P A141 - Nociceptin antagonist analogues: structure-activity studies

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Among the opioid receptors family, the cloning of μ, κ and δ receptors was followed by an other member, named LC_{132}/ORL_1 [1]. Searching for an endogenous ligand for this receptor resulted in the identification of a 17-amino acid peptide [2] called nociceptin/orphanin FQ (FGGFTGARKSARKLANQ). In vitro and in vivo studies have demonstrated that nociceptin mediates a variety of biological actions. Results from structure-activity experiments suggest that the whole sequence of nociceptin is not required for binding to the LC_{132} receptor and for full biological activities. noc(1-13)-OH seems to be the minimum and essential sequence for good interaction with the receptor [3]. This neuropeptide is not resistant against enzymatic degradation. Some previous experiments refer to that the C-terminal amidation [3] may protect the peptide against degradation. We have synthesized carbamoyl analogues of noc(1-13)-NH_2, hoping that these derivatives retain the ability to bind LC_{132} receptor and are resistant against enzymatic degradation. We built the carbamoyl-group into the N-terminal message-sequence of Noc(1-13)-NH_2. The first step in the synthesis of the carbamoyl analogues was the preparation of the building block R-CO-NH-CO-NH-HC(R')-COOH by the classical method and then it was incorporated into the peptidechain by solid phase peptide synthesis.Screening of combinatorial library containing more than 52 million different hexapeptides resulted in the identification of a high affinity ORL_1 ligand. This hexapeptide (Ac-RYYRIK-NH_2) proved to be a partial agonist in different biological tests [4]. Enzymatic degradation of peptide derivatives can be suppressed by the formation of C-terminal peptide alcohols, as Ac-RYYRIK-ol was a potent antagonist in the receptor binding assays. Interestingly this peptide has only a minimal structural similarity to noc, and so far there is no explanation for the binding of this hexapeptide to ORL_1. On the basis of structural homology between citrullin and arginine we have synthesized a series of the hexapeptides - with amid and alcohol C-terminal - containing citrullines instead of arginines. Beside of the synthetic details, the biological characterisation of the synthesized analogues will be presented.

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P A142 - Novel approaches for synthesizing azapeptides

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Azapeptides are peptide mimics possessing amino acid analogs in which the central α-carbon is replaced by nitrogen [1]. Electronic repulsion between the adjacent nitrogens has been suggested to account for turn geometry adopted by azapeptide analogs [2,3]. Peptides bearing aza-amino acids include the drug Zoladex for treating prostate cancer [1]. They can exhibit longer duration of action, presumably because of increased resistance to proteases[1]. The synthesis of azapeptides on solid-support has, however, proven difficult using the common Boc and Fmoc nitrogen protection strategies because of intramolecular hydantoin formation [4]. Our presentation will illustrate an alternative approach for the synthesis of azapeptides on solid-phase that avoids this issue. Targeted on the synthesis of calcitonin gene related peptide (CGRP) antagonists, we are exploring azaptides to scan for turns in the antagonist C-terminal peptide fragment (FVPTDVGPFAF) of this potent vasodilator.

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P A143 - "Il mondo è bello perché è vario", expanding azabicycloalkanone amino acid diversity. Progress on the synthesis of pyrrolizidinone and piperidinoazepinone amino acids.

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Azabicycloalkane amino acids 1 can mimic natural peptide secondary structures in order to probe spatial requirements for recognition events in peptide chemistry and biology. Suitable for parallel synthesis as platforms that orient the display of pharmacophores, they can also introduce features, such as protease resistance, for improved metabolic stability. We have reported previously the syntheses of azabicyclo[X.Y.0]alkane amino acids 1 having fused 5,6-, 6,6-, 6,5- and 7,5-ring systems with stereocontrol and capacity for appending side-chains onto the heterocycle. Our conformational analyses using computational, spectroscopic and crystallographic methods, all have shown that the heterocycle size of these amino acids can influence peptide conformation and suggest the use of sets of these analogs for systematic study of SARs of native peptides. We will present progress on the syntheses of the fused 5,5- and 7,6-ring systems 2 and 3. Employing aspartate as an inexpensive chiral educt, we have synthesized effectively linear precursors that may now be converted to the respective pyrrolizidinone and piperidinoazepinone amino acids 2 and 3 using our reductive amination and lactam cyclization strategy.

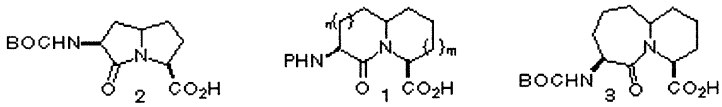

Figure 1.

