring point for the optimized basic P3 residue, epsilon-amino hexanoic acid, to exploit interactions with the negatively charged Glu-217 within the S3 pocket of beta-

tryptase. On the other hand the S1 ligand present in the natural product, (*S*)-3-amino-6-guanidino-2-oxo-hexanoic acid, was substituted by the structurally related beta-homoarginine shifting the binding from a covalent into a fully reversible mode.
Previously, we have investigated 3-aminomethyl phenylalanine as S1 ligands in bivalent beta-tryptase inhibitors [3]. It turned out that the derivative of this non-proteinogenic amino acid, Ac-Phe(3-H_2N-CH_2)-OMe, is a moderately potent though surprisingly selective inhibitor of beta-tryptase (K_i (beta-tryptase) 14 µM *vs*. K_i (trypsin) 400 µM). To further improve the selectivity profile of our lead structure, we have substituted the S1 ligand, beta-homoarginine, by the corresponding beta-homo amino acid of 3-aminomethyl phenylalanine. The synthesis, the inhibitory profile, and stability against proteolytic degradation of this novel beta-tryptase inhibitor will be presented.
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P249. Abstract number: 278
Design, synthesis and anti-influenza activity of peptidomimetic artificial ribonucleases
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Despite the advances in understanding of the molecular and cellular aspects of influenza, the disease remains the major cause of respiratory diseases among patients. The World Health Organization recommended vaccination as the main means of fighting influenza, but this approach showed a rather limited efficacy because the influenza virus is subject to rapid and unpredictable variations. Several viral molecular targets have been identified for drug intervention including hemagglutinin, neuraminidase, M2 protein, and endonuclease. The chemotherapy of influenza is based mostly on drugs of the adamantine groups (amantadine and remantadine) and neuraminidase inhibitors (zanamivir and ozeltamivir). The search for new antiviral agents remains urgent task.
Here we represent design and synthesis of novel anti-infectives based on peptidomimetic artificial ribonucleases: Xaa-diamine-Xaa, where Xaa- Lys, Glu, Ser, His, Pro, Thr, Trp, Leu.
Anti-influenza activity and cytotoxicity of peptidomimetics was investigated. Tested compounds exhibited significant antiviral effect and low cytotoxicity.
This work was supported by RFBR-09-04-01483, Grant of SB RAS-88, Carl Zeiss-2010.

P250. Abstract number: 292
Solid phase synthesis and in depth analysis of the tumour targeting peptide DOTATOC for clinical applications
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Peptides are useful tools for the targeted delivery of radionuclides and/or chemotherapeutic drugs to their site of action within an organism. Due to its excellent performance the peptide derivative (1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid)-Tyr3-octreotide DOTATOC is the gold standard of peptide radiopharmaceuticals. DOTATOC binds to somatostatin receptors (receptor subtypes SSTR2 and SSTR5) and can be used for the diagnosis and therapy of tumours expressing these receptors. Somatostatin receptors are expressed on neuroendocrine tumours, including carcinoid tumours, pituitary adenoma, pheochromocytoma, and medullary thyroid carcinoma. Somatostatin receptors are also positive on the cell surfaces of other types of tumours, such as small cell lung carcinoma, meningioma, astrocytoma, and neuroblastoma. When labelled with 68Ga DOTATOC allows the diagnosis by positron emission tomography (PET), 90Y labelled DOTATOC has been proven to be effective for endoradiotherapy. The basis for the clinical application of this peptide is an efficient solid phase synthesis.
Even though Tyr3-octreotide contains only eight residues, the synthesis is complicated by several structural features. The conjugation of the chelator sets further hurdles to obtain a product with essentially no side products. Here we describe a synthetic protocol which allows the solid phase formation and purification of DOTATOC at pharmaceutical quality. Eventual side products caused by racemisation of the two cysteine were studied using the synthetic peptide standards. The product is characterized in detail by high resolution LC/MS using orbitrap technology and a complete assignment of its 1H and 13C NMR spectra. In addition conformational features of the peptide were evaluated using 1D and 2D NMR experiments.

P251. Abstract number: 297
Solid-Phase Synthesis and analytical characterization of Myrcludex B, a potent new entry inhibitor for the treatment of HBV infections
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Myrcludex B is the lead substance of a new class of HBV entry inhibitors derived from the *N*-terminus of the large HBV envelope protein. Chronic HBV infection is the main cause of liver cirrhosis and HCC. These synthetic peptides have been shown to efficiently block HBV entry. Myrcludex B, a 47-mer lipopeptide, is currently progressing to clinical application. For the industrial production according to GMP standards an efficient and cost-effective synthesis of this peptide is required. Consequently, the solid phase synthesis of Myrcludex B was studied in detail. The peptide and the side products arising in this synthesis have been characterized by LC-MS analysis.
The peptide described consists of the 47 *N*-terminal amino acids of the human hepatitis B virus surface protein and is myristoylated at the *N*-terminus. The Fmoc cleavage pattern obtained by the peptide synthesizer and HPLC analysis of the crude product reveal an uncomplicated manufacturing process with already very good purity. Nevertheless, after the coupling of the myristic acid and the purification step it became obvious that some impurities could not be separated by the use of standard preparative HPLC protocols. To identify the main side products, the purified Myrcludex B was characterized under optimized analytical HPLC conditions and HPLC-MS measurements. Failure sequences lacking the amino acid asparagine or water (aspartimide formation) were considered to constitute the main impurities. By further analyses using enzyme degradation protocols the exact positions within

the peptide sequence were defined and attempts were undertaken to avoid the formation of these side products.
By critical observation of the solid phase synthesis of Myrcludex B and localizing the main difficulties within the synthesis, it was possible to obtain the peptide described in high yields and purity.

P252. Abstract number: 298
Conformational analysis of hemopressin, a novel peptide endowed with selective CB1 receptor antagonist activity
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Hemopressin, a novel alpha-hemoglobin-derived endogenous peptide , is a CB(1) receptor-selective antagonist/inverse agonist.1,2 Herein we report the conformational analysis of Hemopressin and its N-terminal fragment hemopressin(1-6) in membrane mimic environment composed of DPC/SDS 90:10 M/M mixed micelles. Circular dichroism and Nuclear magnetic resonance conformational studies show the presence of regular type I □-turn structures on different triads of hemopressin sequence, whereas in hemopressin(1-6) a preponderant presence of □-turn centered on the residues F4-F6 is detectable. Molecular docking studies in the presence of a modeled CB1 receptor target show that the conformational characteristics of hemopressin at the binding site are consistent with with the statements of the current CB1 antagonist farmacoforic model; even so possibilities for new structural motifs may be identified.

P253. Abstract number: 300
Synthesis of a PEG conjugated HIV Gp41 MPER fragment: a new Gp41 helix bundle mimic
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Human Immunodeficiency Virus (HIV), responsible for acquired immunodeficiency syndrome (AIDS), perform cell entry via a molecular mechanism involving viral Env glycoproteins with the final fusion mediated by the surface glycoproteins Gp41. The global architecture of Gp41 consists of a transmembrane (TM) region separating the cytoplasmic domain from the ectodomain enclosing the membrane-proximal external regions (MPER) directly involved in the virus cell fusion. In particular the fusion mechanism occurs through conformational changes of three Gp41 with the interaction of three Gp41 N-terminal helices forming a highly stable six helix bundle and the MPER effecting the events that occur after merging of the viral and cell membrane.
Peptides deriving from MPER in the ectodomain of TM glycoprotein are able to prevent HIV cell membrane fusion [1], and one of these, formerly peptide T-20, is clinically used as anti HIV fusion inhibitors [2]. It was recently shown that polyethylene glycol (PEG) conjugated dimeric and trimeric fusion inhibitors, selected from a combinatorial peptide database, to mimic the Gp41 trimeric helix bundle, were characterized by a significant increased in antiviral potency [3].
We recently synthesized and tested for antiviral activity, EPK249 a Trp rich peptide corresponding to residues 666-681 of Gp41 MPER. EPK249 displayed weak in vitro antiviral activity.
Here we present the synthesis of PEG conjugated dimers and trimers of EPK249 designed in the attempt to achieve, according to the previous reported strategy, an improvement in the antiviral, fusion inhibitory activity.
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P254. Abstract number: 318
Investigation on Inhibition of the two Active Sides of ACE by Modified Prolyl Peptides
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Many bioactive peptides derived from food proteins or artificial synthetic product inhibit Angiotensin I converting enzyme (ACE) and Ang II type 1 receptor (AT1) in the cardiovascular system contribute to the prevention and treatment of hypertension. Angiotensin I converting enzyme (ACE) belongs to the class of zinc proteases and has a two distinct active catalytic sites, called the N- and C-domains. Recent investigations have revealed that some ACE inhibitors display different inhibitory potency toward the two active sites. For example, captopril appears to be about 16 times more efficient for blocking the N-domain than the C-domain, whereas lisinopril is equally potent toward the two active sites.
The aim of this paper is to study the inhibitory potency of modified prolyl peptides by using selective fluorescence substrate Ant-Gly-Phe(NO2)-Pro-OH for C-domain and Ant-Ser-Arg-Lys(Dnp)-Pro-OH for N-domain of the active sides of enzyme. A differential selectivity may help to elucidate some peculiar properties observed with some peptides and artificial synthetic inhibitors of ACE.

P255. Abstract number: 339
Targeting Stat3 in tumors & chronic inflammation: rS3-PA, a peptide-based inhibitor, efficiently blocks Stat3 function in vitro and in vivo
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rS3-PA is a peptide aptamer that was identified in our laboratory by yeast-two-hybrid screening of a random peptide library. Its short sequence is embedded in a scaffold structure and thereby this peptide can serve as competitive inhibitor of protein recognition modules. Applications of this mechanism depend upon the binding specificities and affinities of peptide aptamers to their target structures, recombinant expression and purification of the peptide- scaffold protein and the sufficient delivery of the purified protein into the cell.
The rS3-PA peptide aptamer is directed against the dimerisation domain of Stat3 and efficiently inhibits intracellular Stat3 signalling functions and the associated phenotypes *in vitro*.
The transcription factor Stat3 is one of seven members of the signal transducer and activator of transcription family. It is not only indispensable during embryonic development

but is also widely expressed in various tissues after birth e.g. in the skin and in the thymus.
In addition to its multiple functions in normal cells Stat3 has been found to be constitutively activated in many tumors. Constitutive activation of Stat3 induces the expression of target genes supporting various processes like enhanced cell proliferation and survival, angiogenesis, migration and immune evasion.
Besides its effectiveness *in vitro*, my group was able to show that systemic application of rS3-PA in a glioblastoma mouse model clearly affected the kinetics of tumor growth in a negative fashion without side effects.
Despite its important role in cancer cells, increased activation of Stat3 also plays a crucial role the development of chronic inflammation. We are studying this phenomenon exemplarily in Psoriasis. This skin disease affects approximately 2% of the worlds population and although not life threatening is reducing the living quality of the patients to a high extend.
Psoriatic lesions are characterised by high IL-6 and IL-23 levels activating Stat3 in keratinocytes and Th17 cells, causing uncontrolled skin proliferation and chronic inflammatory responses.
For further investigations of this disease we induced local inflammation with the immune response modifier Imiquimod in mouse skin. This drug causes a local immune reaction, associated with a strong activation of Stat3.
As we were able to show that rS3-PA is able to penetrate and transduce mouse skin we now want to investigate the therapeutic effects of topical rS3-PA treatment in this disease model.

P256. Abstract number: 355

Bioinformatics-assisted SPOT peptide array screening for successful design of anti-hyperlipidmic short peptides

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Hyperlipidemia, is a significant risk factor associated with life threatening multi-factorial diseases, such as cardiovascular disease. To diminish the risk of such lifestyle-related diseases, early prevention through chronic treatment with low-cost medicines has attracted increased attention. Peptides are an attractive molecule for designing new therapies because of their biocompatibility and its safety assured by dietary experiences. Certain peptide fractions from protein hydrolysates, such as hydrolysates from soybean, potato, milk, and egg white, have been shown to mitigate hypercholesterolemia.
One of the recent strategies to obtain anti-hypercholesterolemia peptides is to target bile acids for the inhibition of intestinal cholesterol absorption [1, 2]. Bile acids are synthesized from liver cholesterol, and secreted into the lumen of the small intestine to encapsulate cholesterol by micelle, and facilitate cholesterol absorption via cholesterol transporters. Inhibition of such bile acid micelle formation or destruction of micelles can suppress intestinal cholesterol absorption. Furthermore, it is also known that the decreased bile acid re-absorption by bile acid micelle destruction triggers the consumption of stored cholesterols in the liver for the synthesis of lacking bile acids. Therefore, bile-acid binding peptides have potential to be used as cholesterol lowering agent.
In the 30EPS conference, we reported the bioinformatics-assisted peptide screening [3] to enhance the efficacy of conventional hydrolysate-based peptide exploration by combining bioinformatics and SPOT array technique [4] to obtain bile-acid binding peptides. The disadvantage of the limited library size of array-based screening was effectively compensated by ”model-based screening”or ”clustering-based screening”. In this work, we report the detailed analysis of *in vitro* and *in vivo* evaluation of peptide candidates found from the bioinformatics-assisted screening. By oral administration, the novel 6-mer peptides inhibited intestinal cholesterol absorption. We also report the commonly found peptide structural feature for higher bile-acid binding affinity from the further bioinformatics-assisted screening of more than 2,000 random peptides.
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P257. Abstract number: 362

Chimeric natriuretic peptides for heart failure treatment

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Natriuretic peptides are body fluid volume modulators, termed natriuretic peptides due to a role in natriuresis and diuresis. This peptide family is characterised by a 17-residue ring structure formed by a single disulfide bond with a highly conserved internal sequence. The three mammalian natriuretic peptides, atrial or A-type natriuretic peptide (ANP), brain or B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), have been extensively investigated for their use as therapeutic agents for the treatment of cardiovascular diseases including congestive heart failure. This is due to their fundamental role in cardiovascular homeostasis through the regulation of electrolyte balance and vascular smooth muscle tone. Although effective, short half-lives and renal side effects limit their use. This identifies a need for effective drugs for congestive heart failure treatment.
TDT is a chimeric natriuretic peptide consisting of the N- and C-terminal tails of TNPc (isolated from the venom of *Oxyuranus microlepidotus*) and the 17-residues comprising the characteristic internal ring from DNP (isolated from *Dendroaspis angusticeps* venom). The cGMP-stimulating ability of TDT was evaluated at the human natriuretic peptide receptor A (hNPR-A), which mediates the majority of the biological effects of natriuretic peptides. TDT, with an additional two point mutations had a greater potency than hANP with EC_{50} values of 0.35 ± 0.1 nM and 0.84 ± 0.1 nM respectively. PEGylation did not significantly alter the potency of the peptide at hNPR-A. The resistance of TDT to degradation through natriuretic peptide receptor C (NPR-C) mediated internalisation and lysosomal degradation, and neutral endopeptidase is presently under investigation. TDT represents an attractive lead for congestive heart failure treatment.

P258. Abstract number: 369

Protection against heat stress injury of deuterohemin peptide in C. elegans

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Heat stress will has a profound impact in *Caenorhabditis elegans* (*C. elegans*). Mild heat stimulation can cause hourmesis and lead to extend the longevity. In the other hand, continued heat stress would cause reduced life expectancy. Our group has invented a novel deuterohemin

containing peptide deuterohemin-AlaHisThrValGluLys (DhHP-6), which has various biological activities including protection of heat stress injury, improving cell survival and preventing apoptosis. We hypothesized that DhHP-6 is beneficial on the lifespan of *C. elegans* and increases their resistance to heat stress(30' and 35'). *C. elegans* were treated with different concentration of DhHP-6. Survival time and sensitivity to heat was investigated. We further demonstrated that stress resistance genes such as *hsp-16.1* and *hsp-16.49* were regulated by DhHP-6. Combining with other gene data suggest that a possible explaination for the protection against heat stress injury of DhHP-6 in *C.elegans*.

P259. Abstract number: 374

Modified Cyclic Peptides as Inhibitors of HIV-1 Integrase Activity

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Human immunodeficiency virus (HIV) is a retrovirus which is the causal agent of acquired immunodeficiency syndrome (AIDS). Viral replication is driven by three viral enzymes, namely reverse transcriptase, protease and integrase (IN). IN catalyses the insertion of viral DNA into the DNA of the host cell.

Lens epithelium derived growth factor (LEDGF) is a host cell protein which interacts directly with HIV IN and has been identified as a cellular cofactor for effective HIV integration. LEDGF binds directly to IN and dramatically stimulates the strand transfer activity.[1]

The aim of this work is to develop cyclic peptide mimics of the segment of LEDGF involved in HIV IN binding. Peptides have been designed with the intent of inhibiting the LEDGF-IN binding interaction and consequently reducing IN activity. These peptide sequences are based on the sequence of LEDGF itself, most notably retaining the reverse-turn observed in LEDGF which appears critical to its' ability to bind to IN. The use of cyclic peptides offers the possibility of increasing potency and stability relative to a linear peptide (in this instance LEDGF) contingent upon the successful mimicry of the bioactive conformation.

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P260. Abstract number: 419

Natural products as a source of inspiration for the discovery of novel POP inhibitors with blood-brain barrier transport capacity

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Natural products can often be a source of inspiration to discover novel drug candidates with properties of interest. The natural product chlorogenic acid [1], the main component of Hypericum brasiliense, shows potent inhibitory activity against prolyl oligopeptidase enzyme (POP) [2]. Starting from this compound, we have discovered a novel family of POP inhibitors based on a modified diketopiperazine scaffold. This new family of compounds also has the capacity to cross the blood-brain barrier (BBB).

The solid-phase peptide synthesis and the evaluation of the inhibitory activity and BBB transport capacity of these novel compounds have been explored in order to select the best candidate. This compound was then further studied in terms of stability in human plasma and undesired binding to relevant human receptors.

Once again, the synthesis of novel compounds inspired on ones found in nature can contribute to the discovery of new pharmaceutical agents.

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P261. Abstract number: 421

Mechanism in Inhibition of Histone Deacetylase by Cyclic Tetrapeptides with Various Functional Groups

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Class 1 and 2 histone deacetylases (HDACs) involve zinc atom at the active center to employ metalloprotease-like mechanism in cleavage of acetyl group. As natural inhibitors of these HDAC enzymes, two different type of metabolites have been known . One group includes tricostatin A, which bears hydroxamic acid functional moiety at the end of the molecule. Cyclic tetrapeptides in another group have typically epoxyketone moiety as a part of quite unusual amino acid in the cyclic peptides. Having epoxyketone is not necessary as the natural inhibitors. Simple ketone and hydroxymethylketones are also found as HDAC inhibitors so far. In last decade, a number of HDAC inhibitors have been reported in literatures and patented as the possible candidate of cancer drug. However, they have randomly different type of so-called zinc ligand which is expected to bind to the enzyme strongly at the active site. The functional groups are, for instance, hydroxamic acid, retro-hydroxamic acid, o-aminoanilide, ketone, trifluoromethylketone, hydroxymethylketone, methoxymethylketone, mercaptan, disulfisde, thioether, thioacetate, borate, phosphate, and so on. In order to evaluate such functional groups in the same molecular condition as HDAC inhibitory activity and elucidate the role of them in the mechanism of HDACs, we introduced new (azide, click product, acryloyl, and carboxyl) and known functional groups to the chlamydocin framework, cyclo(-L-AA-Aib-L-Phe-D-Pro-), (where AA bears different possible zinc ligands at the end of side chain) as standard of comparison. By examining the profiles of inhibition of HDAC1, HDAC4, and HDAC6, we could divide the many cyclic tetrapeptide HDAC inhibitors into several groups for insight into the enzymatic mechanism.

P262. Abstract number: 424

Exploiting disulfide-rich peptides in drug discovery

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Disulfide-rich peptides are ideal scaffolds for drug development and have favorable features for diverse clinical applications. They posses a disulfide-strained core that imparts extraordinary chemical and biological stability characteristics, but also sequence flexibility, allowing the introduction of novel functionalities. We have developed robust design, synthesis and display methods to exploit this scaffold class. In this presentation we will describe their potential drugability by developing a series of

analogues, based on several diverse scaffolds, as antagonists of TNF, IL-6 and IL-6R with favorable affinity, PK and in vivo activity. We will describe an innovative combination of small molecule drug discovery approaches and protein-based molecular evolution strategies guided by structure based drug design to exploit this scaffold class.

P263. Abstract number: 429

Sar Study Of P5u And Urantide Analogues Modified At Position 9

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In order to elucidate the importance of Tyr residue in biological activity and for receptor interaction recently we have synthesized new analogues where Tyr9 was replaced with the unnatural amino acid. The modifications were performed in both agonist P5U and antagonist Urantide sequences. On these new ligands we evaluated the biological activity at hUTII receptors and performed preliminarly NMR conformational studies. These 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-Trp-Lys-Xaa-Cys]-Val-OH
Xaa = unnatural aminoacids

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P264. Abstract number: 441

P53/TFIIH complex: structure-guided design of peptides to modulate apoptosis mediated by p53-dependent pathways

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The tumour suppressor protein p53 plays a key role in a number of biological pathways and approximately 50% of all human cancers are associated with mutations in p53 that result in loss of function. As a result, considerable efforts have been made to design drug therapies that increase levels of p53 function as potential cancer treatments. One key function of p53 is its role in inducing the apoptotic response at both the transcription and non-transcription levels. However, recent evidence suggests that up regulation of p53-dependent transcriptional activation may be detrimental in the apoptotic response associated with certain blood and neuronal cells as up regulation of p53 has been found in certain leukemias and neuronal dysfunctions such as Alzheimer's disease, Parkinson's disease, epilepsy and multiple sclerosis. As a result, there has been an effort to design small molecules that could selectively inhibit p53-dependent transcriptional activation as possible therapies for select leukemias and neurodegenerative diseases. P53 transcriptional activation function is associated with its amino-terminal transactivation domain (TAD). The interaction between the TAD of p53 and the general transcription factor TFIIH is correlated with the ability of p53 to stimulate both the initiation and elongation phase of transcription. Starting from our NMR structure1 of the p53/TFIIH complex, the aim of this research is to design short peptides and small molecules that are able to modulate the interaction as potential drugs treatment of some leukemias and neurodegenerative diseases.

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P265. Abstract number: 442

Anti-Plasmodium Effects of Angiotensin II Analogues

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Malaria is a major parasitic disease affecting around 300-500 million people in the world. The efforts to control this disease are hampered by drug resistance in parasites, insecticide resistance in mosquitoes, and the lack of an effective vaccine. Recently, we performed a study, which showed that the Angiotensin II (AII) and some analogues are highly active against immature and mature sporozoites of Plasmodium gallinaceum. In an attempt to increase the biological activity, we synthesized and tested two series of AII analogues with an i-(i+2) and i-(i+3) lactam bridge scaffold. Analogues were synthesized by solid phase method on Merrifield's resin and cyclized using activator BOP reagent. Peptides were cleaved from the resin using HF anhydrous, purified by RP-HPLC and characterized by mass spectroscopy, amino acid analysis and capillary electrophoresis. In the bioassays experiments the sporozoites showed nuclear fluorescence, indicative of cell damage, after 60 minutes incubation with cyclic analogues VC-5, VC-17 and VC-19, which present the introduction of the side-chain to side-chain bridging element in the N-terminal portion, and with the linear peptides VC-12, VC-26 and VC-28, that present the insertion of Asp and Lys residues in the C-terminal portion. These results suggest that the position of the lactam bridge in the sequence is important for the association of the molecule with the sporozoite membrane; and that biological effect could be increase with the addition of charged residues. This kind of approach may offer the basis for development the new drugs for malaria prevention and chemotherapy.

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P266. Abstract number: 443

CAL-selective PDZ Inhibitors to Treat Cystic Fibrosis: A SPOT-Synthesis Based Study

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The cystic fibrosis transmembrane conductance regulator (CFTR) is an epithelial chloride channel mutated in patients with cystic fibrosis. Its expression and functional interactions in the apical membrane are regulated by several PDZ domain containing (PSD-95, disc large, zonula occludens 1) proteins. In particular, the CFTR-associated ligand (CAL) limits cell-surface expression of the most common disease-associated mutant ΔF508-CFTR by lysosome targeting [1, 2] whereas the Na+/H+

Exchanger-3 Regulatory Factors 1 and 2 (NHERF1, 2) increase CFTR-mediated chloride efflux [3]. We are looking for a molecular inhibitor of the ΔF508-CFTR/CAL interaction, which would significantly increase the plasma membrane lifetime of ΔF508-CFTR and therefore reduce the pathogenicity of Cystic Fibrosis. To be most effective, such a bioactive peptide should competitively bind the CAL-PDZ domain binding pocket without interfering with the favourable interactions between the ΔF508-CFTR and NHERF1 and NHERF2, respectively. Starting from a library of 6223 peptides with free C-termini synthesized by our modified SPOT synthesis, we found new CAL ligands which indeed have higher binding affinities as the wt-CFTR. By combining different peptide libraries (e.g. combinatorial libraries, substitutional analyses, profile libraries), we could further optimize the CAL ligands to CAL specific inhibitors, which are able to increase Cl--efflux on the plasma membrane. In vitro pull-down assays and mass spectrometry analysis indicate this peptide inhibitors are potent and selective for endogenous CAL. Finally, the validations of our experiments were performed by means of fluorescence polarisation assays (Kd-/Ki-measurements). The received results lead to the assumption that we are heading in the right direction towards reducing the pathogenicity of Cystic Fibrosis by a peptide based pharmacological approach.

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P267. bstract number: 446

Development of peptide immunogens for anti-HCV candidate vaccine

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Hepatitis C virus displays a high genetic and antigenic variability thus presenting a great challenge for a vaccine elaboration. A joint application of bioinformatics and proteomics approaches and chemical synthetic methods has been required for the development of synthetic peptide immunogens for a candidate vaccine against hepatitis C. Data mining regarding sequences of hepatitis C virus (HCV) envelope proteins, analysis of their primary structures allowed us to reveal several conserved sites that could be used as antigenic determinants able to raise isolate-non-specific antibodies. Though these conserved sites were shown to possess a weak immunogenicity as parts of full-size HCV envelope proteins, their immunogenicity was improved by linking to a T helper epitope. Sites with predicted overlapped T helper epitopes specific towards different human II class Major Histocompatibility Complex allele variants were revealed in HCV envelope proteins. Six artificial peptide constructs each composed of one putative B-epitope and one predicted T helper epitope linked with a short spacer were designed and synthesized. These peptide constructs demonstrated rather high immunogenicity in laboratory animals and the ability to raise antibodies reactive with HCV envelope protein E2 and E1E2 heterodimer. The peptide constructs were shown to form multimers in buffer solutions thus improving the stability of peptide preparations and enhancing their immunogenicity.

P268. Abstract number: 465

Targeted platinum peptide complexes holding diamino-dicarboxylic coordination modes

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Applying of biologically active carrier ligands into platinated drug moieties generates a significant challenge in anticancer chemotherapy. Such outcome offers presented in the past [1, 2] potential chance for selective delivery systems and targets presumed cancer pain reduction. Deliberatelly pointed overexpression of opioid receptors at cancer cells [3] traces an address for pharmacophore carrier ligand *ipso facto* introduces potential selective activity. Therefore previously proposed combining of opioid pharmacophore with platinum ion chelating units presumably creates a promising bifunctional tool maintaining anticancer and antinociceptive properties.

Implementing verified conjugating chains, combining both fragments, may modulate discovered activity aspects. Proposed modification is expected to retain drug affinity to opioid receptors however may change cytotoxic properties of proposed bifunctional molecules consisiting of two fragments described above.

Hereby we present parallel systems bearing hybride molecules with contrary (lipophilic or hydrophilic) linkers combining pharmacophore fragment with inorganic moiety of ligand chelating platinum(II) ion. Proposed modification is expected to influence drug properties and activity. Additionally coordination mode through dicarboxylic acid's moieties should contribute to increased solubility in water systems and is believed to affect lability of stable diamino-platinum unit in the designed bifunctional structures.

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P269. Abstract number: 484

Tachykinin receptors' ligands as platinum ion carriers for anticancer therapy

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Tachykinins belong to the family of neuropeptides and are characterized by a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH2, where X is lipophilic amino acid either an aromatic or an aliphatic. Substance P, neurokinin A, neurokinin B and hemokinin are examples of these peptides. They interact with specific membrane proteins, belonging to the family of G protein-coupled cell membrane receptors. Tachykinins are involved in broad spectrum of normal neuromodulatory functions as well as pathological cancer self-activation. Additionally substance P responsibles for transmission of pain information in the central nervous system. Therefore, tachykinin receptors' antagonists are promising, therapeutically relevant agents.

In reference to the fact of tachykinin receptors' overexpression in tumor cells, applying of these compounds may contribute to improved drug selectivity, the same decrease toxicity.
Our aim is to synthesize platinum (II) complexes with analogues of substance P receptors' antagonists as an address part of our molecule. Such compounds could serve as carriers for platinum ions. The general molecule consists of pharmacophore for substance P receptors and fragment responsible for platinum ion complexation.

P270. Abstract number: 492

Preclinical pharmacokinetics of myrcludex B, a novel entry inhibitor for the treatment of HBV infections

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The currently approved therapies for chronic Hepatitis B either block reverse transcription of the HBV-pregenomic RNA in infected hepatocytes (reverse transcriptase inhibitors) or stimulate the immune system by IFN□/pegIFN□. Both strategies show limitations as they are non-curative and in the case of nucleoside analoga the application provoke the development of drug-resistant virus strains. We have previously shown that HBV large envelope protein-derived lipopetides efficiently block HBV entry into hepatocytes in vitro and in vivo. As these peptides address a cellular substructure they show promise to inhibit the infection by a different pathway. Myrcludex B, the lead substance, is a first-in-class HBV entry inhibitor currently progressing to clinical application.
For pharmacokinetic studies Myrcludex B was radioactively labeled and the organ distribution in mice was investigated. The results obtained reveal a rapid and exclusive distribution of Myrcludex B and related preS lipopeptides to the liver. Mutational analyses showed that both, myristoylation and a conserved seven amino acid sequence motif are crucial for the exclusive liver accumulation. The process is highly specific and differs from constitutive hepatic delivery via the blood, since single amino acid exchanges within the conserved motif resulted in a total loss of the specificity.
In vivo studies in rats and dogs again demonstrated the exclusive liver targeting, obviously encompassing a species-independent determinant of hepatotropism. Surprisingly, cynomolgus monkeys did not show any preference of the radiolabelled peptide for the liver. It was uniformly distributed and rapidly renally excreted. In correlation, in vitro experiments confirmed highly specific binding of Myrcludex B to mouse, rat, dog and human hepatocytes whereas cynomolgous and rhesus monkey hepatocytes did not show any specificity. As human hepatocytes show a strong binding of Myrcludex B and because of the coherence of in vivo and in vitro data we assume a rapid and exclusive accumulation of Myrcludex B to the human liver both after intravenous and subcutaneous injection.
Myrcludex B is a new antiviral drug that targets and inactivates the HBV-preS1-specific receptor. Its high potency to block a HBV infection combined with its excellent pharmacokinetic properties and its low toxicity makes it a promising therapeutic option for acute and chronic HBV infections.

P271. Abstract number: 493

DhHP-6 protects H9c2 cells cardiomyocyte against oxidative injury by up regulating SIRT1 expression and deacetylase activity

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SIRT1, an NAD+-dependent (nicotinamide adenine dinucleotide) deacetylase, can protect cells from stress-induced apoptosis and delay aging in lower eukaryotes. DhHP-6 (Deuterohaemin-AlaHisThrValGluLys) is a porphyrin-peptide designed and synthesized in our laboratory. DhHP-6 has been demonstrated to exert anti-cataract activity in vitro and significant cardio protective effects against myocardial ischemia reperfusion injury in rats previously. Here, we investigate the protective effects and potential molecular mechanism of DhHP-6 on cardiomyocytes against oxidative injury. We found that the mRNA expression level of *sirt1* was much higher in treatment groups by real time RT-PCR. Western blot analysis and deacetylase activity test showed that SIRT1 expression and deacetylase activity was higher in DhHP-6 treatment groups than in control groups. P53 is a very important nature substrate of SIRT1, and Western blot analysis showed that p53 acetylating was decreased by DhHP-6 treated. It indicates that DhHP-6 treatment can increase SIRT1 deacetylase activity in H9c2 cells cardiomyocyte. DhHP-6 can not protect H9c2 cells cardiomyocyte against oxidative injury anymore when NAM (nicotinamide, SIRT1 inhibitor) was added, showing that this protection by DhHP-6 was related with the activity of SIRT1 protein. In summary, these data indicate that DhHP-6 protect H9c2 cells cardiomyocyte against oxidative injury by up regulating SIRT1 expression and deacetylase activity.

P272. Abstract number: 521

Synthesis of a PEG conjugated HIV Gp41 MPER fragment: a new Gp41 helix bundle mimic

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Human Immunodeficiency Virus (HIV), responsible for acquired immunodeficiency syndrome (AIDS), perform cell entry via a molecular mechanism involving viral Env glycoproteins with the final fusion mediated by the surface glycoproteins Gp41. The global architecture of Gp41 consists of a transmembrane (TM) region separating the cytoplasmic domain from the ectodomain enclosing the membrane-proximal external regions (MPER) directly involved in the virus cell fusion. In particular the fusion mechanism occurs through conformational changes of three Gp41 with the interaction of three Gp41 N-terminal helices forming a highly stable six helix bundle and the MPER effecting the events that occur after merging of the viral and cell membrane.
Peptides deriving from MPER in the ectodomain of TM glycoprotein are able to prevent HIV cell membrane fusion [1], and one of these, formerly peptide T-20, is clinically used as anti HIV fusion inhibitors [2]. It was recently shown that polyethylene glycol (PEG) conjugated dimeric and trimeric fusion inhibitors, selected from a combinatorial peptide database, to mimic the Gp41 trimeric helix bundle, were characterized by a significant increased in antiviral potency [3].

We recently synthesized and tested for antiviral activity, EPK249 a Trp rich peptide corresponding to residues 666-681 of Gp41 MPER. EPK249 displayed weak in vitro antiviral activity. Here we present the synthesis of PEG conjugated dimers and trimers of EPK249 designed in the attempt to achieve, according to the previous reported strategy, an improvement in the antiviral, fusion inhibitory activity.

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P273. Abstract number: 526

Chemical modification of natural immunomodulators tuftsin and muramyl dipeptide significantly influence their biological activity

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Tuftsin and muramyl dipeptide (MDP) are well known particles displaying various immunomodulatory properities. Although those compounds are known since 1970s, they are still among the most interesting elements exerting influence on the activity of immune system. Many different analogues of both immunomodulators have been already designed, synthesized and examined in terms of their biological activity. Due to all those analysis it was possible to confirm that even small changes in the chemical structure can significantly influence immunostimulatory properties of synthesized analogues of natural biologically active compounds.

For many years we have been interested in analyzing analogues of tuftsin and MDP as potent immunostimulators with possible antibacterial properties. The newest group of synthesized compounds are conjugates of MDP or it's less toxic analogue - nor-MDP with either tuftsin or retro-tuftsin.

The aim of this study was to asses the influence of the synthesized compounds on the viability of white blood cells: either heterogenous population of peripheral blood mononuclear cells or peripheral blood leukocytes and isolated monocytes. The next step was to asses the observed influence on immune cells by determining the cytokine profile stimulated by the examined compounds. We analyzed three cytokines: two proinflammatory - interleukin 6 and tumor necrosis factor alpha, as well as anti-inflammatory -interleukin 10.

The preliminary viability studies show, that conjugates with retro-tuftsin do not influence the viability of white blood cells in comparison to unstimulated cells. Moreover, those conjugates express a tendency to slightly increase percentage of viable cells in tissue cultures. Interestingly, the highest concentration of all examined compounds - 1mg/ml - is the most cytotoxic one, as the viability of blood cells incubated with this concentration was visibly decreased.

We assume that the analysis of the cytokine profile will confirm our assumption that examined conjugates of tuftsin and MDP are capable of activating antibacterial mechanisms by switching on Th1 immune response.

The above mentioned analysis of new immunomodulating molecules is extremely important in a continuing pursuit for potent antibacterial compound with potential usefulness in treatment of severe bacterial infections.

P274. Abstract number: 528

D-Amino Acids Containing Temporin L Analogues

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Temporins are antimicrobial peptides (AMP'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 two members of this AMP family Temporin L and Temporin A [1,2]. At the same time, we developed new analogues of these peptides, among which Pro3TL (FVPWFSKFLGRILNH2) exhibiting a higher antimicrobial activity and a lower hemolytic activity than the native peptide TL. New analogues of Pro3TL, where C-terminal residues were replaced one-by-one by D-amino acid, are now tested and analysed by NMR techniques.

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P275. Abstract number: 529

A novel tetrabranched antimicrobial peptide that neutralizes bacterial lipopolysaccharide and prevents septic shock in vivo.

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A novel synthetic antimicrobial peptide selective for Gram-negative bacteria is described. The peptide (KKIRVRLSA; M33) was obtained by the selection of a large phage library and sequence optimization by rational aminoacid modification. These improvements allow to produce a very stable peptide with high antibacterial activity for Gram-negative bacteria.

M33 was synthesized in the branched Multiple Antigen Peptide form, that we had previously demonstrated to induce general peptide resistance to proteolysis, making this kind of molecules very suitable for in vivo use. Branched M33 resulted markedly selective for Gram-negative bacteria showing low Minimal Inhibitory Concentrations for multi-drug resistant clinical isolates of Pseudomonas aeruginosa, Klebsiella pneumoniae, Acitenobacter baumannii and other bacteria. We demonstrated that M33 shows a poor toxicity for eukaryotic cells, it binds LPS and DNA and it does not produce appreciable haemolysis even upon prolonged incubation. We also evaluated M33 toxicity in vivo and we demonstrated that it is not immunogenic upon repeated injections in animals.

We also report that M33 is able to neutralize LPS derived by Pseudomonas aeruginosa and Klebsiella pneumoniae preventing TNF-alpha release from LPS activated macrophages. This is of great interest because main sepsis symptoms are triggered by LPS release from lysated Gram-negative bacteria. In this respect, peptides

which are not only bactericidal, but also effectively neutralize LPS, are of considerable importance in combating sepsis.
We also describe results on tetra-branched M33 in vivo activity. The peptide resulted capable to avert septic shock symptoms, and consequently death, in animals infected with reference strains and multi-drug resistant clinical isolates of E. coli and Pseudomonas aeruginosa, when administered in doses comparable to traditional antibiotics and compatible to a clinical use.
M33 peptide is currently under investigation for the cure of severest symptoms due to Pseudomonas lung infections in Cystic Fibrosis.
Pini et al., Antimicrob Agents Chemother, 2005
Pini et al., J Pept Sci, 2007
Falciani et al., Chem Biol Drug Des, 2007
Pini et al., FASEB J, 2010

P276. Abstract number: 531

Conjugates of tuftsin and muramyl dipeptide as stimulators of monocyte-derived dendritic cells

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Dendritic cells, as professional APC (antigen presenting cells) play a role of a linkage between innate and adaptive immune response. Those cells have been widely and extensively studied as a potential tools in therapy of several severe diseases. They also seem promising in solid organ transplantology as cells able to delay or even prevent transplant rejection. But before those cells are used in above mentioned fields of medicine, they need to be properly stimulated to exert the desired properties. There are many studies trying to determine the stimulators of dendritic cells, among them there are natural immunomodulators. It has been already proven that muramyl dipeptide and its analogues can accelerate maturation and activation of dendritic cells. But we were curious whether conjugates of muramyl dipeptide and tuftsin synthesized in our laboratory can be more powerful in modulating activity of that APC population.
To acquire more knowledge about the influence of the conjugates of muramyl dipeptide and tuftsin on the reactivity of dendritic cells, we expanded those cells from human isolated monocytes by tissue culture supplemented with GM-CSF and IL-4. The obtained immature dendritic cells were then stimulated with the examined compounds and their level of maturation and activation was assessed by flow cytometry the next day. We compared all our results to a known dendritic cell stimulator - lipopolysaccharide.
Our preliminary results show, that muramyl dipeptide is comparable in terms of activating dendritic cells, to strong stimulator - LPS. Tuftsin proves to be much less active then both muramyl dipeptide and lipopolysaccharide. The results obtained for tuftsin are extremely important, as no one before has published any observation of dendritc cells stimulated with this tetrapeptide. The other examined compounds, the conjugates of muramyl dipeptide and tuftsin, seem to keep balance between the activity of both native immunomodulators. The most promising compound are to be examined more extensively in nearest future, probably both in vitro and in vivo.

P277. Abstract number: 534

Synthesis Of Peptide Analogs Of The A2 Subunit (Sequence 558-565) Of The Factor FVIIIa Of Blood Coagulation

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It is known that platelets aggregation causes clotting in the blood vessels during the blood circulation due to several reasons. The clots formation is prevented by using anticoagulant drugs. On the other hand the coagulation of blood is important for the precaution of an organism from bleedings and takes place through a process of thrombin production. Factor VIII (FVIII) is a key component of the fluid phase of the blood coagulation and is comprised of a heavy (A1-A2-B) and a light (A3-C1-C2) peptide chain, which are efficiently cleaved by proteases at three sites, two within the heavy and one within the light chain resulting in alteration of its covalent structure and conformation. This proteolytic cleavage is caused by thrombin or by factor Xa. Thrombin production is depended on FIXa, which plays a crucial role in curtailing of thrombin generation and accordingly on the additional activation of platelets. Peptides which are expected to inhibit selectively the maximization of thrombin production are based on the regions in which the FVIII interacts with the FIX. These both factors are essential for normal coagulation and deficiency of either is associated with the bleeding diathesis. Based on the acceptance that the sequence 558-565 of the A2 subunit domain of FVIIIa interacts with FIXa, our research efforts focus on the synthesis of linear and cyclic head to tail peptides and peptidomimetics, analogs of this sequence, aiming at the inhibition of interaction between FVIIIa and FIXa, in order to suspend the platelets adhesion and furthermore the thrombin production.
The synthesized peptide analogs are investigated for their inhibitory activity and tested for clotting deficiency by measuring their activated partial thromboplastin time (APTT) in seconds and the reduction of the % value of the FVIIIa that they generate in samples containing recombinant FVIIIa, *in vitro*.
Ser558-Val559-Asp560-Gln561-Arg562-Gly563-Asn564-Gln565-OH (10.7)
Ser^{558}-Val^{559}-Asp^{560}-Gln^{561}-Arg^{562}-Gly^{563}-Asn^{564}-Gln^{565}-NH2 (15.7)
Ser^{558}-Val^{559}-Asp^{560}-Gln^{561}-Arg^{562}-Gly^{563}-Asp^{564}-Gln^{565}-OH (12.2)
Ser^{558}-Val^{559}-Asp^{560}-Gln^{561}-Arg^{562}-NPhe^{563}-Asn^{564}-Gln^{565}-OH (5.4)
Cyclo[-Ser^{558}-Val^{559}-Asp^{560}-Gln^{561}-Arg^{562}-Gly^{563}-Asn^{564}-Gln^{565}] (10.8)
Gln^{561}-Arg^{562}-NPhe^{563}-Asn^{564}-Gln^{565}-OH (7.3)
Arg^{562}-NPhe^{563}-Asn^{564}-Gln^{565}-OH (13.1)
NPhe^{563}-Asn^{564}-Gln^{565}-OH (5.5)

P278. Abstract number: 540

Synthesis and biological evaluation of novel peripherally active morphiceptin analogs

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Morphiceptin (Tyr-Pro-Phe-Pro-NH_2), a tetrapeptide present in the enzymatic digest of bovine β-casein, is a selective ligand of the μ-opioid receptor. In the present study, we describe the synthesis of a series of novel morphiceptin analogs modified in positions 1, 2 and 3. Two

of the obtained analogs, [Dmt[1], D-Ala[2], D-1-Nal[3]]morphiceptin and [Dmt[1], D-NMeAla[2], D-1-Nal[3]]morphiceptin (Dmt - 2',6'-dimethyltyrosine and D-1-Nal - 3-(1-naphthyl)-D-alanine) displayed very high μ-receptor affinity, resistance to enzymatic degradation, and remarkable supraspinally-mediated analgesia, as shown in the hot-plate test after intracerebroventricular but not intravenous administration, which indicated that they could not cross the blood-brain barrier. Therefore, these two analogs were further tested in vitro and in vivo towards their possible peripheral analgesic activity and inhibitory effect on gastrointestinal (GI) motility. We report that both peptides showed strong antinociceptive effect in the writhing test after intraperitoneal administration, inhibited smooth muscle contractility in vitro and GI motility in vivo. Taken together, these findings indicate that the novel morphiceptin analogs which induce peripheral, but not central antinociception, inhibit GI transit, and possess exceptional metabolic stability, may provide an interesting approach to the development of peripherally-restricted agents for the treatment of GI motility disorders, such as diarrhea or diarrhea-predominant irritable bowel syndrome (IBS-D). To date, no therapeutic agent was shown to be consistently effective in the treatment of IBS-D and the majority is directed mainly at the relief of individual symptoms, which include altered intestinal motility and visceral hypersensitivity. Hence a need for new treatment options for this highly prevalent disease.

P279. Abstract number: 541

Peptides versus Nonpeptides as Therapeutics: An Exciting Challenge for Big Pharma

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Peptides were abandoned by Big Pharma in favor of small molecule approaches to drug discovery, almost two decades ago. During this period, the peptide field has been under constant siege by the illusionary promise of non-peptides: highly touted by Big Pharma and endorsed by non-expert opinion makers in countless derivative reviews in clinical journals, claiming that non-peptides are superior to peptides as therapeutic agents; thereby obscuring, in Board Rooms, Study Sections and Grant Review Panels alike, the exciting re-emergence of peptides as therapeutic agents. The need for the peptide community to finally set the record straight and to dispel, for once and for all, the myth of the superiority of non-peptides over peptides, (which may had some merit twenty years ago but clearly is no longer tenable), has been addressed, with examples from the vasopressin/oxytocin field, in a recent issue of the EPS Newsletter (1). In this presentation, the authors will illustrate the superiority of peptides over non-peptides as therapeutic agents and as pharmacological tools, by more fully documenting the failures of non-peptides in the clinic during the past two decades, while up-dating the striking advances in the development of peptides as therapeutic agents (2,3). These developments represent an exciting opportunity for Big Pharma; to once again embrace the challenge offered by the enormous, emerging therapeutic potential of peptides. Quo vadis now Big Pharma?

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P280. Abstract number: 581

Synthesis and Bioanalysis of Insulin Analogs with Altered Secondary Structure

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Insulin is a hormone of central importance in physiological management of blood glucose. It is the primary therapeutic agent in managing Type1 diabetes, and it is also commonly used in treatment of Type2 diabetes. The central α-helix of the insulin B-chain and the two smaller A-chain helixes are crucial for the structural stability of the hormone and its biological activity. Previous studies have investigated numerous amino acid changes with a particular emphasis on natural amino acids. The reported results while informative are highly restrictive in the depth and breath of secondary structural change explored given the limited structural diversity of natural amino acids. However, these analogs are easier to obtain since the vast majority can be prepared by rDNA methods where the native conformation is directed by biosynthesis of the native three disulfides. We report here results of our synthesis and bioanalisis of novel synthetic insulin analogs. Insulin analogs with select helix stabilizing elements such as substitution with an Aib residue, salt bridge insertion and backbone stapling were synthesized via a 1+2 chain combination procedure. In the first step partially protected B-chain (B19Cys-Acm) is treated with DNTP to form the Npys-activated derivative. The chain combination is achieved by reacting the activated B-chain with selectively protected A-chain derivatives (A6,11,20Cys-Acm). The insulin analog is obtained by iodine-mediated oxidative deprotective-folding, in concentrated acetic acid. Sequence dependent, synthetic difficulties were encountered at various synthetic steps and most notably for macrocyclized analogs. Most of the insulin analogs demonstrated increased helical content. No clear, consistent correlation between bioactivity, biostability and secondary structure was observed.

P281. Abstract number: 16

Structural and Functional Analyses of Repeating Resion of Apidaecin

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Drug resistance is a major obstacle to successful antibacterial chemotherapy. With the emergence of antimicrobial-resistant bacterial strains, the current drug families start to fail, and there is an urgent need for alternative agents, preferably with novel modes of action that will prevent bacteria from mounting a quick response and building resistance. Small cationic antimicrobial peptides (AMPs) are evolutionarily ancient components of the host defense system of many different unicellular and pluricellular organisms, from bacteria to plants, insects, fish, amphibians, birds, and mammals, including humans. Apidaecins are the largest group of proline-rich antimicrobials known and major humoral components induced in honeybee lymph upon bacterial infection. They were the first to be studied in detail with respect to the mechanism and the identity of the amino acid residues responsible for the antibacterial action. The pharmacophore delivery unit architecture has been proposed to be a general feature of the proline-rich antibacterial peptide family. However, despite a wealth of information on the amino acid residues important for function, little is known about the pharmacophore delivery unit architecture of Apidaecin. So, in this research, full sequence of apidaecin and fragments that were gradually removed from N-terminal to C-terminal were synthesized and studied their growth inhibitory ability against microbial organism in order to identify the functional region and secondary structure. The results obtained from the fragments PR-1, PR-2 and PR-3, synthesized based on the repeating region in apidaecin (Pro-Arg-Pro, Pro-His-Pro) show that the PR-2 (trimer of the repeating region) has similar antibacterial activity like the full sequence of apidaecin. Moreover, PR-2 and PR-3 show more potent activity even in other gram-negative bacteria like P.aeruginosa and P.vulgaris.

P282. Abstract number: 22

Fluctuations and the rate-limiting step of peptide-induced membrane leakage

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Peptide-induced vesicle leakage is a common experimental test for the membrane-perturbing activity of antimicrobial peptides. The leakage kinetics is usually very slow, requiring minutes to hours for complete release of vesicle contents, and exhibits a biphasic behavior. We report here that, in the case of the peptaibol trichogin GA IV, all processes involved in peptide-membrane interaction, such as peptide-membrane association, peptide aggregation, and peptide translocation, take place in a time-scale much shorter than the leakage kinetics. On these bases, we propose a stochastic model in which the leakage kinetics is determined by the discrete nature of a vesicle suspension: peptides are continuously exchanging among vesicles, producing significant fluctuations over time in the number of peptide molecules bound to each vesicle, and in the formation of pores. According to this model, the fast initial leakage is caused by those vesicles which, after the random distribution of peptides among liposomes, already contain at least one pore, while the slower release is associated to the time needed in an intact vesicle to occasionally reach the critical number of bound peptides necessary for pore formation. Fluctuations due to peptide exchange among vesicles represent therefore the rate-limiting step of such a slow mechanism.

P283. Abstract number: 23

Effect of helix kink on the activity and selectivity of an antimicrobial peptide

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Antimicrobial peptides (AMPs) are small molecules (usually less than 40 residues long) with strong bactericidal activity, linked to their ability to perturb the permeability of bacterial cells. For this reason, these peptides are investigated as lead compounds for the development of a new class of antibiotic drugs, to fight the insurgence of drug-resistant bacteria. AMPs often show an amphiphilic composition and a cationic character. From the structural viewpoint the most frequent conformation (after membrane-association) isα-helical Moreover, many helical AMPs have a kink or a hinge in the middle of their structure, caused by Pro or Gly residues. In order to understand the role of this kink, and its relevance to peptide activity and selectivity, we designed a series of analogues of the amphipathic, helical and cationic AMP P5 (KWKKLLKKPLLKKLLKKL), in which the central Pro residue was moved from its central position, or removed altogether. Displacement of the Pro residue resulted in a dramatic increase in the toxicity against erythrocytes, with the most toxic peptide being the analogue lacking the Pro residue (analogue P5F). Circular dichroism experiments and molecular dynamics simulations indicate that both P5 and P5F are helical when associated to lipid bilayers. By contrast, in water the fraction of helical structure is significantly reduced for P5, while P5F maintains its helical conformation. Fluorescence experiments showed that the kinked P5 peptide, which exhibits the highest selectivity for bacterial cells, has a dramatically higher affinity for negatively charged vesicles (mimicking the composition of bacterial membranes) than for neutral liposomes (which are similar to mammalian cells). On the other hand, analogue P5F exhibits comparable affinities for anionic and neutral membranes. HPLC retention times and theoretical calculations indicate that in water the helix-breaking Pro residue allows P5 to attain a closed conformation, in which its hydrophobic residues are partially shielded from the solvent. This property might explain its low affinity towards neutral bilayers, since in this case the hydrophobic effect is the main driving force of peptide-membrane association. The observed differences in the biological and biophysical properties of the two analogues highlight the role of the central Pro-induced kink in the selectivity of AMPs, and provide hints for the design of new, highly selective compounds.

P284. Abstract number: 24

Membrane insertion of para-cyanophenylalanine-labeled alamethicin analogues.

Correlation of fluorescence and infrared absorption data

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Different classes of peptides, such as antimicrobial, cell penetrating and fusogenic peptides, exert their bioactivities by interacting with cellular membranes. Therefore, the determination of their location and orientation inside a lipid bilayer is a fundamental step in the characterization of their mechanism of action. In this respect, the α-amino acid analogue *para*-cyanophenylalanine (*p*CNPhe) is a very promising probe, since it can be employed both in fluorescence and in IR absorption experiments. Its fluorescence quantum yield is 0.11 (as compared to 0.025 for Phe). The stretching vibrational transition of the cyano group is located at around 2230 cm^{-1} (i.e. in a convenient spectral window) and is sensitive to the medium polarity. In this study, we exploited the peculiar properties of *p*CNPhe to investigate the membrane interaction of the [Glu(OMe)^{7,18,19}] alamethicin analogue by synthesizing three peptides in which Ala4, Val9 or Val15 were substituted by *p*CNPhe. Liposome leakage kinetics data indicate that the label does not perturb significantly the peptide activity. Furthermore, *p*CNPhe fluorescence is sensitive to the fluorophore environment, allowing a characterization of peptide aggregation and water-membrane partition. The position of the fluorophore in the membrane was determined by fluorescence, depth-dependent quenching experiments, performed as a function of the peptide to lipid ratio. Correlation of these data with the IR absorption spectrum of the cyano group allowed a determination of its dependence on the depth of insertion in the bilayer, while polarized ATR-FTIR experiments provided indications on peptide orientation in the membrane. Overall, these data offer a picture of alamethicin insertion in the membrane and confirm that *p*CNPhe is an extremely useful probe in fluorescence and IR absorption studies of peptide-membrane interactions.

P285. Abstract number: 39

Cell-penetrating Peptides as Adenovirus Vector Carrier

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A Pro-rich peptide derivative, acetyl-Val-Arg-Leu-Pro-Pro-Pro-Val-Arg-Leu-Pro-Pro-Pro-Val-Arg-Leu-Pro-Pro-Pro-Gly-Cys amide, and an octaarginine derivative, acetyl-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Gly-Cys amide, were prepared. Each peptide was coupled to the heterobifunctional cross-linking reagent, 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester, and then conjugated with Adenovirus vector. The both conjugates exhibited gene transfer efficiency in CAR-negative cells (B16BL6).

P286. Abstract number: 40

Jelleine-I analogues with increased antibacterial activity

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Jelleine-I is a octapeptide amide, H-PFKLSLHL-NH_2 isolated from Royal Jelly of honeybees (*Apis mellifera*) [1]. The peptide is active against Gram-positive and Gram-negative bacteria. However, Jelleine-I is also hemolytic towards human erythrocytes.In the present paper, we report the synthesis and antibacterial activity of 19 analogues of Jellleine I including an Ala-scan and single residue substitutions.The analogues were synthesized by Fmoc SPPS, purified by preparative HPLC and characterized by MALDI-TOF-MS. A stock peptide solution in 1% DMSO was prepared and the concentration was determined by amino acid analysis. The Jelleine-I analogues were tested for antibacterial activity against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Furthermore, the cytotoxity towards red blod cells was evaluated. Alanine positional scanning showed restrictions on 6 out of the 8 residues, and a moderate drop in MIC-activity for the [Ala^1] and [Ala^5] Jelleine-I analogues.Based on the above results, two series of analogues were designed and tested. Pro^1 was replaced with either Ala or 3-(2 naphthyl)-L-alanine and Ser^5 was replaced with the more hydrophobic amino acids Val, Leu, Ile, Trp and Phe. Our most promising candidate was [Ala^1, Ile^5] Jelleine-I which displayed improved MIC-activities towards *S. aureus* and *E. coli* (1.56 µM both strains) as compared with Jelleine-I, which showed 6.125-12.5 µM and 1.25 µM against *S. aureus* and *E. coli*, respectively. Furthermore, [Ala^1, Ile^5] Jelleine-I showed no haemolytic activity.Reference:1. R. Fontana et al. (2004) Peptides, 25, 919-28.

P287. Abstract number: 45

The designer antibacterial peptide A3-APO is the last resort in mouse models of multidrug-resistant Gram-negative nosocomial infections

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Multidrug-resistant (MDR) Gram-negative bacteria causing nosocomial infections are nearly as common in hospitals as methicillin-resistant Staphylococcus aureus strains. Especially problematic are Acinetobacter baumannii and Klebsiella pneumoniae strains unaffected even by carbapenems, the last therapeutic resort. For example, between 2005 and 2007, resistance to imipenem (a carbapenem-type antibiotic) increased by 33% in military hospitals. Equally disturbing, resistance to the second best agent, the quite toxic yet powerful cyclic peptide antibiotic colistin, similarly increased.

Antimicrobial peptides rarely exhibit activity in animal models of systemic infections, or their therapeutic index is too low for future clinical applications. A3-APO [(Chex-Arg-Pro-Asp-Lys-Pro-Arg-Pro-Tyr-Leu-Pro-Arg-Pro-Arg-Pro-Pro-Arg-Pro-Val-Arg)2-Dab] is a representative of the designer proline-rich antibacterial peptide family that shows in vitro activity against MDR Enterobacteriaceae alone or in combination with conventional antibiotics. A3-APO was inactive when added subcutaneously to mice infected with a beta-lactamase producing Escherichia coli strain, but upon intraperitoneal (ip) administration at 3x10 mg/kg exhibited survival and bacterial count reduction figures comparable to imipenem added at 3x40 mg/kg. However, the low 50 mg/kg ip lethal dose limits its utility as a clinically viable antimicrobial agent.

Nevertheless with intramuscular (im) administration, only low levels of systemic toxicity could be observed at a 75 mg/kg bolus dose as opposed to colistin which was toxic already at 25 mg/kg. When administered either intravenously at 3x2.5 mg/kg or im at 2-3x5 mg/kg to mice systemically infected with a carbapenem-resistant A. baumannii strain, peptide A3-APO reduced the bacterial counts by at least two log10 units and increased the survival rate compared to untreated animals or mice treated 2-3x with 40 mg/kg imipenem. Even more impressively, in an A. baumannii burn infection model representing the real-life situation during hospitalizations following combat injuries, A3-APO at 5 mg/kg given once reduced the bacterial counts in the wounds at day 5 by a factor of 18,400 (untreated), 600 (imipenem) or 60 (colistin) relative to other treatment forms. Four consecutive daily doses of 5 mg/kg A3-APO im sterilized the blood of all mice studied.

P288. Abstract number: 47

Antimicrobial activity of small 3-(2-naphthyl)-L-alanine containing peptides

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Antibiotic-resistant pathogens have become a very serious problem world-wide. Most alarming is the increasing occurrence of vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, infections due to resistant fungi such as *Candida albicans* and *Cryptococcus neoformans* are a major concern. Antimicrobial peptides (AMPs) are a part of the innate immunity in all multicellular organisms and are promising lead structures for developing new antimicrobial agents. However, AMPs often display undesirable pharmaceutical properties, such as susceptibility to proteolytic breakdown. This may be circumvented by incorporation of non-natural building blocks into the peptide, such as 3-(2-naphthyl)-L-alanine. In the present study, we investigated the antimicrobial activities of 6 peptides containing 3-(2-naphthyl)-L-alanine: H-XFXLKKK-NH_2 (**1**), H-FXLKKK-NH_2 (**2**), H-XFLKKK-NH_2 (**3**), H-XKFKXKLKK-NH_2 (**4**), H-KFKXKLKK-NH_2 (**5**), H-XKFKKLKK-NH_2 (**6**), X being the non-proteinogenic amino acid. The analogues were synthesized by Fmoc SPPS, purified by preparative HPLC and characterized by MALDI-TOF-MS. A stock peptide solution in 1% DMSO was prepared and the concentration was determined by amino acid analysis. The peptides were tested against clinically relevant bacteria and fungi.The most active compound, **4**, displayed excellent against activity against the Gram-positive bacteria methicillin-resistant *S. aureus* (3.12 μM); vancomycin intermediate *S. aureus* (6.25μM), vancomycin resistant *E. faecium* VRE (≤1.25 μM), the Gram-negative bacteria *E.coli* (≤1.25 μM), *P. aeruginosa* (3.12 μM), *S. typhimurium* (≤1.25 μM), *K. pneumonia* (25μM) and the fungi amphotericin B resistant *C. albicans* (12.5 μM) and *C. neoformans* (25 μM). However, compound **4**, was also haemolytic towards red blood cells (91%).The results presented here suggest that small 3-(2-naphthyl)-L-alanine containing peptides are promising lead structures for developing future antibacterial agents.

P289. Abstract number: 57

Antimicrobial peptides containing D-amino acids with in vivo activity against plant pathogenic bacteria

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Phytopathogenic bacteria are responsible for a wide range of plant diseases causing large economic losses in fruit and vegetable crops. Their control is mainly based on chemical pesticides, such as copper derivatives and antibiotics. Although these compounds are highly efficient to treat these diseases, many of them are not allowed in Europe due to environmental concerns. In addition, it has been reported the emergence of antibiotic-resistant strains, which limits the effectiveness of these compounds [1]. In recent years, antimicrobial peptides have been described to be effective against plant pathogens. However, their use *in vivo* is limited because of enzymatic degradation problems associated with the presence of plant proteases. One strategy used to protect peptides against enzymatic hydrolysis is the incorporation of D-amino acids in the sequence. It has been described that selective substitutions by D-amino acids not only provide more stable sequences to protease degradation, but also reduce the hemolysis while maintaining the antimicrobial activity.
Up to now, we have identified linear undecapeptides with high *in vitro* activity against plant pathogenic bacteria [2]. The best peptide, KKLFKKILKYL-NH2 (BP100), was also effective *in vivo* to prevent infections of *Erwinia amylovora* in flowers. However, the *in vivo* dose was 20 to 40-fold higher than the MIC. This loss of activity could be attributed to peptide degradation by plant proteases. In the present study, we designed and synthesized thirty-one BP100 analogues containing D-amino acids. These sequences were first evaluated *in vitro* against *E. amylovora*, *Xanthomonas vesicatoria*, and *Pseudomonas syringae*, and assayed for their hemolytic activity and stability to protease degradation [3]. Sequences with a better biological profile than BP100 were identified and were analyzed for their inhibitory activity *in vivo*.
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P290. Abstract number: 77

Inhibitory effects of novel AMP on biofilm forming clinical isolates of Pseudomonas aeruginosa

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Biofilms are microbial communities attached to solid surfaces and enclosed in a self-produced polymeric matrix. Resistance to conventional antibacterial agents is a key feature of biofilm infections, which highlights the necessity for the development of novel compounds able to effectively control them. Accordingly, antimicrobial peptides represent a promising class of molecules with which to combat these pathogens, and among those is the analogue peptide P5, which was designed and synthesized from a hybrid peptide, CA-MA [derived from cecropin A (1-8) and magainin 2 (1-12)]. In the present study, P5 and several conventional antibiotics were applied to biofilm-forming clinical isolates of Pseudomonas aeruginosa for susceptibility testing. All of the strains were found to be resistant to the tested compounds. Nonetheless, P5 inhibited the formation of biofilms at very low concentrations and also showed positive synergy when

applied at its MBIC50 concentration in combination with vancomycin. P5 also inhibited production of such extracellular matrix components as exopolysaccharides, proteins and nucleic acids, as evidenced by epifluorescence microscopic and spectrofluorometric analyses. The results obtained using this multiparametric approach suggest that P5 could potentially be used as an agent for the prevention of biofilm formation by drug-resistant strains.

P291. Abstract number: 78

Antibiotic effect of P5-18mer peptide against antibiotic resistant microorganisms isolated from patients with cholelithiasis

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Pseudomonas aeruginosa has developed resistance against flomoxef sodium, isepamicin and cefpiramide. Therefore, in this study, the antibacterial activity and synergistic effects of the amphipathic-derived P5-18mer antimicrobial peptide were tested against pathogens associated with cholelithiasis that have developed resistance against commonly used antibiotics. The results were then compared with the activities of the amphipathic-derived peptide, P5-18mer, melittin and common antibiotics. Growth inhibition of planktonic bacteria was tested using the National Committee for Clinical Laboratory Standards (NCCLS). The bactericidal activity of the antimicrobial peptides was measured using time-kill curves. Synergistic effects were evaluated by testing the effects of P5-18mer alone and in combination with flomoxef sodium, isepamicin or cefpiramide at 0.5 x MIC. P5-18mer peptide displayed strong activity against pathogens and flomoxef sodium, isepamicin and cefpiramide-resistant bacteria cell lines obtained from a patient with gallstones; however, it did not exert cytotoxicity against the human keratinocyte HaCat cell line. In addition, the results of time-kill curves indicated that P5-18mer peptide exerted bactericidal activity against four strains of P. aeruginosa. Finally, the use of P5-18mer and antibiotics exerted synergistic effects against cell lines that were resistant to commonly used antibiotics. These results indicate that this class of peptides has a rapid microbicidal effect on flomoxef sodium, isepamicin and cefpiramide-resistant strains of P. aeruginosa. Therefore, these peptides may be used as a lead drug for the treatment of acquired pathogens from patients with cholelithiasis who are affected with antibiotic-resistant bacteria.

P292. Abstract number: 79

Synthesis and preliminary conformational analysis of TOAC spin-labeled analogues of the medium-length peptaibiotic tylopeptin B

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The achiral tetrasubstituted α-amino acid 4-amino-1-oxyl-2,2,6,6,-tetramethylpiperidine-4-carboxylic acid (TOAC) has been used as paramagnetic probe to study, by electron spin resonance (ESR), the mode by which peptides insert into membrane. In particular, detailed information about the location, orientation and aggregation of membrane-active peptides in the phospholipid bilayer has been obtained for the TOAC-labeled peptaibiotics alamethicin F50/5 [1,2] and trichogin GA IV [3], in which one or two α-aminoisobutyric acid (Aib) residues in the sequence were replaced by the free radical-containing amino acid. Considering that the detailed mechanism of membrane permeabilization by medium-length peptaibiotics, e. g. tylopeptin B (Ac-Trp-Val-Aib-Aib-Ala-Gln-Ala-Aib-Ser-Aib-Ala-Leu-Aib-Gln-Lol), is largely unknown, we have planned to synthesize a series of analogues in which the TOAC amino acid replaces the Aib residue at one of the three positions (4, 8 or 13) throughout the tylopeptin sequence, and to investigate their interaction with model membranes by a combination of ESR techniques. We have recently shown by a solution conformational analysis performed by the combined use of FTIR absorption, CD, NMR and molecular dynamics calculations that tylopeptin B is largely helical, with a predominance for the α- or 3_{10}-helix type structure, depending upon the nature of the solvent [4]. Here, we present our approach to the solid-phase peptide syntheses of TOAC-tylopeptin analogues. In addition, we report a preliminary solution conformational analysis, performed by FTIR absorption and CD spectroscopy, that shows that the replacement of the Aib residues by TOAC in the three selected positions does not alter the overall molecular conformation of the peptaibiotic.

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P293. Abstract number: 80

Porphyrin-antimicrobial peptide conjugates: synthesis, conformational studies and preliminary light activated biocidal activity

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Photodynamic therapy (PDT) is a very promising approach for killing bacteria [1]. It is well establish that singlet oxygen is produced as the main species responsible for cell death. During PDT multiple cellular targets are damaged and this strongly reduces the probability of developing the resistance phenomena which frequently occur after repeated antibiotic treatments. Porhyrins are commonly used as photosensitizers, that can generate reactive oxygen species upon exposure to light in the presence of oxygen. It is known that Gram-positive bacteria can be efficiently killed by light after incubation with a number of photosensitizers, whereas Gram-negative bacteria are less susceptible to phodynamic killing and only cationic porphyrins can induce their photoinactivation. Short cationic antimicrobial peptides (CAMP) are components of the innate defense of many organisms [2]. Their overall positive charge ensures accumulation at the poly-anionic microbial cell surfaces that contain acidic polymers, such as lipopolysaccharides, and wall-associated teichoic acids in Gram-negative and Gram-positive bacteria, respectively. Beyond the presence of several cationic amino acids, a substantial proportion of hydrophobic residues permit most of CAMP to fold into an amphipathic structure, that allows

them to insert into the phospholipid bilayer. After insertion, antimicrobial peptides act by either disrupting the physical integrity of the membrane or translocate across the membrane and act on internal targets. By conjugating a porphyrin to an antimicrobial peptide we can expect to direct the photosensitizer against specific bacterial targets and increase the efficacy of PDT. Here, we present the synthesis of two conjugates in which a neutral 5(4'-carboxyphenyl)-10,15,20-triphenylporphyrin has been covalently attached to the N-terminal end of apidaecin 1b or of a magainin analogue. The compounds were characterized by HPLC, MS and UV-vis spectroscopy and their conformational preferences were preliminarily investigated by CD. The bactericidal activity of the conjugates against selected Gram-positive and -negative bacteria will be reported and compared to that of the parent peptide and of the photosentizer alone, in the dark or under light activation.

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P294. Abstract number: 84

Design of Helical Antimicrobial Peptides with exceptional Therapeutic Indices against Gram-negative Pathogens

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Due to the rapid increase in bacteria resistance to classical antibiotics, antimicrobial peptides (AMPs) have become important candidates as potential therapeutic agents. In previous studies we introduced the concept of a 'specificity determinant' where substitution of a lysine residue in the center of non-polar face of amphipathic α-helical AMPs dramatically reduced toxicity and increased the therapeutic index [1-4]. We also showed that our L- and D-enantiomeric AMPs had equivalent biological and biophysical properties and their sole target was the bacterial membrane [2]. The excellent stability of the D-enantiomers to proteolysis highlights their potential as clinical therapeutics. In this study, we introduced a second lysine specificity determinant in the center of the non-polar face to further reduce toxicity. We then investigated the role of systematic increases in hydrophobicity and varying the type of hydrophobic side-chain (Leu, Ile, Phe and Trp) had on the biological and biophysical properties of AMPs. All six 26-residue peptides maintained the identical polar face and two specificity determinants (K13 and K16) on the non-polar face. We evaluated these peptide analogs for their antimicrobial activity against eleven clinical isolates of *Acinetobacter baumannii* and six clinical isolates of *Pseudomonas aeruginosa*, hemolytic activity to human red blood cells, structure in aqueous and hydrophobic media, overall hydrophobicity and self-association ability. To our surprise, we obtained a new antimicrobial peptide with outstanding overall properties, excellent antimicrobial activity and a therapeutic index (3,355), 26-fold better than our previous lead peptide.

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P295. Abstract number: 104

Sequences of the Polypeptide Antibiotics (Peptaibiotics) Acretocins

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Screening of filamentous fungi for the production of Aib and Iva (1,2) had revealed that *Acremonium crotocinigenum* (CBS 217.70) was a potent producer of peptides comprehensively named peptaibiotics (3). The mold was cultured submers in a malt extract medium. A peptide mixture named acretocins (ACR) was isolated from the culture broth by XAD and Sephadex LH-20 chromatography. Individual peptides of ACR were fractioned using semi-preparative RP-HPLC and binary gradient elution using mixtures of MeCN/MeOH/water with addition of 0.1% TFA. Individual peptides of ACR were analyzed by direct infusion ESI-MS as well as on-line analytical HPLC-ESI-MS. In a previous report (4) we could not assign a characteristic mass fragment counting for 140 Da. Here we show that it represents the sequence positions Gly^{8}-Acc^{9} in all ACR peptides. Only few peptides are known to contain Acc, but it is also a constituent of peptaibiotics named neoefrapeptins (5). Amino acid analysis of total hydrolysates of ACR and comparison with reference amino acids by GC-MS on Chirasil-Val revealed the presence of Aib, Acc, ß-Ala and Gly as well as L-Leu, L-Pip and D-Iva. No L-Iva was detected in acidic total hydrolysates (6 M HCl, 110 ^{o}C, 24 h) of ACR. Comparison of the MS data from ACR and related efrapeptins indicated that both peptaibiotics have the same *C*-terminal heterocyclic residue PIHPPE. A major sequence is exemplified with ACR-2: Ac-Pip-Aib-Pip-D-Iva-Aib-Leu-β-Ala-Gly-Acc-Aib-Pip-Aib-Gly-Leu-Aib-PIHPPE (Ac, acetyl; Pip, L-pipecolic acid; Aib, α-aminoisobutyric acid; Acc, 1-aminocyclopropane-1-carboxylic acid; PIHPPE, *N*-peptido-1-isobutyl-2-(2,3,4,6,7,8-hexahydro)-1-pyrrolo[1,2-α]**p**yrimidinio)**e**thylamine. The sequences of ACR peptides are distinguished by exchange of Iva/Aib or Gly/Ala.

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P296. Abstract number: 114

Conformational Studies Of Toac-Analogues From New Cytolytic Peptide Isolated From Hypsiboas Albopunctatus

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Antimicrobial peptides have been isolated from several species of microorganisms. This work aimed to evaluate 3 analogues containing TOAC of a new antimicrobial peptide extracted from the skin secretion of the frog *Hypsiboas albopunctatus* called Hylin (*hy* - GWLDVAKKIGKAAFNVAKNFI/L). This sequence has shown identity with ceratotoxins. The synthesis of sequence containing Ile in C-terminus and addition of TOAC in position 0 or substitution of Trp^2 and Ala^{13} allowed studying the conformational properties and its interaction with membrane models by EPR. The SPPS using the Fmoc/tBu protocol was feasible to obtain the peptides. All synthetic peptides exhibited hemolytic, antimicrobial and antifungal activity. The hemolytic activity of analogues was bigger than wild type peptide, expect by $TOAC^2$-*hy*. Permeabilization studies in vesicles containing DPPC:DPPA:SM and DPPC:DPPA:DPPE (80:5:15; w:w:w) showed that $TOAC^2$-*hy* has the lowest activity, followed by $TOAC^0$-*hy*, $TOAC^{13}$-*hy* and wild type peptide. The CD studies demonstrated that peptides in water performed a random coil structure, except by $TOAC^{13}$-*hy*, which had an α-helix structure. This is in accordance to the TOAC properties, which exhibits tendency to strongly promote helical conformations. In the presence of TFE or membrane mimetic, all peptides acquired high amount of α-helix. The order of α-helix content was $TOAC^{13}$-*hy* ≈ wild type peptide > $TOAC^0$-*hy* ≥ $TOAC^2$-*hy*. Fluorescence and EPR assays showed that $TOAC^{13}$-*hy* had the highest interaction with LPC micelles and both vesicle models described above. Apparently, $TOAC^0$-*hy* had no interaction, locating outside these systems; $TOAC^2$-*hy* is located in the interface between the vesicles and the aqueous solution and finally $TOAC^{13}$-*hy* is fully immersed in the membrane. These findings allowed the description of the peptide topology in the membrane, where the N-terminal region is not immersed; the position 2 is in the interface, and 13 is fully inserted. Additionally, the results suggest a model where the N-terminal region is responsible for starting the pore formation.

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P297. Abstract number: 117

Increasing amphiphilicity in peptaibiotics: Gly to Lys replacements in trichogin GA IV

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Trichogin GA IV, isolated from the fungus *Trichoderma longibrachiatum*, is the prototype of lipopeptaibols, the sub-class of short-length peptaibiotics exhibiting membrane-modifying properties. The primary structure of the 10-amino acid peptide trichogin GA IV is as follows: 1-Oct-Aib-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Lol, where 1-Oct is 1-octanoyl and Lol is the 1,2-amino alcohol leucinol. In previous papers we have shown, using a variety of physico-chemical techniques including X-ray diffraction, that this peptaibol is predominantly folded in a mixed 3_{10}-/α- helical conformation with a clear, albeit modest, amphiphilic character (1). In this work we have synthesized by the SPPS methodology, purified, and fully characterized a set of trichogin GA IV analogues in which the four Gly residues at positions 2, 5, 6, 9, lying in the poorly hydrophilic face of the helical structure, are substituted by one (position 2 or 9), two (positions 5 and 6), three (positions 2, 5, and 9), and four (positions 2, 5, 6, and 9) Lys residues. The analog with the triple Lys replacement is additionally characterized by the incorporation of a helix-inducing Aib residue at position 6. The conformational preferences of selected Lys-containing analogs were assessed by CD and 2D-NMR techniques in aqueous, organic, and membrane-mimetic environments. The role played in the analogs by the markedly increased amphiphilicity (one face is, partially or totally, positively charged) was further tested by fluorescence leakage experiments in model membranes, protease resistance, antibacteral and antifungal activities, citotoxicity, and hemolysis.

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P298. Abstract number: 118

Synthesis, preferred conformation, and membrane activity of heptaibin, a medium-length peptaibiotic

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The medium-length peptaibiotics are characterized by a primary structure of 13-15 amino acid residues and include *inter alia* some samarosporins, stilbellins, bergofungins, and emerimicins. Despite the interesting antibiotic and antifungal properties exhibited by these membrane-active peptides, their exact mechanism of action is still unknown. Recently, we focused our study on one of these peptaibols (1), aiming at investigating their conformational properties and bioactivity. Here, we present our results on heptaibin, extracted from the culture of *Emericellopsis* sp. BAUA8289 and chemically characterized by Ishiyama *et al.* ten years ago (2). The heptaibin primary structure is as follows: Ac-Phe-$(Aib)_3$-Val-Gly-Leu-$(Aib)_2$-Hyp-Gln-Aib-Hyp-Aib-Phol, where Phol is the 1,2-aminoalcohol (S)-phenylalaninol. The SPPS of the 13-mer bergofungin D, the sequence of which is similar to that of heptaibin, has been recently reported (3). Here, we present the SPPS of heptaibin itself, using the Fmoc-protection/HATU C-activation methodology. Special attention was devoted to prevent 2,5-dioxopiperazine formation, particularly when the N-terminal sequence of the growing chain is the H-Aib-Hyp- dipeptide. The final product was purified and fully characterized. A detailed conformational analysis was performed by use of FT-IR absorption, CD, 2D-NMR combined with MD calculations, and X-ray crystallography (the latter technique on an N-terminal segment). Fluorescence leakage experiments revealed that heptaibin is a membrane-permeabilizing compound.

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P299. Abstract number: 195

Synthesis, characterization and cytostatic effect of new pemetrexed-peptide conjugates

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Pemetrexed is used as a folate-antimetabolite in the treatment of pleural mesothelioma and non-small cell lung carcinoma. This drug inhibits at least three enzimes (thymidilate synthase, dihydrofolate reductase and glycinamide-ribonucleotide formyl transferase) involved in purine- and pirimidine synthesis. The pemetrexed (pem) treatment could be accompanied by various side-effects like nausea, vomiting, diarrhea etc. Several examples show that the conjugation of an anticancer drug (eg. daunomycin, metothrexate) with peptide could decrease or even eliminate side effects (1,2). Oligoraginines are de novo designed cell penetrating peptides capable to translocate covalently attached cargoes (eg. enzymes, antitumor agents) (2). It was observed that peptide IELLQAR used as selective inhibitor could prevent the attachment of E-selectin to the cell surface-carbohydrates and thus limit the risk of metastases formation (3). Based on these findings we have prepared new pemetrexed conjugates with octaarginin and/or IELLQAR peptide. Conjugates were characterized by RP-HPLC and mass spectrometry. Their antitumor effect were determined in vitro by MTT assay on HL-60 human leukemia and NCI-H358 human non-small cell lung carcinoma cell lines. We report here our findings on the comparative analysis of the conjugates with different peptide components on two different cell lines. In contrast our previous observation with daunomycin-peptide conjugates (4), we found that pemetrexed-conjugates were as effective as free pemetrexed. Interestingly IELLQAR-conjugates were even more effective as compared with octaarginine-conjugates on both cell lines studied.

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P300. Abstract number: 205

Discovery of antimicrobial cyclic and dendritic peptides by off-bead screening of self-encoded TAGSFREE combinatorial libraries

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We showed recently that combinatorial one-bead-one-compound libraries of linear, cyclic or dendritic peptides designed using the TAGSFREE algorithm can be decoded by determining the amino acid composition of the beads, an inexpensive analysis which also provides a useful quality control of the libraries (J. Kofoed, J.-L. Reymond, *J. Comb. Chem.* **2007**, *9*, 1046). Our recently reported off-bead screening protocol originally demonstrated to identify catalytic activities (N. Maillard et al., *J. Comb. Chem.* **2009**, *11*, 667) was adapted to screen combinatorial libraries of cyclic and dendritic peptides for antimicrobial activity, leading in both cases to the identification of potent antimicrobial compounds with activity against MRSA. The compounds show MIC values in the range of 2-32 ug/ml but show only weak hemolytic activity (> 1 mg/ml). The assay is generally applicable to screen one-bead-one-compound combinatorial libraries and should greatly facilitate research for new antimicrobial agents.

P301. Abstract number: 211

Towards lasso peptide engineering: Insights into the maturation mechanism of microcin J25

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Lasso peptides are a group of gene-encoded bioactive peptides produced by bacteria. They adopt a remarkable knotted fold, the lasso structure, which is characterized by an N-terminal backbone-to-side-chain macrolactam ring that tightly traps a threaded C-terminal tail. Such a compact structure confers extraordinary stability to the peptide toward protease degradation and denaturing conditions. Known bioactivities of lasso peptides are diverse including antibacterial, anti-HIV and receptor antagonist properties. Therefore, lasso peptides provide a promising scaffold for drug development by peptide engineering. In this study, microcin J25 (MccJ25), an antibacterial lasso peptide inhibitor of bacterial RNA polymerase, was used as a model system to characterize the maturation of lasso peptides. MccJ25 is generated from the ribosomally-produced precursor McjA further processed by the two maturation enzymes, McjB and McjC [1]. Site-directed mutagenesis on the genes encoding mcjA, mcjB or mcjC permitted confirming the respective roles of the maturations enzymes and identifying their catalytic sites and the key regions in the precursor McjA. In addition, the molecular mechanisms of the maturation enzymes were studied in vitro using recombinant proteins. Taken together, our data permit delineating the mechanisms that govern lasso peptide maturation, which is a key step towards lasso peptides engineering.

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P302. Abstract number: 212

Enterocins L50A and L50B from Enterococcus durans A511: conformational studies and antibacterial activities

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Bacteriocins produced by lactic acid bacteria present a strong interest as potential food preservatives, given their antibacterial activity against human pathogens such as Listeria. Two antibacterial peptides secreted by Enterococcus durans A5-11 isolated from Mongolian airag [1] were purified by salting out and RP-HPLC. Their amino acid sequences were determined by enzymatic digestions together with nano-ESI-MS/MS fragmentations. They were identified as enterocins L50A and L50B isolated previously from Enterococcus faecium [2]. Despite 72% of sequence identity, they showed different resistances to proteases, L50A and L50B being resistant and sensible to trypsin, respectively. Contrary to two-peptide bacteriocins, each isolated peptide exhibited potent activity on a variety of Gram-positive bacteria (MIC in the 100-500 nM range). L50A was in most cases twice more active than L50B. Weak synergy of the two peptides was observed on the Lactobacillus sakei subsp. sakei strain. CD spectra revealed an helical conformation for the two peptides in phosphate buffer at pH 7.0, both in the absence and in the

presence of SDS micelles. For L50B only, the helix content increased in the presence of micelles. Stability of the L50A helical structure in the presence and in the absence of SDS micelles could be responsible for the exceptional resistance to proteases and stronger antibacterial activity exhibited by L50A, as compared to L50B. The predominance of helical structure of L50A and L50B allowed assigning the regions critical for the helix stability and for the antibacterial activity, taking into account their amino acid sequence differences. Our results show that enterocins L50A and L50B are well structured in aqueous media and do not exhibit strong synergistic activity, two characteristics that differ from those shared by two-peptide bacteriocins and contribute to make them class IId bacteriocins [3, 4].

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P303. Abstract number: 229

Revealing the lytic mechanism of gomesin by optical microscopy of giant unilamellar vesicles and isothermal titration calorimetry

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Gomesin (Gm) is a potent cationic antimicrobial peptide from a Brazilian spider. Here we use optical and fluorescence microscopy to study the interaction of Gm, its low active linear analogue, [Ser^{2,6,11,15}]-Gm (GmL) and a fluorescent labeled analogue, Gm-Rh, with giant unilamellar vesicles (GUVs, 10 µm) composed of mixtures of the neutral lipid POPC with the negatively charged lipid POPG or cholesterol, so as to mimic bacterial and mammalian cell membranes, respectively. We observed the effect of injecting a peptide solution with a micropipette close to GUVs. As a result of peptide-lipid interaction, GUVs burst suddenly. Stable pores, which result in leaky vesicles, were not observed. Fluorescence microscopy of Gm-Rh injected on GUVs confirmed the high peptide/lipid affinity. These facts lead us to conclude that Gm and GmL disrupt the membrane via the carpet model. GmL exhibited lower lytic activity as compared to Gm, but this difference vanished at high POPG molar fraction. Additionally, the interaction of Gm and GmL with large unilamellar vesicles (LUVs, 100 nm) of various POPC:POPG ratios was investigated with isothermal titration calorimetry. Binding of GmL to negatively charged vesicles is an exothermic process for all POPC:POPG ratios investigated. On the other hand, the binding of Gm entails an exothermic and an endothermic component; the latter is more pronounced at low POPG ratio and vanishes for 50 mol% POPG. Supported by FAPESP and CNPq.

P304. Abstract number: 235

D-Maurocalcine, a new potent cell penetrating analogue devoid of pharmacological activity

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Maurocalcine (MCa) is a 33-mer peptide isolated from the venom of the scorpion Scorpio maurus palmatus. MCa possesses three disulfide bridges connected according to the pattern cys3-cys17, cys 10-cys21 and cys16-cys32 and contains three β-strands running from amino acid residues 9-11 (strand 1), 20-23 (strand 2), and 30-33 (strand 3), respectively, with β-strands 2 and 3 forming an antiparallel β-sheet. MCa has proven to be a highly potent modulator of the skeletal muscle ryanodine receptor type 1 (RyR1), an intracellular calcium channel target. The peptide stimulates the binding of [3H]-ryanodine onto purified RyR1 or RyR2 present in sarcoplasmic reticulum (SR) vesicles. In addition, MCa can be used as vector for the cell penetration of various compounds. Herein, we sought a novel strategy for the design of an analogue of MCa that would lose its pharmacological activity while retaining most of its cell penetration efficacy. These goals have been reached by synthetising a MCa with D-amino-acids. The peptide retains intact cell penetration properties, and completely loses pharmacological regulation of RyR1. In addition, it is protease resistant. The 3D structure of this analogue will be presented.

P305. Abstract number: 243

Peptide constructs that interfere with the Sec pathway can reduce the secretion of bacterial pathogenicity factors

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The development of novel strategies for antimicrobial drugs against bacterial infections is confronted with fast development of resistance. The emergence of antibiotic-resistant strains of Staphylococcus aureus like MRSA and VISA demands modification in the strategy of antimicrobial agents aiming to reduce the selective pressure. In our study, we focus on the major bacterial secretion pathway Sec as a novel target. Through the Sec pathway most of the bacterial virulence factors are secreted or displayed on the surface. We aimed to disarm S. aureus from its virulence factor V8 protease (SspA) in a co-translational phase, trying to reduce its secretion through the membrane nano-translocon by synthetic peptides, avoiding at the same time to kill the bacteria and inhibit their growth. For that purpose we investigated the bacterial uptake of several known cell penetrating peptides (CPPs) in their fluorescein-conjugated form. Uptake efficiency was quantified by confocal laser fluorescence microscopy and the HIV Tat derived peptide showed the most promising properties.

Based on this result, a competitive peptide (40 AA), that consists of (1) a mimic sequence of the V8 protease secretion signal sequence (29 AA long) and (2) the HIV Tat sequence (11 AA long), was synthesized by Fmoc chemistry and also tested for internalization into S. aureus.

Incubation of growing bacteria with the peptide construct at 10 µM reduced secretion of the V8 protease after 24h of up to 50%. A scrambled control sequence did not show activity. As a conclusion, engaging peptides as ”disarming tools”of microorganism virulence can open a new strategy of antimicrobial intervention.

P306. Abstract number: 262

Integrin Cytoplasmic Tail Derived Peptide Conjugated To A Cell Penetrating Sequential Carrier Inhibits Human Platelet Aggregation

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Integrin $\alpha_{IIb}\beta_3$ is the major adhesion receptor on platelets, playing a key role in the final step of platelet aggregation and thrombus formation during hemostasis and thrombosis. In resting platelets, the receptor $\alpha_{IIb}\beta_3$ exists in a low affinity closed conformation. 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. Previous results have demonstrated that the RPPLEED (residues 997-1003) sequence of the αIIb cytoplasmic tail is important for the $\alpha_{IIb}\beta_3$ activation and platelet aggregation (Koloka V et al. 2008). Aiming to disrupt the inside-out signalling pathway and inhibit platelet thrombus formation peptide analogues derived from α_{IIb} 997-1003 cytoplasmic region (RPPLEED, CRPPLEED) were synthesized in their N-terminus iodoacetylated form and N-terminus acetylated form correspondingly. These peptide sequences were conjugated to a recently developed Cell Penetrating Sequential Carrier (CPSC) (Ac-[Lys-Aib-Cys]$_4$-NH_2) (Papas et al. 2007), which incorporates the cysteine residue for anchoring the bioactive molecules through thioether or disulfide bond, in order to investigate their membrane permeability, as well as their inhibition potency on the platelet aggregation. From the biological assays in PRP platelets we concluded that the modified peptides penetrate platelet membrane and inhibit human platelet aggregation, in contrast to the corresponding free peptide analogues. *References: Koloka V. et al. (2008), Platelets 19, 502-511. Papas et al. (2007), J. Pept. Sci. 13, 662-671.*

P307. Abstract number: 265

CARGO DELIVERY INTO HUMAN PLATELETS USING THE Tat(48-60) CELL-PENETRATING PEPTIDE

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Translocation of proteins and other therapeutic macromolecules, which do not go through the cell membrane by themselves, into the cell, is a recently developed novel technology for the intracellular drug delivery. One of the most often used membrane-permeable carrier peptide is the arginine-rich cationic peptide derived from Human Immunodeficiency Virus type 1 (HIV-1) Tat protein (position 48-60, G^{48}RKKRRQRRRPPQ^{60}). Although the membrane permeability of the above cationic peptide and the intracellular cargo delivery has been investigated in multiple aspects, it is not yet clear how such a cationic and hydrophilic peptide is able to penetrate the hydrophobic lipid bilayer of cell membrane and to bring bioactive molecules into cells. Several uptake studies demonstrate that the internalisation occurs either directly across the plasma membrane or endocytotically and the uptake mechanism depends on both the type of peptide and the cell culture model that was used. Studies based on cellular toxicity induced by the Tat-derived peptide, showed very low toxicity in various types of cells and little perturbation of the plasma membrane despite of its highly cationic nature. However, there are not data relative either to the ability of Tat-derived peptide to penetrate the platelet membrane or its effect on the platelet activation. To this aim, we synthesized various peptide analogues derived from the β_3-cytoplasmic domain of $\alpha_{IIb}\beta_3$ integrin (Koloka V et al. 2008), tagged with the Tat(G^{48}RKKRRQRRRPPQ^{60}) sequence. Using confocal microscopy and flow cytometry assays we conclude that although the cell-penetrating peptide Tat(48-60) facilitate the entrance of exogenous macromolecules into the platelets, it exhibit stimulatory effect on platelet at concentrations higher than 62.5 μM. This result may impose restrictions on the in vivo use of the Tat carrier for intracellular cargo delivery. *References: Koloka V. et al. (2008), Platelets 19, 502-511.*

P308. Abstract number: 266

Comparison of the Mechanism of Action of Antimicrobial Peptides on Giant Unilamellar Vesicles Via Optical Microscopy

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Antimicrobial peptides (AMPs) are important components of the innate defense system of plants and animals against microorganisms, such as bacteria and fungi. Most AMPs are cationic and amphipathic, features which are essential for their non-specific interaction with the lipid phase. Initially, the cationic aspect of AMPs ensures an accumulation at the membrane surface of microorganisms, rich in negatively charged lipids. Subsequently, the amphiphilic character of the AMPs facilitates their insertion into lipid bilayers, with formation of pores and/or disruption of membranes. Therefore, the interaction of AMPs with lipid bilayers has been extensively studied as a means to widen the knowledge on the mechanism of membrane destabilization by AMPs [Domingues, T. M. et al., Langmuir. DOI: 10.1021/la100662a]. In particular, giant unilamellar vesicles (GUVs; diameter around 10 μm) have been used recently in such studies because their morphology and dynamics can be directly followed with more detail by using optical microscopy. This allows distinguishing among different mechanisms of membrane destabilization. Formation of stable toroidal pores results in leaky, yet integer vesicles, whereas disruption via the carpet mechanism cause burst of GUVs. Here we use optical microscopy to study the interaction of different AMPs with giant unilamellar vesicles (GUVs) composed of mixtures of the neutral lipid 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC) with the negatively charged lipid 1-palmitoyl 2-oleoyl phosphatidylglycerol (POPG) or cholesterol (1:1), so as to mimic bacterial and mammalian cell membranes, respectively. Peptides were synthesized by SPPS, purified by RP-HPLC and characterized by LC/ESI-MS. Our results suggest that the AMPs are discussed in terms of the different mechanisms involved in the destabilization of the lipid membrane. We show that gomesin and polyphemusin 2 act via the carpet model which causes the burst of GUVs, whereas magainin 2, protegrin 1 and tachyplesin 1 are able to form stable toroidal pores across the membrane. Supported by FAPESP, CNPq and CAPES.

P309. Abstract number: 269

Conformational Comparative Studies of Gomesin and its Analogues by Using Circular Dichroism and Fluorescence Spectroscopies

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Gomesin (Gm) is a potent antimicrobial peptide that was isolated and characterized from the Brazilian spider Acanthoscurria gomesiana. Due to its large range of antimicrobial and antifungical activities, Gm seems to be an interesting lead compound in the development of alternative new drugs for human therapy. With the aim of understand the interaction of this peptide with biomimetic membranes systems, we studied Gm and its cyclic and linear analogues conformational behavior in the presence of SDS (monomer and micellar form). Conformational studies were performed by Circular Dichroism (CD) and fluorescence spectroscopies. CD spectra obtained for all peptides in 1 or 15 mM SDS displaied a profile that is characteristic of a β-turn structure. In water, linear analogues exhibited CD spectra typical of a random coil conformation. Cyclic analogues had the same behavior of Gm in water and also showed a β-turn conformation. Fluorescence spectra showed changes in the maxima fluorescence emission of about 10 nm when the peptides were transferred from the aqueous medium to the less polar micellar environment, caused a blue-shift from around 350 nm to 340 nm. These data corroborate previous results that suggest that electrostatic interactions play an important role in the mechanism of action of gomesin. Moreover, the results show that hydrophobic interactions also contribute to membrane binding of this antimicrobial peptide. Supported by FAPESP and CNPq.