P A144 - Structural analysis of the β-turn inducing (S)-[3-amino-4-oxo-2,3-dihydro-5H-benzo[b][1,4]thiazepin-5-yl] acetic acid (DBT) motif

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Conformationally restrained surrogates have been used in the synthesis of enzyme inhibitors and antagonists of peptide hormone receptors. In the design of bradykinin (BK) and HOE140 (a potent B_2 receptor antagonist) analogues, constrained non-peptidic moieties have been introduced at positions 7 and 8, which are thought to be important for the agonist/antagonist activity and represent the major cleavage site for angiotensin converting enzyme (ACE). For example, in a series of B_2 receptor analogues, the dipeptide Pro-Phe in BK or D-Tic-Oic in HOE-140 was replaced by several ACE inhibitor cores, among which the constrained (*S*)-[3-amino-4-oxo-2,3-dihydro-5*H*-benzo[*b*][1,4]thiazepin-5-yl] acetic acid (DBT) moiety. However, it has been suggested that the high affinity of BK analogues (including HOE140) for B_2 receptors is related to their high propensity to adopt a C-terminal β-turn conformation. We then investigated the conformational properties of Boc-DBT-NH_2 **1** in the solid state by X-ray diffraction, and in organic solution (CH_2Cl_2, $CHCl_3$ and DMSO) by IR spectroscopy and proton NMR. The 7-membered benzothiazepinone ring in **1**, which is composed of the two planar amide and aromatic fragments, may adopt two symmetrical conformations allowing an axial (A) or equatorial (E) orientation of the Boc-amino group. They can be discrimated by the rotational state of the Cys C^{α}-C^{β} bond, and therefore the H^{α}/H^{β} vicinal coupling constants, corresponding to the Cys-χ^1 value of about -60° (A) or 180° (E).

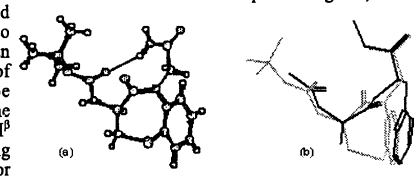

Figure 1. Crystal molecular structure of 1 in the βII'-folded conformation (a) and superimposition in the mean squares approximation of 1 with the βII'-folded Ac-D-Ala-L-Phe-NHMe dipeptide with Phe-χ^1 = -40° (b).

In the solid state, the benzothiazepinone ring in **1** assumes the E form (χ^1 = -168°) while the peptide backbone is β-folded by an i+3 i H-bond (N···O = 3.12 Å). The main torsional angles (ϕ_1 = 66°, ψ_1 = -144°, ϕ_2 = -83°, ψ_2 = 7°) indicate a type II' β-turn (Figure 1a). Superimposition of the peptide backbone atoms for **1** and the type I' and II' β-folded Ac-D-Ala-L-Phe-NHMe dipeptide reveals a good agreement with the βII'-folded sequence (rmsd = 0.67 Å), with an orientation of the Phe aromatic ring corresponding to χ^1 = -40° (Figure 1b). On the basis of the small N-H_{trans} stretching frequency (3347 cm^{-1} in CH_2Cl_2) and the low solvent sensitivity for the H_{trans} carboxamide proton resonance (δ = 7,16 ppm in $CDCl_3$ and 7,29 ppm in DMSO-d_6) the folded conformation of **1** is retained in solution. The medium and large H^{α}/H^{β} vicinal coupling constants (6.8 and 11.2 Hz) are in favor of conformation E for the benzothiazepinone ring, with the sulfur in the anti position with reference to the Cys-nitrogen, and are only compatible with a type II' β-turn (Figure 1a). Other DBT-derivatives, and especially DBT oligomers are under investigation.

P A145 - Experimental structural analysis of model urea-containing gamma-peptide analogues

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The use of peptide backbone mimetics giving defined secondary structures *via* non-covalent interactions is an area of major interest in peptidomimetic chemistry. In this context, unsymmetrical ureas, because they contain both hydrogen bond donors and acceptors, have recently emerged as a promising class of peptide surrogate. The NH-CO-NH urea motif has revealed interesting conformational properties due to the capacity of the urea CO-NH bonds to adopt the E or Z conformation. For example, model compounds of the general formula Boc-NH-CHR-NH-CO-NHR' **1** are extended in the solid state with a ZZ urea engaged in intermolecular H-bonds, but are folded in non polar organic solvents, where the EZ urea participates in an 8-membered γ-like turn. In oligoureas of the H_2N-(CHR-CH_2-NH-CO-NH)$_n$-H type **3**, the urea link adopts the ZZ structure and participates in H-bonds stabilizing a helical structure. Then it appears that the structure of N,N'-disubstituted urea may depend both on the molecules investigated and whether the molecules are in solution or in the solid state.

In order to gain more information on the NH-CO-NH urea motif, we have investigated the following molecules containing one or two urea links and two consecutive sp^3 carbons, so that they can be considered as γ-peptide analogues: (*S*)-Boc-NH-CHR-CH_2-NH-CO-NR'R" **3** and (*S*)-R'NH-CO-NH-CHR-CH_2-NH-CO-NHR" **4**. X-ray diffraction experiments on single crystals of **3a** (R = Bn, R' = H, R" = Me), **3b** (R = Bn, R' = R" = Me) and **4** (R = R' = Bzl, R" = Me) have shown extended molecules where the ZZ urea motifs exchange intermolecular H-bonds in a way similar to peptide chains in β-parallel extended structures (Figure 1). In CH_2Cl_2 or $CHCl_3$ solution, preliminary NMR results on **3** are in favor of rapidly exchanging EZ and ZZ urea motifs and, contrary to **1**, the percentage of conformers folded by an intramolecular H-bond is quite small on the basis or the IR spectra. Experiments on oligoureas with two sp^3 carbons are in progress in order to determine the critical number of urea motifs for a conformational transition between extended and helical structures.