P310. Abstract number: 288

Lasiocepsin: Novel antimicrobial peptide from the venom of the eusocial bee Lasioglossum laticeps

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We have already shown that the venom of wild bee is a rich source of pharmacologically interesting antimicrobial peptides (AMPs). Particularly, three AMPs named lasioglossins were identified recently by us in the venom of wild eusocial bee *Lasioglossum laticeps* [1]. Further detailed inspection of the venom extract of this wild bee revealed the presence of other interesting AMPs. One of those, which we named lasiocepsin, is 27 residues and two intramolecular disulfide bridges containing peptide. Its primary sequence was determined by ESI-QTOF mass spectrometry and by Edman degradation as GLPRKILCAIAKKKGKCKGPLKLVCKC. The pattern of disulfide bridges (Cys8-Cys25, Cys17-Cys27) was determined by MS analysis of the fragments resulting from trypsin digestion. The SPPS of lasiocepsin using the standard protocol of N^{α}-Fmoc chemistry on 2-chlorotrityl chloride resin with trityl protection of all Cys residues resulted in a crude peptide showing HPLC profile dominated by the peak of required product. The oxidative folding of purified linear peptide performed under open air resulted however in the mixture of three peptides differing in positions of disulfide bridges. The amount of required product in the mixture represented roughly half of the distribution. Synthetic lasiocepsin showed potent antimicrobial activity against both Gram-positive and -negative bacteria, especially against *Pseudomonas aeruginosa* and no hemolytic activity against rat and human erythrocytes. Lasiocepsin also possess antifungal activity against *Candida albicans*. CD study of the lasiocepsin secondary structure indicates significant proportion of α-helical conformation. This proportion even slightly increases upon addition of helix supporting 2,2,2-trifluoroethanol.

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P311. Abstract number: 363

Antimicrobial activity of dipeptides with 3-[2-(8-quinolinyl)benzoxazol-5-yl]alanine

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3-[2-(8-quinolinyl)benzoxazol-5-yl]alanine is fluorescent unnatural amino acid with some antimicrobial [1] and cytotoxic [2] potential. To determine an influence of modification in position 5 of the benzoxazole, the series of dipeptides with above-mentioned derivative were synthesized. All proteinogenic amino acids, except cysteine, were attached to C- and N-terminus of the benzoxazolylalanine, respectively. Dipeptides were synthesized in solution using Boc/Bzl tactics. The activity of all obtained peptides was screened against model Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria whereas antifungal activity was tested against yeast *Pichia pastoris*. The minimal inhibitory concentrations were determined using two-fold serial dilution technique. Additionally, the active derivatives were then tested against most important pathogens.

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P312. Abstract number: 375

Synthesis of macrocyclic antifungal peptides: Towards novel antifungal agents against invasive fungal infections

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Echinocandins are a class of antifungal agents that act by inhibiting the β-(1,3)-D-glucan synthase complex, which is responsible for the biosynthesis of the major cell wall biopolymer β-(1,3)-glucan. Glucan is absent in human cells, making inhibition of its biosynthesis a selective antifungal approach. By blocking the synthesis of β-(1,3)-glucan, the cell wall is weakened, leading to lysis of the cells. Different fungi have varying amounts of chitin, glucans, mannoproteins, and other cell wall constituents making some species more susceptible to the echinocandins than others. 1,3-β-D-glucan is a major cell wall component of Candida and Aspergillus species, rendering them more vulnerable to these antifungals.
Echinocandins are highly oxygenated cyclic hexapeptides with a lipophilic side chain.
Due to the large number of hydroxyl groups in these lipopeptides most structure activity relationship studies were based on semisynthetic derivatives starting from the natural compounds. In 1992 the first total synthesis of simplified echinocandin analogues was reported by Merck. Structure activity relationship data from this work showed

that several of the functional groups, primarily the hydroxyl groups, were not necessary for its antifungal activity.
Therefore, the aim of this project was to investigate more extensively the cyclic peptide ring structure on the antifungal activity and evaluate if additional more simpel derivatives could be obtained.
By designing a simplified hexapeptide; cyclo[Hyp-Orn-hTyr-Hyp-Thr-εOrn] with a lipophilic terphenyl side chain, the importance of ring size was studied.
Moreover, we were interested in the synthesis and activity of echinocandin analogs obtained by alternative cyclization methods such as ring closing metathesis or disulfide formation. This also allowed the study of modification of the C-terminus.
The specific minimum inhibitory activity for each mimic was determined by testing with Candida (fungal).

P313. Abstract number: 381
Synthesis and biological investigation of (bis)intercalator-functionalized cell penetrating peptides
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Bioorganometallic chemistry has become more important in several fields, especially in the development of new drugs for cancer treatment. Over the last years numerous promising new organometallic lead structures have been developed exhibiting highly active cytostatic properties. However, the efficiency of such chemotherapeutics in the treatment of tumors is often limited by their low air-stability, water solubility and bioavailability. Recently, so called cell-penetrating peptides (CPP) have emerged as potent tools to introduce substances into cells. CPP are an inhomogenic group of peptides that share the ability to translocate in a large number of different cell-lines without the need of a receptor or transporter molecule. Thereby they are capable to transport various cargos inside cells, like proteins, oligonucleotides, nanoparticles or small organic drugs.
This work describes the synthesis of (bis-)intercalator-functionalized cell-penetrating peptides sC18 based on an antimicrobial peptide cathelicidin CAP18 which should act as a transporter for the metal complexes. In previous studies we could show, that this peptide is able to transport different covalently coupled organometallic compounds into tumor cell lines, enhancing the cytotoxic properties of the compounds.[1] Here, as metal complex iridium(III)/rhodium(III) polypyridyl complexes were chosen, which showed an intercalative binding with DNA and represent a promising class of potent cytostatic agents.[2] Synthesis of the peptide was achieved by solid phase peptide synthesis using standard Fmoc chemistry and activation by HOBt/DIC. The metal complexes were attached to the peptide either by complexation or by covalent coupling. Then products were characterized and tested with respect to their cytotoxicity against tumor cells.
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P314. Abstract number: 382
Selective membrane interactions of nucleolar-targetting peptides
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Nucleolar-targeting peptides (NrTPs) were recently designed by structural dissection of crotamine, a toxin from the venom of a South-American rattlesnake [1]. At μM concentration, NrTPs penetrate different cell types and exhibit exquisite nucleolar localization. The aim of this work was to decipher the molecular mechanism for the translocation of NrTPs into cells. Quantification of partition into membranes was carried out based on intrinsic tyrosine fluorescence [2] using biomembrane model systems (large unilamellar vesicles). The role of the bilayer phase, anionic lipids, reducing agents and peptide concentration on the extent and kinetics of partition were studied. The results for NrTP1 and NrTP2 partition, evaluated by steady-state fluorescence spectroscopy, revealed that both peptides have high partition to POPC (zwitterionic) and POPG (anionic) lipid vesicles. The peptides showed a decrease in partition for POPC:cholesterol (liquid ordered state) or DPPC (gel) membranes. Time-resolved fluorescence spectroscopy confirmed these observations. Quenching experiments with acrylamide showed that the Tyr residues are exposed to acrylamide and that there are no major peptide aggregates in solution. In the presence of lipid vesicles there is a reduction of the exposure to acrylamide of the Tyr residues. The reducing agent TCEP (tris(2-carboxethyl)phosphine) was used to assess dimerization, as these peptides contain one Cys residue, which can promote the formation of disulfide bonds. Results revealed the presence of some disulfide bond. In order to determine the ability of NrTP1 and NrTP5 (D-enantiomer of NrTP1) to translocate into giant unilamellar vesicles (GUV), rhodamine B labeled peptides were used. Confocal microscopy results showed that both peptides translocate membranes, even into mulitlamellar systems. However, the extension of the translocation is apparently higher when lymphocytes (either primary or cell lines) were used instead of GUV. The above results suggest that, although peptide translocation is enhanced for cell membranes, the entry is not dependent on a specific receptor.
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P315. Abstract number: 400
Towards a new anionic Cell-Penetrating Peptide: SAP(E)
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Cell-penetrating peptides (CPPs) are powerful tools to internalize cargoes in cells without damaging their membrane, thus allowing cell-impermeable molecules to penetrate the cytoplasm in order to exert a desired action [1-5]. The vast majority of these peptides described until now share, among other properties, that of being positively charged at physiological pH [6]. Furthermore, in several cases a clear correlation between the increasing number of positive charges and the internalization properties has been reported. Here we describe what to the best of our knowledge is the first anionic CPP. This new compound SAP(E) internalizes into several cell lines with good efficiency and shows low toxicity. We also report on the internalization mechanism. The discovery of this new class

of CPPs opens the way to the intracellular delivery of new molecular cargoes.
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P316. Abstract number: 431

Studies towards improved stability and permeability of a cell-penetrating peptide

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Since drug delivery facilitated by cell-penetrating peptides (CPPs) became known some decades ago, research in this field has been intensified in order to solve the issues regarding their practical utility for delivery of biopharmaceuticals. Especially focus concerning further development of CPP sequences with increased in vivo stability is likely to result in higher efficiency of these peptidic carriers.
The aim of the present project is to improve the membrane translocation properties of the naturally derived CPP penetratin mainly by increasing the stability against enzymatic degradation without loss of membrane activity, and thus potentially improved delivery efficiency.
Novel unnatural amino acids as well as D-amino acids are incorporated into penetratin and the stability of the resulting analogs is assayed against intestinal enzymes. In addition the transepithelial delivery will be correlated to physicochemical and structural features of the peptide analogs. The nine unique amino acid building blocks containing additional cationic charges have been synthesized from enantiopure aziridines. The increased the number of net positive charges, may lead to improved interaction of the CPP with the cell-membrane in addition to increased intestinal enzymatic stability of the CPP. By measuring the resilience towards degradation by intestinal rat juice, the enzymatic stability of penetratin is estimated. In order to identify the initial cleavage sites of penetratin exposed to intestinal enzymes, the metabolites were identified by Orbitrap-MS. The initial cleaving sites are considered optimal sites for incorporation of the novel amino acids.
Our results showed that merely a single mutation in one of the initial cleavage sites could increase the stability of penetratin toward intestinal juice from rats by three fold. Further development of stable analogs will be performed by SPOT-synthesis.

P317. Abstract number: 444

Cyclodextrin Scaffolded Alamethicin with highly efficient Channel-forming Properties

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Bacterial resistance to classical antibiotics has become a serious- and growing medical problem. Small antimicrobial peptides represent a potential solution and are increasingly being developed as novel therapeutic agents. Many of these peptides owe their antibacterial activity to the formation of trans-membrane ion-channels resulting in cell lysis. However, to further develop the field of peptide antibiotics a thorough understanding of their mechanism of action is needed.
Alamethicin represents one of these antimicrobial peptides, constructed of 20 amino acids of which eight are α-aminoisobutyric acid Aib. Alamethicin inserts into lipid bilayers, aggregates and forms voltage-gated channels, which accounts for its antibiotic activity. To examine these dynamics of assembly in alamethicin aggregates and to contribute to the development of artificial ion channels, we have synthesized templated alamethicin multimers covalently bound to cyclodextrin (CD) scaffolds.
The peptides are attached to the CD scaffold using the Cu(I)-catalyzed alkyne azide cycloaddition, also known as ”click chemistry”. CD has been functionalized with azide-groups and alamethicin has been synthesized using automated solid phase peptide synthesis. A linker containing the alkyne-group has been coupled on to the peptide. The 1,3-dipolar cycloaddition is very selective and can hence be done with unprotected CD´s and peptides.
By the use of oriented circular dichroism, calcein release assays and single channel current meassurements, the templated multimers have been shown to insert into lipid bilayers forming highly efficient and stable ion-channels.

P318. Abstract number: 466

Delivery of anti-inflammatory agents and a-methylalanine-modified antimicrobial peptides for treatment of Clostridium difficile infection

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The bacterium Clostridium difficile is the main cause of pseudomembranous colitis and diarrhoea associated with treatment by antibiotics. As strains of Clostridium difficile have acquired resistance to many antibiotics, treatment of infection has been limited mainly to the antibiotics metronidazole and vancomycin [1], and new antibiotic treatments need to be developed. Cationic antimicrobial peptides offer potential as new antibiotics against a broad spectrum of pathogens, including bacteria, fungi, and cancer cells [2.] We have designed a method of combined delivery of antimicrobial peptides and anti-inflammatory agents for treatment of infection and colitis caused by Clostridium difficile [3]. This method of delivery is facilitated by optimisation of the antibacterial potency of the antimicrobial peptide by modification at the ammonium terminal by the amino acid residue alpha-aminoisobutyric acid [4].
This publication has emanated from research conducted with the financial support of Science Foundation Ireland.
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P319. Abstract number: 476

Discovery of novel short AMPs with therapeutic potential by combining medium throughput screening and different QSAR approaches

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Despite decades of intensive research, antimicrobial peptides (AMPs) have not yet revealed all their secrets; in fact, increasingly they are appearing to be more complex than previously imagined. In recent years, it has become clear that they are not only able to kill Gram-positive and Gram-negative bacteria, fungi, parasites and enveloped viruses, but can also alter immune response in mammals. They have been used successfully in animal models, for the prevention of septic shock, they have been shown to be chemotactic, promoting wound healing and angiogenesis, and they have been found to selectively modulate chemokine and cytokine production; however, the mode of action of these AMPs, especially short peptides with a length between 9-13 amino acids, are not yet understood. Little is known about the sequence requirements of short cationic AMPs. With help of our novel technique using an artificially created luminescence producing Gram negative bacterium and peptide synthesis on cellulose (SPOT technology), we investigated the sequence requirements of such peptides. Several thousands of peptides were tested for their ability to kill Pseudomonas aeruginosa. Complete substitution analyses of different indolicidin variants as well as a semirandom peptide library with about 3000 members were studied. The complete substitution analysis gave us information about the importance of each single position whereas the peptide library gave us broader information concerning which composition of amino acids resulted in an active antimicrobial peptide. The data is being analyzed using a different quantitative structure-activity relationship approach (QSAR) to A) increase the percentage of active peptides in a library (100000 peptides were screened in silico) without very complex descriptors and B) understand the rules by using simple descriptors that discriminate between active versus inactive. This is providing us with 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.

P320. Abstract number: 488

Reduced phosphorylation of lipopolysaccharide decreases E. coli susceptibility to the human host defence peptide LL-37

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Antimicrobial peptides (AMPs) contribute to host defence in both animals and plants. In mammals, the most important AMP families are defensins and cathelicidins. The only human cathelicidin, LL-37, is a 37-residue, α-helical, cationic peptide with a direct membranolytic antibacterial activity and with multifunctional immunomodulatory capacities. It kills bacteria primarily by altering the membrane bilayer barrier, like many other AMPs, although the exact mechanism of action is not yet known. To increase our knowledge on the bacterial components involved in its mode of action, we screened an *E. coli* knock-out mutant library in search for mutations conferring altered susceptibility to LL-37. The library was created by random insertion of the Tn5 transposon into the bacterial genome and mutants with decreased susceptibility to LL-37 were selected by plating *E. coli* cells on peptide-supplemented solid medium. The different susceptibility of the wild-type and mutant cells was characterised by determining the effect of increasing peptide concentrations on either the kinetics of bacterial growth or the kinetics of bacterial killing, by observing peptide binding to the cells using confocal microscopy, and by measuring membrane permeabilisation using flow cytometry.

In 15 out of 20 LL-37-resistant mutants, Tn5 insertion was observed to occur in the *waaY* gene, encoding for a specific kinase responsible of the phosphorylation of the Hep II residue in the core region of bacterial lipopolysaccharide (LPS). This modification decreased the peptide's ability to inhibit bacterial growth or to kill bacterial cells at micromolar concentrations, correlating with reduced binding to and permeabilisation of bacteria. Susceptibility to several other AMPs of different structural classes, including helical ones, was however unaffected, indicating that binding may relate also to LL-37's particular structure. *WaaY* inactivation thus determines a decreased anionicity of the outer membrane due to a missing phosphorylation of a specific sugar residue, which in turn causes a significant but selective reduction in peptide binding to the cell surface and a decreased antibacterial activity. The results reveal a putative LPS-binding site for LL-37 and stress the importance of the electrostatic properties of the cell surface for binding of and susceptibility to LL-37.

P321. Abstract number: 489

Combination of a Host Defence Peptide with a classical cytotoxic agent and their delivery to cancer cells

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Chemotherapy combinations have been the mainstays in the treatment of most types of cancer at advanced stages in the past half century. Multi-drug therapies may circumvent mechanisms of tumour resistance to single agents and potentially provide greater clinical benefit if the selected agents act synergistically in combination.

Host defence peptides are multifunctional molecular effectors of innate immunity, the first line of defence against infection in multicellular organisms. They are recognised as promising candidates for the development of novel antimicrobial but also anticancer agents, owing to their original mode of action which may circumvent the classical mechanisms of drug resistance, their low propensity to select resistant mutants [1] and their additive or synergistic activities with traditional antitumour agents [2]. Development of combination therapies require the selection of the correct drug combination, the development of reliable assays for pre-clinical and clinical evaluation and the efficient delivery of these agents.

Preliminary studies for the delivery of a classical cytotoxic agent and a molecular effector of innate immunity to tumour cells have been completed. The approach developed can also be exploited to facilitate the delivery of host defence peptides as single agents.

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P322. Abstract number: 496

A Far UV CD study of de novo designed antimicrobial coiled coils upon interaction with model membranes

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Multiresistant bacteria have been the cause of growing interest in antimicrobial peptides (AMP) as an alternative to conventional antibiotics. AMPs show activity towards a wide range of pathogens but are often unable to differentiate between bacteria and eukaryotes. Antibacterial activity and selectivity depends on various parameters one of which is the ability to fold into amphipatic structures upon interaction with membranes[1]. In the present paper, we studied 5 peptides in different membrane models by Far-UV CD-spectroscopy. The peptides were designed to be antibacterial and form coiled-coil structures. Peptides **1-5**, were synthesised using standard Fmoc SPPS and tested for antibacterial activity against *E.coli* and *S.aureus*. The hemolytic activity of the peptides was tested on human erythrocytes. Far-UV CD-spectroscopy experiments were analyzed in 150mM NaF, and three membrane models mimicking an erythrocyte, gram-positive and negative bacteria. The peptides showed activity towards *E.coli* with MIC values of 25-50μM. **2** also showed activity towards *MSSA* with a MIC of 50μM. All the peptides, except **4**, showed high hemolytic activity. Far-UV CD experiments on **1-5** in NaF revealed that **1**,**3** and **5** formed a coiled-coil structure with characteristic negative bands at 222 and 208nm with $[\theta]_{222}/[\theta]_{208}\geq 1$ and a positive band at 190nm. **2** and **4** showed no distinct structure. When introduced to the *E.coli*-mimick **2** and **4** both achieved a coiled-coil like structure with a $[\theta]_{222}/[\theta]_{208}=1$. When **3** was introduced to the mimic the ratio $[\theta]_{222}/[\theta]_{208}$ changed from 1 in NaF to 1.3, indicating further stabilization of the coiled-coil structure. When the peptides were introduced to the *S.aureus*-mimic no changes occurred with **2** compared to the CD-spectrum obtained in the *E.coli*-mimick. The CD-spectrum of **3** resembled that run in NaF whereas the most significant changes occurred for **4** where a characteristic non-interacting α-helical structure was obtained with a $[\theta]_{222}/[\theta]_{208}=0,8$. When **2**,**3** and **4** were introduced to the erythrocyte mimick both **2** and **3** formed coiled-coils whereas **4** formed a non-interacting α-helical structure. In conclusion higher-ordered structures are important for membrane activity of AMPs. Furthermore, structural flexibility could be a key aspect in designing peptides with potent antibacterial activity and low hemolytic activity.

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P323. Abstract number: 497

Interaction of cell-penetrating peptides with giant plasma membrane vesicles

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The plasma membrane areas where the translocation of cell-penetrating peptides (CPP) eukaryotic cells is initiated has remained unclear in spite of intense research since the definition of CPPs as a specific class of a potential delivery vehicles.

We have characterized the interaction of various CPPs with the giant plasma membrane vesicles (GPMV) derived from the different cell lines as a model of membrane with differing compositions. Results obtained with nonaarginine, Tat peptide, Penetratin, MAP, Transportan and TP10 corroborate the classification of CPPs by their amphipathic properties. The most amphipathic peptides Transportan and MAP have the highest affinity to membranes whereas the secondary amphipathic penetratin concentrates in model membrane to a lower extent. The membranes of vesicles can be induced to segregate into liquid-ordered and liquid-disordered membrane domains which can be easily distinguished by fluorescence microscopy after labeling with cholera toxin and annexin V respectively. The above-mentioned amphipathic CPPs showed a clear preference towards liquid-disordered areas suggesting the involvement of these domains in the cellular uptake of amphipathic CPPs. The kinetics of peptide's association with GPMVs was estimated both by fluorescence microscopy and FACS analysis on order to analyze the efficacy of used CPPs.

P324. Abstract number: 530

Antimicrobial Oligopeptides And Rapid Alkalinization Factors In Chilean Grape

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One only grape oligopeptide primary structure was extracted and characterized before [1] whereas information on numerous grape uncharacterized proteins has to be found in different protein databases. Thereupon we have carried out a theoretical structure-function analysis of uncharacterized grape protein amino acid residue sequences in order to elucidate new primary structures of grape regulatory oligopeptides.

The data of DEGECHIVID (Chilean Grapewine Genomic Project) [2] database containing primary structures of unrecognized grape proteins, EROP-Moscow (**E**ndogenous **R**egulatory **O**ligoPeptides) database containing information on more than 600 plant regulatory oligopeptides [3], and special computer programs [4, 5] were used for the comparison of DEGECHIVID information with EROP-Moscow data. Six putative antimicrobial oligopeptides and four rapid alkalinization factor (RALF) sequences have been identified:

AMP1
RTCESQSHRFKGTCVRQSNCAAVCQTEGFHGGNCRGF
RRRCFCTKHC
AMP2
RPCESQSPRFKGTCVRQSNCAAVCQTEGFHGGNCRGF
RRRCFCTKHC
AMP3
KTCESQSHRFKGTCVRHSNCAAVCQTEGFHGGNCRGF
RRRCFCTKHC
AMP4
RTCDSQSHRFKGTCVTHINCAAVCHTDGFHGRNCRGF
RRPCFCTKHC
AMP5
RLCESQSHWFRGVCVSNHNCAVVCRNEHFVGGRCRG
FRRRCFCTRNC

AMP6
RVCESQSHKFEGACMGDHNCALVCRNEGFSGGKCKGL
RRRCFCTKLC
RALF1
ATSKYISYGALQRNSVPCSRRGASYYNCQPGAQANPYN
RGCSTITRCRS
RALF2
ASKRYISYGALSRNSVPCSRRGASYYNCRPGAQANPYT
RGCSAITRCRR
RALF3
AQRRRYISYGALRRNQVPCNRRGRSYYNCRRGGRANP
YRRGCSVITKCHRFTD
RALF4
VMQKKYISYETLKKDMIPCARPGASYYNCRASGEANPY
NRGCEVITGCARGVRDINS
Four AMPs and all RALFs were described by us earlier [4-6]. However two AMPs (AMP3 and AMP4) had unique amino acid residue sequences not described before. It has been noted also that RALF oligopeptides contain many positively charged residues (K and R) as well as a wide variety of antimicrobial oligopeptides. Such similarity serves as proof of poly-functional properties of RALFs.
Our thanks to H.Peña-Cortes for idea proposal and kindly providing access to DEGECHIVID data. This study was supported by Chilean National Science and Technology Research Fund FONDECYT, Grant No. 1080504.
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P325. Abstract number: 544

Microcalorimetric and spectroscopic studies on the mechanism of interaction between novel peptidomimetics and lipid bilayers

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In recent years biomacromolecules such as proteins and nucleic acids have been developed into promising drug candidates. However, one problem with biomacromolecules is that they usually have to pass the cell membrane in order to exert their effect. Utilization of cell penetrating peptides (CPPs) might be a way to enhance their transport across the cell membrane. It is becoming increasingly evident that CPP uptake pathways may vary depending on the physico-chemical properties of the CPP and the cargo that they deliver as well as on the specific cell types and the specific experimental conditions. Nevertheless, the interaction between CPPs and the membrane is one of the first steps of the internalization. A way to elucidate the mechanism(s) of cell membrane interaction is to analyse the CPP´s interaction with model liposomal membranes.
Previously, we described a new class of CPP mimics, which seemed to show superior biological effect compared to well-known CPPs. The molecular design of these α-peptide/β-peptoid chimeras is based on alternating repeats of α-amino acids and β-peptoid residues designed to benefit from the structure-promoting effects and lipophilicity of the unnatural chiral β-peptoid residues, as well as the cationic properties of the α-amino acid residues. The chimeras are very stable toward proteolysis, non-hemolytic, possess antibacterial activity and promising cell-penetrating potential.
The aim of the current study was to characterize the binding between a series of novel CPPs and anionic liposomes by a thermodynamic and spectroscopic investigation.
Results from ITC experiments showed that an increased number of basic residues in these novel sequences results in a more favorable interaction with the anionic liposomes, corresponding to data obtained from ellipsometry experiments on binding of the mimics to anionic lipid bilayers. Additional experiments revealed that hydrophobic interactions also contributed to the binding. However, from CD spectra it was concluded that the CPP mimics do not undergo any major structural changes in the presence of anionic liposomes. The ranking obtained by calorimetric and spectroscopic methods correlated well with that obtained with live cells.
In conclusion, when comparing the CPP mimics with results obtained for well-known CPPs it seems, that the binding to anionic liposomes is more favorable for all novel CPP mimics investigated, and that the mechanism of binding can be described by biophysical studies.

P326. Abstract number: 557

Glycine And Histidine-Rich Antifungal Peptides: On The Way To The Mode Of Action Of Shepherin I

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Shepherin I (*Shep I*) is a 28-mer antimicrobial peptide, isolated from the roots of shepherd's purse *Capsella bursa-pastoris*, with almost exclusive glycine and histidine contents and six tandem repeats of the motif GGH. Aiming to understand its structure activity relationship and mode of action, we studied *Shep I* and its truncated, amidated, fluorescently labeled and/or tryptophan-containing analogues. Peptide assembly was done at 60°C using conventional heating and customized protocols. The final peptides, purified by RP-HPLC, were characterized by LC-MS, amino acid analysis and bioassays. The fluorescently labeled analogues were also submitted to confocal microscopy and FACS analysis.
Shep Ia, *Shep I (3-28)a* and *Shep I (6-28)a* were as active as *Shep I* against *Candida albicans* strains, *C. tropicalis* Squibb 1600 and *Saccharomyces cerevisiae* ATCC 2601, indicating that *Shep I (6-28)a* may be the minimal fully active portion of *Shep Ia*. Anticandidal activity of *Shep Ia* was inhibited in high ionic strength, but significantly enhanced in the presence of the Zn^{2+} ion. Such analogue killed *C. albicans* MDM8 cells at 62.5 μM in 30 min, caused low hemolysis in human erythrocytes in isotonic glucose phosphate buffer (IGP) and was not hemolytic in phosphate buffered saline (PBS). The amidated fluorescently labeled analogues *Fluo-Shep Ia*, *Fluo-Shep I (3-28)a* and *Fluo-Shep I (6-28)a* were more active against *C. albicans* ATCC 90028 than their respective unlabeled-analogues. Except for the *[Trp3]-Shep I (3-28)a* analogue, all amidated Trp-containing analogues were 2-fold more active than *Shep Ia* against *Candida albicans* ATCC 90028. All of them were equally or 2-fold less active than *Shep Ia* against *C. parapsilosis* ATCC 22019 and equally or 2-fold more active against *C. krusei* ATCC 6258. None of them was hemolytic in IGP or PBS. *Fluo-Shep Ia*, *Fluo-*

Shep I (3-28)a and *Fluo-Shep I (6-28)a* were rapidly internalized into *Candida albicans* in an energy- and temperature-dependent manner that suggest internalization by endocytic process.
Altogether, these results indicate that: (i) amidated Trp-containing analogues of *Shep I* can act as anticandidal drugs; (ii) *[Trp6]-Shep I (6-28)a* is the best analogue obtained so far; (iii) the fluorescently labeled analogues have the potential to act as vector for the delivery of macromolecules and/or drugs into cells.
Acknowledgments: FAPESP, CNPq. *present address: Special Laboratory of Pain and Signalling, Butantan Institute; Sao Paulo, Brazil.

P327. Abstract number: 568
Identification of antimicrobial domains in proteins by a novel in-silico approach
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Discovery of novel leads against antibiotic-resistant bacterial pathogens has become an important issue for the medical-pharmaceutical communities, in a permanent struggle to develop potent, non-toxic antibiotic drugs to combat infection. A promising source in this context is the ever-growing class of antimicrobial peptides identified in eukaryotes over the last three decades. In addition to peptides whose main function is antimicrobial, domains with distinct antimicrobial properties have long been observed in proteins better known for other biological functions. To identify these antimicrobial determinants, we have developed a theoretical approach that can predict antimicrobial behavior from amino acid sequence and thus map proteins for antimicrobial domains. Using a training set of 50 proteins of known antimicrobial activity plus 50 other proteins lacking such activity, our algorithm has allowed identifying and structurally locating novel putative antimicrobial regions in the practical totality of the first group. Furthermore, the predictions of the algorithm have been successfully corroborated experimentally by the corresponding set of derived antimicrobial peptides, all of them with hitherto unreported activities. In these validation experiments, our method has correctly identified active antimicrobial domains with an accuracy of 85% and a sensitivity of 90%, and can thus be viewed as a useful tool for the design of new antimicrobial drugs.

P328. Abstract number: 578
A Novel Branched Cell-penetrating Peptide for the Delivery of Pharmaceutical Compounds
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The efficient therapeutic application of biologically active compounds is often hampered due to their size or low water solubility. In order to eradicate this drawback, cell-penetrating peptides (CPPs) have emerged as a promising tool for efficacious drug delivery owing to their capacity to autonomously translocate into the cell with high efficiency and to confer water solubility. We designed a new branched cell-penetrating peptide, (sC18)2, based upon two units of CAP18106-121, which is derived from the 18-kDa cationic antimicrobial peptide and was shown to effectively internalize into various cell lines without being cytotoxic to them.
The peptide was obtained highly pure (> 99 %) after preparative RP-HPLC. CD spectroscopy indicated formation of an α-helix in trifluoroethanol, which is also the case for the parent peptide sC18 and is thought to be a key structural motif for translocation. By means of fluorescence microscopy and flow cytometry after introduction of a carboxyfluorescein label, we observed that the novel CPP highly effectively internalizes into various cell lines even at a concentration of 1 µM, which constitutes a remarkable improvement compared to sC18. Most importantly, the peptide did not show any cytotoxic effects in this concentration range as verified by a resazurin-based cell viability test. Since the high content of basic amino acids and thus a net charge of +17 renders (sC18)2 a candidate for non-covalent oligonucleotide delivery, we assessed its potential to form stable electrostatic complexes with nucleic acids by electromobility shift assays. We observed full retention of plasmid DNA in an agarose gel after complexation with (sC18)2 even at very low charge ratios.
In conclusion, we designed a highly efficient CPP which has the yet-to-be-investigated potential of being a powerful tool for cellular delivery of oligonucleotides, small organic molecules or pharmaceutical compounds.

P329. Abstract number: 13

Experimental observation vs. in silico prediction of disordered regions and O-glycosylation sites in N-terminal (NT) proANP and NT-proBNP.

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The mature forms of the cardiac natriuretic peptides, ANP and BNP, are proteolytically processed to active hormone, 28 and 32 residues, respectively and NT-pro fragments, 67 and 76 residues, respectively. Far-UV CD, 1D and 2D- H1 NMR, SE-HPLC, and AUC sedimentation equilibrium (AUCSE) data are described. Two metaserver disorder predictor algorithms, MeDor and metaPrDOS, were applied to the fragments for comparison to empirical data. As NT-proBNP is known to be O-glycosylated, it was also of interest to apply O-glycosylation prediction programs, NetOGlyc and YinOYang, to each.

CD data showed a large negative molar ellipticity for NT-proANP of -14,400 at 200nm and -19,000 at 199nm for NT-proBNP . Estimated 2º structure %'s were 9 helix, 27 beta, 28 turn and 36 coil and 6 helix, 15 beta, 32 turn and 47 coil, respectively. The amide region 1D-H1 NMR resonances were confined to 8-8.6 ppm. There were no cross-correlation peaks from 2D-H1 NOE spectra for NT-proANP and only a few for NT-proBNP. Peak elution of these fragments by SE-HPLC was 20.4' for NT-proANP, 20.6' for NT-proBNP and 20.4' for 17K Myoglobin. No higher molecular weight oligomers were seen in the AUCSE experiments. Collectively, the physical data demonstrate that NT-proANP and NT-proBNP are mainly disordered, monomeric proteins.

The metaserver programs are designed for consensus description of disordered regions. NT-proANP MeDOR analysis gave residues 9-51 or 64% of sequence and for NT-proBNP residues 2-54 or 70% of sequence. At a false positive rate of 5%, metaPrDOS predicted the entire sequence of NT-proANP to be disordered and residues 1-21;32-51;53;55-56;62-63;66;73-76 or 70% for the NT-proBNP sequence. Thus, the in silico results are consistent with the empirical data.

NT-proBNP has 4 consensus O-glycosylation sites: S37;S44;T48;T58. NetOGlyc predicted 4 sites, 2 of which were S44;T48 and YinOYang found 2 sites, S37;S44. For NT-proANP, similar analysis gave S9;S20;T29;S33;T65 and T29;S33;S49;S50;T65, respectively. This suggests that NT-proANP may also be O-glycosylated.

P330. Abstract number: 34

ANALOGS OF CONTULAKIN-G, AN ANALGETICALLY ACTIVE GLYCOPEPTIDE FROM CONUS GEOGRAPHUS

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Cone snails are marine predators who use immobilizing venoms for catching prey. Chemical analysis of the venoms has revealed a variety of biologically active small and intermediate size peptides rich in post-translational modifications (modified amino acids, glycosylation). Contulakin-G (structure, see 2 below) is a potent analgesic from Conus geographus venom. The in vivo activity of synthetic Contulakin-G was previously found[1] to be significantly higher compared to that of a peptide lacking the glycan. These observations touch on the general question of the function of glycans of glycopeptides and glycoproteins in Nature. We believe that conus glycopeptides are among the best model compounds available to address this question, since they are comparatively small molecules which can be readily prepared and modified by chemical synthesis. In order to further investigate the importance of the glycan of Contulakin-G, we have now synthesized glycopeptide analogs where the glycan chain has been altered. The glycopeptides were prepared by a combination of solid-phase peptide synthesis and enzymatic synthesis.

P331. Abstract number: 50

Characterization of prolactin-releasing peptide binding, signaling and hormone secretion in rodent pituitary cell lines

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Recently discovered prolactin-releasing peptide (PrRP) binds to the receptor GPR10 both in central nervous system and periphery and is involved in endocrine regulation and energy metabolism but its physiological role is up to now mostly unknown. In organism, two biologically active isoforms of PrRP of either 31 (PrRP31) or 20 (PrRP20) amino acids containing C-terminal Phe amide sequence are present. C-terminal PrRP(25-31) heptapeptide was found to be the minimal active agonist fragment (however of lower potency compared to PrRP31).

In the present study, we demonstrate PrRP receptor status of three rodent tumor pituitary cell lines: somatotrophs and lactotrophs containing rat GH3 cells, adenocorticotrophs containing mouse AtT20 cells and rat RC-4B/C cells consisting of all types of pituitary cells: somatotrophs, lactrophs, adenocortitrophs and gonadotrophs. Saturation binding of radiolabeled PrRP31 to intact plated cells resulted in Kd in 10-9 M range and various Bmax in particular cell lines. In competitive binding experiments, both PrRP31 and PrRP20 competed with 125I-PrRP31 binding to RC-4B/C cells with similar Ki. C-terminal analogue PrRP13 showed lower binding potency compared to PrRP31 and PrRP20.

In addition, we examined also cell signaling (extracellular-regulated kinase phosphorylation) and prolactin release induced by PrRP. As stable tumor-derived cell lines are much more convenient for receptor pharmacology studies than either primary cell preparations or cells transfected with PrRP receptor, our aim was to identify and characterize binding and functional activity of endogenously expressed PrRP receptor in a physiologically relevant cell lines.

Finally, food intake after peripheral and intracerebroventricular administration of PrRP analogues in fasted mice was also followed.

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P332. Abstract number: 124

Design of PAC1/VPAC1 selective analogs as multifunctional drug candidates for the treatment of Parkinson's disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder after Alzheimer's disease, with almost 6.5 million cases worldwide. The precise mechanism leading to a massive degeneration of dopaminergic neurons of the substantia nigra pars compacta is unknown but compelling evidence demonstrated that apoptosis and inflammation participate in the pathogenesis and progression of PD. So far, no effective treatment has been developed and available drugs are essentially symptomatic and unable to stop the progression of the disease. Thus, a potent and innovative strategy to treat PD could be the use of a multifunctional drug exhibiting neuroprotective and anti-inflammatory properties. In this regards, the neuroprotective and neurotrophic pituitary adenylate cyclase-activating polypeptide (PACAP), with its unique ability to cross the blood-brain barrier, could be a promising candidate. As a neurohormone belonging to the VIP family, PACAP is able to interact with three distinct GPCRs, namely VPAC1 (modulation of inflammation) and VPAC2, which recognizes both PACAP and VIP with similar affinity and a specific PACAP receptor named PAC1 (inhibition of the caspases pathway). The goal of our study was to design selective PAC1/VPAC1 analogs that might be able to modulate both the apoptotic and inflammation processes involved in PD. To do so, chemical and structural modifications (Ala-scan, D-scan, N-methylation, constrained amino acids) were incorporated in the N-terminal segment (region 1-7) of the peptide, known to be involved in the recognition and activation processes. Peptide derivatives were pharmacologically characterized using a radioligand binding assay and an intracellular calcium mobilization assay, using three cell lines transfected with the PAC1, VPAC1 and VPAC2 receptors, respectively. Analogs with an improved pharmacological profile were further evaluated in an in vitro model of PD. Two compounds, [Ala7]- and [Hyp2]PACAP27, with an improved selectivity toward PAC1 and VPAC1, were identified and their propensity to protect dopaminergic neuroblastic cells (SH-SY5Y) against the toxicity of MPP+ was evaluated. Results showed that in pre- and co-treatment experiments, these two compounds were able to protect SH-SY5Y cells, with the same efficacy as PACAP. These results open new perspectives for the development of a promising peptide-based multifunctional drug for the treatment of PD.

P333. Abstract number: 143

A non-peptide mimetic insect kinin agonist interacts with an expressed receptor and an in vitro diuretic assay in the mosquito Aedes aegypti

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Insect neuropeptides regulate critical processes and behaviors in insects, though they are unsuitable as tools to arthropod endocrinologists and/or as pest management agents due to poor biostability and/or bioavailability characteristics. Peptidomimetic analogs can overcome these limitations and either over-activate or block critical neuropeptide-regulated functions. The diuretic insect kinin class of neuropeptides share the evolutionarily conserved C-terminal pentapeptide motif Phe-X1-X2-Trp-Gly-NH2, where X1 = His, Asn, Ser, or Tyr and X2 is generally Ser or Pro. Structure-activity studies on an expressed insect kinin receptors from the mosquito Aedes aegypti and tick Boophilus microplus demonstrate that the most critical residue for activity is the Trp and that the peptide ligands adopt a cisPro, type VI beta turn conformation. A rationally-designed combinatorial library based on the imidazopyridoindole motif, which contains an indole moiety representing the side chain of the critical Trp residue, was developed and evaluated on the expressed mosquito and tick receptors. This effort led to the identification of an active non-peptide agonist analog. The non-peptide analog, designated TA-1, also demonstrates significant agonist activity in an in vitro mosquito Malpighian tubule diuretic assay at a concentration of 0.1 umole. Analog TA-1 represents the first non-peptide mimic of the insect kinin neuropeptide family and provides a lead for the generation of an agent capable of disrupting diuresis in arthropods that vector important human and animal diseases.

P334. Abstract number: 157

Characterisation of the Human INSL5 Solution Structure

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Insulin-like peptide 5 (INSL5) is a member of the insulin/relaxin super family. Members of this family are structurally characterized by two peptide chains (A and B) that are held together by three disulfide bonds, one intra A-chain and two inter-chain between the A and the B-chain. INSL5 was recently discovered through searches of expressed sequence tag databases. INSL5 is expressed in peripheral tissue with high expression levels in the colon and in the brain. The function of INSL5 is still being elucidated but is suggested to have a role in gut contractility and glucose and fat metabolism. The endogenous receptor for INSL5 is relaxin family peptide receptor 4 (RXFP4) which has an overlapping expression pattern to INSL5.

To gain further knowledge about structural features of the ligands of the relaxin peptide hormone family, synthetic human INSL5 was subjected to solution-state NMR to determine the three dimensional structure. The structure reveals a insulin/relaxin like fold with two parallel helices in the A-chain, a central helix in the B-chain and enclosing a hydrophobic core. However, the central helix in the B-chain, which holds the primary receptor binding site, is longer in INSL5 than compared to its closest relative relaxin-3 and extends all the way to the C-terminus. This results in a different position of the residues ArgB23 and TrpB24, which is the key receptor activating domain. This difference may be an important contributor to the different biological activities of relaxin-3 and INSL5. Relaxin-3 is a high affinity agonist for both its endogenous receptor RXFP3 and RXFP4, whereas INSL5 acts as a low affinity antagonist on RXFP3. INSL5 was also subjected to temperature and pH-titrations providing a detailed picture of the relaxin hydrogen bonding network and key electrostatic interactions stabilising the fold.

P335. Abstract number: 190

Synthesis and bioactivity studies on the C-terminally expressed heptapeptide orthologues of various Proenkephalin A sequences

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Since the isolation of enkephalins a number of other opioid peptides have been discovered, including heptapeptide with the sequence of Tyr-Gly-Gly-Phe-Met-Arg-Phe (Met-enkephalin-Arg6-Phe7, YGGFMRF or MERF). The heptapeptide MERF is a potent opioid cleft from the sequence of proenkephalin A (PENK), the common precursor of Met- (ME) and Leu-enkephalin (LE).
Our bioinformatic analysis exposed chemical biodiversity at the heptapeptide region of PENK among 56 animals. Moreover, with alignment outcome, it became clear that the C-terminal heptapeptide domain of the PENK was far more conserved, compared to the octapeptide region, which was also noticed in our previous study (Bojnik E. et al., Neuroscience, 2010, 165:542-552) The mammalian consensus structure MERF was found to be the most frequent in searching protein databases. Nevertheless, four novel orthologous sequences were found among 11 animals, such as YGGFMGY (Zebrafish), YGGFMRY (Newt), YGGFMKF (Hedgehog) and YGGFMRI (Mudpuppy); being the YGGFMGY the most widespread sequence among all.
Each novel heptapeptides, together with the human ME and MERF, were synthesized using solid phase peptide synthesis method (some of them were prepared containing oxidized methionine) and subjected to functionality studies, using receptor binding and G-protein activation assays. Equilibrium binding affinities changed from good to modest measured by various [3H]opioid radioligands in rat barin membranes, while unlabelled homologous ligands exhibited the highest affinities. The relative affinities of the heptapeptides reveal rather mu-receptor preference over the delta-receptors. Interestingly enough, the mudpuppy sequence (YGGFMRI) displayed moderate affinity towards the kappa receptors, indicating a possible influence of the terminal isoleucine (F'I) side chain for kappa receptor selectivity. [35S]GTPgS assay, which measures the agonist-mediated G-protein activation, has demonstrated that these novel heptapeptides are also potent in stimulating the regulatory G-proteins.
As a conclusion, we have put forward our recent results of the bioinformatic studies that revealed novel endogenous heptapeptide structures within PENK sequence of various animal species. All of the newly identified orthologues were able to bind and to activate mammalian opioid receptors and G-proteins, with quite good affinities and potencies.
Supported by OTKA-NKTH CK-78566 grant, Hungary

P336. Abstract number: 197
Substitution of various p-N-alkylcarboxamidophenylalanine analogues for Tyr1 in TIPP opioid peptides.
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We recently reported that an analogue of the δ opioid antagonist TIPP (H-Tyr-Tic-Phe-Phe-OH; Tic=tetrahydroisoquinoline-3-carboxylic acid) containing 4`-[N-((4`-phenyl)phenethyl)carboxamido]phenylalanine (Bcp) in place of Tyr 1 showed potent δ opioid agonist activity (I.Berezowska et al., J.Med.Chem.52,6941(2009)). Here we describe analogues of TIPP in which the Tyr1 residue was replaced by derivatives of 4`-carboxamidophenylalanine (Cpa) containing various alkyl substituents at the 4`-carboxamido group, including 4`-[N-(hexyl)carboxamido]phenylalanine (Hcp), 4`-[N-(decyl)carboxamido]phenylalanine (Dcap), 4`-[N-(diethyleneglycolyl)carboxamido]phenylalanine (Dgcp) and 4`-[N-(methoxypolyethyleneglycolyl)carboxamido]phenylalanine (Mgcp).
These amino acids were synthesized by coupling the respective alkylamines to the -COOH group of Boc-Phe(4`-COOH)-OMe followed by ester hydrolysis. Peptides were prepared by solid-phase synthesis. The in vitro opioid activities of the compounds were determined in μ-, δ- and κ-opioid receptor binding assays and in the functional guinea pig ileum(GPI) and mouse deferens(MVD) assays. Whereas [Cpa1]TIPP was a potent δ opioid antagonist, [Hcp1]TIPP showed potent δ partial agonist activity and subnanomolar δ receptor binding affinity. The analogue containing a further extended alkyl chain, [Dcap1]TIPP displayed 4-fold lower δ receptor binding affinity than [Hcp1]TIPP and turned out to be a full δ agonist. Interestingly, the ethyleneglycol-containing analogue, [Dgcp1]TIPP, was a weak δ opioid antagonist. Finally, the analogue carrying the methoxypolyethyleneglycol moiety at the 1-position side chain was found to be inactive. Taken together, these results indicate that the length and the polarity of the carboxamido substituents in these TIPP analogues have a profound effect both on the δ receptor binding affinity and on the intrinsic efficacy at the δ receptor.