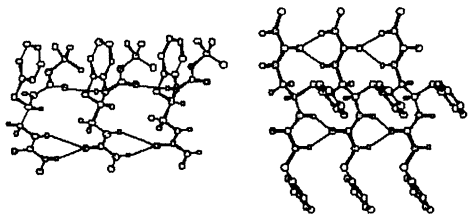

Figure 1. Crystal molecular structure of (S)-Boc-NH-CH(Bn)-CH_2-NH-CO-NHMe (left) and (S)-BnNH-CO-NH-CH(Bn)-CH_2-NH-CO-NHMe (right).

P A146 - Organocopper-mediated stereoselective synthesis of multi-substituted alkene dipeptide isosteres and the application to cyclic RGD peptides

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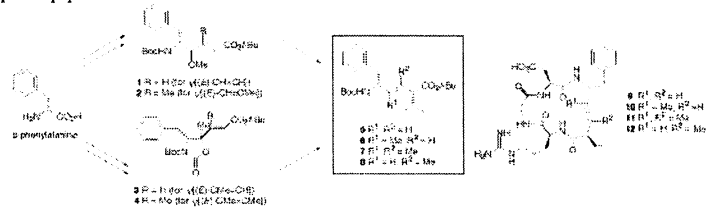
A number of peptidomimetic motifs have been developed for restriction of local and/or global conformation of bioactive peptides. Alkene dipeptide isosteres, in which an amide bond of a dipeptide is replaced by (*E*)-alkene or (*Z*)-fluoroalkene, have been widely utilized for structure-activity relationship studies and elucidation of reaction mechanism of peptide-based catalysts, and thus the synthetic methodologies for the isosteres have been intensively investigated by us and other researchers [1]. Tri- and tetra-substituted alkene dipeptide isosteres (ψ[(*E*)-CMe=CH]- and ψ[(*E*)-CMe=CMe]-type isosteres (**6**,**7**)) are reported as more potent type II and type II' β-turn promoters than the corresponding disubstituted alkene dipeptide isosteres (ψ[(*E*)-CH=CH]-type isosteres (**5**))[2]. In addition, ψ[(*E*)-CH=CMe]- (**8**) and ψ[(*Z*)-CH=CMe]-type isosteres are also useful tools for conformational research of *N*-methylamino acid-containing peptides by restriction of a peptide bond to *trans*- and *cis*-conformer, respectively. Therefore, we engaged in stereoselective synthesis of these multi-substituted alkene isosteres from a chiral amino acid. In a key reaction for synthesis of ψ[(*E*)-CMe=CH]- and ψ[(*E*)-CMe=CMe]-type isosteres (**6**,**7**), alkylation of β-(oxazolidinon-5-yl)-α,β-unsaturated esters (**3**,**4**) with organocopper reagents led to α-alkylated products regio- and stereo-selectively. ψ[(*E*)-CH=CMe]- (**8**) and ψ[(*Z*)-CH=CMe]-type isosteres were synthesized via regio- and stereoselective organocopper-mediated alkylation of γ-mesyloxy-β-methyl-α,β-unsaturated esters (**2**) utilizing the same strategy for ψ[(*E*)-CH=CH]-type alkene dipeptide isosteres.These multi-substituted alkene dipeptide isosteres were applied to the substructure of type II' β-turn motif of highly active $\alpha_v\beta_3$-integrin antagonists [3], *cyclo*(Arg-Gly-Asp-D-Phe-Val) and *cyclo*(Arg-Gly-Asp-D-Phe-MeVal) to evaluate the biological activities of the pseudopeptides. The comparative structure-activity relationship study on the cyclic pseudopeptides will be discussed.



Synthesis of multi-substituted alkene dipeptide isosteres and cyclic RGD pseudopeptides

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P A147 - Synthesis of several substituted at position 3 analogues of the naturally accurring peptide Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2)

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Tyr-MIF-1 antagonizes both endogenous and exogenous opiate-induced analgesia and has been suggested to belong to an endogenous antiopiate system. The incorporation of unnatural amino acids is a well-known approach to design biologically active peptide sequences with increased lipophilicity and metabolic stability. As part of our ongoing effort for systematic investigations in structure elucidation for a potential role of peptides with antiopiate properties in opiate tolerance and dependence, we synthesized several analogues of Tyr-MIF-1 using the classical solution phase technique. Two groups of peptide analogues modified at position 3 in the molecule of Tyr-MIF-1 were synthesized. In the first one, the leucine residue was replaced with unnatural S-substituted cysteinsulfonamides:

Tyr-Pro-sLeu-Gly-NH_2 [sLeu3]-Tyr-MIF-1; Tyr-Pro-sIle-Gly-NH_2 [sIle3]-Tyr-MIF-1; Tyr-Pro-sNle-Gly-NH_2 [sNle3]-Tyr-MIF-1

The incorporation of these structural sulfoanalogues of the natural amino acids leucine, isoleucine and norleucine - cysteic acid S-(N,N-dimethyl)amide (sLeu), cysteic acid S-(N-methyl,N-ethyl)amide and cysteic acid S-(N-propyl)amide (sNle) was accomplished in the similar manner using fragment condensation approach (2+2) with IBHF and DCC/HOBt as coupling agents. The second group of Tyr-MIF-1 analogues modified at position 3 was assumed to increase basicity in the side chain in the molecule icorporating non-protein amino acids involved in arginine metabolic pathway:

Tyr-Pro-Cav-Gly-NH_2 [Cav3]-Tyr-MIF-1 Tyr-Pro-Can-Gly-NH_2 [Can3]-Tyr-MIF-1 Tyr-Pro-Cit-Gly-NH_2 [Cit3]-Tyr-MIF-1

Because of the reactive chain functionality, each of the three amino acids - canavanine (Cav), canaline (Can) and citruline (Cit) were incorporated as suitable protected derivatives. The synthetic route to this group tetrapeptides involves the stepwise synthesis of N-terminal tripeptide with Gly-NH_2 using different coupling reagents as DCC/HOBT, BOP, TBTU. A formal work-up involving exposure to TFA followed by RP-MPLC purification yielded the desired tetrapeptides. Biological tests are in progress and we hope that these substituted analogues of the endogenously occurring Tyr-MIF-1 can produce potent and long-lasting effects on nociception.

P A148 - The role of glutamic acid and its malonate analogs in cyclic peptide antagonists of Grb2-SH2 domain protein

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Receptor tyrosine kinases, such as the EGF-receptor family, play important roles in the growth and progression of human malignancies. EGF-receptor and its oncogenic analog, erbB2, are overexpressed in a variety of malignancies. Tyrosine phosphorylation sites on these receptor proteins provide a docking site for the SH2 domain of the Grb2 adaptor protein, hence transmitting the cell's proliferating signals. We have developed cyclic peptides that do not contain a pTyr mimic, but inhibit this cellular signaling pathway. We have shown that the lack of pTyr mimic is compensated for by a glutamic acid, located 2-amino acids N-terminal to a nonphosphorylated tyrosine. Structure / activity studies have shown that replacement of Glu by (S)-2-amino-adipate or gamma-carboxy-Glu (Gcg) potentiated binding affinity 6-fold and 22-fold, respectively, using Biacore based competitive binding assays. Heteroatoms in these side chains are disfavored within these glutamic acid congeners, and extension of the Gcg side chain, provides an even better antagonist with an IC_{50} of 780 nM. Molecular modeling studies indicate that the Arg side chains within the Grb2-SH2 binding pocket that normally provide binding to the pTyr of the growth factor receptors, bind to acidic side chain of Gcg, and the turn conformation of the cyclic peptide provide the necessary conformation for these interactions. In cellular assays, the cell permeabilized conjugate of one of the analogs showed inhibition of erB2 receptor/Grb2 interactions at 2 microM concentration. These studies provide a unique pharmacophore model for Grb2 antagonist development.

P A149 - Design and synthesis of new benzodiazepine based turn mimetics incorporated in Ang II

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A large number of biologically active peptides have been discovered, but peptides are in most cases not suitable as drugs. A transformation of peptides into small organic molecules, peptidomimetics, could give important information of the bioactive conformation and solve some of the basic pharmacokinetic problems. The hypertensive octapeptide angiotensin II (Ang II) has been used as a target peptide in this study. A turn conformation around Tyr in Ang II has been suggested. According to conformational analysis the non-peptide benzodiazepine derivative **1** will mimic a β-turn or, alternatively, an extended γ-turn. To obtain more information about Ang II and its interaction with the receptor we are developing a synthetic route from the substituted benzaldehyde **2**, where different side chains will be introduced to give a number of turn mimetics. The turn mimetic **1** has been synthesized with different R2 substituents and incorporated into Ang II as an extended γ-turn, fig 2. The synthesis and results from the biological testing will be presented.

Fig. 1. The suggested turn mimetic 1 can be synthesized from the benzaldehyde 2.

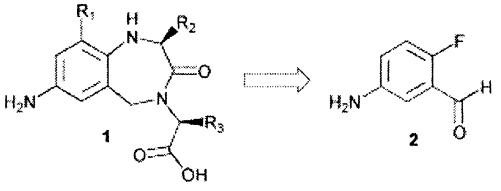
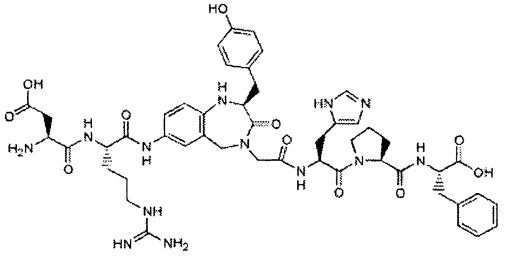


Fig. 2. The suggested turn with a tyrosine side chain incorporated in Ang II.