P337. Abstract number: 207
NMR studies of vasopressin analogues modified with indoline-2-carboxylic acid in position 2 in the dodecylphosphocholine micelle
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Arginine vasopressin, the neurohypophysial circulating nonapeptide CYFQNCPRG-NH_2 (AVP), plays major roles in regulating renal function in mammals. It exhibits renal antidiuretic effects by supporting conservation of body fluid osmolality and volume, controlling urine volume and renal sodium excretion [1]. In this project, we study the effect of the substitution of L-indoline-2-carboxylic acid for Tyr^2 and Mpa (3-mercaptopropionic acid) for Cys^1 in AVP analogues on their conformation. Proper orientation of the Tyr^2 side chain is necessary for activity. Although the conformation of the N"terminal part of vasopressin analogues is so important for their pharmacological activity [2], new analogues showed affinity to the OT receptors. All the new compounds exhibited only negligible antidiuretic activity. With regard to pressor test, all the analogues were devoid of the pressor potency (unpublished data). In the interaction of peptide hormones with their membrane receptors a significant role plays lipid bilayer, therefore we have carried out our studies of new vasopressin analogues in a dodecylphosphocholine (DPC) micelle. DPC micelles are considered to be good models of eukaryotic cell membrane [3]. For these studies we use 2D nuclear magnetic resonance (NMR) supported with theoretical methods. Conformation-activity considerations will be attempted.
This work was supported by the Polish Scientific Research Committee Grant No. N N204 181736 grant. The calculations were carried out in the Academic Computer Centre (TASK) in Gda'sk, Poland.
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P338. Abstract number: 225

Synthesis Of New Lgnrh-III Analogues And Studies On Prostate Cancer Cell Proliferation

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Several agonists and antagonists of gonadotropin-releasing hormone (GnRH) have been identified as effective compounds in breast and/or prostate cancer therapy. However, chronic administration of GnRH analogs desensitizes the pituitary (chemical castration), results in arrest of gonadotropin secretion, and thereby suppresses ovarian and testicular function.
Lamprey Gonadotropin-releasing hormone (pGlu-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2, lGnRH-III), is a variant of the hypothalamic neurohormone GnRH that has been isolated from the brain of the sea lamprey (Petromyzon marinus). lGnRH-III stimulates the release of estradiol and progesterone in the adult female sea lamprey in similar concentrations to that by lGnRH-I, but has negligible endocrine activity in mammalian systems. Data concerning the superior direct antitumor activity of lGnRH-III were published in the last decade. This ability of lGnRH-III to inhibit proliferation of cancer cells combined with the absence of endocrine activity at the concentrations effective against growth of cancer cells makes it an excellent starting compound for the development of constrained peptide analogues with increased and potentially selective anticancer activity.
In order to study the structure-activity relationship of lGnRH-III on prostate cancer cell proliferation, we synthesized eight new peptide analogues of lGnRH-III with modifications at position 1, 5, 6 and 8. Asp in position 6 of lGnRH-III was substituted by Asn, Asn(OMe), Glu and Gln. $pGlu^1$ was replaced by Glu or Ac-Glu and His^5 and Lys^8 switched places. Cyclopeptides are of great importance both in pharmaceutical and chemical respect. These molecules, with designed sterical hindrance, exhibit often increased biological activity and selectivity. Furthermore, they are more stable in metabolism than the parent linear molecules. Taking into consideration of these potentials we synthesized four cyclic peptides and studied their effect on prostate cancer cell proliferation. Results demonstrate that the side chain of Asp^6 is important for anticancer activity and that modifications in position 1 are well tolerated. On the contrary, replacement of His^5 by Lys and Lys^8 by His reduced the biological effect of the analogues while, 1-8 cyclization led to peptide analogues with enhanced antiproliferative effect.

P339. Abstract number: 244

New analogues of arginine vasopressin and its selected agonists modified at position 2 with (S)-1-adamantyl-glycine

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Since the original synthesis of arginine vasopressin (AVP) in 1954, thousands of analogues of this hormone have been synthesized in the course of extensive investigation of structure - activity relationships. Despite huge efforts in many laboratories, the design of analogues, which are very active either as agonists or antagonists and truly selective for individual receptors, still remains an area of great interest.
Steric restrictions in the N-terminal part of neurohypophyseal hormone analogues might result in a significant change in the interaction of the compounds with AVP and oxytocin (OT) receptors, as expressed by altered biological activities. In this study we present the synthesis and some pharmacological activities of five new analogues of AVP, deamino AVP, [Val^4]AVP, [D-Arg^8]VP and deamino [D-Arg^8]VP, carrying at position 2 a conformationally constrained non-proteinogenic alpha-amino acid, (*S*)-1-adamantyl-glycine (Adg). It should be noticed that the modification, apart from reducing the flexibility, also changed the character of the molecule from aromatic to aliphatic.
All the peptides were tested for their *in vitro* uterotonic and *in vivo* pressor and antidiuretic activities as well as for their affinity to human oxytocin receptor. None of the analogues displayed significant biological activity. A low level of antiuterotonic activity was found in the case of peptide, deamino [Adg^2]AVP with pA_2 = 6.64, while the other compounds showed a slight agonistic activity in the uterotonic test (values ranging from 0.20 to 0.46 IU). With respect to antidiuretic activity, only deamino [Adg^2,D-Arg^8]VP, showed a weak agonism. Regarding the pressor activity, all the analogues were inactive over the concentration range tested. On the basis of these results, we are undertaking further SAR studies using NMR and theoretical molecular modelling methodology.

P340. Abstract number: 246

Interaction of curcumin with a-synuclein and its relationship to curcumin's ability to inhibit fibril deposit

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Lewy bodies and Lewy neurites in the brain constitute the main histopathological features of Parkinson's disease (PD), and are comprised of amyloid-like fibrils composed of a small protein named α-synuclein (AS). As the aggregation of AS in the brain has been implicated as a critical step in the development of the diseases, the current search for disease-modifying drugs is focused on molecules that can act on the process of AS deposition in the brain. Recently, curcumin, a constituent of the Indian spice Turmeric, structurally similar to Congo Red, has been demonstrated to bind Aβ amyloid and prevent further oligomerization of Aβ monomers onto growing amyloid β-sheets. Reasoning that oligomerization kinetics and

mechanism of amyloid formation may be similar in Parkinson's disease and Alzheimer's disease, some authors suggested the use of curcumin to prevent AS aggregation and/or to reduce AS fibrils. Although the mechanisms by which this molecule inhibits Aβ fibril formation and destabilizes preformed fibrils are still unclear, it could be an effective starting template for the development of preventive and therapeutic drugs for PD.
With the aim to unravel the mechanism of action of curcumin, we investigated the interactions of curcumin with wild-type AS by NMR, CD and fluorescence spectroscopies. The chiroptical properties of curcumin make this molecule an ideal compound to perform interaction CD studies both in the far-UV and in the visible region of the spectrum. On the other hand, the presence of four tyrosine residues in the AS sequence provide a useful in-site fluorescence probe for quenching studies. The preliminary results of the studies on the curcumin-AS interactions and curcumin-mediated fibril destabilization will be reported and discussed.

P341. Abstract number: 253

Synthesis, Antiproliferative Activity Prostate Cancer Cells, Enzymatic Stability And Conformational Studies Of New Gnrh Analogues

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Analogues of GnRH, including [DLeu^{6}, desGly^{10}]-GnRH-NHEt (Leuprolide®, commercially available), have been widely used in oncology to induce reversible chemical castration. Several studies have provided evidence that besides their pituitary effects, GnRH analogues may exert direct antiproliferative effects in tumor cells. In order to study the effect of modifications in position 4 and 6 of GnRH on prostate cancer cell proliferation we synthesized twelve new GnRH analogues. To improve enzymatic stability, NMeSer was incorporated in position 4 and the rate of hydrolysis by a-chymotrypsin and subtilisin was investigated. Our results demonstrate that incorporation of NMeSer in position 4 increases significantly the enzymatic stability of analogues compared to their counterparts with Ser^{4}. The antiproliferative effect of the analogues on PC3 and LNCaP prostate cancer cells was similar to that of leuprolide while, we did not observe considerable differentiations on the effect of analogues with Ser or modified Ser. Conformational studies have been performed in an attempt to elucidate structural changes occurring upon substitution of native residues and to study structure-activity relationship for these analogues. The solution models of [DLeu^{6}, desGly^{10}]-GnRH-NHEt (leuprolide), [NMeSer^{4}, DGlu^{6}, desGly^{10}]-GnRH-NHEt, [Glu^{6}, desGly^{10}]-GnRH-NHEt and [DGlu^{6}, desGly^{10}]-GnRH-NHEt peptides have been determined through 2D NMR spectroscopy in DMSO-d6. NMR data provide experimental evidence for the U-turn like structure preserved in all four analogues, which could be characterized as β-hairpin conformation.

P342. Abstract number: 254

Analogues of neurohypophyseal hormones, OT and AVP, conformationally restricted and acylated in the N-terminal part of the molecule

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It is generally accepted that the conformation of the N-terminal part of neurohypophyseal hormones (oxytocin (OT) and arginine vasopressin (AVP)) analogues is important for their pharmacological activity. In this work, we decided to investigate how the substitution of position 2 with bulky cis-1-amino-4-phenylcyclohexane carboxylic acid (cis-Apc) would alter pharmacological properties of OT, [Mpa^{1}]OT and [Cpa^{1}]OT (Mpa = 3-mercaptopropionic acid; Cpa = 1-mercaptocyclohexaneacetic acid). Moreover, we decided to learn how acylation of the N-terminus of [cis-Apc^{2},Val^{4}]AVP with different acyl groups would affect biological potency of the new analogues. The [cis-Apc^{2},Val^{4}]AVP peptide was chosen as a reference compound owing to its interesting pharmacological profile. This analogue turned out to be a potent oxytocin antagonist (pA_2 = 8.22), weak pressor antagonist (pA_2 = 6.85), while its antidiuretic potency was lower than that of AVP but with significantly prolonged action. The new analogues were obtained by acylation of the N-terminus of the peptide with 1-adamantanecarboxylic acid (Aca), 4-tert-butylbenzoic acid (t-Bba), 4-hydroxybenzoic acid (Hba) and 4-aminobenzoic acid (Aba). All the peptides were tested for their *in vitro* uterotonic and *in vivo* pressor and antidiuretic activities in the rat.

P343. Abstract number: 268

Design and functional evaluation of [Cmpi21,aza-β3-Hht23]26RFa(21-26), a pseudopeptidic analog of 26RFa

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26RFa, a neuropeptide of the RFamide family, is the endogenous ligand of an orphan GPCR named GPR103. Consistent with the hypothalamic distribution of both 26RFa and GPR103 mRNAs, intracerebroventricular injection of 26RFa and 26RFa$_{(20-26)}$, the strictly conserved C-terminal heptapeptide (GGFSFRF-NH_2), was found to stimulate food intake in rodents. Moreover, it has been recently shown that GPR103-knockout mice suffer from osteopenia. *In vitro*, 26RFa increases $[Ca^{2+}]_i$ in GPR103-transfected cells with an EC_{50} of 10.2 ± 1.1 nM, while 26RFa$_{(20-26)}$ is about 75 times less potent than 26RFa. In competition experiments, 26RFa displaced [^{125}I]-26RFa with an IC_{50} of 1.26 ± 0.23 nM, whereas 26RFa$_{(20-26)}$ was a poor competitor (IC_{50} = 8730 ± 4330 nM). Molecular modeling under NMR constraints of 26RFa showed that, in DPC micelles, the N-terminal region encompasses an α-helix while the C-terminal region adopts a γ-turn conformation. In the same medium, 26RFa$_{(20-26)}$ exhibits major distortions of this turn that may be responsible for its weak potency. In order to develop potent and physiologically stable ligands of GPR103 with low molecular weight, we have recently designed the pseudopeptide [Cmpi^{21},aza-β^{3}-Hht^{23}]26RFa$_{(21-26)}$, which combines a reduced peptidic bond Ψ^{20,21}[CH_2-NR] and an aza-β^{3} bond Ψ^{22,23}[CO-NH-NR]. In fact, it has been shown that aza-β^{3} residues are γ-turn inducers. *In vitro*, the pseudopeptide [Cmpi^{21},aza-β^{3}-Hht^{23}]26RFa$_{(21-26)}$ was 5 times more potent in displacing [^{125}I]-26RFa than 26RFa$_{(20-26)}$. Moreover, [Cmpi^{21},aza-β^{3}-Hht^{23}]26RFa$_{(21-26)}$ was 10-

fold more potent than $26RFa_{(20-26)}$ in stimulating $[Ca^{2+}]_i$ in GPR103-transfected cells. The half-life time of [Cmpi^{21},aza-β^{3}-Hht^{23}]$26RFa_{(21-26)}$ in human serum was 3 times higher than $26RFa_{(20-26)}$. Finally, intracerebroventricular injection of [Cmpi^{21},aza-β^{3}-Hht^{23}]$26RFa_{(21-26)}$ in food-restricted mice provoked a dose-dependent increase of food intake that lasted longer than that induced by $26RFa_{(20-26)}$. In conclusion, the pseudopeptide [Cmpi^{21},aza-β^{3}-Hht^{23}]$26RFa_{(21-26)}$ is more potent, more stable and exerts a longer lasting effect than $26RFa_{(20-26)}$. This study constitutes an important step towards the development of new GPR103 analogs that could prove useful for the treatment of feeding disorders and/or osteoporosis. *Supported by the LARC-Neurosciences network, FEDER, INSERM (U982), IFRMP23 and the Région Haute-Normandie.*

P344. Abstract number: 275

Side-chain to side-chain cyclization of opioid peptides enhances proteolytic stability of their exocyclic peptide bonds

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The side-chain to side-chain cyclization can significantly affect a proteolytic stability, which is an important factor in designing of the peptide-based drugs [1]. The synthesis and the biological activity of deltorphin and enkephalin analogs restricted by cyclization *via* the urea bridge was described by us previously. The analogs contained a carbonyl bridge, which linked the two side-chain amino groups to form an ureido moiety. Several of these compounds showed a very high δ-receptor agonist potency [2].

Here we present our studies on the proteolytical stability and the degradation products of the cyclic peptides incubated in the presence of chymotrypsin and pepsin. Identification of the degradation products was based on the analysis of the high resolution mass spectra (HR-MS) and tandem mass spectrometry (MS/MS) of the main digestion products.

It appeares that the cyclization *via* the urea bridge increases the resistance of the peptides against the proteolytic digestion by pepsin and chymotrypsin. Notable enhancement of stability to proteolysis was observed not only for the peptide bonds located within the formed ring but also in the N-terminal exocyclic linear peptide segments. The observed stability depends on the ring size but also on the position of the urea bridge within the ring. One of the intriguing observations is the proteolytical stability of a potent opioid, {[H-Tyr-D-Lys(&^{1})-Phe-Dap(&^{2})-NH_2][&^{1}CO&^{2}]}, whereas its isomer with the {[H-Tyr-D-Lys(&^{1})-Phe-Dap(&^{2})-NH_2][&^{1}CO&^{2}]}sequence hydrolyzed rapidly in the presence of chymotrypsin.

A possible explanation of this phenomenon will be presented.

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P345. Abstract number: 290

Tritium labeling of d[Leu4, Lys8]vasopressin

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The pituitary peptide hormon arginine vasopressin (AVP) is a cyclic nonapeptide, which contains an intramolecular S-S bridge. This peptide is responsible for various physiological effects both in human and mammals, including the regulation of the blood pressure, diuresis and the ACTH release. Besides, AVP has multiple effects on the regulation of memory, synaptic transmission, body temperature, anxiety and depression. The physiological effects of AVP are connected with the activation of the G-protein coupled receptors V1a (vascular), V1b (pituitary), V2 (renal) and OT (uterine). For the autoradiographic study of the V1b receptors in rat brain, the V1b receptor selective agonist dezamino-[Leu4, Lys8]vasopressin was prepared in tritium labelled form. Labelling was achieved by the incorporation of tritiated amino acids to the nonapeptide sequence. The labelled amino acid derivatives [3H]Boc-Leu-OH and [3H]Boc-Phe-OH were prepared from Boc-dLeu-OH and Boc-pI-Phe-OH, respectively by catalytic tritium gas reduction. The protected labelled amino acids were introduced to the vasopressin sequence by manual solid-phase peptide synthesis using the in situ neutralization protocol. The Cys side chain was protected with Npys disulfide that was exploited for the intramolecular on-resin disulfide bond formation. The de novo labelling strategy resulted in [3H]dezamino-[Leu4, Lys8]vasopressin with a specific activity of 4107 GBq (111 Ci)/mmol.

P346. Abstract number: 324

VCD spectroscopic analysis of m-opioid peptides.

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The determination of structural requirements for binding of ligands to the m-opioid receptor is a key aspect of the development of novel analgesics. Many natural and synthetic peptides and peptidomimetics were shown previously to bind to the m-opioid receptor with high affinity and selectivity, but there is no consensus about what structure is responsible for such biological activity. Previously, ten well known m-opioid receptor ligands, DAMGO, Tyr-W-MIF-1, morphiceptin, endomorphin-1 and 2 and their analogues, possessing different opioid receptor affinity and selectivity were examined by using molecular dynamics (MD) in aqueous and dimethyl-sulfoxide environment and four structural parameters were found to correlate with receptor binding data. In the present study IR and VCD spectroscopies were applied to determine the solution structure of these molecules. Theoretical simulation of vibrational spectra utilizing DFT quantum chemical calculations was carried out using structural data obtained from previous MD simulations. In this approach, the theoretical spectra is built from spectra calculated for the representative structures of dominant conformational families. The optimal number of such consituent spectra was determined and the effect of explicitly included solvent molecules was investigated as well. Comparison of theoretical and experimental spectra confirmed that the solution conformational ensemble of all molecules consists of turn and extended structures in various ratios. In agreement with previous observations, this suggests that, besides other structural properties, bent backbone structure may be important for m-opioid receptor activity. Furthermore, the strategy applied here could be a useful alternative for solution conformational analysis of short, highly flexible peptides and could help to identify VCD features which are yet unknown.

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P347. Abstract number: 335

Synthesis, Evaluation And Conformational Solution Studies Of Cysteine-Based A4β1 Integrin Ligands

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The integrin family is an extensive group of structurally related receptors for extracellular matrix (ECM) proteins and immunoglobulin superfamily molecules. They are heterodimers consisting of α and *β* subunits. Until now, 18 *α* subunits and 8 *β* subunits have been discovered that form 24 different integrins. Inhibitors of integrin function include function-blocking monoclonal antibodies, peptide antagonists and small molecule peptide mimetics matrix. An integrin with particular interest is α4β1 (very late antigen-4, VLA-4), which is vital for the inflammation process, while it also seems to participate in tumor angiogenesis. The primary ligands for $\alpha_4\beta_1$ are the endothelial surface protein vascular cell adhesion molecule-1 (VCAM) and the extracellular matrix protein fibronectin. A number of cyclic peptide antagonists of $\alpha_4\beta_1$ have been reported in the literature, either based on the LVD or RGD motifs and cyclised through cysteine disulphide linkages. The aim of this research is the development of $\alpha_4\beta_1$ cyclic peptide ligands and their study as inflammation and tumor angiogenesis inhibitors. The synthesized analogues have the basic cyclic sequence X_1-X_2-cyclo(Cys-Asp-Pro-Cys)-COY, where X_1=L- or D-Tyrosine, D-Tyrosine(OEthyl) [D-Tyr(Et)], Salicylic acid (Sal), 1-Naphtylalanine (1-Nal), L-*β*-(2-thienyl)-alanine [Thi], X_2=L- or D-Arginine, Tryptophan, *β*-alanine and Y=OH or NH_2. The analogues were synthesised by Fmoc/But solid phase methodology utilizing Rink Amide MBHA and 2-chlorotrityl-chloride resin to provide C-terminal amide and carboxylic acid, respectively. At present, selected analogues are tested in chick embryo chorioallantoic membrane (CAM) model, while biological experiments will be performed for their potential use as anti-inflammatory agents. Furthermore the conformation of three peptide analogues is studied in solution environment, by NMR spectroscopy techniques and molecular modeling.

P348. Abstract number: 336

Synthesis And Biological Evaluation Of New Linear And Cyclic Analogues Of Neurotensin

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Neurotensin (NT), a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) was first isolated from bovine hypothalamus. NT displays a wide spectrum of biological actions in the central nervous system as well as the periphery of different mammalian species. At central level, NT plays the role of neurotransmitter and/or neuromodulator, whereas in the anterior pituitary it regulates hormone secretion. In addition NT plays an important role in the function of the digestive tract and the cardiovascular system of mammals as well as in the regulation of growth of normal and cancer cells. The plethora of NT actions is mediated through binding to NT receptors (NTRs), which are plasma membrane proteins and belong to the family of G-protein coupled receptors. Binding of NT to NTRs produces a biological effect by activating the Gq-proteins and subsequent stimulation of phosphoinositide hydrolysis. The C-terminal hexapeptide fragment of NT [NT(8-13)], which contains the necessary structural requirements to bind and elicit biological effects at NT receptors, is an obvious lead compound for development. However NT(8-13) is rapidly degraded by peptidases. Therefore, it is important to synthesize functional NT analogues with stabilized bonds against metabolic deactivation. Based on the above, we herein report the synthesis of twelve new linear and cyclic analogues of NT with modifications in the basic structure of the C-terminal part of the molecule needed for function in order to improve the metabolic stability. The analogues contain D-Tyrosine(Ethyl) [D-Tyr(Et)] or D-1-naphtylalanine [1-Nal] in position 11, D-Arginine in position 8 or 9, L-Lysine in position 8 or 9 and 1-[2-(aminophenyl)-2-oxoethyl]-1*H*-pyrrole-2-carboxylic acid (AOPC) in position 8 or 7. They were synthesized by the Fmoc/But solid phase methodology utilizing 2-chlorotrityl chloride resin and they are now being tested for their functional properties.

P349. Abstract number: 337

Influence Of Non Natural Amino Acids At Position 3 Of [Mpa1, D-(Et)Tyr2] or [Mpa1, D-1-Nal2] Oxytocin On Their Pharmacological Properties

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Oxytocin (OT), a physiologically important nonapeptide hormone and neurotransmitter containing a 20-membered tocin ring (from Cys-1 to Cys-6) and an acyclic tripeptide tail (from Pro-7 to GlyNH_2-9), regulates several physiological functions, such as milk ejection, uterine contractions, vascular and cardiac relaxation, interferes with salt and water balance, and is known to play a role in social and reproductive behaviour and emotions. The multiple established and proposed actions of OT are all mediated by one type of OT receptor. The effect on uterine contractions is of major pharmacological importance because OT is the strongest uterotonic agent known and is commonly used in obstetrical practice to speed up labor. The role of OT in preterm labor led to the search for and design of synthetic peptide antagonists as potential tocolytic agents. OT receptor antagonists represent relatively new class of tocolytics under investigation. They afford greater specificity and can be expected to exhibit improved efficacy and risk profiles. The design of new OT

antagonists is based on data from structure-activity studies. Antagonistic activity depends on the configuration and the hydrophobicity of the amino acid at position 2. In continuing our work aimed at the design of selective OT antagonists we investigated the effectiveness of modifications in position 3 by unnatural amino acids. Here we present the synthesis of eleven new [Mpa[1], D-(Et)Tyr[2]]OT or [Mpa[1], D-1-Nal[2]]OT analogues which contain in position 3 the following residues: L- or D-1-naphtylalanine [1-Nal] or L- or D-allylglycine. We also studied the effect of modified *C*-terminal amide on biological potency of the new OT analogues. The peptides were prepared by standard SPPS methods, analyzed by analytical HPLC and their identity confirmed by Electro-spray MS. The analogues were tested for uterotonic activity in the rat uterus *in vitro* test, for pressor activity in the rat pressor assay and for the affinity to human OT receptor.

P350. Abstract number: 340

Molecular Recognition in Amyloid Protein Cross- and Self-Association: Hot Regions of the Abeta-IAPP Cross- and Self-Interaction Interface

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In vivo protein aggregation is linked to cell degeneration and the pathogenesis of a number of fatal cell degenerative diseases including the Alzheimer's disease (AD) and type 2 diabetes (T2D). Increasing evidence supports the suggestion that cross-amyloid interactions may play an important role in the pathogenic self-association pathways of amyloidogenic polypeptides and proteins. Such cross-amyloid interactions include for example the Aβ-α-synuclein, the Aβ-prion protein, and the IAPP-insulin interaction.

The two key amyloid forming polypeptides of AD and T2D, the 40- to 42-residue polypeptide beta-amyloid peptide (Aβ) and the 37-residue islet amyloid polypeptide (IAPP) (T2D), are intrinsically disordered but highly amyloidogenic polypeptides and share a 25% degree of sequence identity and a 50% of sequence similarity. Recently, we have shown that non-fibrillar and non-toxic Aβ and IAPP species bind each other with low nanomolar affinity and that Aβ-IAPP hetero-association suppresses cytotoxic self-association and amyloidogenesis by both peptides (Yan et al., Angew. Chem. Int. Ed. (2007)). Thus, the Aβ-IAPP cross-amyloid interaction might be a molecular link between AD and T2D. In fact, clinical and pathophysiological studies suggest that the two diseases might be linked to each other.

Here we will present our studies on the cross- and self-interaction interface of Aβ and IAPP using synthetic peptide arrays and fluorescently or biotin labeled peptides. We identify short Aβ and IAPP peptide sequences as hot spot regions of the Aβ-IAPP interaction interface or the shortest sequences which are able to bind IAPP or Aβ with affinities in the nano- to low μM range. Most importantly, the identified hot spot regions bind with high affinity both IAPP and Aβ. These results show that the high affinity cross-amyloid interactions between Aβ and IAPP are mediated via their amyloidogenic self-recognition domains (Andreetto et al., Angew. Chem. Int. Ed., (2010)). Our results support the idea that common molecular recognition features underlie both amyloidogenic self- and the non-amyloidogenic hetero-association processes of Aβ, IAPP, and likely other amyloidogenic polypeptides and provide a novel mechanistic basis for understanding protein interactions involved in amyloid disease and toward developing compounds to modulate these processes.

P351. Abstract number: 376

Conformational behaviour of vasopressin-like peptides in the membrane-mimetic environment

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It is believed that lipids are implicated in interactions of peptide hormones with their membrane receptors. These interactions, through the change of peptide conformation, is likely to facilitate the entry of the hormone into the microenvironment of the receptor. For this reason, in the present study, we made a combined experimental and computational approach to learn how vasopressin-like peptides interact with a dodecylphosphocholine (DPC) micelle. The micelle simulates eukaryotic cell membranes, which are generally rich in zwitterionic phospholipids.

We studied the structures and interactions of a variety of vasopressin analogues modified with bulky aromatic residues at position 2 with membrane mimic by concerted use of NMR and restrained time-averaged molecular dynamics simulation techniques employing an explicit (united atom) DPC micelle model. Molecular dynamics simulations were carried out with the parm99 force field in the AMBER9.0 package. The analysis of conformational properties and interactions of the peptide with the membrane surrogate provides a basis for understanding the mechanism of interactions of vasopressin-like hormones with their receptors.

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

P352. Abstract number: 411

A complete substitutional analysis of GLP-1 leads to potent and proteolytically stable analogues

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One of the major drawbacks of peptides is their short half-lives in vivo were they are rapidly degraded by proteases. Thus, measures to stabilize peptides towards proteolysis are needed while still maintaining the peptide efficacy.

Herein we present a complete substitutional analysis of GLP-1 based on the well-established method of automated SPOT peptide synthesis (Frank R, *Tetrahedron* **1992**, *48*, 9217-9232.). By anchoring target peptides via a photolabile linker or an ester linkage to the cellulose support in 96-array format, peptide analogues can be assayed in solution using a high-throughput functional assay thus allowing for fast generation of structure-activity relationships. Furthermore, by submitting fluorescently labelled, overlapping fragments of the target peptide to purified enzymes detailed information about degradation patterns can be obtained.

In combination, these techniques allows for the rapid generation of new lead structures.

P353. Abstract number: 453

M1154 - A novel galanin ligand to delineate the galaninergic system

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The galanin family currently consists of four members, namely galanin, galanin-message-associated peptide

(GMAP), galanin-like peptide (GALP) and alarin. The galanin peptide family and its three receptors (GalR1-3) have with compelling evidence been implicated in a variety of human disorders. The co-localization with other neuromodulators and the distinct up-regulation during and after pathological disturbances has drawn attention to this neuropeptide family. Here we present data on receptor binding and preliminary data in functional response for the novel M1154 peptide, including a comparison with several GalR2 selective peptides. M1154 retains high affinity binding to GalR1 and GalR2 although it has no detectable binding to GalR3. We believe that utilizing this selective galanin ligand together with previous published M1145 raise the possibility to delineate the galanin system.

P354. Abstract number: 463

Oxytocin Agonist Design - A selenocysteine mimetic reveals a functional selectivity switch for the human oxytocin receptor

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Oxytocin (OT) and vasopressin (AVP) are closely related, highly conserved, multifunctional nonapeptides with a N-terminal cyclic 6-residue ring structure stabilized by an intramolecular disulfide bond, and a flexible C-terminal 3-residue tail. They act via one oxytocin receptor (OTR) and three vasopressin receptors (V1aR, V1bR, V2R), which are all members of the G-protein-coupled receptor (GPCR) family. The structural similarity of OT and AVP combined with the high extracellular receptor homology results in significant cross talk, and physiological function is essentially controlled by receptor up/down regulation, enzymes, plasma half-life, controlled release mechanism and receptor location rather than by ligand selectivity. The development of therapeutics and diagnostic tools however requires a high degree of ligand selectivity to avoid side effects and to delineate individual receptor function. Although many analogues have been synthesized, there remains a shortage of receptor selective agonists and antagonists, in particular for the human receptors.
This work illustrates the impact of C-terminal and disulfide bond modifications of OT on activity, plasma stability and receptor selectivity. It also describes the novel chemistry developed for this study, including on-resin thioether formation and new cysteine/selenocysteine protecting groups. The lead compound identified has increased stability and is a highly selective and potent peptide agonist for the human OTR. This ligand should not only facilitate future OTR studies, but also shows great promise for therapeutic development.

P355. Abstract number: 469

Design, synthesis and biological evaluation of novel endomorphins with multiply structural modifications

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Endomorphins (Tyr-Pr-Trp/Phe-Phe-NH2) are μ-opioid agonists with significant analgetic acivity.1 14 new analogues were designed and synthesized. In all cases two or three amino acids were substituted with unnatural amino acids in order to obtain proteolytically stable, pharmacologically active compounds, and on the other hand to investigate the mode of receptor-ligand interactions favourable for opioid binding. On he basis of our earlier works2 dimethyltyrosine (Dmt), 2-amino-cyclohexanecarboxylic acid (Achc), (2S,3S)beta MePhe and p-FPhe were used for the substitution. SPPS was used for the peptide synthesis. Radioreceptor assays using rat brain membrane preparation, 3H-DAMGO and 3H-Ile5,6-deltorphin-2 as selective opioid ligands and [35S]GTPgamma S binding were done for biological evaluation. In consistent with earlier findings, the derivatives manifested low to high binding potencies, selectivities and efficacies (all retained agonist properties) depending upon the chiralities of the incorporated amino acids and their positions in the structure. The most effective analogue was Dmt-(1S,2R)Achc-Phe-pFPhe-NH2 with subnanomole affinity and high selectivity to μ-opioid receptor and very stable against proteolytic enzymes.

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P356. Abstract number: 558

A neuropeptidomics study of the bovine hypothalamus reveals novel endogenous peptides and processing pathways.

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The hypothalamus is the central regulatory region of the brain that links the nervous system to the endocrine system via the pituitary gland. It synthesises and secretes neuropeptide hormones which in turn act to stimulate or inhibit the secretion of pituitary hormones. In our laboratory, we have undertaken a detailed investigation of the peptide profile of the bovine hypothalamus after thermal stabilisation (using a novel tissue stabilizing system) to inhibit post-mortem degradation. Optimisation of acquisition conditions specific to the study of endogenous peptides, combined with iterative and comprehensive database searching strategies, uncovered neuropeptides that would otherwise have remained undetected. The untreated samples contained large numbers of protein degradation products that interfered with the analysis of the neuropeptides. In the treated samples, we identified whole suites of neuropeptides that are known to be expressed in the bovine hypothalamus. Furthermore, we have characterised a range of post-translational modifications that are indicative that we have correctly processed mature neuropeptides in the stabilized tissue samples, whereas we detected many trimmed or truncated peptides resulting from post-mortem degradation in the untreated tissue samples. Altogether, using this optimised workflow, we were able to detect 118 bovine neuropeptides, some of which we report the first spectral evidence for and many of which that have not been annotated in databases to date. The identification of a range of novel neuropeptides and the characterisation of post-translational modifications give clues about non-conventional prohormone processing potentially involving a family of carboxypeptidase enzymes.

P357. Abstract number: 53

Implications of replacement of amino acids at positions B24 and B26 of insulin by the non-aromatic residues

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Insulin is a polypeptide hormone with a broad impact on a wide range of biochemical processes in the organism; especially on the lowering of plasma glucose levels and lipid and protein metabolism. Two main requirements are necessary for a correct accomplishment of the insulin mission: the ability of the hormone to bind to the insulin receptor (IR) and the ability of the IR to translate to insulin binding into signaling processes in the cell. Insulin consists of 21 amino acids A-chain of and 30 amino acids B-chain, covalently linked by two disulphides. Insulin is released from pancreatic cells to the bloodstream as a hexamer, which then splits into dimers and monomers depending on the insulin concentration. The insulin is active only as a monomer.

Our study is focused on two amino acids from C-terminus of the B chain: phenylalanine B24 and tyrosine B26. The aromatic triplet PheB24-PheB25-TyrB26 belongs to invariant residues and it is probably the first place of the contact of insulin with the receptor. Each of these residues provides its own distinct contribution towards insulin aggregates and insulin - receptor interactions. The PheB24 and TyrB26 are involved in dimer interface; contributing towards four hydrogen bonds that form antiparallel β-sheet between insulin molecules. Although non-direct interactions between PheB24 and TyrB26 with receptor are presumed, they play an important role in binding affinity.

Using enzymatic semisynthesis, we prepared eleven new insulin analogues. Three analogues were prepared with the PheB24- and four analogues with TyrB26 substitutions. Amino acids with the envisaged impact on the main chain structure were selected here: Pro, D-Pro, Sar and Gly. PheB24 was also replaced by His and D-His as these insertions yielded analogues with interesting properties (1-3). Additionally, we also prepared of three novel analogues with simultaneous substitutions at positions B24 and B26. Binding affinities of all analogues will be presented and discussed.

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P358. Abstract number: 54

Binding affinities of insulin analogues substituted at the position B26 with glutamine, asparagine and aspartic acid.

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Insulin regulates blood glucose levels with a widespread impact on lipid and protein metabolism. It is a molecule of major therapeutic importance in the treatment of diabetes. The mature form of insulin has a B chain (PheB1-ThrB30) and an A chain (GlyA1-AsnA21), stabilized by three disulphide bonds. Insulin's metabolic actions are expressed through binding as a monomer to the insulin receptor (IR). The assumed place of the first contact of insulin with IR is located to the C-terminus of the B chain, particularly to the aromatic triplet PheB24-PheB25-TyrB26. These amino acids belong to invariant residues and each of them provides its own distinct contribution to insulin-insulin and insulin-IR interactions. PheB24 and TyrB26 are involved in monomer-monomer contacts in the insulin dimer; four hydrogen bonds form antiparallel β-sheet between insulin molecules. Although position B26 of insulin is probably not involved in direct interaction with the insulin receptor, replacement of the TyrB26 with various amino acids resulted in full-length analogues with binding affinities ranging from less active [D-ProB26]-insulin (0.9%) (see our second abstract) to more potent [GluB26]-insulin (125%) (1). To our knowledge, [GluB26]-insulin is the only full-length analogue substituted at the position B26 with enhanced binding affinity compared with human insulin (100%). Moreover, some shortened B26-substituted analogues are "superpotent"; [D-AlaB26]-DTI-NH2 (1250%) (2) or [SarB26]-DTI-NH2 (1100%) (3).

The present study deals with the B26 position in full-length insulin analogues. For the design of new analogues we were inspired by [GluB26]-insulin; B26-position was substituted with glutamine, asparagine, and aspartic acid. Binding affinities of the analogues will be presented and discussed.

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P359. Abstract number: 61

Peptide-peptide recognition of a-helices to dissect the mechanism of docking and fusion using SNARE derived peptides

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SNARE-proteins catalyze the fusion of membranes in all intracellular membrane fusion reactions except mitochondrial fusion. Membranes destined to fuse contain complementary sets of SNARE proteins each containing a conserved stretch of 60-70 amino acids (SNARE motif) that is usually connected to a *C*-terminal transmembrane domain via a short linker domain. Upon membrane contact, the SNARE motifs assemble into a stable four-helix coiled-coil recognition complex combining three helices of the membranes (t-SNAREs) with a single helix from the other membrane (v-SNARE).[1, 2] However, it is not clear yet whether fusion is caused by force exerted on the membranes (transmitted to the transmembrane domain by a stiff linker[3]) or via other mechanisms. We attempt to clarify these biological questions with a chemical approach replacing the SNARE motifs of neuronal SNARE proteins by an artificial recognition unit. The well-known coiled-coil forming peptides $(E\text{-}I\text{-}A\text{-}A\text{-}L\text{-}E\text{-}K)_3$ and $(K\text{-}I\text{-}A\text{-}A\text{-}L\text{-}K\text{-}E)_3$ were chosen as recognition unit.[4] The corresponding artificial SNARE biooligomers were synthesized using microwave-assisted Fmoc-SPPS. Fusion experiments with vesicles containing these artificial SNARE biooligomers and various lipid compositions showed in vitro fusion

activities with comparable kinetics to those of neuronal SNARE proteins. Control experiments with the coiled-coil forming peptides as soluble additives showed no or at least reduced fusion activity with respect to their excess. Further experiments are to be carried out to investigate the mechanism of the fusion process.

Financial support by the Deutsche Forschungsgemeinschaft (SFB 803) is gratefully acknowledged.

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P360. Abstract number: 75

Peptide inhibitors of HIV-1 integrase: from mechanistic studies to improved lead compounds

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One of the most important steps in the replication cycle of HIV-1 is the integration of the viral DNA into the host cell genome. This step is catalyzed by the viral enzyme integrase (IN), making it an attractive target for the development of anti-HIV drugs.

We previously developed peptides that inhibit IN in vitro and as well as HIV-1 replication in cells. In this work we focused on one of these peptides, IN1 and studied its mechanism of IN inhibition as a basis for improving its activity. Here we present the design, synthesis and evaluation of several derivatives of the 20-mer IN1. The peptide corresponding to the N-terminal half of IN1 (IN1 1-10) was easier to synthesize and much more soluble than the original IN1. IN1 1-10 bound IN with improved affinity and inhibited IN activity in vitro and HIV replication and integration in infected cells. While IN1 bound the IN tetramer, its shorter derivatives bound dimeric IN. Mapping the peptide binding sites in IN provided a model that might explain this difference. We conclude that IN1 1-10 is an improved lead compound for further development of IN inhibitors.

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P361. Abstract number: 97

Antibodies Against Synthetic Peptides For Detection Of Anti-Apoptotic Proteins Survivin And Nucleophosmin In Tumor Cells

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Several proteins such as survivin and nucleophosmin, are known to inhibit apoptosis in tumor cells. These proteins play an important role in a tumor cells, because their quantity, intracellular localization and the structure status are changed during cells malignization. Detailed characteristics of these factors are necessary for differential diagnostics of tumor diseases and for an optimal radio- and chemotherapy scheme selection. There are no antibodies which would be able to specifically detect different forms of these proteins. Recent data show that not only the significant increase in the amount of these proteins but also their new forms are detected within the tumor cells. New forms of the proteins result from changes either on the genetic or protein level. Changes in monomer-oligomer state of both survivin and nucleophosmin are detected. At the same time there are no antibodies specific to different forms of the proteins.

More than 20 peptides were synthesized to raise antibodies against tumor-specific forms of nucleophosmin and survivin. Peptides were chosen from immunoactive fragments of survivin 2B, nucleophosmin isoform NPM1.1 and its mutated forms: NPMm A and NPMm '. Rabbits have been immunized with synthetic peptides either in a free non-conjugated state or conjugated with a protein carrier. BrCN-activated Sepharose 6B conjugated with a corresponding peptide was used for affinity purification of anti-peptide antibodies. Using ELISA, immunoblotting, immunocyto- and immunogistochemical methods we studied the ability of the affinity purified antibodies to detect different forms of nucleophosmin and survivin in tumor cell cultures, lymphoid cells from patients with hematopoietic malignancies cancer and in tissue samples of different solid tumors. Specific anti-peptide antibodies detecting either nuclear or cytoplasmic survivin have been obtained. Correlation between a quantity of survivin in tumor and the degree of malignanancy in breast cancer samples has been established. Investigation of 40 tissue samples of bladder cancer allowed to reveale the correlation between high concentration of survivin and a low degree of tumor cells differentiation.. Therefore, the obtained antibodies can be applied for development of new approaches to tumor diagnostics development and to increase the effectiveness of the antitumor therapy.

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P362. Abstract number: 120

Using peptides to study the protein-protein interactions involved in Agrobacterium infection in plant cells

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The plant pathogen Agrobacterium is able to genetically transform several plant species. This is the only known event of trans-kingdom DNA transfer in Nature. This way, Agrobacterium induces a tumor growth on the plant (known as 'Crown Gall' disease), creating a favorable environment for its growth. Agrobacterium transfers its DNA to the infected plant by using a T-plasmid, which contains a T-DNA sequence that is integrated into the host cell genome. The T-DNA is enveloped by the bacterial VirE2 protein, which interacts with the plant VIP1 protein. In addition, VIP1 interacts with numerous other proteins, both of the bacterium and of the plant. VIP1 contains a nuclear localization signal (NLS), and by interaction with importin alpha, it directs the whole complex of T-DNA and associated proteins to the cell nucleus where integration into the host genome takes place.

Despite its importance, no structural or quantitative information is available regarding VIP1 or its interactions with other proteins. Our research aim was to characterize

these interactions in detail, in order to gain insight into the mechanism of the Agrobacterium infection and its DNA integration. We developed a protocol for expression and purification of the VIP1 protein. To study the VIP1 interactions, we designed a peptide array containing partly overlapping peptides derived from bacterial and plant VIP1-binding proteins. Screening the array for VIP1 binding resulted in selection of several peptides corresponding to the VIP1-binding domains of these proteins. The peptides were synthesized and their VIP1 binding properties were quantified using fluorescence anisotropy. The peptides penetrated cells, which allowed us to study their effect on the intracellular VIP1-VirE2 and VIP1-importin alpha interactions.

P363. Abstract number: 134

The oligomerization equilibrium of p53 is modulated by peptides that bind its C-terminal domain

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p53 is a homotetrameric protein that has a pivotal role in the cellular anti cancer defense mechanism. It is a transcription factor that is active in the nucleus as a tetramer, but is inactive in its dimeric and monomeric forms. Oligomerization of p53 is mediated by a structurally independent tetramerization domain (residues 326-355). Stabilizing the p53 tetramer is an important goal in anti-cancer therapy, since it could result in activated p53 that can cause increased apoptosis of cancer cells.

In our lab we recently developed the Shiftides approach, in which short peptides are used to modulate the oligomerization equilibrium of proteins (Hayouka et al., PNAS 2007). Here we implemented this approach to discover p53-activating shiftides that will stabilize the p53 tetramer. We screened an array of peptides derived from proteins known to bind the p53 C-terminal domain (p53CTD, residues 293-393) for binding to recombinantly expressed p53CTD. We identified 10 peptides that bound p53CTD, and quantitatively characterized their binding to p53CTD using fluorescence anisotropy. All peptides showed binding in the micromolar range. We tested the effect of the peptides on the oligomerization equilibrium of full length p53 using fluorescence monitored analytical ultracentrifugation sedimentation rate analysis (Rajagopalan et al., Nucleic Acids Res. 2008; Brandt et al., BMC Genomics. 2009). Several peptides shifted the oligomerization equilibrium of p53 towards the tetramer and other high order oligomeric species. The peptides that stabilize the tetramer may serve as basis for the development of anti-cancer compounds that activate p53 by stabilizing the active tetramer in a shiftide mechanism.

P364. Abstract number: 135

Affinity binding studies of a-synuclein and anti-a-synuclein antibody by online combination of SAW biosensor and ESI-MS

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The surface acoustic waves (SAW) biosensor is a newly developed method able to detect the bioaffinity interaction between two molecular species [1]. Here we describe the combination of SAW biosensor and electrospray ionisation mass spectrometry (ESI-MS), as a highly sensitive technique recently developed in our laboratory [2]. In the present work, this method has been applied for the characterisation of several antigen- antibody systems related to Parkinson's disease. The anti-α-synuclein specific antibodies (200 nM, 150 µl) were covalently immobilised on the gold coated surface of the quartz chip, using as a linker a self-assembled monolayer (SAM) of 16-mercaptohexadecanoic acid. The interactions with α-synuclein (α-Syn), α-Syn (59-80), (1-23), (61-140), (96-140) peptide antigens were determined. Further, the affinity bound antigens were eluted under acidic conditions (pH 2) and analysed by ESI mass spectrometry using an intermediate sample desalting and concentration step on a guard column. Molecular interaction kinetics between α-Syn (10 µM) immobilised on a gold chip and different concentrations of anti-α-synuclein mono/ polyclonal antibodies, ranging from 5 to 200 nM, were determined by SAW biosensor. The obtained KD values are in the low nano-molar range. The bioaffinities were comparatively investigated by Dot Blot, Elisa, SAW biosensor and SAW-ESI MS, and the results were found to be in good agreement.

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P365. Abstract number: 138

Dengue Virus Infection and Assembly - Unravelling the Role of the Capsid Protein

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Aim: Dengue virus (DV), a member of the Flavivirus family, is responsible for around 100 million infections worldwide. Although some models have been proposed to explain its infection mechanism, this process remains unclear. Recent studies point to the capsid protein (DenC) participation in the viral assembly and infection. This is supported by the identification of two distinct domains in DenC, one highly positively charged and other highly hydrophobic that may interact with the viral genome and with the host membrane, respectively. With this work we intend to study the interaction of DenC with lipid bilayers, using large membrane vesicles (LUV) as biomembrane models, in the presence of nucleotides. For this purpose we studied two peptides (Peptide R and Peptide M) derived, respectively, from the DenC RNA-affinity domain and membrane-affinity domain.

Materials and Methods: Dynamic Light Scattering (DLS) and ζ-potential results on negatively charged membranes (POPC:POPG) show LUV aggregation by Peptide R and membrane interaction is predominantly electrostatic. Vesicle aggregation by Peptide M is slower and hydrophobic interactions govern its interaction with membranes. DLS results with oligonucleotides showed the formation of high affinity supramolecular complexes of Peptide R with DNA and less with Peptide M.

A model was developed to assess the interaction of each peptide in a ternary system (Peptide+nucleotide+membrane) by Fluorescence Spectroscopy techniques [1][2]. This model determines the membrane partition coefficient (Kp,c) of the complex PeptideR/M+nucleotide. It is considered that there is an aqueous equilibrium of the peptide and the nucleotide with a binding constant Kb, determined by Life Time Fluorescence quenching. A double partition can occur (one

by the free peptide (Kp) and other by the complex (Kp,c)) which was observed on LUVs probed with a membrane potential-sensitive probe, DI-8-ANNEPS.