P A150 - Synthesis and conformational analysis of distinctin a bioactive peptide from *phyllomedusa distincta*

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Distinctin is a bioactive peptide purified from crude skin glands extracts of the tree-frog *Phyllomedusa distincta* [1]. Combined mass spectrometry and Edman degradation analysis demonstrated that this molecule presents an heterodimeric structure constituted of two different polypeptide chains connected by an intramolecular disulphide bridge [1]. Preliminary antimicrobial assays showed that this peptide is active against pathogenic *E. coli, S. aureus, E. fecalis and P. aeruginosa* strains and its minimal inhibitory concentration was determined [1]. Its strong lytic activity against large unilamellar vesicle suggested a possible action directed to cellular membranes. CD and FTIR spectroscopy studies demonstrated that this molecule adopts in water a conformation containing a mixed helical and β-sheet elements [1]. In order to definitively ascertain its mechanism of action, we synthesized this peptide and studied its tridimentional structure in aqueous and membrane-like environment by NMR spectroscopy techniques. The synthesis of two peptides was achieved by solid phase methods using the Fmoc strategy, while the disulphide bond formation to give the heterodimeric structure was achieved by air oxidation. This reaction gave the heterodimeric polypeptide in high yield and good purity. Its identity was confirmed by MALDI-MS. NMR data indicate the presence of contacts between the helical regions in the two chains. Refinement of the structure is now in progress.

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P A151 - Development of small molecules to mimic the action of ω-conotoxins.

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Cone snails (*Conidae*) are ordinary marine predators with some extraordinary features. Their venom contains a hundred or more peptides that target several different ion channels and receptors in mammals. This feature makes them interesting as potential therapeutic lead molecules.
ω-Conotoxins is one class of peptides found in the cone snail venom. They are 25-27 residue peptides with a rigid 4-loop cysteine framework. They target the N-type voltage-sensitive calcium channel and one peptide is currently used clinically in pain management under the name Ziconotide. However, the drawbacks of using this peptide as a drug are unwanted side effects (othostatic hypotension) and painful administration (intrathecal injection). There is therefore a need to develop small molecular equivalents of Ziconotide to improve pharmacokinetics and minimise side effects. Loop 2 of the ω-conotoxins, comprising residue 13 has been shown to be crucial for exhibiting maximal effect at the target channel. We used a minimal version of the proposed pharmacophore containing only residues 9-14 as a query in a database searching strategy. The sidechain comprising this query were simplified to cα-cβ vectors. This vector describes the initial sidechain position. Using an in-house database searching program and commercial ad in-house virtual databases of molecules, we identified numerous candidates that matched the topography of the cα-cβ vectors of the query. The hits were filtered based on the number of vectors that were matched how well they were matched (RMSD) and finally visually sorted using Insight II. From this process a series of cyclic pentapeptides, with one or more D-amino acid, were designed.
Synthesis of the cyclic peptides and initial results of the radioligand binding assays will be discussed. test.

P A152 - Macrocyclic hairpin mimetics of the cationic antimicrobial peptide protegrin I - a new family of broad spectrum antibiotics

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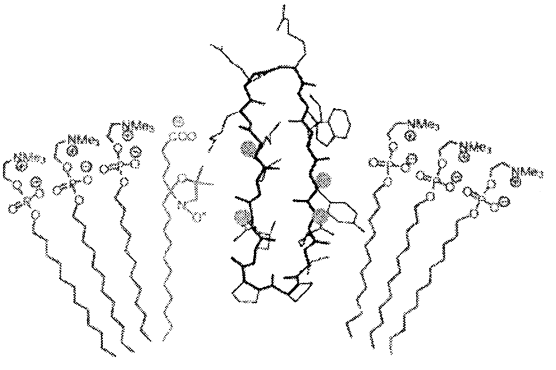
The peptides protegrin I, tachyplesin and RTI-1 represent a new classe of antimicrobial natural products, which adopt β-hairpin-like structures. We report here an approach to novel peptidomimetics patterned on these natural products. The mimetics were designed by transplanting the cationic and hydrophobic residues onto a β-hairpin-inducing template, either a D-Pro-L-Pro dipeptide or a multiply substituted xanthene derivative. The family of 12 residue mimetics showed good antimicrobial activity against a range of gram- positive and gram-negative bacteria (MIC~12-25μg/mL). From a small library prepared by parallel synthesis, analogues with improved selectivity for microbial rather than red blood cells (1% hemolysis at 100μg/mL) were identified, showing that it is possible to separate the antimicrobial and hemolytic activities in this class of mimetics. NMR studies on one mimetic revealed a largely unordered structure in water, but a transition to a regular β-hairpin backbone conformation in the presence of dodecylphosphocholine micelles. The mimetics were further shown to insert into the micelle with the template positioned in the hydrophobic interior, and the largely cationic hairpin tip most likely in contact with the aqueous phase. This family of mimetics may provide a starting point for the optimization of antimicrobial agents of potential clinical value in the fight against multiple drug resistant



P A153 - Peptidomimetics and their incorporation into peptide opioids

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The design and synthesis of several peptidomimetics and their incorporation into peptide opioids are described. The constrained tyrosine analogs were designed based upon preliminary results by Mosberg et al [1] Mosberg et al. synthesized a constrained tyrosine using a proline derivative to constrain the phenolic side chain. In our design a cyclopentane ring is used to constrain the phenolic side chain (Figure 1). By using a cyclopentane ring the amine function remains primary and not secondary as in the proline mimetic. Another constrained tyrosine was synthesized without the amino functional group. The des-amino constrained tyrosine analog was synthesized based on the observations by Schiller et al. [2] that removal of the terminal amino functionality converts opioid agonists into antagonists. The synthesis of these constrained tyrosine analogs are described. The constrained tyrosine derivatives were incorporated into enkephalin analogs. Their biological activity are being determined. Recently a potent δ-selective opioid was designed and synthesized in our laboratories. This enkephalin analog contains an α-methyl D-cysteine residue in position two or five and is constrained by a disulfide bridge. In an attempt to improve potency and selectivity of this lead compound the synthesis of a lanthionine derivative is underway.

The key building block for the lanthionine synthesis is the peptidomimetic α-methyl iodoalanine (Figure 2). The synthesis of α-methyl D-iodoalanine, its incorporation into a peptide opioid and its biological activity are described.

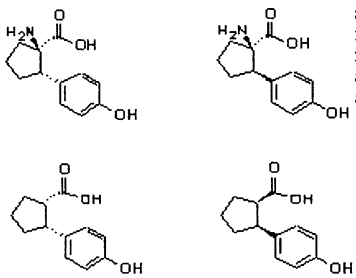

Figure 1: Constrained tyrosine analogs

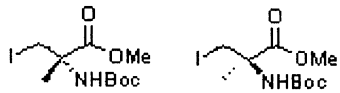

Figure 2: α-methyl iodoalanine

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P A154 - Benzyloxycarbonyl protection of the indole moiety in the synthesis of the bicyclic β-turn peptidomimetics.

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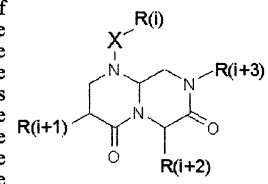
Tryptophan is often a key pharmacophore in many peptide ligands binding to GPCR's. Therefore, the synthetic access to peptidomimetics presenting the indole moiety is important to the rational, "peptide to small molecule" drug discovery process. During the development of our methodology for the solid-phase synthesis of the bicyclic β-turn mimetics (see Figure below) with four sites of diversity (*i* ~ *i* +*3*) [1] we have encountered the exclusive formation of the Pictet-Spengler-type tetracyclic products, occurring during the acidic cleavage of the linear precursors including tryptophan at the *i* + *2* position. Similar type of side-reaction observed while the tryptophan was residing at the *i* + *1* position of the mimetic was reported by Vojkovsky et al. [2]. We also experienced that the purity of the desired, bicyclic products derived from sequences containing the indole moiety at the *i*, or *i* + *3* position was lower than expected due to the presence of the oxidation and (or) dimerization byproducts. Those facts pointed to the need of applying the acid-stable protection of the indole moiety, which could be removed in the post-cleavage step without the need for additional purification.



X: NHCO, OCO, SO_2

We have investigated the use of the benzyloxycarbonyl (Z) protection [3], which is known to be stable to mildly acidic conditions (formic acid, TFA) and is readily removed by hydrogenolysis. In order to successfully apply the chosen protection to the high throughput format we have found that the nucleophilic conditions (methylamine) and transfer hydrogenation with the immobilized catalyst [4] cleanly remove the indole protection. Overall, our method allowed obtaining desired, unprotected peptidomimetics with the LCMS purity consistently above 80%. The synthetic details and examples will be presented.

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P A155 - Conformational properties of cyclic peptides containing 2-aminocycloalkane carboxylic acids

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Integrins are membrane-bound heterodimeric receptors with more than 20 known combinations of about 16 α- and 8 β-subunits which bind to numerous proteins of the extracellular matrix and take part in the adhesion of cells to the matrix [1]. Interaction with their natural ligands form the molecular basis of physiological or pathophysiological processes such as tumour-cell adhesion ($\alpha_v\beta_3$) or thrombocyte aggregation ($\alpha_{IIb}\beta_3$). $\alpha_4\beta_1$-Integrin (very late antigen 4, VLA-4) is expressed on human lymphocytes, monocytes, eosinophils, basophils and mast cells. Binding of vascular cell adhesion molecule 1 (VCAM-1) or of the CS-1 form of fibronectin is responsible for leukocyte adhesion. Inhibitors of VLA-4 binding to fibronectin or VCAM-1 should be useful in the treatment of some inflammatory diseases [2].

The amino acid sequence -Thr-Gln-Ile-Asp-Pro-Leu-Asn- (TQIDSPLN), which is located in a surface-exposed loop connecting two β-strands (the CD-loop), has been proposed as a binding epitope for VCAM-1 [3].

A series of cyclic peptides containing proline or the non-proteinogenic amino acids 2-aminocyclopentane carboxylic acid and 2-aminocyclohexane carboxylic acid as proline mimetics has been synthesized and investigated by NMR with respect to the conformational properties.

In the proline molecule the ϕ angle is restricted towards -60°, so proline is predominantly found in the i+1 position of a βI- or βII-turn. The targeted application of unnatural building blocks such as β-amino acids or D-amino acids in cyclic peptides allows the control of the peptide backbone conformation [4].