P366. Abstract number: 139
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P367. Abstract number: 145
Iap-Based Peptide Inhibitors Of Apoptosis
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The aim of this study was to detect the binding sites of IAP with caspase-7 and caspase-9, to predict and to synthesize their peptide analogs, to estimate their ability to inhibit apoptosis.
To perform computer modelling of the binding sites the proteins from IAP family with caspases, the following spatial structures were selected from the ProteinDataBank (PDB; http://www.pdb.org/pdb/home/home.do): 3D9T - caspase-9/IAP complex; 1KMC, 1I51, 1I40 and 1NW9 - caspase-7/IAP complex; 2AR9 - caspase-9 dimer; 2QL9, 2QLB and 2QL5 - caspase-7 dimer.
For detection the binding sites of proteins we have developed a program complex which allows to build a molecular protein surfaces, to detect cavities on the protein surface and to carry out the analysis of hydrophobic and hydrophilic properties of detected cavities. With the help of the developed by us program complex and using free software BLAST and ALIGN from UniProt consortium (http://www.uniprot.org/) for alignment of primary sequences, binding sites of IAP with caspase-7 and caspase-9 were modelled in silico. The following peptides were proposed, which model the inhibitory function of IAP: Asp-Gln-Gly-Arg-Ser-Thr for caspase-7 and Asp-Glu-Gly-Arg-Trp for caspase-9.
The peptides were synthesized by classical methods in solution using hydrogen-labile protection of side functional groups and Boc-protection for α-aminogroups by the subsequent elongation of the peptide chain starting from the C-end. The C-end amino acids were introduced in the synthesis in the form of methyl esters, C-end fragments were coupled with the Boc-derivatives of the corresponding amino acids. Diisopropylcarbodiimide with the addition of N-hydroxybenzotriazole was applied as the coupling agent. The target peptides Asp-Gln-Gly-Arg-Ser-Thr and Asp-Glu-Gly-Arg-Trp were identified by quantitative amino acid analysis and mass-spectrometry. Their homogeneity was confirmed by analytical HPLC.

The ability of peptides to inhibit apoptosis was demonstrated using glioma C6 cells as a model. The activity of caspases 7 and 9 was detected using chromogenic substrates Ac-Asp-Gln-Thr-Asp-pNA and Ac-Leu-Glu-His-Asp-pNA, accordingly.

P368. Abstract number: 191
Structural studies of Neutrophil Elastase-dependent Host Defence Peptides
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Peptides with broad-spectrum antimicrobial activities play a crucial role for developing drugs for variety or diseases. In particular Host Defence peptides related to Neutrophil Elastase have excited interest as candidates for development into therapeutically valuable antimicrobial and anti-inflammatory agents. However, their use as an effective agent is restricted due to various known and unknown chemical and biological factors. In order to understand the structural basis for their difference in biological activity, authors have investigated the solution NMR structure of peptide D-P18 and its propeptide, E4-A3-D-P18, by NMR spectroscopy and molecular modelling in aqueous conditions and in TFE-water mixed solvent systems. In aqueous solution conditions, both peptides lack formation of secondary structural character. In a 50% TFE-water mixed solvent system, D-P18 peptide is characterised by a left-handed short α-helical conformation between residues Ile8-Leu14 whereas the propeptide, E4-A3-D-P18, adopts a long extended α-helical conformation between residues Lys8-Ile15 and Lys17-Lys24. The data provide the basis for the design of further analogues of the D-P18 peptide with improved therapeutic properties. This structural study has demonstrated that the greatly reduced haemolytic activity of the propeptide analogue, E4-A3-D-P18, compared with the D-P18 peptide is possibly a consequence of the increased α-helicity of the analogue as well as the concomitant decrease in hydrophobic character.
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P369. Abstract number: 196

N-linked homodimers of the kinin B1 receptor antagonist R-715

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The kinin B1 receptor (B1R) may play an important role in pathological conditions. We previously developed a highly potent and selective kinin B1R antagonist, R-715 (Ac-Lys-[D-βNal7, Ile8]desArg9-bradykinin). However, R-715 is only partially resistant against enzyme degradation, which may explain its relatively short half life in vivo. It is contemplated that covalent peptide dimers may improve stability, circulating half-life and make more potent therapeutics than monomers. With this in mind, we designed and synthesized on solid phase N-linked homodimers of R-715 comprising linkers with different acyl-chain lengths such as 6 (adipoyl) (NG2049), 8 (suberyl) (NG2035), 10 (sebacoyl) (NG2050) and 12 carbons (dodecanedioyl) (NG2051). Synthesized homodimers were cleaved from the resin and purified by HPLC. Peptides identities were confirmed by mass spectrometry. We initially determined the antagonistic potencies (pA2 value) of the synthesized homodimers by using rabbit aortic strip contraction assays. Results showed that all dimeric analogues of R-715 impaired the agonist-induced contractile activity although not to the same extent. In comparison with the parent monomer antagonist R-715 (pA2: 8.40±0.12), the peptide NG2049 (pA2: 8.43±0.17) and NG2035 (pA2: 8.66±0.19) maintained similar inhibitory effects while peptides NG2050 (pA2: 7.69±0.14) and NG2051 (pA2: 7.75±0.12) showed reduced potencies. We concluded that the optimum length of spacer arms of N-terminal dimeric R-715 analogues for B1R antagonism is with acyl chains of 6 and 8 carbons, which corresponds to a maximal length of 7.47 Å and 9.79 Å. Further studies are needed to test whether the strategy of R-715 dimerization translates to greater potency and long-lasting in vivo activity.

P370. Abstract number: 234

Design and Synthesis of Novel Peptidic Apoptosis Mimetics

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Apoptosis or programmed cell death is a critical process in both development and homeostasis of multicellular organisms. In appropriate regulation of apoptosis is associated with many human diseases, such as cancer. Targeting critical apoptosis regulators is an attractive strategy for the development of new classes of therapies for the treatment of cancer and other human diseases. Caspases are a family of cysteine proteases, which play essential roles in apoptosis. Meanwhile, Smac peptides promote at least in part through antagonizing inhibitor of apoptosis proteins (IAPs) including XIAP. Apoptosis is promoted by the release of Smac from the mitochondria into the cytosol, where it interacts with multiple IAPs and relieves their inhibitory effect on caspases. Studies have shown that Smac promotes the catalytic activity of Caspace for the same binding pocket on the surface of the BIR3 domain. Molecular studies have shown that some decapeptides could affect the apoptosis pathway. In this work, some decapeptides were synthesized using solid phase peptide synthesis method, and their anti-cancer activity was investigated. The details about the active sites of the synthesized peptides will be discussed.

P371. Abstract number: 245

Synthesis and comparison of new citrullinated epitopes the early diagnosis of rheumatoid arthritis

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Anti-citrullinated protein antibodies (ACPA) are sensitive and specific markers for diagnosis and prognosis in rheumatoid arthritis (RA). Citrullination is a post-translational modification of arginine by deimination, induced by peptidylarginine deiminase (PAD). Citrullination has been observed in different synovial proteins, including fibrinogen, filaggrin, vimentin and collagen. Antibodies specific for cyclic citrullinated filaggrin peptides were detected in RA sera and anti-CCP positivity is widely used for diagnostic purposes. However, to determine the ACPA-reacting epitopes on vimentin and new epitopes on filaggrin would be useful in the diagnosis of anti-CCP2 seronegative patients.

Our aim was to develop new tools for the detection of ACPA and thus for the early diagnosis of RA by the use of clearly defined epitopes on filaggrin, vimentin and collagen.

First we used conventional solid-phase peptide synthesis (Fmoc strategy) carried out on ”MULTIPIN NCP”(Chiron Mimotopes Peptide System) non-cleavable kit. Citrullinated peptides and the unmodified counterparts containing arginine were synthesized on the pins in order to compare their respective reactivities. We have used these peptides-on-pins in an ”indirect”ELISA and ACPA was determined in the sera of RA and non-RA patients using anti-IgG plus IgM secondary antibodies. Comparing short sequences of filaggrin, vimentin and collagen we have found that only two 5-mer and a 19-mer citrullinated filaggrin peptides were recognized by the RA sera, with a sensitivity and specificity that was comparable with the currently used tests. To validate the data obtained by multi-pin ELISA, selected peptides were synthesized in a C- or N-terminally biotinylated form by SPPS, according to Fmoc/tBu strategy. These peptides were fixed to ELISA plates pre-coated with neutravidin. The RA sera samples specifically recognized the C terminally biotinylated filaggrin peptides, while showed no reaction with the N-terminally biotinylated ones. We could not find any correlation between the reactivity profile of the 5-mer peptide and the values of anti-CCP test, suggesting that the 5-mer C-terminally biotinylated filaggrin peptide represents a new epitope recognized by ACPA.

Identification of new filaggrin epitopes could be important for the development of novel diagnostic tools for RA.

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P372. Abstract number: 273

Structure-Activity Relationships of hNPFF2 Receptor

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The G protein coupled $NPFF_{1/2}$ receptors were discovered in 2000/2001 [1]. They belong to an opioid-modulatory system, interact with the inhibitory G_{α} protein and are stimulated by neuropeptide FF (NPFF, **F**LFQPQR**F**-NH_2). Three additional peptides with related sequences are known in humans: NPAF, NPVF and NPSF. All peptides exhibit a conserved structure, the RFamide peptide motif and are $NPFF_{1/2}$ receptor ligands with different nanomolar affinities [2].

As all RFamide peptides contain the RFamide motif, the contribution of Arg^7 of the NPFF for ligand affinity and the role of Phe^8 of the RFamide motif has been investigated.

Accordingly, the cDNA of human $NPFF_2$ receptor was cloned into an eukaryotic expression vector. Furthermore, NPFF and several analogues at positions of the Arg^7 or Phe^8 were synthesised by solid phase peptide synthesis. Scores of artificial amino acids with differences in charge, aromaticity, length, or size of the side chain as well as backbone modifications were used to identify the structure-activity relationships. To investigate the ligand receptor interaction, an IP_3 signal transduction assay was performed, applying a chimeric $G_{\alpha qi4}$ protein. We could confirm that Arg^7 and Phe^8 of the NPFF play a major role in ligand binding to the $hNPFF_2$ receptor and its signal transduction. Our results indicate on a molecular level, which ligand modifications have an impact on binding affinity. Especially for the Arg^7 solely minor alterations were tolerated and Phe^8 seems to interact with its distinct size and length more than with its aromaticity.

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P373. Abstract number: 309

4-Methylpseudoproline analogues of cyclolinopeptide A: synthesis, conformation and biology

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Pseudoprolines (ψpro, 1,3-oxazolidines and 1,3-thiazolidines) derived from Ser, Thr or Cys, have been applied as a versatile tool in a structure-activity relationship studies due to enhanced content of *cis* isomer, depending on stereochemistry and degree of substitution at the C-2 atom position of the proline ring [1].

4-Alkylpseudoprolines derived from α-alkyl-α-hydroxymethyl amino acids possess alkyl substituent at the C-4 atom of the ring which changes their chemical and conformational properties in comparison to pseudoprolines derived from serine or threonine [2].

Now we present synthesis, conformation and biological activity of new analogues of cyclolinopeptide A (CLA), containing 4-methylpseudoproline instead of proline residues in position 6 or 7. It is known that immunosuppressive activity of CLA, comparable with cyclosporine A, is connected to the presence of the tetrapeptide Pro-Pro-Phe-Phe fragment containing Pro-Pro *cis* amide bond [3].

The linear precursors of modified CLA analogues were prepared manually by standard solid-phase procedure ”step by step”on Wang resin. N-Fmoc derivative of 4-methylpseudoproline was obtained by one pot cyclocondensation of the corresponding α-methylserine with formaldehyde and N-(9-fluorenylmethoxycarbonyloxy)succinimide in alkaline solution. The NH-group in 4-methylpseudoproline residue was acylated ('difficult coupling') by using BTC (*bis*-(trichlomethyl)carbonate). The cyclization of linear precursors has been made in solution using EDC/HOBt as a coupling reagents.

The compounds strongly suppressed mitogen-induced proliferation of T and B cells to a similar degree as well as the humoral immune response *in vitro*. The suppression was comparable to that of cyclosporine A at 10-100 μg/ml concentration. A moderate toxicity with regard to mouse and human lymphocytes, comparable to that of unmodified peptide - CLA together with a strong antiproliferative properties suggest their potential application in prevention of graft rejection and limitation of tumor growth.

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P374. Abstract number: 314

Design, synthesis and stability studies of potent inhibitors of pro-protein convertases (PCs).

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Pro-protein convertases (PCs) are a mammalian family of nine serine endoproteases responsible for post-translational processing of inactive precursors of many regulatory proteins. Seven structurally related PCs, known as furin, PACE4, PC1/3, PC2, PC4, PC5/6 and PC7 cleave various precursors at the motif $(K/R)-(X)_n-(K/R)'$. Two additional members, namely SKI-1 and PCSK9 are implicated in cholesterol and lipid metabolism. During the past few years, several important pathologies have been linked with PC-like activity such as cancer, viral and bacterial infections, neurodegenerative disorders, diabetes, and atherosclerosis. Thus, PCs inhibitors might represent potential drugs for the treatment of these diseases. The aim is to obtain specific inhibitors for individual PCs because not all of them are implicated in a given disease state. Many peptide inhibitors have been investigated such as those derived from PC prodomains, endogenous inhibitors of PC2, PC1/3 or identified from a combinatorial library. However, the development of peptides as clinically useful drugs is greatly limited by their poor metabolic stability and low bioavailability. Recently, our group has developed a relatively specific inhibitor of PACE4, PC5/6 and PC7. The introduction of multi-Leu residues in the P8-P5 positions made the peptide more selective for these enzymes in comparison to Furin and PC2. This inhibitor has potent effects on cell proliferation and tumor progression, especially in cell models of prostate cancer. However, further stabilization of the structure is necessary. Thus the aim of this work was to introduce a series of chemical modifications in the potent PC inhibitor

AcLLLLRVKR-*NH2* . Several approaches including the use of D-amino acids, unusual amino acids, click chemistry have been used to improve the pharmacokinetic properties of the model peptide. In the present study, we report synthesis of new peptides and their inhibitory potency against furin, PC2, PACE4, PC1/3, PC5/6 and PC7. Furthermore, we determined the stability and bioavailability of selected compounds. Our results showed that position P6 (Leu^3) of inhibitors based on AcLLLLRVKR-*NH2* sequence is crucial for their activity. Modification of this position by D-Leu, Nle resulted in suppression of inhibitory potency. On the other hand, substitution of position P8 or introduction of triazole ring between P8 and P7 (Leu^1-Leu^2) did not affect peptide activity.

P375. Abstract number: 321

A new class of somatostatin analogues with antiproliferative activity in human cancer cells

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Somatostatin [SS-14: H-Ala-Gly-*c*(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH] suppresses growth of cancer cells that lead to efforts to develop antitumor drugs based on its structure of SS-14. Despite efforts, difficulties exist on separating two specific functions: namely, the inhibitory action on tumor cell growth, and the regulatory effect on endocrine and exocrine systems. A derivative of SS-14, TT-232 [H-D-Phe-*c*(Cys-Tyr-D-Trp-Lys-Cys)-Thr-NH_2][1], had potent antiproliferative activity in human cancer cells without antisecretory action mediated SS-14 receptors (SSTRs). Toward this goal, we prepared small peptides related to TT-232 and screened for their biological activities.

Protected peptides were synthesized by a solution method using Boc-chemistry. Final products were identified by MALDI-TOF mass spectrometry and elemental analyses. The SS-14 receptors binding activity was assessed using a radioligand and recombinant cells expressing SSTR1-SSTR5. Antiproliferative activity in human cancer cells and *in vitro* DNA polymarase inhibition by the compounds were evaluated by the MTT test and the dTTP incorporation to DNA template-primer such as poly(dA)oligo(dT), respectively. All compounds exhibited 1000-fold less binding toward SSTR1-SSTR5 than SS-14 (K_D = 0.034-0.9 nM); however, H-Tyr(BrZ)-D-Trp-1-Ada (**1**) had the most potent antiproliferative activity in human colon carcinoma cell line, HCT116 (< 5% viability at 10^{-6} M) and induced cell cycle arrest at S phase. Furthermore, **1** inhibited rat DNA polymerase beta (>95% inhibition at 10^{-6} M). These findings suggested that small peptides related TT232 are a new class of somatostatin analogues exhibiting antiproliferative activity without interaction to SSTRs.

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P376. Abstract number: 323

Affinity ionic liquid for biomolecular interaction analysis

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Based upon specific biomolecular recognition, an efficient technology platform namely affinity ionic liquid (AIL) was developed for conveniently isolating and purifying peptide and proteins such as His-tagged green fluorescent protein (GFP) from bacterial cell lysates. This AIL is composed of a chemically stable bicyclic imidazolium ionic liquid covalently conjugated with a Ni(II)-chelated nitrilotriacetic acid (NTA) used as the key recognition element (**1**). In our hands, this AIL was capable of quantitatively extracting the peptide such as Fmoc-His_6-NH_2 (**2**) from aqueous buffer phase into the ionic liquid layer. Results of titration experiments showed that the stoichiometry of binding interaction of **1** with **2** is 1:1. Additionally, our result of protein interaction analysis using **1** clearly demonstrated that the GFP retains its native conformation in ionic liquid.

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P377. Abstract number: 328

Analogues of trypsin inhibitor SFTI-1 modified in absolutely conservative P'1 position by synthetic or non-proteinogenic amino acids

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As reported in the literature, in canonical serine proteinase inhibitors Ser residue present in P1' position is absolutely conservative and therefore crucial for inhibitory activity. In the case of isolated from sunflower seeds a trypsin inhibitor SFTI-1 - the smallest (14 amino acid residues homodetic peptide containing also one disulfide bridge) among the most potent inhibitors of the Bowman-Birk family, this residue is located in position 5. It was shown that hydroxyl group of this amino acid residue is involved in the hydrogen network comprising also Thr4 and Ile10 and the interaction between Ser6 and Thr4 appears to be instrumental in projecting the substrate specificity P1 side chain outward for the interaction with the enzyme S1 subside. Only few attempts have been made to obtain synthetic analogues of natural occurring inhibitors modified in this position. Having in mind our promising studies on peptomeric analogues of SFTI-1 and limited experimental data that support the statement about the role of the inhibitor P1'position, we decided to focused our attention on role of hydroxyl group of Ser6 in the inhibitor - enzyme interaction. Here we report chemical synthesis and determination of α-chymotrypsin inhibitory activity of a series of linear and monocyclic (with disulfide bridge only) analogues of SFTI-1 modified in the P'1 position by other than proteinogenic amino acids: Hse (L-homoserine), Nhse (N-(2-hydroxyethyl) glycine), Sar, Ala, Pro and Hyp (L-hydroxyproline). In the substrate specificity P1 position Phe or its mimetic N-benzylglycine (Nphe) were introduced. The performed kinetic studies of analogues synthesized with cognate enzyme shown that in monocyclic (but not linear) analogues, Hse, Nhse and Hyp are tolerated yielded potent inhibitors. Interestingly, the introduction peptiod monomer Nhse in position P'1 produced analogue with the activity of almost one order of magnitude higher than naturally occurring (and highly conserved) Ser or Hse. This is the first evidence that absolutely conservative Ser present in the inhibitor P'1 position can be successfully replaced by synthetic derivative. In addition, analogues

with Nphe in position P1 displayed full proteolytic resistance.
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P378. Abstract number: 329
The influence of the disulfide bridge of trypsin inhibitor SFTI-1 for the enzyme - inhibitor interaction
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The trypsin inhibitor SFTI-1 isolated in 1999 from the sunflower seeds is currently the smallest naturally occurring peptidic proteinase inhibitor. Because of its small size and well defined structure, SFTI-1 has been chosen by several groups to be a lead structure in a design of new inhibitors of serine proteinases. One of the first studied aspects of SFTI-1 structure was the role of cyclic elements (disulfide bridge and head-to-tail cyclisation) in maintaining its rigid structure and the influence it has on its high inhibitory activity. In our first paper on SFTI-1 we showed that the elimination of one cycle yielded very potent inhibitors. It was also proved by other groups that a disulfide bridge is essential for maintaining the structure of the inhibitor and is responsible for its proteolytic stability. In order to obtain more proteolytic-resistant analogues of the SFTI-1, several attempts have been made to modify the disulfide bridge, including its replacement by ethylene and olefin bridge. Such analogues appeared to be metabolically stable. Based on these interesting results, we decided to synthesize two series of SFTI-1 monocyclic analogues. In the first one, a disulfide bridge was formed by combination of Cys, Hcy, Pen and Nhcy (N-sulfanylethylglycine)). The second series contained monocyclic SFTI-1 analogues in which a ring formation was achieved via a ureido group incorporating the side-chains of amino groups of L-2,3-diaminopropionic acid (Dap), L-2,4-diaminobutyric acid (Dab), Orn and Lys placed in positions originally occupied by Cys residues. Our results have clearly shown that Pen and Nhcy are not acceptable in position 3, yielding inactive analogues, whereas second residue (Cys11) can be substituted without significant impact on affinity towards proteinase. On the other hand, lengthening of Cys3 side chain (introduction of Hcy) does not affect inhibitory activity and the analogue with Hcy - Hcy disulfide bridge was more than twice as effective as the reference compound. It is worth noticing that all analogues that contained carbonyl bridges of a different size, appeared to be potent trypsin inhibitors. The obtained results show that this redox stable modification is well tolerated in the structure of proteinase inhibitor.
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P379. Abstract number: 344
A novel diagnostic-prognostic tool for diabetes: UPLC analysis of glycated metabolites
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Urine and blood of each individual contain a unique pattern of metabolites, among which are free amino acids and short peptides. A definite metabolite pattern can also be associated to several pathological conditions and thus becoming a biomarker of a given disease1. Herein we report our approach to novel diagnostic-prognostic assays for diabetes based on the analysis of glycated amino acids/or peptides in biological fluids.
Glycation of proteins through non-enzymatic reactions between glucose or other reducing sugars and reactive amino groups represents one of the most abundant process involved in aberrant modifications of proteins2. Spontaneous and reversible condensation of reducing sugars and a free amino group forms an aldimine (Schiff base) that undergoes a rearrangement into the more stable ketoamine (Amadori product)3. In the case of glucose, the initially formed Schiff base rearranges into the more stable 1-deoxyfructopyranosyl moiety, leading to the irreversible formation of advanced glycation end products (AGEs) that are associated with numerous diseases4,5. Abnormally high glucose concentration in blood, characteristic of many diabetes forms, increases the frequency of glycation in diabetic patients. In fact glycated albumin in blood is already used as diagnostic parameter for diabetes. Nevertheless, this methodology suffers from some limitations such as accuracy and impossibility to identify ”pre diabetic”conditions. We speculate that hyperglycaemia may generate a higher concentration of glycated metabolites, in the form of glycated amino acids and peptides present in diabetic patients’ biological fluids compared to healthy individuals. Thus we propose to follow such glycated molecules as specific biomarkers of disease progress. To this aim we set up an UPLC analytical method of amino acid/peptide analysis to evaluate the presence of free glycated Lysine derivatives in diabetes patients’ biological samples. We recently reported the first synthesis of an Nε-Amadori-containing Nα-Fmoc-Lys-OH derivate for SPPS (Fig. 1a),6 we applied for the synthesis of a deprotected glycated Lysine derivate (Fig. 1b). This product can be used as reference in amino acid analysis. In addition we generated a free glucito-Lysine (Fig. 1c), representing the reduced form of product b, to be used for the analysis of glycated metabolites derived from protein hydrolysis. The latter reduction is necessary to make the sugar moiety stable to hydrolytic conditions.

P380. Abstract number: 351
Enhancement of aggregation energetics and neurotoxicity by site-specific modification of amyloid beta peptides with oxidized cholesterol
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Aggregation and accumulation of amyloid bata peptide (Abeta) linked to age-associated deficiencies in protein homeostasis appear to cause Alzheimer’s disease (AD), which is one of the most common amyloid diseases. However, how Abeta can aggregate in vivo even though its physiological concentration (which is in the nanomolar range) is much lower than its critical concentration for aggregation (which is in the micromolar range) remains unexplained. We have proposed that covalent modification of Abeta by lipid oxidation products can explain how Abeta can form amyloid at physiological concentration. The cholesterol oxidation product 1(2) with an aldehyde group,

which can modify Abeta by Schiff-base formation, is an example of the products that could affect AD onset. Here we examine several significant questions about the modification of Abeta by 1(2), including: Does modification by 1(2) lower the critical concentration of Abeta aggregation into the physiological concentration range?; Is Abeta modified by 1(2) able to aggregate at low concentrations on a biologically relevant time scale?; and are the aggregates formed by Abeta-1(2) toxic to primary neurons? These questions were addressed by studying chemically synthesized analogs of Abeta that were site-specifically modified with 1(2) at Asp1, Lys16, or Lys28 [1]. Although these distinct modifications have a similar effect on the thermodynamic propensity for aggregation, enabling aggregation at low concentrations, the modification site differentially influences the aggregation kinetics; Lys-16-modified Abeta formed amorphous aggregates fastest and at the lowest concentration. The influence of modification site on the nature of the aggregates suggests that amorphous aggregation and fibrillization place different conformational demands on Abeta. Furthermore, the aggregates resulting from Lys-16-modified Abeta were more toxic to primary rat cortical neurons than treatment with unmodified Abeta under identical conditions and at the same concentration. These studies may partially explain how Abeta can form neurotoxic aggregates at nanomolar physiological concentrations when the critical concentration of unmodified Abeta is in the micromolar range.

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P381. Abstract number: 356

Lwg1A: A novel scorpion toxin that adopts an ancestral motif

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Scorpion venoms and their component peptides have been studied for almost 50 years, with over 200 peptides discovered to date. A novel peptide named Lwg1A containing two disulfide bonds was isolated and sequenced from the venom of the Australian scorpion *Liocheles waigiensis*. It was synthesised using soild phase Boc chemistry and found to have insecticidal activity against mealworms, crickets and the pest species, the Australian sheep blowfly. Structural determination using NMR revealed it adopts a hitherto hypothetical ancestral fold, the disulfide-directed hairpin, or DDH. This is the first example of a native peptide that adopts this fold. The DDH can be considered the precursor from which the inhibitory cysteine knot (ICK) and the cystine-stabilised alpha beta (CSAB) motifs evolved, both of which are present in scorpion venom peptides. The ICK motif is also found in toxin peptides from other evolutionarily divergent organisms, including spiders, cone snails and plants. In addition to providing a structural precursor to the ICK and CSAB motifs, Lwg1A has the potential to be developed into a novel pesticide.

P382. Abstract number: 357

Tryptophan defines a key interaction between μO-conotoxin MrVIB and the Voltage-Gated Sodium Channel

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μO-conotoxin MrVIB (MrVIB) is a 31-residue peptide isolated from the venom of the predatory cone snail *Conus marmoreous*. It is of great research interest due to its exquisite selectivity on the subtypes of the voltage-sensitive sodium channel (Na_v). To give further insight into the structure-activity relationship of MrVIB, we synthesised the first analogue of MrVIB - [W6A]MrVIB, to evaluate the contribution of tryptophan makes toward channel selectivity and affinity. In this study, a selective disulfide bond protection strategy was applied to the synthesis of both MrVIB and its analogue. Disulfide bridge patterns of the MrVIB analogues were characterized by pepsin digestion and partial reduction/alkylation. The solution structures of MrVIB and [W6A]MrVIB were compared by NMR. Synthetic MrVIB showed identical potency and selectivity as native MrVIB on $Na_v1.8$ over $Na_v1.2$ channels in an oocyte expression system. However, replacement of tryptophan with alanine led to the activity complete abolition at both $Na_v1.2$ and 1.8 channel subtypes. NMR of [W6A]MrVIB revealed disrupted structure over the well resolved region of native MrVIB. It appears that tryptophan residue has a significant influence on the structure of MrVIB, therefore plays a critical role in receptor recognition.

P383. Abstract number: 387

B-cell epitope mapping of immunodominant proteins in Pemphigus Vulgaris: prediction, synthesis, and immunoserological evaluation

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Pemphigus Vulgaris (PV) is an autoimmune, intraepithelial, blistering disease affecting the skin and mucous membranes and is mediated by circulating and tissue-bound autoantibodies (of the IgG1 and IgG4 subclasses) directed against keratinocyte cell surfaces. Pemphigus antibody binds to desmosomal proteins: desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3). These are transmembranous components of desmosomes, adhesion units specialized in conferring epidermal keratinocyte cohesion and linked to intercellular molecules of the desmosomal plaque, which in turn interacts with components of the cytoskeleton. The binding of antibody to desmoglein 1 and 3 may have a direct effect on desmosomal adherents or may trigger a cellular process that results in acantholysis[1].

In order to localize the potentially antigenic sites, we have predicted beta-turns of Dsg1 and Dsg3 protein segments using the Chou-Fasman secondary structure prediction method[2] and hydrophobicity predictions by Eisenberg et al[3]. Segments with high probability of beta-turn secondary structure ($P_{beta\text{-}turn}>1$) and low probability of hydrophobicity ($P_{hydrophobicity}<0$) were selected. We also used the PredictProtein website in order to make our prediction results more accurate. According to these results pentadecapeptides overlapping in five amino acid residues were synthesized in duplicates on hydroxypropylmethacrylate pins with Fmoc/^{t}Bu chemistry. The side chain protecting groups were removed with trifluoroacetic acid in the presence of scavengers, but the peptides remained covalently attached to the pins. To detect the interaction between the autoantibodies and the synthetic peptides modified ELISA (Enzyme Linked

Immunosorbent Assay) tests were performed with the serum of a group of patients with PV and the serum of healthy individuals.

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P384. Abstract number: 391

RGD containing mono- and oligomeric polyprolines as large molecular rulers

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Cell adhesion is an essential process in every complex life form, but its understanding is also important for medical applications. Migrating primary tumor cells forming metastases in distinct tissues as well as bone cell attachment to implants are two known examples. To date, this process is not fully understood yet on a molecular level. Cells adhering to a given surface change their morphology by forming filapodial extensions and stable focal adhesion points at their ends.[1; 2] Within these anchoring points a class of proteins, the integrins, are responsible for the interaction, surface recognition and attachment of the cells.[3] It is unknown how many integrins have to cluster to form a stable focal adhesion point.

The question of geometrical alignment of the integrins in focal adhesion points was approached by former studies with RGD-modified surfaces and oligomeric RGD containing molecules. These molecules feature short, flexible spacers based on polyethyleneglycol or aminohexanoic acid. Due to the high flexibility of these kinds of spacers their absolute length in solution cannot be determined. Furthermore, binding moieties might be buried within these molecules. Therefore no consistent results could be obtained.

To overcome the problem of undefined spacer lengths the polyproline helix was chosen. This spacer forms in aqueous surroundings a very rigid helix which can be used as a molecular ruler, thereby overcoming the disadvantages of the spacers mentioned above. Helices between 9 Å and 65 Å have been synthesized. A cyclic RGD sequence was ligated by ”click-chemistry”to the ends of the mono- and dimeric polyproline helices. The other end of the spacer consists of a lysine core which can link helices. Additionally, this core can be functionalized to attach the whole construct to a surface, e.g. gold. The longer scaffolds can cover the distance between two integrins (approx. 42 Å) and therefore direct them close to each other.

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P385. Abstract number: 393

T-cell epitopes in autoimmune bullous skin disorders

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Autoimmune bullous skin diseases, such as bullosus pemphigoid (BP) and pemphigus vulgaris (PV), are severe, sometimes life-threatening skin disorders. Serologically they are characterized by autoreactive serum antibodies directed against different adhesion molecules of the epidermis and the dermoepidermal basement zone. The antibody binding affects the adhesive function of these molecules resulting in detachment of the cells and subsequent blister formation.

In the pathogenesis of both pemphigus and pemphigoid, autoreactive T-cell response also plays a crucial role, because initiation and perpetuation of B-cell response needs the recognition of T-cell epitopes; and also the involvement of $CD4^+$ T-lymphocytes in PV has been suggested by the strong association with distinct HLA class II alleles. In both types of diseases the $CD4^+$ T-cells recognize epitopes from the extracellular domain of desmoglein 3 or BP180 proteins, and produce both Th1 and Th2 type cells producing different cytokines (e.g. INFγ).

Based on prediction methods and the literature we have selected several potential T-cell epitopes within the BP180 antigen (collagen XVII). We have synthesized the peptides by solid phase peptide synthesis method using Fmoc/tBu chemistry. The peptides were HPLC purified, then characterized by ESI-MS and amino acid analysis.

For *in vitro* functional studies PBMC from healthy donors as well as patients with active diseases were stimulated with synthetic peptides and T-cell proliferation and IFN-γ production were measured.

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P386. Abstract number: 396

Elucidation of sequence-specific collagen recognition mechanisms by anti-angiogenic factor; pigment epithelium-derived factor

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Pigment epithelium-derived factor (PEDF) is a collagen-binding serpin that abundantly distributes in various tissues including the eye. It exhibits various biological functions, such as anti-angiogenic, neurotrophic, and neuroprotective activities. PEDF also interacts with extracellular matrix (ECM) components such as collagen, heparan-sulfate proteoglycans (HSPG), and hyaluronan. The collagen-binding property has been elucidated to be important for the anti-angiogenic activity in vivo. Here, we investigated the collagen-recognition mechanism by PEDF. We first narrowed down the candidate PEDF-binding sequences by taking advantage of previously reported structural requirements in collagen. Subsequent searches for PEDF-binding sequences employing synthetic collagen-like peptides resulted in the identification of one of the critical-binding sites for PEDF. Further analysis revealed that collagen-recognition by PEDF is sequence- and conformation-specific, and the high-affinity binding motif in

the triple helix. The PEDF-binding sites were significantly overlapped with the proposed heparin/HSPG-binding sites in collagen. The interaction of PEDF with collagen I was specifically competed by heparin, but not by chondroitin sulfate-C or hyaluronan. The results suggested a mechanism for the anti-angiogenic activity of PEDF through modulation of a triangular interaction between collagen, HSPG and endothelial cells. To reveal biological significance of PEDF-collagen interactions, we examined for collagen I-induced endothelial tube formation. In this assay, wild-type PEDF and the heparan sulfate-binding-null mutant inhibited tube formation, but collagen-binding-null mutant did not. This result indicated PEDF- binding to collagen was essential for anti-angiogenic activity. The PEDF-binding motif was also found to be overlapped with covalent cross-linking sites between collagen molecules. This implied the tissue capacity for PEDF, and hence its biological activities might be altered by the molecular aging of collagen.

P387. Abstract number: 399

Molecular recognition of family 18 chitinases by helix-loop-helix polypeptides

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Family 18 chitinases are enzymes that hydrolyze the glycosidic bonds of chitin, a β-(1,4)-linked homopolymer of N-acetylglucosamine, and they play an essential role in many pathogenic organisms.1 Therefore, family 18 chitinases have been considered as potential chemotherapeutic targets for the design of drugs against asthma, malaria and fungal diseases.2,3 So far, several chitinase inhibitors including synthetic xanthine derivatives and naturally occurring allosamidin, argifin and argadin have been studied and the structures of their complexes with chitinase have been elucidated.4,5,6 These molecules are all binding to the active site of chitinase. However, few synthetic molecules that bind to the protein surface outside of the active site have been studied.7,8 Here, we report 42-residue polypeptides that bind to family 18 chitinases in a unique manner. These polypeptides are potential components in diagnostics and biosensors targeting chitinases.

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P388. Abstract number: 401

The Transcriptional Activator PhoB: Chemical Synthesis of Epitopes and Functional Studies

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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 on peptide and on protein level.

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 and an AT-rich minor groove.[1]

Different epitopes representing parts of the DNA binding domain of PhoB ($PhoB^{DBD}$) were chemically synthesized using microwave assisted solid phase peptide synthesis. The binding contributions of these molecules were compared to the complete DNA binding domain (127-229). This protein was purified using intein mediated protein splicing.

The protein/DNA or peptide/DNA interaction was determined using different methods like surface plasmon resonance. Structural investigations of peptides, proteins and DNA/protein complexes were performed using circular dichroism measurements.[2]

In order to investigate the binding mechanism of $PhoB^{DBD}$ the specific recognition of different oligonucleotides based on the sequence of the *pstS*-regulon was analyzed. Surface plasmon resonance results indicate, that *pho*-regulons varying in the AT-rich minor groove sequence exhibit different affinities concerning the transcription factor. These findings reveal that both sections, the major and the minor groove sequence, are essential for the binding process.

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P389. Abstract number: 427

Assay of Histone Methyltransferases using Peptidyl-MCA as Substrate.

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Histone methyltransferases (HMTs) play an important role in controlling gene expression through site-specific methylation of lysines in core and linker histones within chromatin. Histone lysine methylation can either activate or repress transcription, depending on the residue modified and the degree of methylation of its ε-amino group. As the typical HTMs, G9a and Set9 have been intensely studied so far. G9a is specific to the methylation of H3K9 and H3K27 and repress transcription, while Set9 methylates at H3K4 and others. In order to establish high through put and specific assay systems for these HTMs, we utilized the poor susceptibility of K(Me)n toward tryptic hydrolysis. Namely, we prepared various Ac-peptidyl-MCA (7-amino-4-coumarinamide) related to histone tail peptide including Ac-ARTK-MCA, Ac-QTARK-MCA, Ac-KAARK-MCA, and Ac-ARTKQTARK-MCA. In addition, several peptide sequences from methylation proteins are applied to the substrates, such as Ac-LKSK-MCA and Ac-KRSK-MCA, sequences from p53 and estrogen receptor-alfa, respectively. The most sensitive and specific substrates to G9a and Set9 are Ac-ARTKQTARK-MCA and Ac-KRSK-MCA, respectively. As conclusion, these fluorogenic

peptides are the most convenient substrates for screening HMT inhibitors.

P390. Abstract number: 432

Interactions of the transcription factor PhoB from E. coli with DNA

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Specific molecular recognition between DNA and proteins is essential for the regulation of fundamental biological processes, including DNA replication, repair and transcription. The purpose of this study is to enhance the understanding of the interaction between DNA and DNA binding proteins. The DNA binding domain (DBD) of the transcription factor PhoB from *E. coli* was investigated as a model system. Physiologically, PhoB serves as a regulator of phosphate metabolism[1]. Upon activation and dimerization, the PhoB DBD binds to its cognate TGTCA sequence located within specific Pho-Box DNA motifs.

The entire DBD of PhoB was generated by recombinant expression in E. coli and intein-mediated protein purification[2]. Different point mutants were generated, in which selected amino acids that were supposed to be involved in DNA binding were replaced by alanine residues, including valine 197, histidine 198 and arginine 203. The selection of these specific amino acid residues was based on the previously published X-ray crystal structure of the DNA:protein complex[3].

The consequences of DNA:protein complex formation were investigated by circular dichromism (CD) spectroscopy. Structural changes of DNA upon binding of the PhoB (wild type) and several mutants (V197A, H198A and A02C) were observed, while mutant R203A did not induce structural change of the DNA upon binding.

Using surface plasmon resonance measurements (SPR), the formation of DNA:protein complexes was investigated. Based on DNA molecules with two binding sites, the binding properties of the DBD PhoB towards DNA were analyzed and suggest a negative cooperativity of binding. The equilibrium response values at different protein concentrations and the equilibrium dissociation constants were determined and compared for the different mutants. In the case of the mutant PhoB V197A, the binding affinity was comparable to wild type PhoB. Mutant PhoB H198A displayed an increased DNA affinity, while PhoB R203A did not bind to DNA in any of the experiments performed[4].

The data resulting from these studies provide insight into the molecular recognition of specific DNA sequences by proteins and their binding mechanisms.

P391. Abstract number: 433

Peptides stimulating prostate-specific antigen (PSA) enhance the activity of PSA towards protein substrates

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Prostate-specific antigen (PSA) is a serine protease expressed in prostate and secreted into seminal fluid. In prostate cancer PSA is leaking from the malignant tissue into serum making PSA a useful biomarker for cancer diagnosis. The slow growth of prostate cancer is potentially dependent on high PSA-levels around the tumor, since PSA has been shown to exert anti-angiogenic activity. Therefore, it may be possible to control prostate cancer growth by modulating the proteolytic activity of PSA. We have developed peptides that stimulate the enzymatic activity of PSA towards a small chromogenic peptide substrate. Here, we have characterized the effect of these peptides on the activity of PSA towards protein substrates.

We have identified by phage display a 12-amino acid long cyclic peptide (B2) and a 13-amino acid long double cyclic peptide (C4). The effect of these peptides on PSA activity towards peptide substrates and proteins, such as IGFBP-3 and fibronectin, was studied using colorimetric and fluorometric assays, and SDS-PAGE followed by silver staining.

Peptides greatly enhanced the activity of PSA towards the peptide substrates, C4-peptide being more active. Peptides also enhanced the activity of PSA towards protein substrates, but their relative efficiency was different from that with peptide substrates. These results suggest that the peptides would be useful in stimulating the activity of PSA towards physiological substrates and, therefore, they could be useful lead molecules for drug development.

P392. Abstract number: 434

Molecular modeling, design and structural studies of a new class of peptide inhibitors of bacterial topoisomerases

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The bacterial toxin-antitoxin (TA) systems contribute to plasmid stability by a mechanism that relies on the differential stabilities of the toxin and antitoxin proteins and leads to the killing of daughter cells that did not receive a plasmid copy at the cell division. In the last years we have considered these systems as models for the design of peptide inhibitors of bacterial topoisomerases. Bacterial protein ParE is an example. ParE is the toxic component of a TA system whose three-dimensional structure is not yet available and only rare in vitro studies have been demonstrated that the target of toxin activity is *E. coli* DNA gyrase. So, firstly we built a 3D Model for *E. coli* ParE toxin by comparative modeling using MODELLER program. The validate Model showed a similar architecture for the general structure of the RelE superfamily and structural similarity search employing DALI server showed RelE and YoeB families as the best matches. Structural prediction from ParE primary structure using PSIPRED server showed the β-α-α-β-β-β-α sequence as secondary structure, as inferred in the proposed 3D Model. As an approach for structure-function studies and based on proposed Model, we have designed and synthesized by solid-phase methodology a series of linear peptides and its activity studied by supercoiling and relaxation assays. For the design, initially we considered the 25 residues C-terminal region, indicated as the functional region of the toxin and that contain the predicted β-sheet and α-helix structures. Six fragments of the natural ParE were obtained and the predicted C-terminal secondary structures confirmed by FTIR studies. In standard supercoiling and relaxation assays at 37°C and 200 μM of synthetic peptides, EcParE1 (L93-R100) and EcParE2 (F87-R100) peptides did not inhibit the DNA gyrase and Topo IV activities. EcParE4 (L93-S105) and EcParE6 (P80-S105) peptides showed only partial inhibition for both enzymes. On the other hand, EcParE3 (P80-R100) and EcParE5 (F87-S105) peptides, were able to inhibit completely the DNA gyrase activity and ATP dependent relaxation reaction of Topo IV with a concentration required for complete inhibition (IC_{100}) value of 20 and 50 μM, respectively. Our findings revealed a new class of peptide inhibitors of bacterial topoisomerases and suggest that

DNA gyrase as well as Topo IV may be effective targets of the natural ParE toxin.
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P393. Abstract number: 435

Structural aspects and biological evaluation of new mannose derived immunomodulating admantyltripeptides

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The aim of this study was to synthesize, characterize and investigate the influence of mannose derived adamantyltripeptides on humoral immune reaction in mice. The adamantyltripeptides are chemically characterized compounds and in our previous investigations we have demonstrated their different biological activity, especially adjuvanticity. Also, it is well known that glycosylation affects several cell functions such as proliferation and differentiation and also the immune response in mammals. It is well documented that mannose receptors are present on the cell surface of macrophages and other immunocompetent cells.
D-Mannose was coupled to D/L-(adamant-1-yl)Gly-L-Ala-D-*iso*Gln (Ad1TP1 and Ad1TP2) and D/L-(adamant-2-yl)Gly-L-Ala-D-i*so*Gln (Ad2TP1 and Ad2TP2) via chiral linker ($HOCH_2CH(CH_3)COOCH_3$), and the resulted several diastereoisomers obtained were characterized by NMR and their purity was tested by HPLC. All the examined compounds are water-soluble, non-toxic and non-pyrogenic substances. Their adjuvant effect was tested on CBA mice with ovalbumin as a model antigen. The results revealed that the adjuvant activity of examined compounds was changed in comparison to the parent adamantyltripeptide molecules. The statistically significant difference in induction of total specific IgG antibodies was found for the D-(adamant-1-yl)Gly-L-Ala-D-isoGln and D-Man-$OCH_2CH(CH_3)$CO-D-(adamant-1-yl)Gly-L-Ala-D-*iso*Gln, respectively, when the chiral carbon atom in the linker molecule had the *R*- absolute configuration. Also, the significant difference was observed between two examined molecules of mannose-adamantyltripeptides regarding the *R*- and *S*- absolute configuration on chiral carbon atom in the linker molecule. The analyses of specific IgG subclasses IgG1 and IgG2a revealed the influence of mannose derived adamantyltripeptides on Th1 and Th2 type of immune reaction. OVA given without adjuvants induced IgG1 predominantly, reflecting the induction of Th2 immune response. The effects of examined compounds on IgG1 induction were basically the same as those observed for total specific IgG. Regarding IgG2a, the significant difference was again found between mannose-adamantyltripeptides coupled by *R*- and *S*- linker, respectively. From all examined compounds only mannose-Ad1TP2 with *S*- linker had significant impact on switching the immune response towards more pronounced Th2 response specific for OVA. However, the immune response to each compound will be discussed in detail.

P394. Abstract number: 436

N/Ofq(1-13)Nh2 Analogues With Aminophosphonate Moiety: Synthesis And Analgesic Activity

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Nociceptin/orphanin FQ is a neuropeptide (heptadecapeptide: Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln) that selectively interacts with the opioid-like receptor (ORL1 or NOP), a novel member of the opioid receptor family. It has been reported that the nociceptin-NOP system modulates several biological functions, including pain transmission, stress and anxiety, learning and memory, locomotor activity, food intake. N/OFQ(1-13)NH2 is the minimal sequence maintaining the same activity as the natural peptide nociceptin. Structure-activity studies demonstrated that N- and C-terminal modifications of nociceptin/orphanin FQ generate highly potent NOP receptor ligands.
Pain is frequently observed symptom of various diseases. Some of the greatest achievements in medicine in theoretical and in clinical aspect are connected with the research on pain and especially on the development of analgesic drugs. α-Aminophosphonic acids and aminophosphonates have reached a position of eminence in the research works intending to discover, to understand and to modify physiological processes in the living organisms. They are also a potential source of medicinal lead compounds.
Aiming to develop ligands for the NOP that possess stronger analgesic activity, new series of N-modified analogues of the N/OFQ(1-13)NH2 with aminophosphonate moiety were prepared and tested for the nociceptive effects. The new analogues have been synthesized including cyclic α-aminophosphonates at position 1, using SPPS by Fmoc (9-fluorenylmethoxycarbonyl) chemistry. Analgesic activity was examined by two nociceptive tests - paw-pressure (PP) and hot-plate (HP). All compounds were injected intraperitoneally in male Wistar rats at a dose of 10 μg/kg. The obtained results showed that some of the newly synthesized analogues significantly increased the pain threshold in PP and prolonged HP latency. Thus modification of N/OFQ peptide with cyclic aminophosphonate moiety may be useful for developing of new analgesic drugs.
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P395. Abstract number: 437

Structural basis of cyclic non-phosphorylated peptide interactions with Grb7 SH2 domain implicated in cancer.

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Grb7 is an adapter protein, aberrantly overexpressed in several cancer cell types, that mediates the coupling of tyrosine kinases with their downstream signalling pathways. It does this via its SH2 domain that recognises upstream partners at specific phosphotyrosine residues. The current study aims to develop potent and specific cell permeable peptide inhibitors to the Grb7 SH2 domain. These will permit the role of Grb7 in tumour progression to be assessed and may provide proof of concept for future therapeutics.
Here we report structural and biophysical studies of non-phosphorylated cyclic peptides that have been identified as specific for Grb7 SH2 using phage display (Pero et al., 2002). The lead peptide (G7-18NATE) has already been shown to be biologically active - inhibiting cellular proliferation in several breast cancer cell lines that

overexpress Grb7 (Pero et al., 2007), blocking the interaction between Grb7 and FAK and attenuating the migration of pancreatic cancer cells (Tanaka et al., 2006). We have solved the 3D structure of the G7-18NATE/Grb7-SH2 complex - allowing us to identify the key features of the interaction. Interestingly, these features are also reflected in the other peptide sequences selected by phage display methods. Characterisation by isothermal titration calorimetry and surface plasmon resonance (SPR) techniques are being used to assess second generation peptide affinity and selectivity. Together these studies will facilitate the optimization of inhibitor peptides targeted to Grb7.

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P396. Abstract number: 448

Fluorescent and luminescent fusion proteins for detection of amyloid beta peptide localization and aggregation

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It is recently suggested that aggregation and accumulation of amyloid bata peptide (Abeta) linked to age-associated deficiencies in protein homeostasis cause Alzheimer's disease (AD) [1], which is one of the most common amyloid diseases. However, how Abeta can aggregate in vivo and how Abeta aggregates affect the disease development are still questions to be solved. Thus understanding the behaviors of Abeta in vivo, such as where Abeta accumulate and what structure (monomers, oligomers or fibrils) Abeta adopts at each localized point in internal and external cells, is promising clue to these questions. From this point of view, construction of molecules that can bind to Abeta and can provide some output signals depending on Abeta structural states would be indispensable. In this study, fluorescent and luminescent fusion proteins were designed to analyze Abeta localization and aggregation in internal and external cells. The fusion proteins were composed of Abeta sequence, and fluorescent and luminescent proteins (EYFP; Enhanced Yellow Fluorescent Protein, and hRluc; humanized Renilla luciferase) at both termini of the Abeta sequence. The Abeta sequences in the fusion proteins were employed to provide some affinities and co-aggregations with Abeta. Fusion of EYFP and hRluc with the Abeta sequence was applied to allow detecting Abeta localization and monitoring structural changes. First of all, expression vectors for the fusion proteins were constructed and transfected into HeLa cells. Using the protein-overexpressed HeLa cell lysate, fluorescent and luminescent changes during incubation with Abeta were monitored. Additionally, intra- or extracellular localization of aggregated Abeta was analyzed using the protein. Throughout these experiments, we have shown the development of the detection system with hRluc luminescence giving Abeta conformational information and with EYFP fluorescence providing Abeta localization information. With more improvements of binding specificity and detection sensitivity to the Abeta, this system would be one of the powerful tools for diagnosis and study of amyloidogenic protein behaviors.

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P397. Abstract number: 454

Phakellistatins: are they true active natural products?

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Modern pharmaceutical discovery programmes owe much to natural products. These compounds have long been a traditional and prolific source of drug molecules that interact with a wide range of biological targets. In addition to the critical issue of structure elucidation, after isolation and biological evaluation, key steps are the design and execution of total synthesis, thus allowing confirmation of the proposed structure and the preparation of libraries of analogues. The present experimental work, focused on Phakellistatins, addresses these issues.

Phakellistatins are members of a family of proline-rich cyclic peptides isolated from marine sponges of the genus Phakellia that show biological activity when isolated from the natural extracts. Unexpectedly, when biological assays are performed with the corresponding synthetic molecules, no activity is detected. Here we present a new member of the phakellistatin family that has shown significant cell growth inhibitory activity against a mini-panel of three cancer cell lines and also mitosis inhibitory activity with an IC50 in the high nanomolar range. We describe the synthesis, structure elucidation and biological evaluation of this new compound, as well as different attempts to explain its unexpected biological behaviour.

P398. Abstract number: 478

The 'Chemical Reverse Approach' to identify autoantibodies in Systemic Lupus Erithematosus patients.

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We previously reported the use of an innovative 'Chemical Reverse Approach', based on aberrantly modified peptides, for the detection of autoantibodies as specific biomarkers in sera of patients affected by autoimmune diseases [1,2]. We successfully applied this strategy to the identification of autoantibodies in different autoimmune diseases, i.e. multiple sclerosis [3] and rheumatoid arthritis [4].

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of different autoantibodies directed against a wide spectrum of nuclear, cytoplasmic, and cell membrane autoantigens. Among these, antibodies against ribosomal proteins are detected in 10-20% of patients affected by SLE. It is accepted that their targets are three acidic protein components of the 60S ribosomal subunit, termed P0, P1, and P2. Previous studies demonstrated that the main antigenic determinant is located in the highly conserved C-terminal region. In particular the last 22 amino acids of the ribosomal protein eL12 of Artemia Salina [5]. A multiple antigenic peptide (MAP) bearing the last 13 C-terminal amino acids have been used to detect specific autoantibodies in SLE patients' sera [6].

Herein, we report a complete epitope mapping of human P0, P1, and P2 to possibly identify neo-epitopes. The ability of these peptides to detect anti-P ribosomal protein autoantibodies in serum and to discriminate between sub-populations of SLE patients and healthy blood donors was investigated by ELISA. Peptides recognizing antibodies in more than 15% of SLE patients' sera were then selected and developed as MAPs. In total, 25 SLE patients' sera and 68 healthy blood donors sera were tested by ELISA using 25 peptides as putative epitopes. Three peptide fragments recognized autoantibodies in more than 15% of SLE sera, including a limited number of sera not reacting with the C-terminal region.

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P399. Abstract number: 480

Celiac disease: characterization of linear autoantigenic epitopes

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Celiac Disease (CD) is an autoimmune disease characterized by villous atrophy and inflammatory cell infiltration of the lamina propria caused by the gliadin fraction of wheat gluten and similar alcohol soluble proteins (prolamines). The endomysial protein tissutal Transglutaminase (tTG) is the main autoantigen in CD playing a key role in the pathogenesis.1 tTG catalyzes deamidation of Gln residues or protein cross linking through the formation of isopeptide bonds between Gln and Lys residues in gliadin peptides that are rich in proline and glutamine and thus very resistant to proteolysis.

Sera from patients with CD at first diagnosis were demonstrated to have high levels of auto-antibodies recognising distinct functional domains of tTG. The major tTG epitopes are located in N- and C-terminal domains. Tiberti et al.2 showed that there is an evidence of a specific epitope loss of anti-transglutaminase immunoreactivity in gluten free diet celiac sera, which is supposed to be only against protein N-terminal portion. Aim of our study is to characterize linear autoantigenic epitopes by testing in celiac patients' sera the reactivity of different overlappinng synthetic peptide fragments of tTG [aa(1-230)]. The pentadecapeptides were tested with RIA, ELISA and SPR to evaluate the IgA response against tTG in gluten free diet celiac patients sera.

1Dieterich et al. Nat. Med. 1997, 3, 725-726;
2C. Tiberti et al. Clinical Immunology 2006, 121,40-46.

P400. Abstract number: 505

Celiac disease: characterization of amino acids/short peptides finger print for a fast I.V.D.

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Celiac Disease (CD) is an enteropathy characterized by villous atrophy and inflammatory cell infiltration of the lamina propria caused by the gliadin fraction of wheat gluten and similar alcohol soluble proteins (prolamines). The disease is characterized by the presence of specific antibodies recognizing gliadin, food proteins, and the endomysial autoantigen Tissutal Transglutaminase (tTG). CD is classically characterized by gastrointestinal symptoms, atypical forms (dermatitis, iron deficiency anaemia osteoporosis and many others). As gliadins are proteins rich in proline and glutamine they are very resistant to proteolysis and fragmentation in peptides.1

Amino acids and short peptides are important targets for metabolic profiling and for defining a finger print typical of a disease condition.2 In order to achieve a better understanding of this multifactorial disease we are evaluating the free amino acids/peptides profile in serum of newly diagnosed celiac patients compared to healthy subjects by a quantitative AccQ-Tag Ultra UPLC analysis (Waters). Protocol analysis is rather fast and includes a precolumn derivatization, which converts both primary and secondary amino acids into exceptionally stable, fluorescent derivatives as suitable analytes for UV-absorbance, fluorescence, electrochemical, and MS detection. Celiac patients' sera, previously treated with sulfosaliclic acid to induce protein precipitation, were derivatized with a standard solution of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC reagent).

The fast ULPC analysis provides an improvement in resolution compared to conventional HPLC analysis. Baseline separation of all proteinogenic amino acids and short peptides is achieved in less than 10 min.

Applying a statistical analysis to the UPLC-data we found 12 residues (from a total of 43 amino acids/peptides investigated) showing remarkable differences between healthy and celiac population. Moreover, we compared celiac patients before and after gluten free diet.

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2S.V. Suresh Babu et al. Indian Journal of Clinical Biochemistry, 2002, 17 (2) 7-26.

P401. Abstract number: 509

Toward validation of SIMPs as candidates for cancer therapy.

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p53 has been studied intensively as a single protein and more recently as the primary member of a family of transcription factors whose main activities, such as growth arrest, apoptosis, senescence and differentiation, are closely related to tumor suppression.

Protein complexes involving mutant p53 and p73 are readily detectable in tumor cells1.

A pool of peptides, (SIMPs = short interfering mutant p53 peptides) designed by analyzing the sequence of the DNA binding domain of human p73, has been previously shown to effectively disrupt the protein complex mutant p53/p732.

SIMPs activity is specific for mutant p53 and causes no effects on wt-p53 and p53 null cells.

SIMPs action leads to restoration of p73 transcriptional effects that in turns increase the response of tumor cells to anticancer drugs.

Thus, short synthetic peptides can be very instrumental in both the identification of an oncogenic protein complex and the understanding of its pro-tumorogenic effects.

As a contribution in the search for further evidence validating SIMPs as candidates for cancer therapy, here we present preliminary data on the immune response to selected peptides of the pool.
The raised antibodies might become a valuable tool for
- SIMPs pharmacokinetic parameters definition in vivo
- SIMPs immuno-detection in tissues
- SIMPs immuno-detection at the site of the targeted tumor

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2. Di Agostino S. et al. Cell Cycle 2008, 7: 3440-47

P402. Abstract number: 510

Search for inhibitors or activators of human proteasome

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Cell metabolism is strictly depended on number and activity of many different proteins, which are regulated by transcription, translation and post-translative modification, as well as their irreversible proteolysis effectivity. One of the degradation systems, present in eukaryotic cells, is ATP-depended ubiquitin-proteasome pathway [1]. Proteasome is multicatalytic protein complex responsible for intracellular protein degradation. The process start when the chain containing several ubiquitin residues is bonded to the protein. The poliubiquitin-protein complex is transported to proteasome interior, where it is proteolytically degraded [2]. Proteasome activity has major role in homeostasis of the whole organism. Disturbance of regular functioning of proteasome is observed in many diseases, for example neurodegenerative illnesses such as Parkinson, Alzheimer or Huntington [3]. Increase of proteasome activity is also observed in case of immunological and pulmonary illnesses like mucoviscidosis [4].
Due to its function nowadays researchers of the world try to find new regulators of proteasome activity. Peptides, which we have synthesised, are based on the sequences of 11S, PA26, PR39 and HIV-1 Tat proteins, which are the natural proteasome inhibitors [2]. Designed molecules contains the active loop or binding fragment of the mentioned proteins. Biological studies shows that the inhibitory activity of these peptides is not very high. In order to increase biological activity we are searching for compounds with less flexible conformation.

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P403. Abstract number: 513

A surface-plasmon resonance-based label-free in vitro serodiagnostic technology for multiple sclerosis

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Multiple Sclerosis (MS) is the most common cause of neurological disability in young adults. The Laboratory of Peptide & Protein Chemistry & Biology has been involved for a decade in characterizing an antibody-mediated disease pattern of Multiple Sclerosis. We previously demonstrated that the glycopeptide CSF114(Glc), containing a β-D-glucopyranosyl moiety linked to an Asn residue on the tip of a β-hairpin structure, is able to detect specific autoantibodies circulating in blood by Solid-Phase Enzyme Linked Immunosorbent Assay (SP-ELISA) on MS patients' sera. CSF114(Glc) has been selected as specific MS antigenic probe because of its specific and high affinity binding properties to autoantibodies [1,2].
Innovative nanotechnology techniques have been evaluated to set up more reliable and highthroughput diagnostic/prognostic immuno-assays, based on a CSF114(Glc)-modified sensor. In previous studies we described the possibility to apply an optical, real-time, label-free, immunosensor, based on CSF114(Glc), for the detection of autoantibodies in MS patients' sera. The immunosensor viability was studied with anti-CSF114(Glc) antibodies isolated from MS patients' sera. [3]
We present herein the optimization and development of the CSF114(Glc)-based immunosensor for surface plasmon resonance analysis, which is able to distinguish MS patients from normal blood donors. In particular, a large validation study on a statistically significant number of MS compared to normal blood donors' samples confirms these promising results. In conclusion this technology may offer interesting future applications in the development of an in vitro diagnostic device for MS to follow up disease activity and efficacy of therapeutic treatments.

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P404. Abstract number: 515

Characterisation of the minimal epitope detecting autoantibodies in Multiple Sclerosis by surface plasmon resonance

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With the aim of developing efficient tools for autoimmune diseases diagnostics, several studies have been focused on the use of synthetic peptides.
In a previous study, we synthesized a series of glycopeptide shortened sequences derived from the original sequence of CSF114(Glc) (synthetic probe detecting by ELISA antibodies in sera of Multiple Sclerosis patients) containing Asn(Glc) to identify the minimal sequence [1]. All the shortened glycopeptide sequences inhibited anti-CSF114(Glc) Abs in competitive ELISA. The

minimal epitope Asn(Glc) was demonstrated to be fundamental for an optimal antigen-antibody interaction, and a seven amino acids sequence was the minimal one able to inhibit anti-CSF114(Glc) antibodies in competitive ELISA. Nevertheless, short peptides showed poor coating efficiency to the polystyrene of the ELISA plate [2]. Therefore they are not useful as antigens for developing an in vitro SP-ELISA.
In this context we present the study of serum antibody recognition of CSF114(Glc) shortened sequences by surface plasmon resonance. Biacore technology monitors in real time with a microfluidic system on a sensor surface, binding interactions between molecular partners. Using this methodology we can control in real time the peptide immobilization and its interactions with antibodies present in MS patients' sera. Penta and heptapeptide sequences were used as antigens after modification with PEG at the N-terminus as a spacer to favour peptide immobilization. Binding interactions with autoantibodies in MS patients' sera were measured using all the synthetic shortened glucosylated peptide sequences.
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P405. Abstract number: 519

Antigenic probes for autoantibody detection in Multiple Sclerosis: synthetic peptides versus recombinant proteins

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Autoimmune diseases are a class of disorders that need early diagnosis and efficient prognosis for setting up therapeutic treatments. The identification of autoantibodies as specific biomarkers is a relevant target and up to now most of the assays are based on native antigens as immunological probes. However, aberrant post-translational modifications of proteins can play a fundamental role in triggering an autoimmune response by creating neo-Ags.
We previously demonstrated that a 'chemical reverse approach' is efficient in developing specifically modified synthetic peptides, able to fishing out Abs from patients' biological fluids. In particular a specific antigenic probe, termed CSF114(Glc), was developed to identify autoAbs, as biomarkers correlating with disease activity, in a population of Multiple Sclerosis (MS) patients.1
One of the most studied antigen targets in MS is Myelin Oligodendrocyte Glycoprotein (MOG), a glycoprotein of the myelin sheath.2 In many cases autoantibodies directed against unglycosylated MOG have been considered biomarkers of clinically definite MS, but such idea is still subject of debate. Therefore we focused our attention on recombinant MOG with the aim of comparing the relative merit of this protein versus synthetic modified peptides as antigenic probes for autoantibody detection in ELISA.
We employed a solid-phase ELISA based on rat MOG expressed in E. coli after subcloning the cDNA of the extracellular domain (residues 1-125) into the His-tag expression vector in order to perform the refolding procedure on column to allow affinity purification. The proper refolding was checked by Circular Dichroism spectroscopy. Although an efficient refolding procedure has been applied to rMOGED(His)6, Abs were detected in a few sera.
These results validated the hypothesis that it is necessary to obtain MOG (or other myelin proteins) in the native state with the proper post-translational modifications in order to understand the role of anti-MOG (or anti-myelin) Abs in MS patients' sera.
1 F. Lolli, et al., Proc. Natl. Acad. Sci. USA, 2005, 102, 10273.
2 T.G. Johns, C.C.A Bernard J., Neurochem., 1999, 72, 1.

P406. Abstract number: 522

The metabolisation of different iodinated peptide species.

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Radioiodinated I-123, I-125 or I-131 labelled peptides are often used in biomedical research and diagnostic-therapeutic clinical practice. Moreover, iodine is a bio-essential element with specific physiology and biochemistry. Two enzymatic systems are known to handle iodinated organics: iodotyrosinedeiodinase (IYD) and iodothyroninedeiodinase (ID). However, at this moment, only iodinated amino acids and the thyroid hormones have been studied, but no systematic study on the metabolic fate of iodinated peptides is available.
Using HPLC-DAD/MS/MS and HALO-columns, we have characterised different iodinated mouse obestatin peptides towards their in vitro metabolisation. The degradation kinetics and metabolites of the different iodinated obestatin-peptides arising from both enzymatic proteolysis and dehalogenation is studied.

P407. Abstract number: 532

Rational design and optimization of CSF114(Glc) analogs to develop a panel of probes for diagnostic/prognostic assay for Multiple Sclerosis

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Circulating autoantibodies are interesting biomarkers of patients affected by autoimmune diseases. These autoantibodies if exclusive can be used as diagnostic, prognostic, and theragnostic tools.
We previously demonstrated for the first time, that a synthetic glycopeptide CSF114(Glc) is able to detect specific autoantibodies in sera of the patients affected with Multiple Sclerosis (MS).[1] This glycopeptide is characterized by a type I" β-turn surrounding the minimal epitope Asn(Glc) that allows an efficient exposure of this moiety for antibody interactions in the solid-phase conditions of the immunoenzymatic assay. Our results confirm the role of peptide conformation in recognition of MS autoantibodies.[2,3]
A primary structure alignment of CSF114(Glc) was done by SwissProt database and some fragments from nervous system proteins containing N-glycosylation consensus site (Asn-Xaa-Ser/Thr) were selected. [Asn[641]]FAN(635-655) (FAN, Factor associated with neutral sphingomyelinase activation), [Asn[192]]OMgp(186-204), (OMgp, Oligodendrocyte myelin glycoprotein), and [Asn[179]]NogoR(173-191) (NogoR, Nogo receptor) displayed 8/21, 3/21, and 5/21 residues homologous to CSF114(Glc), respectively.

With the aim of optimizing the antibody recognition in MS patients' sera, we designed a CSF114(Glc) analog maintaining Asn(Glc) at position 7th and modifying amino acids sequence for increasing homology with the selected fragments of the selected nervous system proteins.
The designed peptide sequence showed increased activity in IgM recognition in MS patients' sera by SP-ELISA as well as by competitive ELISA (IC50 =7.71E-08), thus appearing as a promising tool for increasing sensitivity in biomarkers detection for MS.
1. Papini, A.M. et al. Granted U.S.A. Patent & PCT WO 03/000733 A2.
2. Lolli, F. et al. P.N.A.S. (2005) 102, 10273;
3. (a) Lolli, F. et al. J. Neuroimmunol (2005) 167, 131; (b) Papini, A.M. Nat. Med. (2005) 11, 13; (c) Carotenuto, A. et al. J. Med. Chem. (2008) 51, 5304.

P408. Abstract number: 547
Structure-Function Relationships of a Hexapeptide Fragment of Carcinoembryonic Antigen
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Carcinoembryonic antigen (CEA), 180-200 kD glycoprotein, binds to the heterogeneous ribonucleoprotein M (hnRNP M) which acts as a cell surface receptor in Kupffer cells. The binding induces release of inflammatory cytokines and promotes colorectal cancer metastasis to the liver. The amino acid sequence in CEA which binds the hnRNP M receptor is Tyr-Pro-Glu-Leu-Pro-Lys. In this study, the structure of Ac-Tyr-Pro-Glu-Leu-Pro-Lys-NH2 (YPELPK) was investigated using electronic circular dichroism, vibrational circular dichroism and molecular dynamics simulations. The binding of the peptide and its Ala-scan analogs to hnRNP M was investigated using molecular docking calculations. The biological activity of YPELPK and its analogs were studied using differentiated human THP-1 cells, which express hnRNP M on their surface and secrete IL-6 when stimulated by CEA. YPELPK forms a stable polyproline-II helix and stimulates IL-6 production of THP-1 cells at micromolar concentrations.

P409. Abstract number: 560
Tying Up Loose Ends: A Capping Unit for Associated Beta-Strands and Loops
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As a follow-up to our finding that peptides with an Ac-W-turn-WTG- sequence display unusual fold stability, we have examined the general applicability of Ac-W / WTG as a capping unit for enhancing the stability of small peptide folding motifs. This unit is a highly effective cap for beta-hairpin (and other beta-sheet model) structures. Numerous examples indicate that the capping motif provides 6 - 9 kJ/mol of fold stabilization. In the case of hairpins of the general formula, AcW-strand-turn-strand-WTG, we have prepared numerous examples with strand lengths of 2-, 4-, 6- (and even 8-) residues. Examples with hairpin fold populations in excess of 98% in water (as measured by D_2O-exchange protection of backbone NHs) have been prepared. The AcW / WTG unit is highly effective in reducing end-fraying in hairpins, structuring and cross-strand H-bonding is retained at the termini even in the presence of 'turn sequences' with less than optimal turn forming propensities and the inclusion of alanines in the strand sequences. The N-terminal acetyl function is essential for the capping motif; deacetylation reduces fold stabilities by > 6 kJ/mol.; however, sequence extensions beyond the WTG unit are allowed and do not reduce the hairpin fold stability. The structuring features of this beta-cap that are responsible for these effects will be presented.
Other examples of beta-sheet stabilization utilizing this capping motif will be presented. These include Cys dimers of the general structure $[AcW\text{-}(XX)_n\text{-}C\text{-}(ZZ)_n\text{-}WTG]_2$ where n = 1 and 2. Here, also, fold populations in excess of 98% were achieved. The beta-cap also appears to serve, so long as a small terminal beta-sheet structure results, as an effective "staple"for closing longer loops. This will be illustrated with a series of loop replacements of the general structure: Ac-WITVTI-loop-KKIRVWTG-NH_2. With loop = HG, a simple hairpin, the amides of I2, V4, I11 and V13 displayed (94±8)-fold exchange protection and a large set of diagnostic structuring shifts appeared for the strands and the cap. When HG is replaced by GG the fold population decreases slightly, further decreases in fold population were observed with longer flexible loops; but even with loop = GGGGKKGGGG, all NMR and CD measures indicated that the fold-population of the terminal beta-sheet and the cap is 0.7 at 280K, melting cooperatively on warming. These results suggest the use of this cap for peptide models of biorecognition loops in beta-sheet proteins.

P410. Abstract number: 572
Pharmaceutical Peptide Formulations.
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Synthetic pure peptide drug substances like hormones and hormonal antagonists, enzyme substrates and - inhibitors, DNA intercalators, immunological signal pep-tides, and neurotransmitters are well defined regarding dosing profiles per indication and mode of action, granted the active principles are pharmaceutically well formu-lated. Yet, we are not considering here chemical modifications like in pro-drugs, pe-gylated peptides, and the like. -In most formulation recipes for i.v. peptide infusions and i.m. injectables, eye drop-lets or nasal sprays, there are physiologically ac-ceptable salts of the drug substances lyophilized from plane water or isotonic phos-phate-buffered saline together with glucose or mannitol or HSA for stability en-hancement and surface tension minimization.
For oral applications most of the oral application formulas are in tablets containing both organic and inorganic additives coated with synthetic polymers, derivatives of gelatine, cellulose or starch, or based on gelatine capsules for retarding the meta-bolic decay of the active ingredient. The chemical type of coating materials will define the area of resorption of the peptide drug crossing the digestive tract. E.g., micronized crystalline or lyophilized peptide drug substance in a physiologically acceptable salt form may be suspended in vegetable oils to form a paste to be filled in soft gelatine capsules digestible selectively in the small intestine. For some peptide hormones we have developed slow release formulations, e.g. by microencapsulation or microgranulation of drug salts insoluble in water. These drug forms are applied in depots injected under the skin for a well defined slow release of the drug. It takes place by retarded decomposition of the

embedding depot materials under the physiological conditions of the surrounding tissue.

P411. Abstract number: 576

Two short peptides arising in inflammation demonstrated strong neuroprotective effects in vitro

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Now a number of short biologically active peptides consisting of Gly, Pro, and Hyp amino acid residues have been found in blood and other tissues. Collagen and some related proteins are rich sources for such peptides. However, the physiological functions of these collagen-derived peptides are still poorly studied. Several authors previously have shown that the levels of peptides Pro-Gly-Pro (PGP) and *N*-acetyl-PGP in the body were increased many times in inflammation. Furthermore, the *N*-acetyl-PGP demonstrated even some pro-inflammatory properties in concentrations of 10^{-4}-10^{-3}M. On the other hand, we have previously found that PGP has a marked neuroprotective effect *in vivo*. The aim of this study was to try to better understand the nature of revealed neuroprotective effect by using various models of neuron damage *in vitro*. Primary cultures of neurons from rat cerebellum and cortex, and the rat pheochromocytoma PC12 cell line were used in our study. For investigation of cytoprotective properties three different neuron damage mechanisms were modeled: 1)- oxidative stress-stimulated necrosis (”oxidative stress model”- PC12 cells, 1 mM H_2O_2, 30 min); 2)- growth factors deprivation-induced apoptosis (”deprivation model”- cerebellar granule neurons, 24 h); and 3)- glutamate receptor hyperactivation (”Glu-toxicity model”- cerebellar granule neurons, 100 uM Glu, 1 h) resulting in both necrosis and apoptosis. It was shown that peptides PGP and *N*-acetyl-PGP in concentrations of 1-100 uM increased PC12 cells survival after H_2O_2-indused oxidative stress. It should be noted, that this effect was observed in both naive and NGF-differentiated PC12 cell cultures. Similar protective effects of PGP were obtained on the deprivation and Glu-toxicity models, too. In all models used PGP in concentration of 100 uM increased cell survival by an average of 20-30%. Moreover, PGP in concentrations of 100 uM delayed calcium dysregulation induced by Glu-toxicity in cerebellar granule neurons, and increased BDNF and TrkB mRNA synthesis in cortical neurons. Thus, the results obtained indicate that the peptides PGP and *N*-acetyl-PGP in pathological conditions may be involved in the neuroprotective mechanisms, which are triggered in the brain under the action of various damaging factors. The work has been partly supported by the Russian Foundation for Basic Research (grants 08-04-01760 and 09-04-13813).

P412. Abstract number: 577

Neuroprotective effect of short collagen-related peptides and their ability to interact with ACE reveal structure-activity similarity

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Short collagen-related (SCR) peptides are known to effect cardiovascular, nervous, immune and digestive systems of the body in health and disease. Nevertheless molecular mechanisms of their effects remain uncertain. Therefore it is of great importance to compare structure-activity data for SCR peptides obtained on different models and objects. We have previously demonstrated that several SCR peptides show neuroprotective properties *in vivo* and *in vitro*. The aim of this investigation was to identify structure-activity relationships of SCR peptides for both neuroprotective activity in vitro and their ability to interact with bovine angiotensin-converting enzyme (ACE) as substrates. About twenty peptides consists of 2-6 amino acid residues (predominantly Gly and Pro) have been analyzed. Neuroprotective activity of the peptides has been assessed based on their ability to increase survival of cultivated PC12 cells after H_2O_2 insult. An efficacy of ACE-SCR peptides interaction was evaluated on the formation of specific enzymatic products using HPLC-analysis. Among all peptides tested only peptides with *C*-terminal Gly-Pro sequence, as well as H-Gly-Pro-OH dipeptide, have been shown to possess neuroprotective activity in the range of 1-100 microM. Testing of the same peptides as substrates for ACE revealed that peptides with *C*-terminal Gly-Pro sequence were hydrolyzed as well. The data obtained suggest that the SCR peptides target in PC12 cells, which have not been identified so far, may have the structure of ligand-binding site vary similar to the substrate-binding site of ACE. This investigation has been partly supported by the Russian Foundation for Basic Research (grants 08-04-01760 and 09-04-13813).

P413. Abstract number: 580

Structural Dynamics of human prion protein upon residue modification

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It is known that Arg^{208} to His mutation is responsible for the conformational transition in the human prion protein, PrP, due to the elimination of the associated salt bridge (Asp^{144}'Arg^{208}). In this study, in order to assess the role of the Arginine residue at position 208 in the early stages of the conformational changes, the salt bridge was removed in silico from prion protein. In contrast to the experimental studies, this construct was used to avoid substituting Arginine residue with a neutral Alanine in order to keep backbone perturbations as low as possible. An Alanine-substituted analog also used to elucidate the impacts of residue substitution on the structural determinants. According to the molecular dynamics simulations, deprotonation of Arginine residue induced some reversible structural alterations in the loop area (167-171). However, the Alanine-substituted analog caused irreversible impacts to the structural flexibility, which led to the significant instabilities in the remote hot region (190-195) of the backbone. These data imply that the observed conformational transition upon Arg^{208} to His mutation is mainly related to the mutation-induced structural flexibilities in the backbone, not to the elimination of the associated electrostatic bond.

P414. Abstract number: 5

A modular approach to promote cellular uptake of recombinant proteins

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Cell penetrating peptide-mediated protein delivery into living cells has become recognized as a powerful tool for controlling cellular function. The cellular introduction of the cargo molecules is easily conducted by simply conjugating cargo proteins with a peptide having a carrier function and adding these chimera molecules to the culture media. In this work we show how such chimeras can easily be obtained in a modular strategy. This is illustrated with a green fluorescent protein that has a leucine zipper attached to its C-terminus and can form a strong non-covalent dimer with a complementary zipper peptide. This complementary zipper, functionalized with an acetylene moiety, is ligated to a fluorescently labeled cell-penetrating peptide (Tat) using the well-known copper(I)-catalyzed 1,3-dipolar cycloaddition. We show the Tat-zipper construct is able to promote cellular uptake of GFP modified with the complementary leucine zipper as monitored with fluorescence cross-correlation spectroscopy.

P415. Abstract number: 131

Design and Synthesis of Guanidinium-Rich Molecular Transporters for Drug Delivery

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For many therapeutic applications, it has become more important to find synthetic compounds that have ability to transport a variety of drugs and cargo molecules into cells and tissues.

Short oligomers of arginine, either alone or when conjugated to therapeutic agents or large biopolymers, have been shown to cross readily a variety of biological barriers (e.g., lipid bilayers and epithelial tissue).

Molecular modeling suggests that only a subset of the side chain guanidinium groups of these transporters might be required for transport involving contact with a common surface such as a plasma membrane or cell surface receptor.

To evaluate this hypothesis, a series of oligomers were prepared that incorporated arginine mimetics with either, oxy-guanidino or sulfoguanidino side chains.

To test the cellular uptake of these oligomers, FACS analysis was performed. The interaction with monolayers of zwitterionic and negatively charged phospholipids was also investigated.

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P416. Withdrawn

P417. Abstract number: 182

Neutrophil Elastase-dependent Host Defence Peptide Prodrugs

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Host defence peptides are multifunctional molecular effectors of innate immunity, the first line of defence against infection in multicellular organisms. They are recognised as promising candidates for the development of novel antimicrobial and anti-inflammatory agents, owing to their original mode of action which may circumvent the classical mechanisms of drug resistance and to their low propensity to select resistant mutants. However the clinical development of these 'Nature's antibiotics' has been limited to date and exploitation of their therapeutic potential will require in particular solutions addressing the concerns related to their potential toxicity. Several approaches have been proposed to achieve clinical success with host defence peptides, such as conversion into peptidomimetics, liposomal delivery and a prodrug strategy [1].

Using a prodrug approach analogous to the natural control process exerted on these macromolecules, pro-forms of a host defence peptide were designed for activation by neutrophil elastase (NE), a serine protease involved in chronic airway inflammation and infections associated with cystic fibrosis. Minimum Inhibitory Concentrations performed against two representative cystic fibrosis pathogens, Pseudomonas aeruginosa and Staphylococcus aureus, with exogenous or endogenous NE indicate that the levels of antimicrobial activities obtained from the propeptide can be correlated with, and therefore controlled by, the concentration of NE in airway secretions of cystic fibrosis patients. Moreover, toxicity studies performed by the haemolysis assay showed that the active peptide causes dose-dependent cell lysis, while the propeptide is not toxic to red blood cells up to a concentration of 500 microM. Structural basis of the activity differentials between the propeptide and its active component have been investigated by NMR.

This prodrug approach can be exploited to selectively deliver a host defence peptide to sites of infections.

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P418. Abstract number: 248

Synthesis and Biological Evaluation of Daunorubicin-GnRH- III Prodrug Conjugates

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Tumor targeting with the decapeptide gonadotropin-releasing hormone (GnRH) or its analogues is based on the discovery that GnRH receptors are overexpressed in many tumor cells (e.g. prostate, ovarian, breast, endometrial), compared with their expression in normal tissues. Using these peptides as carriers/targeting moieties in a conjugate with therapeutic agents can increase the selectivity and the stability of the conjugates, or eliminate the toxic side effects of the drug.

The lamprey GnRH-III (<EHWSHDWKPG-NH_2) is especially favoured as a targeting moiety because of its antiproliferative effect and weak endocrine activity in mammals.

The anthracycline antibiotic daunorubicin (Dau) is an effective anticancer drug; however, its use is limited by severe cardiotoxicity. Dau can be conjugated through its

amino group of the sugar moiety or through the oxo group on the 13C atom of the aglicon part[1,2]. However, the free amino group might be essential for the biological activity. Therefore, an enzyme cleavable spacer should be incorporated between the amino group and the drug. Cathepsin B, a lysosomal enzime overexpressed in cancer cells splits peptide sequences such as ALAL, LALARR, YRRL, GFLG[3,4], that can be used as spacers.
The aim of our work was to synthesize new prodrug conjugates of daunorubicin that contain different types of cathepsin B cleavable peptide spacers and GnRH-III derivatives.
GnRH-III derivatives (GnRH-III-ClAc, GnRH-III-IAc, GnRH-III-Ttds-ClAC)[5] were synthesized by solid phase synthesis (SPPS) on a Rink-amid MBHA resin according to Fmoc/^{t}Bu strategy. The peptide spacers (Ac-CYRRL-OH, Ac-CGGFL-OH, Ac-CALAL-OH, Ac-CGFLG-OH, Ac-CPFR-OH) were synthesized by SPPS on a Merrifield resin using Boc/Bzl strategy. Dau was coupled to the linker molecules through an amide bond. The chloroacetylated or iodoacetylated GnRH-III derivatives were conjugated via thioether bond with the cysteine elongated spacer molecules.
The release of dau from the conjugates was determined in the presence of Cathepsin B. The in vitro cytostatic effect of the prodrug conjugates were studied on MCF-7 human breast cancer, HT-29 human colon carcinoma cell lines.
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P419. Abstract number: 249

The [Tc(N)PNP] Metal Fragment Labeled Peptide for MC1 Receptors Imaging: preliminary studies

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Malignant melanoma is the most lethal form of skin cancer: melanoma metastases are very aggressive and no curative treatment exists for it due to its resistance to chemotherapy and immunotherapy regimens. Therefore, development of new melanoma-specific radiopharmaceuticals for early detection of primary melanoma tumors or internal radiotherapy is a subject of great interest.
In this perspective, melanocortin type-1 (MC1R) receptors represent a promising target for the development of effective molecular probes for diagnosis or therapy. MC1 receptors are overexpressed on the surface of melanoma cells and able to selectively recognize the peptide sequence His-D-Phe-Arg-Trp mimicking the melanocyte stimulating hormone (αMSH). Literature described several linear and cyclic radiolabeled αMSH analogs. In particular, the introduction of a cyclic constraint in a lead peptide may restrict the flexibility and favor peptide-receptor interactions, and is an effective way of generating ligands with enhanced potency, receptor selectivity, and enzymatic stability.
In order to improve the affinity of a NAPamide analogue (H-Nle-Asp-His-D-Phe-Arg-Trp-Gly-NH_2) towards MC1 receptors the cyclic peptide H-c[Lys-Glu-His-D-Phe-Arg-Trp-Glu]-Arg-Pro-Val-NH_2 was synthesized. In both peptides the Cys-Ahx-βAla sequence were conjugated to the terminal amino group. The resulting Cys-peptide conjugates were reacted with the $[^{99m}Tc(N)(PNPn)]^{2+}$mojety, a versatile synthon for labeling biomolecules, including peptides, and where PNPn was aminodiphosphines carry different bulky alkoxyalkyl substituents (e.i. $CH_3CH_2OCH_2CH_2CH_2$- or $CH_3OCH_2CH_2CH_2$-). The $[^{99m}Tc(N)(NS\text{-}Cys\text{-}peptide)(PNPn)]^{+}$ complexes were obtained according to either one-step or two-step procedures in high yield and high specific activity. In general, the agents were stable in aqueous solution and in PBS. In vitro challenge experiments with an excess of cysteine and glutathione indicate that no transchelation reactions occurred, confirming the high stability of these compounds. Stability studies carried out in human and mouse sera, as well as in mouse liver and kidneys homogenates, showed that the radiolabeled compounds were stable in sera for prolonged incubation at 37 °C. Meanwhile, in homogenates a partial degradation of the agents was observed. In vivo pharmacokinetics of the different $[^{99m}Tc(N)(PNPn)]$-labeled conjugates in healthy rats were evaluated.

P420. Abstract number: 276

Oligoprolines as Multivalent Scaffolds for Tumor Targeting Vectors

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Herein we present a novel approach towards multi- and heterovalent radiotracers for tumor targeting. Multivalent tumor tracers are envisioned to provide higher affinity and longer residence times on the tumor cell compared to established monovalent agents.[1] Exploiting heterovalency, a more universal targeting might be accessible.[2]
In our approach, azido-functionalized oligoprolines[3] are used as helical, conformational well-defined scaffolds to display peptidic targeting vectors. As targeting vectors, bombesin agonists and antagonists with a high affinity towards gastrin releasing peptide receptors which are abundant in most breast and prostate cancer are employed.[4] Conjugation to the scaffold has been achieved via copper catalyzed ”click chemistry”. The modular synthesis of the molecules is performed on solid support, using a standard Fmoc-protocol. Additionally, a DOTA-chelator was introduced for labelling with different radiometals, which should allow for diagnosis via PET and SPECT as well as radiotherapy.
A series of molecules, differing in type and composition of the targeting vectors (divalent agonists , divalent antagonists and hybrids consisting of one agonist and one antagonist) has been synthesized. To the best of our knowledge, this is the first example of combining known agonistic and antagonistic bombesin peptides within the same molecule. Binding and Internalisation studies with human prostate cancer cells (PC-3 cells) will be reported.
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P421. Abstract number: 284

Kinetic studies on cellular uptake of polyarginine peptide by FRET

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Cell-penetrating peptides (CPPs) are well known to be able to across the plasma membrane into cells. Several endocytotic mechanisms for cellular uptake of CPPs were suggested in live cells, involving clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis. Moreover, some findings suggested the cellular uptake can occur via a non-endocytotic process. In either uptake mechanism, cationic portion of CPPs is thought to initially interact with anionic substances present at the extracellular surface of the plasma membrane, such as proteoglycan. Generally, the cellular uptake of CPPs was examined using FACS analysis and fluorescence microscopy after incubation of cells with fluorescently-labeled peptide. In these methods, cell surface-attached or non-intracellular CPPs should be removed by washing with medium, which led to overestimation of the uptake. To avoid these artifact, trypsin-treatment of the cells after incubation and washing was suggested (J. P. Richard et al., *J. Bio. Chem.*, **278**, 585, 2003)

In the present study, an attempt was made to assess the amount of CPPs in the cytosol of cells using a conjugated peptide by a fluorescence resonance energy transfer (FRET). The FRET peptide consists of FAM-Gaba-Cys-His-His-NH_2 (cargo) and Dabcyl-Gaba-Cys-$(Arg)_6$-D-Arg-$(Arg)_2$-NH_2 (CPP), which are covalently linked via S-S bond. Considering the glutathione-rich environment in the cytoplasm, the cellular uptake is probably detected after translocation into cells as the increase of FRET signal (fluorescence) by reductive cleavage of the disulfide bond.

We examined the cellular uptake of the conjugated FRET peptide into Jurkat cells under different conditions as follows: temperature, concentration of peptide, culture medium, and addition of endocytosis inhibitors. In the FACS analysis, the cellular uptake was clearly estimated without the removal of cell surface-attached conjugated peptide on live cells. From experiments using endocytosis inhibitors, it is likely that the conjugate peptide is incorporated into the cells via macropinocytosis and/or caveolae-mediated endocytosis at 2μM concentration of the peptide, while at higher concentration, the peptide is entering via a non-endocytotic process. Kinetic analysis revealed that fluorescence intensity apparently decreased when cells were incubated with the conjugated peptide in RPMI-1640. The inhibitory effects of FBS on the cellular uptake will also be reported.

P422. Abstract number: 303

Novel molecular dyes for the labelling of synthetic peptides

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Luminescent dyes capable of passive cell delivery may be used as molecular probes for example in live cellular imaging. Ruthenium polypyridyl complexes have unique photophysical characteristics which make them potentially invaluable as probes for cellular imaging by confocal luminescence and resonance Raman microscopy. They are long lived, exhibit polarised luminescence, have good photostability, red emission wavelengths and large stokes shifts and oxygen sensitivity. Although, they have long been muted as potentially useful, application of ruthenium polypyridyl complexes in this regard has been very limited. Recent work by our groups has reported that polyarginine labelled ruthenium complexes efficiently and rapidly transport across the cell membrane into the cytoplasm [1, 2]. Such luminophores provide unique opportunities for imaging dynamic processes in living cells while avoiding limitations associated with fixation.

In this contribution, we describe how we are refining these synthetic strategies to target such metal complexes by conjugating them to synthetic peptides to investigate sub cellular localisation.

We report on the synthesis and characterisation of a ruthenium (II) complex [Ru(dpp)2PIC]ClO4 covalently attached to a nuclear localisation signal peptide AVQRKRQKLMP-NH2 [3]. The spectral (absorption and emission) and photophysical (fluorescence lifetime) properties of this metal-ligand peptide complex are described. The complex exhibits long lived, intense, and oxygen sensitive luminescence. Preliminary results on their application in cell imaging are also presented.

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P423. Abstract number: 306

Antibiotic-Host Defence Peptide conjugates as novel antibiotic and prodrug candidates

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Host defence peptides are multifunctional molecular effectors of innate immunity, the first line of defence against infection in multicellular organisms. Their multiple functions in host defences support the rationale of developing entirely novel peptide-based therapeutics harnessing the effector mechanisms of innate immunity. Their main assets include a low susceptibility to classical mechanisms of drug resistance associated with a low propensity to select resistant mutants.

A method for the conjugation of a host defence peptide to a classical antibiotic has been developed. This approach can be exploited to generate hybrid antibiotics which combine the activity of the two conjugated agents, a novel generation of classical antibiotics with extended effective lives and antimicrobial peptide prodrugs reactivated by an enzyme of antibiotic resistance against the classical agent.

An antibiotic-host defence peptide conjugate based on an 8-mer all-D sequence, derived from the bactenecin protein, has been evaluated against representative strains of gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) organisms. The results of susceptibility testing confirmed that the antimicrobial activities of the peptide component were equivalent or superior to those of the native sequence, with minimum inhibitory concentrations (MICs) ranging from 2 to 5 microM against

S. aureus and of 5 microM against E. coli. MICs of the conjugate were equivalent to those of its peptide component against S. aureus, and slightly inferior against E. coli. Control molecules based on a non- immolative linker between the peptide and the classical antibiotic have also been synthesised and evaluated to assess a potential prodrug mechanism of action.
This publication has emanated from research conducted with the financial support of Science Foundation Ireland and Enterprise Ireland.
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P424. Abstract number: 322
Synthesis And In Vitro Antitumor Activity Of New Daunomycin Containing Gnrh-Ii Derivatives
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In humans two isoforms of GnRH are exist, namely GnRH-I and GnRH-II. The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH-I; <EHWSYGLRPG-NH2, where <E is pyroglutamic acid) is the central regulator of reproductive system through the stimulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from pituitary gonadotropes. The exact function of GnRH-II (<EHWSHGWYPG-NH2) is unknown, but it may play a role in the maturation of sexual behaviour, the regulation of gonadotropin secretion and in the immunological process. It was also indicated recently, that GnRH-II derivatives have more potent antiproliferative activity on human endometrial and ovarian cancer cells in vitro than GnRH-I analogs. Some kinds of tumor cells (e.g. breast, prostate, colon) produce GnRH-I and II and express their receptors. However the presence of functionally active GnRH-II receptors on cancer cells is still not clear.
The sythesis of [D-Lys6]GnRH-II, which is the one of the most potent GnRH-II agonist, was carried out on solid phase using mixed Fmoc/tBu and Boc/Bzl strategies. The drug molecule was coupled to the peptide directly or through an enzyme labile spacer (GFLG) in liquid phase via oxim bond formation.
The in vitro studies of cellular uptake and cytostatic effect of the antracycline containing GnRH-II derivatives were investigated.
The cellular uptake of GnRH-II derivatives was studied by flow cytometry (BD LSR II) on MCF-7 (human breast cancer) and HT-29 (human colon carcinoma) cell lines. The cells were treated with different concentration of GnRH-II analogs and after the treatment were analysed by flow cytometry in order to investigate their cellular uptake.
The cytostatic effect of drug containing GnRH-II derivatives was studied on MCF-7and HT-29 cell lines by MTT-assay.
The drug containing GnRH-II conjugates can be taken up by MCF-7 and HT-29 cells in a concentration dependent manner, and they were effective. The biological data were compared with the results that were measured with GnRH-I and GnRH-III derivatives.

P425. Abstract number: 368
Anthracycline-Gonadotropin Releasing Hormone-III Bioconjugates: Synthesis, Antitumor Activity and in vitro Drug Release
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Chemotherapy is still the main approach for the treatment of advanced or metastatic cancer. However, due to the lack of selectivity for tumor tissues, the application of free anticancer drugs can lead to severe side effects and low cure rates. Therefore, targeted delivery of anticancer drugs is one of the most actively pursued goals in cancer chemotherapy. Bioconjugates with receptor mediated tumor-targeting functions and carrying cytotoxic agents should enable the specific delivery of chemotherapeutics to malignant tissues, thus increasing their local efficacy while limiting the peripheral toxicity. It was found that receptors for peptide hormones, such as gonadotropin-releasing hormone (GnRH), are expressed in a higher amount on cancer cells compared to normal cells. Consequently, GnRH and its derivatives can be used as targeting moieties to deliver cytotoxic agents directly to tumor cells. One of the most promising natural GnRH analogs is GnRH-III (Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH2) which specifically binds to GnRH receptors on cancer cells and has lower hormonal effect in mammals than the human GnRH.
In our work, anthracycline antibiotics daunorubicin (Dau) and doxorubicin (Dox) were attached to the GnRH-III by different linkages such as hydrazone, oxime, amide or ester bond, either directly or by insertion of a spacer moiety. After synthesis and chemical characterization of anthracycline-GnRH-III bioconjugates, their antitumor activity was evaluated by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay on various cancer cell lines. The drug release from the bioconjugates was determined in 90% human serum, in rat liver lysosomal homogenates and in the presence of Cathepsin B by liquid chromatography-tandem mass spectrometry (LC-MS/MS).
This work was supported by grants from the Hungarian National Science Fund (OTKA NK 77485), the Ministry of Health (GVOP-3.2.1.-2004-04-0005/3) and University of Konstanz (Zukunftskolleg and AFF).