β-amino acids have a strong influence on the secondary structure of cyclic peptides. Because β-amino acids contain an additional carbon atom, one more dihedral angle μ is present, which is found to be 60° or -60° in many cyclic peptides. Exchange of proline by 2-aminocycloalkane carboxylic acids fixes the angle μ. The influence of these proline mimetics on the secondary structure is determined by synthesizing cyclic peptides as conformationally restricted analogues of the binding epitopes of VCAM-1 by the following strategy:

Synthesis of linear peptide precursors on 2-Cl-trityl resins using Fmoc/tBu tactics, cyclization under high dilution conditions and finally deprotection.

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P A156 - Synthesis and biological evaluation of highly active nonpeptidic αvβ3 integrin antagonists

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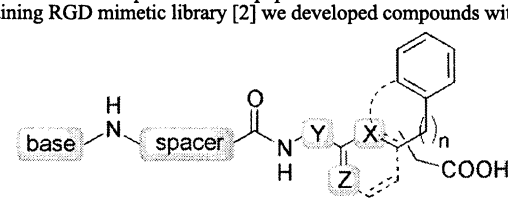
Integrins are a family of heterodimeric transmembrane cell surface receptors which are involved in cell-cell and cell-matrix adhesion processes. The tripeptide sequence RGD is a cell-recognition motif that has been used for the development of different integrin antagonists. The vitronectin receptor, also known as αvβ3, has received increasing attention, due to its potential application as therapeutic target in pathologies as diverse as osteoporosis, restenosis, angiogenesis and acute renal failure. [1] Due to their enhanced metabolic stability, bioavailability, and biological absorption, we focused our research on the development of non-peptidic RGD-mimetics. Based on an aza-Glycin containing RGD mimetic library [2] we developed compounds with higher rigidity and lipophilicity to improve the pharmacokinetic profile. Here, we describe the solid phase and solution phase synthesis of linear RGD-mimetics of the general formula:



X = NH, CH, CH_2 Y = NH, CH_2 Z = O, CH

Parallel synthesis of RGD-mimetics with aromatic acids and different guanidine mimetics yielded in integrin antagonists with high biological activity on the αvβ3 receptor and high selectivity towards the αIIbβ3 receptor.

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P A157 - Solution and solid-phase synthesis of partially-modified retro-peptides incorporating a [$CH_2CH(CF_3)CO$] unit

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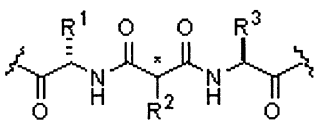
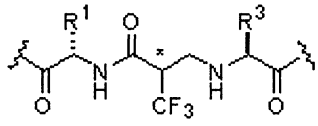
The desire to use peptides as pharmaceuticals is the major incentive for modification. Retro- or partially-modified retro-peptides **1**, in which all or some peptides bond, respectively, are reversed (NH-CO instead of CO-NH), are a particular class of modified peptides [1].
In this communication we will describe the solution and solid-phase synthesis of a novel class of pseudo-peptides **2** incorporating a [$CH_2CH(CF_3)CO$] unit as possible mimics of retro-peptides incorporating trifluoroalanine. It's worth nothing that the synthesis of trifluoroalanyl peptides is extremely difficult due to the instability of the trifluoroalanine at pH>6.

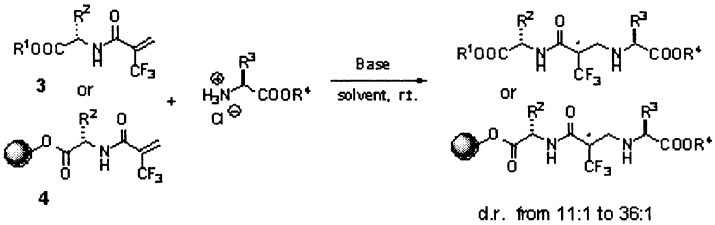


1



2

The key step for the synthesis, both in solution and solid-phase, of pseudo-peptides **2** is the highly stereoselective *N*-type Michael addition of different α-amino esters to the Michael acceptors **3** and **4**.



d.r. from 11:1 to 36:1

Reference
[1] M. D. Fletcher, M. M. Campbell, *Chem. Rev.* 1998, *98*, 763-795

P A158 - Synthesis of bioactive endomorphin analogs containing 1-aminocyclopropane-carboxylic acid

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Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) are very potent endogenous opioid peptides, which exhibit high affinity and selectivity for the μ-opioid receptor [1])
A Pro residue in the position 2 of endomorphins seems to stabilize a bioactive conformation by restriction of peptide-backbone conformation and *cis-trans* isomerization around the Tyr-Pro peptide bond. On the other hand, α,α-disubstituted amino acids are often used as an excellent tool to constrain the backbone conformation of peptides and to control well-defined secondary structures. For example, Aib is expected to exhibit a peptide backbone conformation similar to that observed for *cis* Pro residue [2])
Now we report the synthesis of endomorphin-2 analogs containing various α,α-disubstituted amino acids, such as Aib, Deg, Dcp, Ac_3c, Ac_5c and Ac_3c, at position 2 instead of Pro and the study on the receptor-binding activity. Some of these analogs exhibited considerably high affinity for μ-opioid receptor. In particular, [Ac_3c^2]-endomorphin-2 showed higher affinity than that of endomorphin-2. This is also the case for the similar analog, [Ac_3c^2]-endomorphin-1.
It is concluded that replacement of a Pro residue by Ac_3c may effectively yield a peptide analog with high activity, if the active conformation of the peptide involves a *cis* Pro residue.