P426. Abstract number: 370
Efficacy of Laser-Activated Gold Nanoshells targeting breast cancer cells in vitro
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Gold nanoshells are a class of nanoparticles with tunable optical resonances, which can be designed to absorb near-infrared (NIR) light strongly to generate heat and provide optically guided hyperthermic ablation. Once localized to tumor cells, these nanoshells are extremely efficient at absorbing NIR light and can generate sufficient heat to kill cancer cells upon exposure to laser light. The purpose of this study was to screen peptides that can specifically bind to human breast cancer cell line□MCF-7□using phage display peptide library and to identify its ability of targeting MCF-7 cells in vitro. A peptide 12-mer phage display library was employed and 4 rounds of subtractive panning were performed using MCF-7 cells as the target. After 4 rounds panning, we selected the phages that specifically bound to MCF-7 cells. And the selected phages were proved highly specific affinity to MCF-7 cells by ELISA. After modified with β-amino acid at the specific site, the stability of this molecule was improved, but not change structural requirements for binding MCF-7 cells. We bound gold

Nanoshells with the selected and modified peptide, darkfield imaging successfully demonstrated specific affinity to MCF-7 cells. Therefore, the selected peptide may be a potential candidate for targeted ligand in photothermal therapy and diagnosis of breast cancer.

P427. Abstract number: 373

Design And Synthesis Of Polyfunctional Spacers Based On Biodegradable Peptides

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Researches of drug delivery in cells are actively carried out at present. Release of drug from carrier is necessary after uptake of therapeutic construction in target cells. Peptides - substrates for cathepsins taking part in cancer progression has been described in literature. Application of such peptides as biodegradable linkers let to release the therapeutic in transformed cells. Human serum albumin can be used as a carrier for drug delivery in tumor cells. Modification of albumin on sulfhydryl group of Cys-34 permit to obtain bioconjugate of protein with the therapeutic. Antisense oligonucleotides have a wide application in molecular biology, it gives an opportunity to selective effect on gene expression. We propose such approach to delivery of antisense oligonucleotides to cells.

Synthesis of peptides $(AlaLeu)_2Gly$, $(AlaLeu)_2$, $(AlaLeu)_3$ were carried out in solution using *Boc*-strategy. To obtain bifunctional compounds based on synthesized peptides different constructions has been suggested. The first variant is coupling of 4,9-dioxa-1,12-diaminododecane to the *C*-terminal of peptide and maleimide fragment to the *N*-terminal. The second variant is to couple flexible amino linker with maleimide fragment to *C*-terminal without modification of peptide amino group. Different protection groups enabled us to choose more flexible approach for synthesis of bifunctional compounds.

Available amino groups of obtained compounds let us to connect antisense oligonucleotide to *N*-terminal of peptides. Maleimide fragment in the synthesized peptide-oligonucleotide conjugates permit us to form covalent bond to transport protein of blood. A fluorescent derivative of bifunctional compounds has been synthesized to verify substrate properties of obtained peptides.

The structures of the target compounds were confirmed by NMR spectroscopy and mass spectrometry.

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P428. Abstract number: 398

Amino acids esters of acyclovir-synthesis and antiviral activity

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Aciclovir-9-[(2-hydroxyethoxy)methyl]guanine (ACV), is active against HSV-1, HSV-2, VZV. Aciclovir is poorly water soluble and has poor oral bioavailability (10-20%). Several amino acid esters of acyclovir were developed to overcome this problem, valacyclovir, the valine ester of ACV, being among the first of this series of compounds that were readily metabolized upon oral administration to produce the antiviral nucleoside in vivo, thus increasing several times the bioavailility (three- to four- fold higher bioavailability of acyclovir).

We synthesized and explored antiviral activity (HSV-1, HSV-2) of esters of acyclovir with 4-F-phenylalanine (R, S). The results showed that investigated compounds have the same antiviral activity as acyclovir, but significantly less cytotoxicity. The chemical stability of new esters of acyclovir was studied at pH 1.0 and 7.4 and 37°C.

P429. Abstract number: 402

Enhanced cellular uptake of recently used and in silico identified antituberculars by peptide conjugation

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Mycobacterium tuberculosis is a successful pathogen and it can survive in infected macrophages in dormant phase for over years and decades. The therapy of tuberculosis takes at least six months and the slow-growing bacterium is resistant to many antibiotics. The development of novel antimicrobials to counter the emergence of bacteria resistant to current therapies is urgently needed. *In silico* docking methods and structure-based drug design are useful bioinformatics tools for identifying new agents. The uptake of antituberculars by infected macrophages is limited by extracellular diffusion. The optimization of the cellular uptake by drug delivery systems can decrease the used dosages and the length of the therapy and it can also enhance the bioavailability of the drug molecules.

Therefore functionally active and chemically characterized peptide based conjugates of izoniazid (INH) [1] and new, *in silico* identified drug candidates were synthesized. The *in vitro* antibacterial effect of the compounds was determined on *M. tuberculosis* $H_{37}Rv$ bacterial strain. To study the selectivity of the compounds the cytotoxicity was evaluated on human cells and cell lines. The cellular uptake of the conjugates was measured by flow cytometry and fluorescent microscopy. The conjugation of the TB5 molecule to cationic peptide carriers dramatically enhanced the cellular uptake.

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P430. Abstract number: 405

Across the skin barrier, a comparative transport study of a diketopiperazine-based shuttle.

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Diketopiperazines (DKPs) are very common in nature; they have been isolated from a wide variety of natural sources, including marine sponges, lichens and herbs. DKPs have recently proved to be useful as blood-brain barrier shuttles [1,2]. Here we explored the capacity of these compounds to cross the human skin barrier and to carry therapeutically relevant small molecules.

First of all, we studied the human skin distribution and penetration of the DKP Phe-N-Me2Nal. Then, on the basis of the good results found, DKP-Diclofenac conjugate was prepared using solid-phase peptide synthesis. The conjugate was further studied in two skin models: a human skin in vitro assay and a parallel artificial membrane permeability skin assay (PAMPA-Skin) [3]. The results obtained and the two models are extensively discussed.

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P431. Abstract number: 408

Homobivalent a-MSH derivatives for Melanoma Imaging: 99mTc(CO)3-Labeling and Biological Evaluation

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Melanoma, the most aggressive form of skin cancer, has increased more rapidly than any other malignancy and became a major health issue in most western countries. Early detection of primary melanoma tumours is essential since the current treatments do not enhance substantially the patient survival once metastases have occurred. Therefore, development of new specific radiopharmaceuticals for imaging or therapy of melanoma is of paramount importance [1].

Since most human and murine melanoma cells overexpress melanocortin type 1 receptor (MC1R), radiolabelled α-MSH (α-melanocyte stimulating hormone) analogs have been considered interesting tools for melanoma imaging or therapy [1,2]. We have radiolabelled linear and cyclic α-MSH analogs with *fac*-$[^{99m}Tc(CO)_3]^+$ and evaluated biologically their MC1R-targeting properties [3,4]. Based both on the encouraging results obtained with those monovalent ligands, and on the affinity enhancement expected for the MC1R using a multivalent approach, [5] we are now exploring the utility of multivalent α-MSH derivatives labeled with the same core. Herein, we will report on the synthesis and characterization of a novel homobivalent α-MSH derivative containing a pyrazolyl-diamine chelating unit, as well as on the labeling of this derivative with ^{99m}Tc(I). We will also report in vivo studies with B16F1 melanoma cells and murine melanoma-bearing mice.

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P432. Abstract number: 412

Improved Tumor Imaging and Therapy with Radiolabeled PEGylated Bombesin Analogues

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Bombesin(BN)-based radiopharmaceuticals are interesting molecules for imaging and therapy of tumors that overexpress gastrin-releasing peptide (GRP) or BN-2 receptors, such as breast or prostate cancers. The main limitation of BN and BN analogues is their poor *in vivo* stability. PEG conjugation may help to prevent recognition and degradation by proteolytic enzymes and, in turn, prolong the *in vivo* half-life of peptides and proteins. Radiolabeled BN analogues often show important hepatobiliary excretion, which may obscure the detection of tumor lesions in the abdominal tract. PEGylation increases hydrophylicity and would contribute to improve the pharmacokinetics of BN analogues. New BN derivatives, conjugated to N^{α}His- or DOTA-chelator, were PEGylated, radiolabeled and tested *in vitro* in human prostate carcinoma PC-3 cells and *in vivo* in nude mice with PC-3 tumor xenografts. Binding to GRP/BN-2 receptors in PC-3 cells was very specific for all the labeled derivatives, although the PEGylated analogues showed slower binding kinetics. PEGylation increased the metabolic stability *in vitro*. The ^{99m}Tc-labeled PEG-BN analogue showed preferential renal excretion, much lower liver accumulation and a 3-fold decreased in colon uptake. Tumor uptake was higher and the visualization of the tumors by SPECT/CT clearer. The ^{67}Ga-labeled PEG-BN analogue also showed a more favorable biodistribution compared to its unPEGylated counterpart. Finally, the tumor growth could be importantly inhibited with the ^{177}Lu-labeled BN analogues, being the effect more pronounced for the PEGylated analogue than for the unPEGylated one (decrease in tumor growth of 80% and 60%, respectively, after three weeks).

In conclusion, PEGylated BN analogues showed high binding affinity for GRP/BN-2 receptors and higher stability. PEGylation resulted in favorable biodistribution. The PEGylated analogues were preferentially excreted through the kidneys and their accumulation in the liver and gastrointestinal tract was significantly lower. The tumors were more clearly delineated by SPECT/CT with the PEGylated analogues, which also exhibited higher antitumor activity. Therefore, PEGylation proved to be effective in enhancing the potential of radiolabeled BN analogues for targeted imaging and therapy.

P433. Abstract number: 481

Development of drug delivery systems for targeted cancer chemotherapy based on GnRH antagonist and agonist peptides

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Targeted cancer chemotherapy has been developed to overcome the drawbacks associated with the application of free anticancer drugs (i.e. lack of selectivity, toxic side effects, multidrug resistance of cancer cells). Tumor targeting is achieved by conjugating a chemotherapeutic agent to a targeting moiety which is directed to specific binding sites on cancer cells. Gonadotropin releasing hormone (GnRH) receptor expression was identified on different types of tumors, such as breast, ovarian, colon, endometrial, prostate, renal, brain, pancreatic, melanomas and non-Hodgkin's lymphomas.

The aim of our work was to use GnRH agonist and antagonist peptides as targeting moieties for the attachment of anticancer drugs.
Bioconjugates in which the chemotherapeutic agent daunorubicin (Dau) was attached through an oxime bond to the GnRH analogues MI-1892 (Ac-wxwSkD(LQPa-NH_2)-DEA, where x is D-p-chlorophenylalanine, DEA is diethylamide, and small letters mean D-amino acids) and GnRH-III (<EHWSHDWKPG-NH_2, where <E is pyroglutamic acid) were prepared and their biological properties were investigated. Two different types of enzymatic cleavable tetrapeptide spacers (GFLG and YRRL) were incorporated between the drug and the targeting moiety.
The stability/degradation of the bioconjugates was studied in human serum and rat liver lysosomal homogenates by liquid chromatography-mass spectrometry. The in vitro antitumor effect of the compounds was determined on MCF-7 (human breast) and HT-29 (human colon) cancer cell lines by MTT assay (IC_{50} values of the compounds on MCF-7 cell line were 1-5 μM and on HT-29 cells 10-20 μM) and their in vivo antitumor activity was evaluated on HT-29 human colon carcinoma bearing SCID mice. GnRH-III(Dau=Aoa) and GnRH-III(Dau=Aoa-GFLG) bioconjugates with 15 mg/kg Dau content had significant antitumor activity, (tumor growth inhibition: 41 % and 50 %, respectively); however, a higher daunorubicin content in bioconjugates (30 mg/kg) was also well tolerated.
Our results indicate that GnRH analogue-daunorubicin oxime-linked bioconjugates represent a multivalent system which can be a potential drug delivery system for targeted cancer chemotherapy.
This work was supported by grants from the Hungarian National Science Fund (OTKA NK 77485) and the Ministry of Health (GVOP-3.2.1.-2004-04-0005/3) and the Zukunftskolleg, University of Konstanz.

P434. Abstract number: 482

Miniprotein engineering of the knottin-like scaffold Min-23 - Fmoc-assisted synthesis and oxidative folding strategies

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Owing to their high enzymatical and structural stability, miniproteins are ideal scaffolds for medical application as epitope binding motifs. Min-23 is a miniprotein derived form of the plant-occurring cyclotide ecballium elaterium trypsin inhibitor II (EETI-II). It is a rationally designed 23-mer peptide with a cystine-stabilised beta-sheet (CSB) motif incorporating three beta-sheets, one alpha helix and two disulfide bridges that frame an autonomous folding unit. The plasticity of the CSB motif is tolerant to multiple amino acid substitutions, which allows the integration of a randomized affinity function into the stably folded framework. These characteristics classify Min-23 as a peptide scaffold for lead generation for medial applications using *in vitro* display techniques. However, engineering of cystine-knotted miniproteins is a challenging process, which requires an efficient solid-phase syntheses and an oxidative folding strategy.
Synthetic access to the primary structure of cystine-stabilized knotted peptides was realized by Fmoc/tBu-SPPS using a fully automated peptide synthesiser and dedicated amino acid building blocks. Simultaneous cleavage from the solid support and of the protecting groups was achieved by TFA-treatment. Disulfide formation was performed using a simultaneous or a consecutive oxidative folding strategy. Analysis and characterization of the SPPS-derived peptides and monitoring of the disulfide formation were achieved by HPLC-ESI-MS.
The first solid phase peptide syntheses of Min-23 showed a high aggregation during the chain elongation within the helical section and aspartimide formation. These drawbacks were overcome using specific pseudoproline building blocks. The simultaneous formation of two disulfide bridges was achieved by oxidation in a DMSO-free, buffered solution in less then 24 hours. Using an orthogonal protecting group technique, Min-23 could be folded in different desired conformations. This synthesis strategy is used for the synthesis of peptide motifs identified by phage and ribosome display screening.

P435. Abstract number: 525

Synthesis and application of peptide bioconjugates for diagnosis of M. tuberculosis infection

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Synthetic peptides comprising linear or continuous topographic epitope sequences of proteins are frequently considered as specific and small size antigens. However, it has been demonstrated that short peptides corresponding to the core of the T-cell epitope could possess only limited capability to induce specific T-cell responses. One of the promising approaches to maximize immunoreactivity could be the attachment of optimized epitope in multiple copies to oligo- or polypeptide carriers. Therefore it is important to develop rational chemical strategies for preparation of epitope-conjugates with preserved immunospecificity. In order to study systematically the role of spacial arrangement we have prepared peptide epitope-conjugates with diverse topology like linear, branched, dendrimeric. Our data show that immunorecognition of the epitope could be markedly influenced by the carrier structure and topology. In comparative studies we have studied the T-cell response properties of these bioconjugates containing oligopeptides representing an immunodominant epitope domain of the 38 kDa (Rv0934) and of 16 kDa protein (Hsp16.3, acr1, Rv2031c) of M. tuberculosis. For functional studies PBMC from healthy PPD positive and negative donors as well as treated/non-treated patients with active tuberculosis were stimulated and changes in T-cell proliferation and IFN-□ production were measured. We found that the chemical nature of the carrier, the form of epitope presentation have marked effects on the T-cell responses detected.
These studies were supported by grants of Hungarian Research Fund (K 68285, K68358), GVOP (GVOP-3.2.1 - 2004 - 04 -0352/3.0, 3.2.1 - 2004-04-0005/3.0), of National Office for Research and Technology (NKFP_07_1-TB_INTER) and by Italian-Hungarian Intergovernmental Programme (I-51/2003)

P436. Abstract number: 535

Synthesis and characterization of novel dipeptide ester of acyclovir

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Two dipeptide (Ile-Pro,Val-Pro) ester prodrugs of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, ACV) were synthesized. LC/MS was used to characterize the new prodrugs. Both 1H NMR and 13C NMR spectra of the two prodrugs of ACV were measured and assigned based on spectral comparison with compounds of similar structures.

P437. Abstract number: 4

Nonopioid beta-endorphin receptor: myth or reality

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Even early in the 80s of the last century, the investigators noticed that the effect of β-endorphin on immunocompetent cells does not depend in some cases on the presence of naloxone and hence is not mediated via opioid receptors. Nonopioid receptor for β-endorphin on human cultured lymphocytes was discovered by E.Hazum more than 30 years ago. We synthesized a selective agonist of nonopioid β-endorphin receptor decapeptide immunorphin (SLTCLVKGFY). [^{3}H]Immunorphin was found to bind to nonopioid β-endorphin receptor of mouse peritoneal macrophages (K_d = 2.0 ± 0.1 nM). The [^{3}H]immunorphin specific binding with macrophages was inhibited by unlabeled β-endorphin (K_i = 2.9 ± 0.2 nM) and was not inhibited by unlabeled naloxone, α-endorphin, γ-endorphin and [Met^5]enkephalin (K_i > 10 mM). Thirty fragments of β-endorphin have been synthesized and their ability to inhibit the [^{3}H]immunorphin specific binding to macrophages was studied. Unlabeled fragment 12-19 (TPLVTLFK, the author's name of the peptide octarphin) was found to be the shortest peptide possessing practically the same inhibitory activity as β-endorphin (K_i = 3.1 ± 0.3 nM). The peptide octarphin was labeled with tritium (the specific activity of 28 Ci/mmol). [^{3}H]Octarphin was found to bind to macrophages with high affinity (K_d = 2.3 ± 0.2 nM). The specific binding of [^{3}H]octarphin was inhibited by unlabeled immunorphin and β-endorphin (K_i = 2.4 ± 0.2 and 2.7 ± 0.2 nM, respectively). Octarphin was shown to stimulate activity of murine immunocompetent cells in vitro and in vivo: at the concentration of 1-10 nM enhanced the adhesion and spreading of peritoneal macrophages as well as their capacity to digest bacteria of Salmonella typhimurium virulent strain 415 in vitro; peptide administered intraperitoneally at dose 20 μg/animal on day 7, 3 and 1 prior to the isolation of cells increased activity of peritoneal macrophages as well as T- and B-spleen lymphocytes.

P438. Abstract number: 142

The second extracellular loop of the angiotensin receptor AT1 contains an a helix homologous to the β-adrenoceptors

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In most crystallographically determined 7TM receptor structures some ectodomains of the functionally important extracellular surface (ECS) are not completely resolved. Here, we used an exhaustive methionine proximity assay (MPA) to identify ECS residues of the angiotensin II type 1 (AT1) receptor that are in close proximity to the N-terminus portion of receptor-bound octapeptide angiotensin II (AngII). We identified 38 ligand/receptor contact residues that compose the binding environment of the AT1 receptor for the N-terminal part of AngII. These contacts are in addition to the MPA-derived 12 contacts of the C-terminal residue and the 3 found for residue 7 of AngII. Due to its more favourable structure-activity profile the receptor mutant N111G-hAT1 was used for this study. The obtained set of 58 ligand-receptor contacts allowed for the experimentally-based docking of the AngII molecule in a liganded AT1 receptor homology model. The resulting molecular structure provides a comprehensive view of the AngII-liganded AT1 receptor complex. In particular, the extracellular loop (ECL) 2 features an a-helix similar to those observed in the β-adrenoceptor crystal structures and which is in close contact to residue 3 of Ang II. The here presented finding on the AT1 receptor adds therefore considerable weight to the proposed hypothesis that an ECL 2 helix is a common feature of 7TM receptors that respond to diffusible ligands.

P439. Abstract number: 281

New Socs1-Kir Mimetic Peptides Through The Screening Of Focused Symplified Combinatorial Libraries

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Suppressor Of Cytokine Signalling (SOCS) proteins are negative feedback regulators of several pathways involved in immune response, particularly the JAK/STAT (Janus kinase/Signal Transducer and Activator of Transcription) [1]. Usually their basal levels are low, but they can be selectively induced by cytokines, such as IFNγ. SOCS1 inhibits IFNγ signalling for its capacity to bind and inactivate JAK2 protein and consequently to block the IFNγ-induced tyrosine phosphorylation of IFNγ receptor (IFNγR) and STAT1 activation. It has been demonstrated that keratinocytes avoid the detrimental consequences of an excessive stimulation by IFN-γ over-expressing SOCS1 thus hindering the expression of many pro-inflammatory genes, including those involved in skin diseases, such as psoriasis and allergic contact dermatitis (ACD) [2]. A three-dimensional model of the complex between SOCS-1 and JAK2 [3] shows that the Kinase Inhibitory Region (KIR) of SOCS-1 protrudes towards the catalytic region of JAK2 and occupies the ATP binding site. Here we present new peptides mimicking KIR-SOCS-1 binding activity identified through an ELISA-based screening of a focused simplified combinatorial peptide library [4]. On the basis of an Ala-scanning investigation we have firstly restricted KIR domain (52-67) to a shorter region (52-61) improving binding affinity, then several positions within this sequence have been randomized leading to the selection of new and more potent ligands as antagonists of SOCS-1. These new mimetics bind to JAK2 catalytic site (both in phosphorylated and non-phosphorylated form) in a dose dependent manner providing KD values in the high nanomolar range that are 15-fold lower respect to w-t KIR. Cellular experiments on STAT1 activation signaling suggest their potential application as modulators of disorders involving SOCSs overexpression.

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P440. Abstract number: 487

Molecular Dynamics Simulations of the Angiotensin-II Type 1 Receptor and its N1111G CAM

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The angiotensin-II type 1 receptor (AT1-R) is a family-A GPCR implicated in the regulation of water retention and blood pressure. Recent results obtained from MPA (methionine proximity assay) and SCAM (substituted-

cysteine affinity method) experiments provided information on residues present in the binding pockets of the wild-type receptor (AT1R-WT) and its constitutively active N111G mutant (AT1R-N111G). Those results indicate the existence of a conformational change between the two binding pockets and the two receptors. Characterization of this structural change is of the utmost importance to understand constitutive activation of the N111G mutant. This structural change has been investigated by molecular dynamics (MD) simulations. The AT1 receptor was modelled by homology using the family-A GPCRs crystal structures. MD simulations of the receptors inserted into an hydrated bilipidic membrane were performed with GROMACS. Temperature equilibration was performed for 100ps, followed by a 2ns pressure equilibration. The MD simulations were 21ns in length. Several differences are observed between the WT and N111G receptor. Notably, a stable H-bonds network is observed between residues N111 of transmembrane domain 3 (TMD3), D74 (TMD2), and N295 (TMD7) in the WT receptor. This network is likely stabilizing the inactive state of the receptor. In the constitutively active mutant, the H-bonds network between TMD 2, 3 and 7 is destabilized and a new residue is needed to compensate for the loss of N111 in the stabilization of D74. Two alternate configurations of the network have been observed : either with S115 (TMD3) or N46 (TMD1) completing the D74-N295 network. SCAM results suggest that N46 is the most likely candidate, but the fact that S115 can reach that area of the receptor shows that TMD3 can move more freely in AT1R-N111G than in AT1R-WT due to the loss of stabilization by the N111-D74-N295 H-bonds network. Formation of the N46-D74-N295 network in AT1R-N111G is associated with the formation of an H-bond between R126 (TMD3) of the DRY motif and Y215 (TMD5) at the intracellular part of the receptor. Litterature suggests that this interaction is linked with the coupling of the alpha sub-unit of the G-protein to the receptor. We therefore propose that the improved mobility of TMD3 caused by the destabilization of the N111-D74-N295 network contributes to the constitutive activity of the N111G mutant by facilitating the coupling of the receptor with the G-protein.

P441. Abstract number: 543

The influence of opioids on matrix metalloproteinase-2 and -9 secretion and mRNA levels in MCF-7 breast cancer cell line

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The main cause of failure in cancer treatment is not due to growth of the primary tumor but results from its intensive spread (metastasis) to secondary sites. The critical step of cancer dissemination is migration of cancer cells through the extracellular matrix. Indispensable in this process is the activation of proteolytic enzymes capable of degrading the extracellular matrix. The key proteases that are involved in this process are matrix metalloproteinases MMP-2 and MMP-9. Several studies have shown that MMP expression and activity are increased in several tumor types, especially breast and lung cancer.

Opioids are often administered to cancer patients in order to relieve pain. This study was designed to test the effect of morphine and endomorphin-2 (EM-2) on MMP-2 and MMP-9 secretion and expression levels in MCF-7 breast adenocarcinoma cells, expressing μ opioid receptor.

The obtained results demonstrate that morphine and EM-2 decrease the expression and secretion levels of MMP-2 and MMP-9 in the time- and concentration-dependant manner. However, attenuation of MMP secretion by both opioids was not influenced by opioid antagonist naloxone, indicating that opioid receptors were not involved in the process. This may be explained by other mechanisms, for example by the interaction of opioids with some intracellular molecules, such as nitric oxide (NO). NO is a multifunctional messenger molecule generated from arginine by a family of NO synthases (NOS) . It was shown that production of NO by tumor cells can enhance the angiogenetic and metastatic potential of tumors. Our further experiments showed that morphine and EM-2 inhibited the expression of NOS and secretion of NO, which suggests that opioids exerted their inhibitory effect on MMP-2 and MMP-9 by modulation of NO/NOS system.

Down-regulation of MMP secretion by not only morphine but also EM-2 suggests that administration of EM analogs with improved pharmacological profile and reduced addictive potential may open new possibilities in cancer research and should be further tested on experimental animals.

P442. Abstract number: 18

Utilization of enzymes from the red king crab hepatopancreas to obtain protein-hydrolyzate of cow milk

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The paper presents comparative analysis of molecular-weight distribution of peptide fractions in hydrolyzates of cow milk protein concentrate derived by proteolysis using enzymatic agents "Flavourenzyme", "Pancreatin" and an enzymatic agent from the red king crab hepatopancreas. Suitability of these hydrolyzates as complexing agents with essential microelements was estimated. Formation of microelement complexes, containing peptides with molecular weight of 1,4 kD - 11,2 kD and obtained by proteolysis of cow milk protein concentrate using an enzymatic agent from the red king crab hepatopancreas, is potentially of the highest value.

Waste from fishery and processing of marine invertebrates are used partly, mainly as feed for fur-bearing animals. However, its weight can comprise up to 90 % of a catch.

Sufficient quantity of these raw materials as well as the possibility to use them as sources of enzymes open new prospects for their application. We demonstrated earlier how these enzymatic agents can be used to derive protein hydrolyzates for different purposes.

Nowadays development of new organic forms of digestible essential cationic microelements (EM), such as manganese, zinc, copper and chromium, is an acute problem in medicine, particularly in dietology. One of the ways to solve this problem is to conjugate essential cationic microelements with peptide structures which are formed as a result of enzymic hydrolysis of alimentary proteins.

The efficiency of binding EM by enzymolyzate and, respectively, the content of these microelements in the complex are mostly dependent on fractional composition of the latter. On the other hand, molecular-weight distribution of peptide fractions and the content of free amino acids in enzymolyzate are related to many factors (pH-medium, temperature, duration of enzymolysis and others), but mainly affinity of effects the used enzymatic agents have.

In this connection it is very important to choose an appropriate enzymatic agent with proteolytic activity.

The aim of the paper is to present comparative analysis of molecular-weight distribution of peptide fractions in hydrolyzates of cow milk protein concentrate derived by proteolysis using different enzymatic agents, particularly enzymes of marine origin as well as to estimate suitability of obtained hydrolyzates as complexing agents with essential microelements.

P443. Abstract number: 19

Isovaline-containing peptides: configurational assignment using 2D-NMR spectroscopy

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By 2D-NMR we investigated the naturally occurring and chemically synthesized 16-mer integramides A and B, belonging to a group of bioactive, fungal peptides (peptaibiotics) that are characterized by an abundance of Aib as well as D- and L-Iva residues. The chemical shifts of the C^{α}-alkyl groups in the Iva enantiomers depend on the α-carbon chirality and on the helical screw sense of the peptides, the latter determined by CD. In the full-length, right-handed helical, integramides, as well as in the partial sequences exploited for their total chemical syntheses, the γ-methyl protons of the ethyl side chain of the D-Iva residues located near the C-terminus are significantly more shielded (δ <0.90 ppm) than those of the L-Iva residues (δ >0.95 ppm). The opposite behavior is observed for the left-handed, synthetic, intermediate Z-Aib-L-Hyp-L-Iva^{14}-D-Iva^{15}-OtBu. Here, the γ-methyl protons of L-Iva^{14} are more shielded (0.838 ppm) than those of D-Iva^{15} (0.905 ppm). The chemical shift difference between the diastereotopic β-methylene protons of the Iva side chains in the right-handed helical peptides is much larger for D-Iva than for L-Iva. For D-Iva$^{14/15}$, the values range from 0.38 to 0.63 ppm, whereas for D-Iva1 the value is between 0.26 and 0.31 ppm. In each case, the difference is always larger for the D-Iva than for the L-Iva residues (which is always ≤ 0.19 ppm). Again, an opposite behavior is seen for the left-handed tetrapeptide. Overall, our method enables the non-destructive assignment of the configuration of each Iva residue in peptides of known helical screw sense.

P444. Abstract number: 25

Molecular modeling of novel GnRH analogues using NMR spectroscopy and relation with their anti-cancer activities

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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. Considerable effort has been developed to the synthesis of peptide mimetic structures to overcome the unfavorable properties and also therapeutic deficiencies of peptides.

In a continuation of our research work for the synthesis of pharmaceutical peptides, and in order to study the effect of modification in triptorelin acetate structure, some of new triptorelin analogues were designed, and synthesized via an efficient Ugi-4MCR. The anti-cancer activity of analogues were studied. They showed better anti-cancer activity compared to triptorelin acetate.

Herein, we wish to report the relationship between structure and activity of these novel compounds using conformational analysis and NMR spectroscopy.The NMR studies reveal that GnRH has flexible conformation.

However, it seems that in the solution phase, a turn conformation of triptorelin analogues were occurred and this was supported by computational analysis data.

Results show that the inhibitory effect of GnRH analogues on the cancer cells depends on the nature of the substituent structure.

Our data about the structure of these GnRH analogues using NMR spectroscopy will be described.

P445. Abstract number: 26

Synthesis of Novel Peptides Containing unusual Gamma amino acids and Investigation of their Nanostructures

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The design of molecular building blocks that can self-assemble is an interesting task in multidisciplinary research work. Dipeptide FF and some of its derivatives shows nanostructure and biological activities. Gazit and his colleagues revealed that existence of aromatic residues provide p-p interactions and it may play a significant role in the molecular recognition and self-assembly processes that lead to amyloid formation. Gabapentin as a Gamma amino acid has considerable interest for its unique ability for the increasing the peptide crystallinity.
We wish to report, herein the synthesis of some tri-and tetra-peptides using Phenylalanine, Gabapentin and Baclofen as starting amino acids with different sequences.
All of the desired peptides were synthesized in solution phase and using TBTU as coupling reagent. It seems that the existence of additional aryl group in Baclofen in the final tri- or tetra- peptide could affect the p-p stacking of the aryl groups. The results will be described.

P446. Abstract number: 49

Structural control of diastereomeric Leu-Leu-Aib-Leu-Leu-Aib sequences

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The design of peptides, which are folded well-defined secondary structures, is crucially important in a wide variety of fields such as organic chemistry, biological, and material sciences. The approach to control the conformations of peptides have been made, and we have studied the conformation of peptides composed of α,α-disubstituted α-amino acids in order to achieve this. We have recently reported the attachment of Aib residues at the L-leucine-based hexapeptide (L-Leu-L-Leu-Aib-L-Leu-L-Leu-Aib) stabilized a right-handed 3_{10}-helical structure. Herein, we designed and synthesized three diastereomeric peptides; Boc-L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib-OMe (**1**), Boc-L-Leu-D-Leu-Aib-L-Leu-D-Leu-Aib-OMe (**2**), and Boc-L-Leu-D-Leu-Aib-D-Leu-L-Leu-Aib-OMe (**3**), and studied their preferred conformations in the crystalline state. The crystals of the three peptides were characterized by the X-ray crystallographic analysis, and the dominant conformation of peptide **1** was a left-handed 310-helical structure, that of **2** was a β-turn structure, and that of **3** was an S-type turn structure, respectively.

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P447. Abstract number: 64

Optical spectroscopy and conformational analysis of peptide aggregates: the role of aromatic interactions and conformational flexibility

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Elucidating the self-assembly mechanism of peptides is of fundamental importance for therapeutic reasons (e.g., Alzheimer and Creutzfeld-Jacob diseases) and the development of new peptide-based materials (hydrogels, fibrils, nanotubes). Experimental evidence indicates that peptide aggregation is mainly driven by hydrophobic interactions, where aromatic residues play a specific role. Conformational effects were often claimed to be of secondary importance and conformational disorder was even suggested to be a prerequisite for fibrillization. To tackle this important issue more deeply, the aggregation propensities of oligopeptides functionalized with aromatic groups, <i*e.* naphthyl (N) or pyrenyl (Py) units, and characterized by differing conformational properties were investigated in solution by optical spectroscopy methods and theoretical conformational analysis. In particular, our results concerning two different peptide systems will be presented: i) Peptide foldamers comprising exclusively C^{α}-tetrasubstituted amino acids [$(Aib)_nN$, with n = 6,12,15]. In this case, the extensive use of conformationally-constrained residues forced the oligopeptide to attain a rigid helical structure, that in turn governed the morphology of the peptide aggregates. Formation of J- and H-aggregates in methanol and water/methanol solutions was thoroughly investigated by steady-state and time-resolved fluorescence, optical microscopy and non-linear spectroscopy techniques. Specifically, we focused on the dependence of the aggregation properties on peptide length and secondary structure. (ii) Two Ala-based pentapeptides [Py-CH_2-CO-$(L\text{-}Ala)_5$-OtBu and Py-CH_2-CO-$(L\text{-}Ala)_3$-Aib-L-Ala-OtBu], both N-functionalized with the Py fluorophore. It will be shown that a single Aib *vs.* Ala substitution changed dramatically the aggregation propensities of the peptide investigated, strongly perturbing the conformation and dynamics of the monomeric peptide molecules, and determining the morphology of the aggregates. We emphasize that only the combined application of spectroscopic techniques (CD, fluorescence, FTIR absorption), optical and atomic force microscopies, and molecular dynamics simulations allowed us to characterize in detail the structural and dynamical factors governing the formation and stability of the peptide aggregates investigated.

P448. Abstract number: 65

Mapping charge delocalization in a peptide chain triggered by oxidation of a terminal ferrocene moiety

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Electron transfer is one of the most relevant processes in chemistry and biology. Long-range electron transfer (ET) through proteins and enzymes is a fundamental step for all living organism. The spatial organization of the electron donor and electron acceptor in peptides and proteins, as well as the dynamics of the ET between them, critically depends on the structure-directing and charge-transmitting properties of H-bonds. ET through helices has attracted much attention because their parallel assemblies are universal motifs found in biological ET systems. In this connection, two series of peptides of different length and rigidity, based on the strongly helicogenic α-aminoisobutyric acid (Aib) residue and containing a terminal ferrocene (Fc) unit, Fc-CO-$(Aib)_n$-OMe (A_n, n = 1-5) and Z-$(Aib)_n$-NH-Fc (B_n, n = 1-5), were prepared and investigated. We utilized the oxidation-state sensitive, spectroscopic tags of peptides, the CO and NH groups, to map charge delocalization triggered by oxidation of the terminal ferrocene, induced and monitored by Vis-IR-NIR spectroelectrochemistry. The rigid and well-defined 3_{10}-

helical structure of $(Aib)_n$ homo-peptides is advantageous for the study of the distance dependence of charge delocalization. The orientation of the carbonyl groups in the 3_{10}-helix produces a dipole with the positive end at the N-terminus and the negative end at the C-terminus. Thus, the A_n and B_n peptides series are characterized by an opposite-direction macrodipole towards the redox Fc/Fc^+ probe. The effect of the different orientation of the dipole moment (the latter increasing with the number of Aib residues in the backbone), was investigated by means of electrochemical and spectroscopic techniques. The oxidation potential dramatically depends on the dipole direction and the number of Aib units (the latter tuning intramolecular H-bond formation). Moreover, the dependence of current intensity on peptide length was also explored. Finally, the shift and pattern of the CO and NH vibrations upon oxidation were used for mapping charge delocalization in the peptide chains, and estimating efficacy and time scale of intramolecular ET.

P449. Abstract number: 68

Paternò-Young photocyclization reaction in Bpa/Met 3-10-helical peptides: role of spacer length

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The 3-(4-benzoylphenyl)alanyl (Bpa) residue is extensively used as a photoaffinity label, particularly for studies of *intermolecular* (peptide)ligand-receptor(protein) interaction, where it is believed to most frequently remove a hydrogen atom from the side chain of a Met residue followed by covalent C-C bond formation of the resulting radical pair. We are currently carrying out an extensive and detailed investigation of this Paternò-Young *intramolecular* photochemical reaction in a series of backbone rigidified, 3_{10}-helical hexapeptides of general sequences Boc-$U_xBU_yMU_z$-OMe and Boc-$U_xMU_yBU_z$-OMe, where B=(S)-Bpa, U=Aib, M=(S)-Met, and $U_x+ U_y+ U_z=4$. We aim at determining the effects induced by the spacer length (U_y=0-3) on the chemical and 3D-structures of the resulting products and their ratios. We have already reported our results for the UBU_2MU hexapeptide substrate (1). Here, we describe our recent FT-IR absorption and NMR data on the conformational analyses of other four linear hexapeptides, which support the view of the onset of stable 3_{10}-helical structures for these substrates. Moreover, we demonstrate the significant effects on the isomeric product ratios, and the region- and stereospecificities of the photocyclization reactions played by the distance and relative orientation of the Bpa/Met side chains, and their parallel *vs*. antiparallel alignment with the peptide helical axis.

Moretto, A.; Crisma, M.; Formaggio, F.; Huck, L. A.; Mangion, D.; Leigh, W. J.; Toniolo C. Chem. Eur. J. 15 (2009) 67-70.

P450. Abstract number: 69

Experimental and theoretical spectroscopy study of 3-10-helical peptides using isotopic labeling

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Experimental and theoretical studies of IR absorption, VCD, and Raman spectra have been performed on 3_{10}-helical peptides with two different main-chain lengths: hexapeptides (*i*PrCO-Aib-L-Ala-Aib-L-Ala-L-Ala-Aib-NH*i*Pr) and octapeptides (Ac-Aib-L-Ala-Aib-L-Ala-L-Ala-Aib-Aib-Aib-OMe). These peptides are based on isotopic labeling (^{13}C=O) of the L-Ala residue on the following positions: *A4* and *A5*, (single labeled); *A4A5*, *A2A4* and *A2A5* (double labeled) for the hexapeptide, and *A4A5* for the octapeptide. Aib favors the 3_{10}-helix due to restriction of the backbone (φ,ψ) torsion angles by its α-carbon tetrasubstitution. Theoretical IR absorption, VCD, and Raman simulations were performed on sequences identical to the synthesized ones with φ, ψ torsion angles constrained to an ideal 3_{10}-helical geometry (-60°, -30°), but with all the other coordinates fully optimized. All calculations were performed for peptides *in vacuo* using the DTF BPW91/6-31G* levels of theory. The simulations predicted the conformational dependence of the relative separations of ^{13}C=O and ^{12}C=O features, and the exciton splitting of the ^{13}C=O band in the doubly labeled species to be in agreement with those seen experimentally, with the exception that fraying at the N- and C-termini causing a change in diagonal force field are not well represented in the theoretical modeling. Experimental spectra for the octapeptide (*A4A5*) confirmed the source of deviation for the related hexapeptides. Comparison of IR absorption and VCD intensity patterns helped sort out the vibrational coupling constants sensed in the ^{13}C=O modes. The isotopic labeled group vibrations are coupled to each other most strongly when they are degenerate and are effectively uncoupled from those of the unlabeled groups.

P451. Abstract number: 166

Systematic design and screening of immune modulating innate defence regulator peptides

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With the rapid rise in the incidence of multi-drug resistant infections, there is substantial interest in utilizing natural host defence peptides as templates for the development of novel anti-infective therapeutics. Previously, we reported that the synthetic peptide innate defence regulator peptide-1 (IDR-1) confers protection to bacterial infection in vivo through modulation of host immunity. As the design of IDRs with enhanced immunomodulatory activities is an intriguing approach for developing novel anti-infectives we created a library of IDRs through systematic design. IDR-mediated immunomodulatory activity was assessed by their ability to induce chemokines/cytokines in human peripheral blood mononuclear cells. The enhanced chemokine/cytokine induction activity of the peptides in vitro correlated with the extent of protection they conferred in vivo in a Staphylococcus aureus infection model. Structure activity studies of the peptide library revealed a consensus sequence within the subset of IDRs with enhanced immune protection. By investigating thousands of chemical descriptors characterizing the different peptides in the library, it was also demonstrated that direct antibacterial and immune modulating peptide activities largely depended on separate physicochemical peptide properties.

P452. Abstract number: 221

Peptide regulators of insect reproduction containing D-amino acids

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An introduction of D-amino acids into the peptide chain of pentapeptide (5P) H-Tyr-Asp-Pro-Ala-Pro-OH [1] has maintained or even forced oostatic effect of corresponding analogues in comparison with the parent 5P. The analogues caused a decrease of hatchability in the 1st, and elimination of hatchability in the 2nd gonotrophic cycle of the flesh fly *Neobellieria bullata*. Morphology changes in ovaries are linked to a large resorption of the eggs in treated insect. A degradation of the D-Asp^2 or D-Ala^4 analogues of this 5P proceeded with a significantly lower rate. An incorporation of radioactivity in vitro from the labeled 5P H-Tyr-Asp-[3H]Pro-Ala-Pro-OH into ovaries of the *Neobellieria bullata* [2,3] has been significantly decreased after D-Asp or D-Ala introduction. These differences might be linked to increased stability of the peptide bonds between the central Pro^3 residue and the D-Asp^2 (also connected with N-terminal Tyr^1) or the D-Ala^4 (also connected with C-terminal Pro^5) residues, respectively. Proteolytic enzymes must handle with an opposite orientation of D-amino acid side-chains, shown in our NMR study. Moreover, the different distances of the D-Asp^2 and D-Ala^4 residues from the Tyr^1 residue, at which a very quick metabolic cleavage of the 5P starts in ovaries with a final release of [3H]Pro^3 [4], are responsible for the differences between both the 5P analogues, with the respect to oostatic activity, degradation and radioactivity incorporation in the flesh fly *Neobellieria bullata.* In general, the life prolongation of the oostatic peptides, after the introduction of D-amino acids, resulted in more ovaries influenced during a period of their development, which enhanced the oostatic activity of corresponding analogues.

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P453. Abstract number: 231

Dendrimeric peptides with affinity to opioid receptors - complexation properties

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One of the most promising types of molecules allowing practical accomplishment of functional polyvalency are dendrimers - synthetic macromolecules of nanoscopic dimensions built from several layers of branches assembled around a central core. This affords location of a high number of functional groups at the surface. Unlike other macromolecular compounds, their unambiguous composition, reliability and versatility of their synthesis, make this type of carriers well-suited to various medical and biochemical applications.

Pharmacological characterization of opioid receptor gave evidence of its complex and multiple structure. Therefore, dendrimerc compounds seem to be well suited for design of ligands that interact efficiently with the nervous system receptors.

Here we present convergent synthesis, characterization and biological activity of small library of dendrimers built around Lys(Lys)2 dendron, functionalized with N-terminal fragment of enkephalins. These dendrimers have been used as complexing agents for other peptides known as opioid ligands - biphalin and neurotensin. Application of ESI MS and HPLC methods revealed that in water solution a 1:1 complex is formed between dendrimers and biphalin but not with neurotensin. In addition, solubility of the complex in water was better than biphalin alone. Binding studies to opioid receptors were performed for dendrimers alone as well as for 1:1 complexes with biphalin. It appears that the designed dendrimers might be used as new delivery agents for small opioid peptides.

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P454. Abstract number: 252

NMR-based conformational studies of the C-terminal heptadecapeptide (101-117) of human cystatin C

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Human cystatin C (hCC) is a small (13 kDa, 120 amino acid residues) protein, a member of the superfamily of papain-like cysteine protease inhibitors. It is the most common cystatin in mammalian body fluids involved in various diseases including cerebral amyloid angiopathy, cerebral hemorrhage, stroke and dementia [1]. hCC is a potential target in the Alzheimer's disease; it was found that it binds amyloid β (Aβ) and reduces its aggregation and deposition [2].

Recent in vitro studies including Aβ and hCC found that the C”terminal (101”117) fragment of the latter is the minimal Aβ”binding epitope of hCC [3]. Current communication is an extension of this work and includes NMR-based conformational studies of hCC (101”117) epitope. Structural information derived from sets of 2D NMR spectra will help to understand oligomerization of hCC, possibly including its self-aggregation and interactions with Aβ.

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P455. Abstract number: 272

Beta-Aspartic acid impairs the ability to bind metal ions by immunosuppresory fragment of ubiquitin and somatostatin as studied by ESI-MS/MS

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Recently ubiquitin was suggested as a promising antiinflammatory protein therapeutic [1]. We found that a peptide fragment corresponding to the ubiquitin$^{50-59}$ sequence (LEDGRTLSDY) possessed the immunosuppressive activity comparable with that of ubiquitin. The peptide was much less toxic than cyclosporine, particularly at higher doses [2]. The cyclic

peptide designed to mimic the ubiquitin 50-59 loop strongly suppressed the immune response, suggesting that ubiquitin and its LEDGRTLSDY fragment may interact with the same hypothetical receptors [3].
It is known that the formation of complexes of proteins and peptides with metal ions often plays a significant role in their interaction with the respective receptors [4].
Our earlier studies proved that the mass spectrometry can provide reliable information on the stoichiometry and a possible binding mode of the peptide-metal complexes [5,6]. Therefore, we applied high-resolution mass spectrometry (Bruker apex-ultra FT-ICR spectrometer) to study the interaction of LEDGRTLSDY fragment analogues with with Cu(II) and Zn(II) ions. A series of the peptide analogs, which included acetylated and amidated peptides and sequences with Asp residue substituted by βAsp, was synthesized. Similar approach was also applied to a series of somatostatin fragments analogues.
The analysis of CID spectra for the LEDGRTLSDY peptide and its analogues indicates that the most probable site of the Cu(II) ion binding is the Glu residue. Substitution of Asp residue by βAsp as well as N-terminal acetylation drasticaly reduce the intensity of the complex while C-terminal amidation does not influence the ability to bind Cu(II) ions. We did not observe any peaks for Zn(II)-peptide complexes.
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P456. Abstract number: 274
Influence of adjacent amino acid on photophysical properties of 3-(2-benzoxazol-5-yl)alanine derivatives
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3-(2-benzoxazol-5-yl)alanine derivatives are unnatural fluorescent amino acids which spectral and photophysical properties are in many cases more favourable than natural fluorophores (long-wavelength absorption and emission, high molar absorption coefficient and fluorescence quantum yield). Because of great number of derivatives based on this skeleton, these compounds have various potential applications such as fluorescence probes of peptides and proteins, solvatochromic probes, pH or metal ions sensors as well as antimicrobial agents [1-7]. However, although the photophysical properties of fluorophore itself are quite well-known, there is no detailed information how they change after incorporation of the compound into a peptide chain. Because of that we modified amino or carboxy group of selected benzoxazolylalanine derivatives by all proteinogenic amino acids except cysteine and studied spectral and photophysical properties of obtained series of dipeptides using absorption and steady-state and time-resolved fluorescence spectroscopy to establish influence of the amino acid presence.
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P457. Abstract number: 279
Ensemble fit of conformational equilibria of restrained peptides to the NMR data. Dependence on the force fields: Amber versus ECEPP/3
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The pursuit of this work was an optimal conformational analysis method for short flexible peptides. We used two dermorphin Tyr-D-Daa-Phe-Daa-NH^2 analogs consisting of combinations of D-Dab^2 (α,β-diaminopropionyl) with Lys^4, Analog **1**, and of D-Dap^2 (α,γ-diaminobutyryl) with Orn^4, Analog **2**. The side chain amino groups were coupled in them by a carbonyl, restraining them with 16- and 14-membered rings and resulting in a potent and impotent μ/δ opioid peptide, respectively [1]. The Authors [1] provided us with their NMR NOE- and J-data in H_2O/D_2O, and with the results of fitting conformational equilibria of 1 and 2 to their NMR data, using an ensemble generated with EDMC-ECEPP/3 methodology [2].
We did a similar statistical fitting of conformational equilibria of **1** and **2** to the NMR NOE- and J-data, yet using to this aim an ensemble generated by extensive Amber molecular dynamics in water and the same fitting methodology [2], for comparison. Despite the first being a stiff- and the second a flexible-geometry-method to screening a conformational space, both methodologies provide similar conformational equilibria, in which the restraining rings fit well within 1.4Å both among conformational families within a method and also between the both approaches. On the contrary, the force-field effects to the flexible side-chains of Tyr i Phe were different. Hence, for short peptides, reliable conformational results are obtained from NMR supported with ensemble fit only within limits of a restraint (if there is any), no matter of the force-field used. With no restraint, different equilibria, depending on force field used, may fit common NMR data.
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P458. Abstract number: 296
Understanding and Modulation of the Folding of a Helix-Loop-Helix Dimerization Domain

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The helix-loop-helix (HLH) domain is the characteristic dimerization domain of the HLH transcription factor family. This domain triggers the formation of dimers that display a parallel four-helix bundle. This type of protein-protein interaction is essential for the biological function of the HLH proteins. For example, the Id proteins are HLH transcription factors that inhibit the action of other HLH factors by sequestering them in inactive heterodimers. By using this mechanism, the Id proteins promote cell proliferation and inhibit cell differentiation during development and tumorigenesis. We are interested in the inhibition of Id protein dimerization with their endogenous partners (i. e. E47 and MyoD). Our work focuses on the identification (i) of the structural prerequisites for the correct HLH folding and (ii) of short peptide sequences targeting the Id HLH domain and altering its folding/dimerization properties. Studies based on amino-acid substitutions and backbone modifications have underlined the role of some primary-structure elements on the secondary and tertiary structures adopted by a synthetic Id HLH peptide. Side-chain packing and backbone direction are both crucial for the optimal fold. Further, we have evaluated the ability of short peptides to interact with the Id HLH domain and prepared some peptide constructs that have been tested on smooth muscle cells expressing the Id protein. One construct has been shown to be only moderately active (low-micromolar range), but able to reduce cell growth and migration, stimulate cell differentiation and considerably decrease the cellular Id protein level.

P459. Abstract number: 299

2D IR spectroscopy of oligopeptides conformationally restrained by dialkylated amino acid residues

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Recent advances in ultrafast two-dimensional infrared (2D IR) spectroscopy have demonstrated its unique capabilities in elucidating peptide structures. To develop these techniques with full rigor and to establish spectrum-structure relationships, studies in model systems are needed. To this end, oligopeptides composed of $C^{\alpha,\alpha}$-dialkylated α-amino acid residues serve as ideal model systems because of their well-defined peptide backbone conformations. Here, we report the amide-I/II 2D IR spectra of two series of peptides in $CDCl_3$: (1) unlabeled and isotope labeled Aib-rich hexapeptides, Z-Aib-L-Leu-$(Aib)_2$-Gly-Aib-O*t*Bu (Aib, α-aminoisobutyric acid) and (2) Ac-(Deg)*n*-O*t*Bu (Deg, $C^{\alpha,\alpha}$-diethylglycine, *n* = 2-5). We demonstrate how 2D IR spectral signatures reveal the underlying 3_{10}-helical conformation in (1) and fully-extended (C_5 conformation) structure in (2).
In the study of Aib-rich hexapeptides, a novel isotope labeling scheme was devised to probe vibrational couplings between amide-I and -II modes on specific residues. The 2D IR spectra of $^{13}C=^{18}O$-Leu mono-labeled and $^{13}C=^{18}O$-Leu/^{15}N-Gly bis-labeled isotopologues exhibit isotope-dependent amide-I/II cross peaks, clearly indicating that the second and fourth peptide linkages are vibrationally coupled as they are in proximity, forming a 3_{10}-helical turn. Theoretical calculations, based on an expanded vibrational exciton model, reasonably reproduce the experimental results. In contrast, the semi-extended structure is predicted to exhibit no isotope shifts in the amide-I/II cross peaks. This conformational sensitivity indicates that 2D IR is promising for detecting nascent helix formation.
The Deg homopeptides exhibit unusual spectroscopic features: the amide-II band is more intense than the amide-I band and it red shifts with increasing chain length. We have performed detailed analysis of the peptide conformation through stringent comparisons of measured and simulated amide-I and -II 2D IR spectra. The results indicate that the backbones of these homopeptides are fully extended regardless of the chain length. This conclusion is corroborated by molecular dynamics simulations and density functional theory calculations. The complete characterization of the vibrational properties of the amide-I and II modes in the fully-extended structure will facilitate the conformational analysis of other peptides involving C_5 motifs.

P460. Abstract number: 343

In vitro antiviral properties of alloferon, Any-GS and their analogues against human herpes virus and coxsackie virus B2

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Alloferon (H-His-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH) has been isolated from the blow fly *Calliphora vicina*[1]. Alloferon demonstrates the stimulatory effect on natural killer lymphocytes *in vitro*, and has antiviral and antitumor activity *in vivo*. Pentapeptide *Any*-GS (H-Asp-Ile-Leu-Arg-Gly-NH_2) has been isolated from the wild silkmoth *Antheraea yamamai*[2]. This peptide suppresses proliferation of the rat hepatoma cells. In preliminary investigations we found that alloferon and *Any*-GS inhibited the replication of human herpes virus type 1 (HHV-1) *in vitro*[3].
For further studies on antiviral activity against both DNA and RNA viruses we selected the following peptides:
1/ alloferon (I) and its analogues modified at position 1, [des-His^1]-(II), [Lys^1]-(III), [Arg^1]-(IV) and [Ala^1]-alloferon (V),
2/ *Any*-GS (VI), its shortened derivatives: [1-4]-(VII), [2-5]-(VIII), [3-5]-Any-GS (IX), and the analogues modified at position 1 such as: [Asn^1]-(X), [Arg^1]-(XI), [Gln^1]-(XII), [Gly^1]-(XIII) and [Ala^1]-Any-GS (XIV).
Peptides were synthesized by the standard solid phase method. The cytotoxic properties of investigated peptides were evaluated using Vero and HEp-2 cell lines. The cytotoxicity was determined by the MTT (3-[4,5 dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. In further experiments the antiviral activity was assessed in respect to HHV-1, wild-type HHV-1 and coxsackie virus type B2 using two cell lines, Vero and HEp-2. We found that most of the evaluated peptides presented antiviral activity. Moreover, all the peptides did not show cytotoxic activity against the Vero and HEp-2 cells. The results obtained indicate that the insect peptides may be useful antiviral agents for HHV-1 and coxsackie virus B2.

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P461. Abstract number: 345
Novel calpain inhibitors
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Calpains are intracellular cysteine proteases and are of considerable interest due to their implication in numerous physiological events. Besides physiological functions, they play a key role in some well-studied pathological processes. The overactivation of calpains, which is resulted in by the disorder in Ca^{2+} homeostasis, increases the degradation of the enzyme substrates and could contribute to the development of the Alzheimer and Huntington diseases and also to death of nervous cells caused by traumatic brain injury, spinal cord injury (1). One of the main methods to study the calpain function is to inhibit the enzyme. Unfortunately, there is only one specific calpain inhibitor of native origin is known, the calpastatin protein. Other inhibitors used in the literature are not specific, which might confuse the interpretation of the results (2). The use of calpastatin or its region B, as specific inhibitor is hampered by their low cell-penetration ability. Our aim is to develop new, specific and cell-permeable calpain inhibitor(s), to be used for analysis of calpain function under different conditions and also for the development of drugs for treatment of different calpain dependent diseases. The aza-peptide inhibitors were designed from the substrate, (TPLKSPPPSPR) described by us (3). The novel inhibitors described here contain *N*-terminal part of the substrate and amino acids from P'_1 to P'_2, P'_5 or P'_7. The Lys after which the enzyme cleaves the substrate was replaced by aza-aminoacids. A group of aza-peptides were synthetised by solid phase synthesis using aza-amino acids as hidrazide derivatives and 1,1'-carbonyldiimidazole. The inhibitory effect of new aza-peptides on enzyme Calpain B was measured by fluorimetry with calpain substrate developed in our laboratory (4). The inhibitory effect of aza-peptides was mainly dependent on the C-terminal part of the molecule.
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P462. Abstract number: 354
PNA-peptide conjugates for regulation of DNA and RNA G-quadruplex structures depending on a particular protease concentration
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It has been elucidated in the last few decades that some DNA and RNA secondary structures modulate a variety of cellular events. It is recently suggested that a G-quadruplex structure, which is one of the secondary structures, also regulates cellular events such as transcription, translation, pre-RNA splicing and telomerase elongation and these events are related to serious diseases and aging (1). Therefore, systems that could control DNA and RNA G-quadruplex structures when needed would be able to modulate various cellular events, and as a result the systems could provide biological effects. From this point of view, peptide conjugates of PNA (peptide nucleic acid) to control G-quadruplex structures depending on a particular cellular environment were constructed in this study. First of all, the conjugate sequences were designed. The conjugates consisted of two parts. One part was composed of Guanine PNA-rich sequences to induce G-rich DNA/RNA sequences to form G-quadruplex structures with PNAs. The other part was a switching module depending on a particular cellular environment. In this study, we selected a particular protease concentration as the cellular environment. Consequently, a particular protease substrate sequence was applied to the part. Thus, this system would induce DNA/RNA to form G-quadruplex structure when the protease would not be expressed in cell, and once expressing the protease, these conjugates would be digested and simultaneously loose the induction ability resulting in collapse of a DNA-PNA quadruplex structure. After synthesis of the peptides by Fmoc chemistry, we are checking the G-quadruplex structure induction by CD spectroscopy, electrophoresis and so on. CD melting curves of a target DNA with/without the conjugate indicated that a G-rich DNA could be induced to form PNA-DNA G-quadruplex. Also we are trying to demonstrate the switch function depending on the protease concentration. Throughout this study, these PNA-peptide conjugates would be one of the promising tools for regulation of important cellular events toward cell engineering and tissue engineering.
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P463. Abstract number: 473
Structure of a new stable Cu(III)/cyclopeptide complex by Cu K-edge XAS study
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The study of a 13-membered ring cyclic tetrapeptide *c*(Lys-dHis-Ala-His) (DK13) synthesized by the solid-phase peptide synthesis method revealed the presence, at alkaline pH, of a stable peptide/Cu^{3+} complex formed in solution by atmospheric dioxygen oxidation[1].

Cu K-edge XANES and EXAFS spectra have been collected for three different cyclo-peptides: *c*(Lys-His-Ala-His) (LK13), *c*(Lys-dHis-Ala-His) (DK13), and *c*(Gly-Ala-Gly-Lys) (GK13) to confirm the presence of Cu^{3+} species. Comparison of pre-edge peak features with those of Cu model compounds, allowed to determine the Cu oxidation state in the three peptides: Cu is purely divalent in GK13, purely trivalent in DK13 and present as both Cu^{2+} and Cu^{3+} in LK13. Also edge energies and EXAFS derived Cu-N distances (1.79 Å and 1.90 Å for DK13 and GK13, respectively) are consistent with the XANES determined Cu oxidation states. Theoretical XANES spectra have been calculated by means of the MXAN code. The initial structural model has been calculated according to a DFT code; the structural refinement with the MXAN code allowed to find optimised values of the overlap between Muffin-Tin spheres; to refine the coordinates of the neighbouring N atoms and refine the coordinates of all other atoms.

The good agreement between theoretical and experimental XANES data allows to assume that the refined structure, at least in the first coordination shell around Cu, is a good approximation of the peptide/Cu^{3+} complex structure. These results suggest that the CuN_4 unit is not planar but form a slightly distorted pyramid[2]. Despite no clear explanation can be provided yet, available data strongly suggest that imidazole side chains have an important role in the formation of Cu^{3+} complexes in DK13 peptide.

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P464. Abstract number: 542

CLIPS peptides: high enzymatic stability

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It is well known that linear peptides are not stable against enzymatic degradation. Addition of D-amino acids or beta-amino acids to the selected sequence, modification of the C- and N-termini, or modification of the peptide bonds itself have been shown to improve the stability. However, these modifications require extra, sometimes complex steps in the synthesis of these products.

In our lab, CLIPS™ technology (Chemical LInkage of Peptides onto Scaffolds) was developed for chemical fixation of peptides [1]. This technology was used successfully to select and reconstruct active sites of several proteins [2], but was also expected to improve the enzymatic stability of the construct.

In this study a bicyclic 15-mer CLIPS peptide was synthesized by reacting CSDRFRNCPADEALC with 1,3,5-tris-(bromomethyl)benzene [3]. The enzymatic stability of the peptide was determined in human serum and other enzyme preparations and compared with extended bicyclic sequences, with linear analogues, monocyclic counterparts, and with retro-inverso analogues. Fragmentation products could be identified using LC-MS. The bicyclic CLIPS peptides were found to be extremely stable showing their potential as drug products.

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P465. Abstract number: 561

Tryptophan Interactions That Stabilize Folding Motifs: A Guide to Placement, Dynamics Applications, and Expectations for Fold Stabilization

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A number of interactions provided by the indole ring of tryptophan have emerged as fold stabilizing features in miniprotein and beta-hairpin systems; these include: pi-cation interactions with Arg and Lys sidechains, the indole ring serving as a pi Lewis base in H-bonding interactions with an amide-NH, hydrophobic interactions with other aromatic and aliphatic sidechains, and indole/indole ring interactions of specific geometries. Trp has one of the larger beta-propensities that have been measured in hairpin constructs; this explains some of hairpin stabilization results that have been observed in Trp-containing hairpin models, but the more specific interactions noted must be taken into account to explain the hyperstable constructs that have been obtained in some cases. Each of these indole interactions has rather specific stereostructural requirements that must be understood to design peptide folds. Of these, the indole/indole (W/W) interaction will be presented in this abstract.

In proteins, W/W interactions resulting in CD exciton couplets have been recognized for at least 18 years. The common geometry that results in the CD exciton couplet and diagnostic chemical shifts, the latter arising ring current effects, is an edge-to-face (EtF) alignment of the two indole rings. In hairpin models this geometry, and fold additional stabilization, occurs only when the W/W unit is at a non-H-bonded strand site that is either turn-flanking or near the other termini of the strands. The NMR diagnostics of the EtF interaction are far upfield shifts for the beta-CH_2 and indole H4 hydrogens of the Trp that serves as the ”edge”in the EtF interaction. These structuring shifts are large enough to effect exchange broadening of the NMR signals that can be used to measure folding rates in the l/kF = 200 nsec - 50 microsec range. This will be illustrated with KTW-NXXXXK-WTE peptides; in this series, folding rates can vary by as much as a factor of 20 (depending on the XXXX sequence) for peptides that display the same thermodynamic stability for the hairpin fold.

The indole/cation and amide-NH/indole H-bonding interactions also have specific structural requirements which result in placement requirements in fold design. The amide-NH/indole H-bonding interaction also produces very large structuring shifts, chemical shift deviations as large as 3.9 ppm have been observed. The chemical shift effects noted here allow the determination of fold structures with high precision.

P466. Abstract number: 140

Rational Insights in peptide affinity towards inorganic surfaces

Development of Peptides with High Affinity to Inorganic Surfaces

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Immobilization of specified chemokines or cell attracting motifs is of great interest in the field of ”intelligent”biomaterials. It has been shown previously, that peptides can be discovered that selectively bind to different metal surfaces in non-covalent manner [1]. Interestingly, those peptides can be obtained by solid-phase peptide synthesis [2], which provides high flexibility and sufficient amount to perform structure-activitiy studies. Alternative methods e. g. phage-display-systems, led to the identification of peptides that bind to different surfaces like silicone, gold and titanium.

Here we present an ELISA-like assay for a straightforward quantification of peptide-surface interactions. Peptides were synthesized by solid-phase peptide synthesis and N-terminally extended by biotin. This allows a rapid detection of the peptides bound to different surfaces by using a streptavidin-POD-conjugate. The subsequent oxidation of TMBH2 catalyzed by the enzyme is measured photometrically at 450 nm and is dependent on the amount of surface-bound peptides. We could apply the system to determine the binding of peptides on a multitude of surfaces ranging from TiO2, ZnO to SiO2. Furthermore the tested peptides showed sigmoidal dose-response-curves indicating specific binding. Thus we were able to determine EC50-values ranging from the low µM-range for good binders to values above 1 mM for weak binders.

Considering the advantages of an ELISA-based assay with respect to parallel testing of different peptides on various surfaces we are now able to improve the understanding of peptide-surface interaction. This is necessary to bridge the gap from the common combinatorial procedure to a more rational approach in the design of peptides binding to solid supports. This is crucial to obtain sequences that show a high affinity and an improved selectivity of solid-surface binding peptides.

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P467. Abstract number: 169

Design, Synthesis and Potential Applications of Conformationally Restricted Peptides

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The de novo design of peptides and proteins has elicited considerable interest in recent years. α,β̃-didehydroresidues, in particular α, β didehydrophenylalanine (ΔPhe) are being considered important conformational constraints inducing tools in de novo peptide design. ΔPhe, an analog of phenylalanine with a double bond between Cα and Cβ atoms induces conformational constraint in the peptide structure, and provides the peptide with increased resistance to enzymatic degradation. In small ΔPhe containing peptides, a type II β̃ turn and in longer peptides helical structures are stabilized. Using ΔPhe, super secondary structural motifs like helix turn helix have also been designed and synthesized. Based on these design principles a series of cationic peptides containing ΔPhe residues have been synthesized which showed potent antibiotic activity, fast kinetics of bacterial killing with no hemolytic activity suggestive of their highly specific antibacterial activity. We have also found that small ΔPhe containing dipeptides could self-assemble into stable hydrogels or nanostructures, depending on the peptide motif or assembling conditions. Dipeptide nanostructures could encapsulate drug molecules and release them under suitable conditions. They were stable to proteinase K digestion, noncytotoxic and taken up by mammalian cells. Anticancer drug loaded nanostructures showed enhanced cytotoxicity towards cancerous cell lines. Folic acid functionalized nanoparticles showed enhanced bioavailability and tumour accumulation. Interestingly, Phe-DPhe at higher concentration and appropriate conditions assembled into a nanofibrous hydrogel with high mechanical strength. It responded to changes in pH, temperature, ionic strength and entrap and release bioactive molecules in a sustained and stimuli responsive manner. The hydrogel could be functionalized, through the free amino groups, with cell growth promoting ligands like the tripeptide, RGD. The modified gel supported attachment, proliferation and 3D growth of cells simultaneously preserving their metabolic activities. These interesting properties of stable peptide based nanostructures make them useful templates for further development for future applications.

P468. Abstract number: 184

De novo designed triple-helix bundle proteins on a carbohydrate template

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De novo design and total chemical synthesis of proteins allows the preparation of novel biomolecules to address complex biological questions. It offers the prospect of designing proteins with new tailored properties. The development of highly efficient methods for coupling of unprotected peptides has placed the reliable chemical synthesis of proteins within reach of bioorganic chemistry. We have previously described a class of chimeric model proteins, termed carboproteins, in which carbohydrates are used as templates in de novo design of proteins. The template preorganizes the secondary structure elements and directs the formation of a tertiary structure, thus achieving structural economy.

Here we describe the design and synthesis of novel 3-helix bundle carboproteins. Tri-functional monosaccharides served as templates for anchoring of α-helical peptides to form triple-helix bundle carboproteins. The folding was studied using biophysical methods such as circular dichroism (CD) spectroscopy, size exclusion chromatography (SEC) and, finally, small angle X-ray scattering (SAXS). CD indicated the expected presence of α-helical structure; next, it was used to measure the stabilization of carboproteins to thermal and guanidine hydrochloride denaturation. Then, we analyzed the self-assembly of the carboprotein and the free peptides in solution by SAXS. The most promising self-assembled carboproteins from CD and SAXS analysis were also studied by molecular dynamic simulations (MD). Our result suggest that rational designed de novo protein can be synthesized and analyzed in solution, furthermore structural and dynamics behavior can be predicted and further optimized.

P469. Abstract number: 201

Au25 Nanoclusters Capped by Photoactive Aib-based Azopeptides.

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The study of novel phenomena and processes at the molecular level provides useful knowledge for designing a wide range of tools, materials, devices, and systems with unique characteristics. Monolayer-protected gold nanoclusters of small size (< 1.5 nm) are of particular interest because they are rather complex hybrid systems but still display quasi-molecular features, such as the presence of a well-defined HOMO-LUMO gap [1]. The introduction of different ligands into the nanoparticle-capping monolayer can significantly affect their properties, such as the redox or optical behavior. For this purpose, rigid 3_{10}-helical peptides based on the α-aminoisobutyric (Aib) acid unit are very interesting molecules because they are still and possess a strong dipole moment [2,3]. In this work we used the ligand place-exchange approach to introduce a thiolated Aib-peptide derivative, HS-CH_2CH_2-Ph-N=N-Ph-CO-Aib_3-NHtBu, into the monolayer of preformed phenylethanthiolate-protected Au_{25} nanoclusters. By varying the ligand place-exchange conditions we could vary the load of azopeptide in the monolayer. Upon irradiation at 360 nm, trans-cis isomerization of the -N=N- bond was expected to change the spatial orientation of the peptide dipole moment as well as the distance between the peptide moiety and the nanoparticle core. By using the free ligand, we observed that irradiation leads to the stationary state mixture of the cis and trans isomers and that the peptide 3_{10}-helical structure remains intact. Thermal decay experiments showed that the spontaneous -N=N- cis to trans conversion in the nanoparticle monolayer is about three times faster than in the free azopeptide. The synthesis and the outcome of photochemical and electrochemical experiments will be discussed.

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P470. Abstract number: 440

Biomedical nanotechnology: preparation and characterization of new functionalized gold nanoparticles.

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Over the past decade gold nanoparticles have attracted much interest since they are versatile agents with a variety of biomedical applications including use in highly sensitive diagnostic assays, thermal ablation and radiotherapy enhancement, as well as drug and gene delivery[1]. Several stabilizing agents, that interact with nanoparticle surface, are usually added to improve their stability. In particular, recently we have studied the use of peptide sequences based on the GGC motif as capping agents in the preparation and characterisation of monolayer-protected gold nanoparticles[2]. These capped systems can be considered as a good scaffold for a functionalization with molecules for the targeting of receptor tumor marker such as integrin receptors. The aim of this research is the wet chemistry preparation and characterization by ATR-FTIR, XRD and TEM of new capped gold nanoparticles functionalized with molecules encompassing the RGD motif critical for the integrin binding.

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P471. Abstract number: 460

On-silica peptide probes: innovative in vitro diagnostic devices for detection of antibodies as biomarkers of autoimmune diseases

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The development of in vitro diagnostic devices (IVDD) based on peptides is a challenge for efficient antibody detection of autoimmune diseases. In fact ELISAs have often the great disadvantage not to guarantee efficient exposition of the peptide epitope for efficient autoantibody recognition [1].

The design of nanomaterials with high levels of selectivity is a challenge common to various areas of nanodiagnostics, including sensor development. In the bio-analytical context there are many different approaches used for the detection of key biomolecular interactions in a range of different environments, i.e. serum. The approach we have been focussing on is the use of functionalised silica nanoparticles coupled to peptide antigens as efficient probes to detect autoantibodies as biomarkers in autoimmune diseases patients' sera.

The glucosylated peptide CSF114(Glc) is the first specific Multiple Sclerosis (MS) Antigenic Probe, active molecule of MS PepKit, a peptide-based ELISA diagnostic test developed to recognize specific autoantibodies in sera of a statistically significant MS patients' population [2].

In order to propose an alternative solid support for improving detection of specific autoantibodies in MS patients' sera, silica surfaces modified with CSF114(Glc) have been developed. Because of silanol groups providing binding sites, silica surfaces are attractive solid supports for identifying antigen-antibody specific interaction. Different strategies for silica peptide immobilization have been investigated. Therefore, we elucidated structural properties of the surface linked glycopeptide by different spectroscopic techniques. Moreover, we tested these innovative on-silica glycopeptide probes by an enzymatic assay to evaluate detection of specific autoantibodies in MS patients'sera.

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P472. Abstract number: 485

Topical Vaccination with Nanoparticles

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Vaccines need to be developed that effectively induce antigen-specific CD8+ T cells, the key effector cells in cellular immune responses. These T cells recognise peptides generated by limited proteolysis from cellular proteins that are bound by MHC class I molecules and presented at cell surfaces. The new vaccines should be stable, safe and easy to handle.
To this avail we designed nanoparticle carriers for synthetic vaccine components coated by layer-by-layer technology, and optimised their size and physicochemical properties. The vaccine componenents, T helper and CTL epitopes, and synthetic adjuvants, were synthesised by fully automated solid phase synthesis using Fmoc/tBu-chemistry, purified to homogeneity by preparative HPLC and characterised by HPLC-MS. They were tagged for loading onto the particles and the loading efficiencies established with fluorescein-labelled analogous. The specific immunostimulating capacity of the peptide-functionalised nanoparticle vaccines was investigated and established with bioassays in vitro and in vivo.

P473. Abstract number: 554
Self-assembling Peptides: Biomimetic Fabrication and Application
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Nature has selected and evolved numerous and diverse chemical and molecular structural motifs through billions of years of molecular selection and evolution and these motifs are the basic building blocks of a wide range of sophisticated nanomachines that work at astonishing speed and efficiency with the finest controls. Only now we begin to learn from Nature"'in its finest molecular details and intricate interactions of numerous fine parts: we are learning the basic molecular engineering principles for nano- and micro-fabrication at the exquisitely fine scale through the understanding of molecular self-assembly phenomena.
Molecular self-assembly is ubiquitous in Nature and has recently emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials and engineering. Two complementary strategies can be employed in the fabrication of molecular biomaterials. In the 'top-down' approach, biomaterials are generated by stripping down a complex entity into its component parts. This contrasts with the 'bottom-up' approach, in which materials are assembled molecule by molecule and in some cases even atom by atom to produce novel supramolecular architectures. The latter approach is likely to become an integral part of nanomaterials manufacture and requires a deep understanding of individual molecular building blocks, their structures, assembling properties and dynamic behaviors. Two key elements are required for molecular fabrication, which are chemical complementarities and structural compatibility, both of which confer the weak and noncovalent interactions that bind building blocks together during self-assembly.
We have designed and fabricated a set of self assembling peptides-mediated biomaterials and significant advances have been achieved at the interface of biology and materials science, including the fabrication of nanofiber materials for 3-D cell cultures, tissue engineering and regenerative medicine, the peptide detergents for stabilizing, and crystallizing membrane proteins as well as nanocoating molecular for cell organizations. Molecular fabrications of nanobiomateirals have fostered diverse scientific discoveries and technological innovations.

P474. Abstract number: 14

Beta-Sheet Amphiphilic Peptides Replication; Kinetics and Mechanism

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Non enzymatic molecular replication has been the subject of intense research over the past two decades, and several different replicating systems have been prepared and analyzed, including nucleic acids, fatty acids, peptides, and organic molecules.[1] Several research groups including the authors have studied in the past alpha-helix forming sequences that self assemble to coiled-coil tertiary structures. In these systems, monomeric or dimeric peptides, twenty five to forty amino acids in length, served as templates for substrate binding and thus for enhanced condensation and replication. However, it has been postulated that shorter peptides with simpler sequences may serve as templates for self replication, provided that they are able to arrange themselves into unique and well defined structures. I will present here the design and kinetic analysis of rather simple peptides, close analogs of the synthetic amphiphilic Glu-(Phe-Glu)n peptides that can form soluble one-dimensional beta-sheet aggregates in water, and serve to significantly accelerate their ligation and self replication.[2]

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P475. Abstract number: 63

Photocurrent generation through mono- and bi-component peptide self-assembled monolayers: antenna and junction effects

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Biomolecules have been extensively investigated as possible components of nano-scaled electronic circuits. In particular, hybrid materials obtained by functionalizing metals or semiconductors with biomolecules or bio-inspired molecular systems have been recently synthesized, paving the way for the fast-growing field of bionanoelectronics. In this contribution the photocurrent generation properties of mono- and bi-component peptide-based self-assembled monolayers (SAMs) immobilized on a gold surface were studied by electrochemical, spectroscopic and microscopic techniques. The peptides investigated comprise exclusively C^{α}-tetrasubstituted α-amino acids. These residues, due to their peculiar conformational properties, force the peptide backbone to attain a helical conformation, as confirmed by X-ray crystal structure determinations and CD measurements in solution. An important feature of helical peptides is the electrostatic field generated by the macrodipole resulting from the vector sum of the electric dipoles associated to the peptide bond of each residue (3.5 D). This property imparts a specific polarity to a self-assembled monolayer (SAM) formed by helical peptides thiolated at the N-terminus, with the negative charge (δ^-) at the C-terminus and the positive charge (δ^+) at the N-terminus. It has been shown that such electrostatic field significantly affects the gold surface potential, and the efficiency and direction of the ET process through the peptide chain, stabilizing the charge-transfer state properly aligned along the field. The peptides investigated were suitably functionalized with chromophores strongly absorbing in the UV region with the aim at enhancing the efficiency of the photocurrent generation (*antenna effect*). The influence of the nature of the peptide/gold interface on the ET process (*junction effect*) was analyzed by comparing the photocurrent efficiency in peptide SAMs immobilized on a gold surface through Au-S linkages with that in a bi-component peptide SAM formed by a photoactive peptide embedded into the covalently linked monolayer produced by a thiol N^{α}-functionalized peptide.

P476. Abstract number: 67

Vibrational energy transport in a peptide capping layer over gold nanoparticles

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Gold nanoparticles have found numerous applications in life sciences as labels, where they are often covered by biocompatible capping agents. Thermal relaxation processes in metal nanoparticles and the subsequent energy transfer process into a solvent have reached considerable interest in current research. To obtain site-selective information about different positions in a 3_{10}-helical peptide capping layer we used ^{13}C isotope labeling. The two peptides studied were HS-CH_2CH_2-O-CO-NH-Aib-Ala*-Aib_6-OMe (**1**) and HS-CH_2CH_2-O-CO-NH-Aib_3-Ala*-Aib_4-OMe (**2**), where an asterisk indicates a ^{13}C=O label. The N-terminal HS-groups of these peptides allow covalent attachment to the gold nanoparticles. Due to a difference in the metal core diameter (sample **1**, 1.2 nm; sample **2**, 2 nm) the plasmon resonance in the Vis spectrum of **2** is more pronounced. Because of the size-dependent surface/volume ratio a higher absolute concentration of **2** is needed to reach the same effective peptide concentration as **1**. In a VIS pump /IR probe experiment we deposited vibrational excess energy in the gold nanoparticles by exciting their plasmon resonance and monitored the responses of spectrally resolved C=O ”thermometers”in the backbone of the helical peptides. The sequential appearance of the bleach maxima of the different C=O reporter units nicely showed the propagation of vibrational energy through the capping layer: the further a reporter unit is away from the heated gold nanoparticle, the later and the less vibrational energy arrives. We observed that the cooling behavior depends on the particle size: due to a lower surface/volume ratio, bigger particles cool slower. To analyze the structural distribution and its dynamics, we recorded the 2D-IR spectra of the capping layer. From the distinct tilt of the 2D-IR peaks, in particular the isotope labeled one, we concluded that the transitions are strongly inhomogenously broadened. The increased inhomogeneous broadening, compared to that of the isolated peptides in solution, indicates strong interactions among the aligned peptides in the capping layer or between the peptides and the gold nanoparticles.

P477. Abstract number: 162

Cyclic amino acid-containing alpha-helical peptide-catalyzed enantioselective epoxidation reaction

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Poly L-alpha-amino acids-catalyzed asymmetric epoxidation of chalcone was reported by Julia, Colonna and co-workers. They studied the generality of substrates, reaction conditions, effect of the length of oligomers, and reaction mechanisms. In the reaction, poly L-alpha-amino acids might form alpha-helical structures, and their alpha-helical N-termini are important for asymmetric induction. To stabilize the alpha-helical structure of catalyst, polyethyleneglycol and resin-attached L-Leu oligomers have been developed, and thus, the high molecular weight and insolubility of catalysts were in part overcome.

Replacement of α-hydrogen atom of L-alpha-amino acids results in alpha,alpha-disubstituted amino acids (dAA). An alpha,alpha-disubstituted amino acid, alpha-aminoisobutyric acid (Aib), is well-known, and is widely used to construct helical secondary structures of oligopeptides. Helical secondary structures constructed using Aib are usually not alpha-helices but 310-helices. Oligomers having Aib (310-helices) were used as catalysts for the asymmetric epoxidation of chalcone, but high enantiomeric excess of epoxide was not obtained.

Recently, we have disclosed that cyclic amino acid-containing L-Leu-based peptides preferentially formed right-handed (P) alpha-helices. Thus, we reasoned that cyclic amino acid-containing peptides would catalyze the asymmetric epoxidation of chalcone. We synthesized chiral cyclic amino acid-containing peptide catalysts for enantioselective epoxidation, revealed the relationship between the helical secondary structures and enantiomeric excesses, and re-modeled the peptide catalyst by taking the hydrogen bonding pattern of helices into consideration. Finally, we developed highly enantioselective epoxidation of alpha,beta-unsaturated ketones using chiral cyclic amino acid-containing L-Leu peptide as a helical catalyst.

P478. Abstract number: 383

Rigid, hydrophilic, macroporous, methacrylate functionalized SynbeadsTM polymers for efficient industrial SPPS

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Nowadays many peptide drugs are produced in multi-tons scale on solid support.1

Efficiency of solid phase synthesis is strongly related to supports. Not all the commercial supports are compatible with the different types of solvents and reagents, so the proper support must be selected for each specific application. The general requirements of the support to be used in automated and large scale SPPS are mechanical stability, chemical inertness, consistency of loading, and bead size.

SynbeadsTM are methacrylate functionalised hydrophilic supports with very high physico-chemical stability in several harsh conditions.2,3 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. HR-MAS NMR analysis showed that this technique allows monitoring reaction steps directly on Synbeads; functional group density can also be quantified on the matrix.

We demonstrated that the flexibility of the linkers, once coupled on SP, is directly correlated with chemical accessibility. One of the major advantages of using a rigid matrix as Synbeads is the significant reduction of solvents, reagents and time consuming in the synthesis of peptides of pharmaceutical interest. Different peptides have been synthesized on Synbeads with the aim of comparing yield and purity with standard commercial swelling polymers.

Cheap and reactive reagents and linkers such as DIC/HOBt coupling reagents with Ramage and Rink linkers have been tested to demonstrate that Synbeads can be easily applied also in these disadvantageous reaction conditions allowing peptide production with high yields and product purity. Results demonstrated that peptide synthesis on Synbeads can be easily performed allowing very high yields and product purity, even in comparison with other commercial swelling supports.

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P479. Abstract number: 187

Site-specific lipidation of unstructured peptides sequences as a method for producing amyloid-like peptide assemblies.

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Lipidation of peptides is commonly used to alter their native conformation, improve their proteolytic stability, increase membrane permeability and immunogenicity. Modification of amyloid beta peptides with lipid chains has been shown to influence their propensity for aggregation and their cytotoxicity as a consequence of the increased amphiphilicity.

We have investigated the induced change in the properties of a series of peptides upon introduction of hydrophobic domains. In order to probe the scope of the methodology, native and mutated sequences derived from the known unstructured N-terminus of amyloid beta were selected for the study. Specifically, different numbers and types of lipid chains were introduced at the C-terminus and/or N-terminus of the peptide sequence through the side chain of added lysine residues. The influence of different parameters (type and number of lipid chains, peptide sequence, peptide length and net charge) in the conformation adopted and aggregation state of the resulting lipopeptides was determined by different physico-chemical and spectroscopic techniques.

In summary, several disordered sequences could be tuned into a more ordered amyloid-like conformation suggesting that the method could be general. Importantly the described site-specific lipidation triggered peptide assembly into beta-sheets fibers without any additional external factor (pH, ionic strength, enzymes, temperature, light, etc) and independently of the amino acid sequence. The resulting amphipatic peptides could have application as immunogenic constructs, peptidomimetics, bioactive scaffolds, nanostructure materials, antimicrobial agents, etc.

P480. Abstract number: 280

Design and synthesis of bipyridyl-containing peptide dendrimers as iron protein models

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Peptide dendrimers are hyperbranched molecules which possess suitable properties as soluble protein models, such as a hindered, solvent-shielded core reminiscent of many enzyme active sites and multiple peripheral groups which can improve water solubility as well as display cooperativity. Since peptide dendrimers are composed of amino acids, their chemico-physical properties and hence their activity are easily tunable by modifying their sequence or introducing non-natural amino acids.

In our research on metalloenzyme models, we have employed dendrimers containing bipyridyl-based moieties for the complexation of late transition metals, in particular iron. We have recently reported[1] the synthesis of a combinatorial library of peptide dendrimers carrying a bipyridyl derived amino acid (Bpy) in the focal point. A colorimetric assay of iron binding on solid phase, as well as spectrophotometric binding tests in solution, showed that the ability of the bipyridyl containing dendrimers to bind Fe(II) is strongly modulated by the properties of the amino acid residues in the sequence. In particular, positively charged sequences didn't show any complex formation, but were able to form mixed iron complexes together with negatively charged dendrimers.

We have then prepared dendrimers carrying two Bpy residues, one in each of the two first generation branches. The presence of two bidentate ligands in the same molecule was shown to strengthen metal binding. Some of the dendrimers have shown formation of 2:1 complexes with iron, in which the coordination valence of the metal was saturated by two bipyridyl units from one dendrimer and a third one from a second dendrimer.

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P481. Abstract number: 285

Synthesis Of Self-Assembled Glycolipopeptide And Its Activation Of Peritoneal Macrophages

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The glycoconjugates play important roles, such as intercellular recognition, proliferation control of a cell, the information transmission and so on. Peritoneal macrophage (PEM) was activated by artificial oligomannnose-coated liposome (OML) [1)]. Recently we have reported the synthesis of the self-assembled lipopeptide, Ac-Tyr-Ser-Gln-Glu(Hda)-Gln-Ser-Ser-Ser-Ser-Gln-Glu(Hda)-Gln-Ser-Gly-NH_2, using Fmoc-Glu(Hda)-OH[2)]. This Glu(Had) residue has a long alkyl chain consisting of hexaethleneglycol and decanamine. From AFM and TEM images, we speculated that this lipopeptide might form a cylindrical micelle. In order to develop the alternative of OML, we synthesized a self-assembled glycolipopeptide, Ac-Tyr-Ser-Gln-Glu(Hda)-Gln-Ser-Ser(Man)-Ser-Ser-Gln-Glu(Hda)-Gln-Ser-Gly-NH_2, having Ser(Man) and Glu(Hda) residues. From the AFM and TEM images, we found the synthesized glycolipopeptide might form spherical vesicle and planar bilayer. Next we analyzed production of interleukin (IL)-12 from peritoneal macrophages stimulated with synthesized glycolipopeptide by ELISA. We found this self-assembled glycolipopeptide showed the similar activity to those of OML. Details of these results will be discussed.

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P482. Abstract number: 301

Computational Studies of the Stability and Chirality of Self-assembled Complexes of a Novel Iron(II)-binding Insulin Derivative

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In a concurrent study, we demonstrated experimental evidence for the iron(II) directed self-assembly of a novel insulin derivative (human insulin (B9Asp + B27Glu)) functionalized with bipyridine through the B29Lys side-chain. Here we present the results from computational studies on several putative self-assembled states involving

the chelation of iron(II) by three engineered insulin monomers. In particular, we demonstrate the application of molecular dynamics (MD) simulations in probing the relative stability of four trimeric insulin complexes, each corresponding to one of the two enantiomers (Δ or Λ) of the meridional (*mer*) and facial (*fac*) configurations at the $Fe(bipy)_3$ site. Through application of time-dependent density functional theoretical calculations on the coordination environment around the metal ion, the electronic spectra of the diastereomers have been predicted. By comparison with experimental UV-VIS and circular dichroism spectra, the stereochemistry of the species found in solution can be assigned.

P483. Abstract number: 366

'Super Helix' Formation by Stapled 14-Helical Hexa-β3-Peptides

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β^3-Peptides, (oligomers of β^3-amino acids) are among the most widely studied unnatural peptides at present, due to their ability to adopt discrete and stable secondary helical structures. Of the many types of helices formed by β^3-peptides, 14-helices are particularly interesting as they have 3 residues per helical turn (with a pitch of 5.0Å) and are stabilized by 14 membered hydrogen bonding rings. This allows the side chains to be aligned perfectly in three faces. The residues at positions i/i+3 are not only on the same face of the helix but are also are relatively close to each other. The aim of this study was to design and synthesise novel 14-helical stapled β^3-peptides on which further functionalisation of the stapled face of the helix can be performed and used to create novel peptide assemblies.

β^3-Peptides were synthesised by incorporating O-allyl-β3-serines and allyl-β^3-glycines at i/i+3 positions and efficiently stapling the allyl groups via Ring Closing Metathesis (RCM) on a solid support[1]. The CD and 2D-NMR spectra showed that β3-peptides, stapled as well as unstapled, exhibited 14 helix signatures. Crystallography confirms that the peptides are not only 14-helical but that they assemble into 'super-helices'. These are rod-like structures which self-assemble axially through three inter-molecular hydrogen bonds between adjacent peptides. The crystal packing shows that 'super-helices' are arranged in anti-parallel sheets and are held together by hydrophobic interactions and apparently critical hydrogen bonding. Thus, the binding motifs can be enhanced by attaching functional groups on the stapled face of the β-peptides to generate new molecules of biological significance.

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P484. Abstract number: 449

Tubomicella of gene transfection agent 1,4-dihydropyridine lipid and its binding with DNA

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Artificial lipids are in wide use for gene and drug delivery. In our prior work [1] we showed with molecular dynamics (MD) simulation, that the gene transfection agent, 1,1'-{[3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridin-2,6-diyl]dimethylene} bispyridinium dibromide (1,4-DHP lipid) [2, 3] formed a tubular micellae, if started from a lipid bilayer, and the existence of worm-like structures was confirmed by electron microscopy. In the present work using MD is shown that the 1,4-DHP lipid tubular micellae is stable in a bigger amount of water, as well as the systems of four or five tubular micellaes are stable. The rod of 15-mer DNA was put aside of the five tubular micellaes system in a periodic water box, and by MD, Amber 9.0 force field, was simulated how the 1,4-DHP lipid tubular micellaes 'swallow' the DNA rod. The 1,4-DHP rings tend to take tangential position towards the tubular micellae's surface.

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P485. Abstract number: 468

Increasing immunogenicity of murine vitamin K-dependent protein S through multivalent association of native-folded protein domains.

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Protein S is an essential natural anticoagulant that takes part in the major anticoagulant pathways that regulate thrombin formation. Homozygous deficiencies in protein S are lethal and partial deficiencies result in an increased risk of development of thrombosis. Murine venous and arterial models of thrombosis are widely applied to study the mechanisms of thrombosis development, therapeutics, and the role of coagulation proteins therein. Measuring and/or influencing protein S in these models using antibodies against murine protein S is essential to unravel the mechanisms underlying the anticoagulant properties of protein S. Until now, despite multiple efforts by different groups, it has been found difficult to impossible to raise antibodies against murine protein S.

The goal of this project was the development of an immunogenic structure based on the anticoagulant protein S thrombin sensitive region (TSR: residues 46-75; 1 disulfide) of murine protein S. We tried to increase the immunogenicity of the protein S TSR loop through a dual approach: the single tyrosine in the TSR loop was replaced by para-nitro-phenylalanine ($TSR\text{-}NO_2\text{-}Phe$) and the TSR loop was introduced on a tetravalent dendritic scaffold $(TSR)_4$.

Since the TSR loop contains a disulfide bond, it was necessary to develop a novel strategy to allow the coupling of native folded domains to the scaffold. In practice, this was done by assembling the modules through SPPS, followed by oxidative folding to achieve their native protein domain structure. Next, a thiaproline residue, which had been introduced at the N-termini of the domains as an unlockable cysteine derivative, was reconverted to cysteine. Following HPLC purification, the freed thiol was

used to react the folded domain with a maleimide-functionalized dendritic scaffold. Immunizations are performed to compare the immunogenicity of mono-TSR versus $(TSR)_4$ and $(TSR\text{-}NO_2\text{-}Phe)_4$.
This novel strategy allows the construction of polyvalent dendritic scaffolds decorated with multiple native-folded proteins or protein domains, in a simple, clean and straightforward way.

P486. Abstract number: 508
Functionalized Collagen Model Peptides
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The stability and many functions of collagen, which is the most abundant protein in mammals, depend largely on functional groups attached to its backbone.[1] Aside from hydroxylations other modifications such as, for example, naturally occuring galactosylations are known to influence the conformational stability of the collagen triple helix.[2] Thus, for a deeper understanding of the factors that govern the stability of collagen studies on functionalized collagens are important. Also for the development of new collagen-based materials it is important to understand the impact of modifications on the collagen triple helix. The collagen single strands consist of repeating Xaa-Yaa-Gly units, among them the Pro-Hyp-Gly triplet is the most prevalent. Here we present collagen model peptides (CMPs) with azidoproline (Azp) in the Xaa or Yaa position. The Azp residues provide sites for functionalizing CMPs with a range of different moieties. Functionalization has been achieved both by ”click chemistry”and reduction followed by amidation to simple amides and triazoles but also more complex derivatives such as carbohydrates. The effect of these different moieties on the conformational stability of the collagen triple helix will be discussed.[3]
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P487. Abstract number: 570
Building Blocks for Synthetic Biology: A Basis Set of Coiled Coils of Defined Oligomeric State.
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The recent surge of interest in the field of synthetic biology has given rise to an increased demand for small, independently-folded peptide and protein components which may be used as building blocks for the generation of self-assembling multicomponent biological systems [1]. Arguably, the most thoroughly understood protein fold is the coiled coil: a motif consisting of several (from two, up to seven) alpha helical strands supercoiled around one another with characteristic ”knobs-into-holes”packing around a central hydrophobic core [2]. In order for these peptides to be of broad utility in synthetic biology however, control of coiled coil oligomeric state, as well as orthogonality with respect to other components of the system is vital. To this end, we have recently described a series of six designed peptides which, when combined in solution, form three targeted heterodimers in preference to any other undesired pairing [3]. In the present study however, we have sought to expand these design principles and develop a small family of minimal homo-dimeric, trimeric, and tetrameric coiled coils which are both robustly folded, and capable of folding discreetly in the presence of one another. In this presentation we detail the design, full biophysical characterisation, mutual orthogonality, and x-ray crystal structure of these peptides. It is our aim that these peptides will not only further our understanding of the design rules for specifying coiled coil oligomeric state, but be useful for the preparation of new biomaterials and of general utility to synthetic biologists.
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