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P A159 - Synthesis and antipsychotic activity of conformationally constrained tripeptoid neurotensin analogues

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Recently we developed a series of "tripeptoid" analogues of neurotensin (NT) with antipsychotic activity (T. Gudasheva, 1998). Their conformational properties have been investigated in solution by IR and NMR spectroscopy and the experiments indicated their favored conformational states in this condition are the β-folding modes. The spectroscopy-derived molecular models are very similar to those established by theoretical calculations and analyses. Now we synthesized the NT analogues containing βI-, βII- and βII'-turn constraints. The pharmacological studies revealed an activity of analogue with favorable βI-turn conformation, while the peptides containing the βII and βII'-turn constraints proved to be no active. These results show that the βI-turn could be a bioactive conformation of "tripeptoid" NT analogues.

P A160 - The solid phase fragment condensation using Nsc-amino acids

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Condensations of fully protected peptide fragments on a solid support are an efficient alternative to stepwise solid phase peptide synthesis (SPPS). Usefulness of fragment condensations using Nsc (2-(4-nitrophenylsulfonyl)ethoxycarbonyl) amino acids was investigated. For assembly of fragment peptides, the usual t-butyl or Trt type protected Nsc-amino acids with Nsc-Arg(Pbf)-OH or Nsc-Cys(Acm)-OH as well as 2-chlorotrityl resin were proved to be a practical approach. To minimize racemization the fragment C-terminal amino acids were restricted to Pro or Gly. Enhanced hydrophilicity of Nsc derivatives relative to Fmoc was helpful for purification and analysis of fully protected peptide fragments. Extensive HPLC and Maldi-tof studies were used for optimization of the fragment condensations and cleavage conditions. Fragment condensations were tested in various conditions, such as in the solution, on the solid supports and several condensation conditions. Purifications of fully protected peptide fragment were also investigated. Fragment condensations based on Nsc-amino acids were applied to synthesis of salmon calcitonin(I) and other biologically important peptides.

P A161 - Synthesis and refolding of G20a, a potential antigen for human Respiratory Syncytial Virus (hRSV) vaccine

C. Klinguer-Hamour[1], M. Bussat[1], H. Plotnicky-Gilquin[1], N. Corvaïa[1], J. Bonnefoy[1], T. Nguyen[1], A. Beck[1]

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Human Respiratory Syncytial Virus (hRSV) is one of the most common causes of respiratory infection in infants [1]. Previous attempts to vaccinate children failed. In addition, a severe to fatal pulmonary disease, characterized in part by eosinophilia, was induced after RSV infection of children previously vaccinated with a formalin-inactivated RSV (FI-RSV) preparation [2].
The BALB/c mice model of RSV infection has proven to be a valuable tool since a pulmonary pathology similar to that observed in the human infant FI-RSV vaccinees, characterized by eosinophilia, is induced following RSV challenge and priming with FI-RSV or a recombinant vaccinia virus expressing RSV G protein [3,4]. Eliciting a Th2-type primary response is critical [5]. Furthermore, an association was made between the pulmonary eosinophilia and the induction of CD4+ T cells recognizing the peptide 184-198 of RSV G protein [6]. In this study, we describe the synthesis and the refolding of G20a, a 69 amino acids peptide with two disulfide bridges derived from the RSV G-protein, which comprises multiple B-cell epitopes [7] and was deleted of the T helper epitope. This new potential antigen was evaluated for immunogenicity, protective efficacy and safety in the BALB/c mice model.

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P A162 - Synthesis of MCOTI-I, a cyclic trypsin inhibitor from Momordica cochinchinensis

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2. Vanderbilt University - MCN A5119 - Nashville, TN 37232 - U.S.A.
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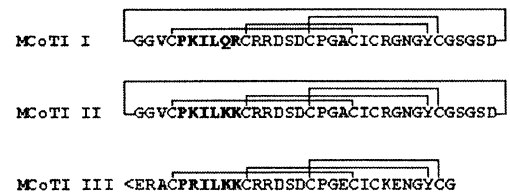
In Vietnam, *Momordica cochinchinensis* (MCo) is a popular cucurbitaceae utilized commonly in cooking and traditional medicine. Its seeds, known for the treatment of various diseases, are very rich sources of protease inhibitors. Isolation and characterization of 3 trypsin peptide-inhibitors (TI) belonging to the squash inhibitors family, namely MCoTI-I, MCoTI-II and MCoTI-III, have been described previously. They all contain the usual 3 disulfide bridges; moreover, MCoTI-I and MCoTI-II are the first end-to-end cyclic squash TIs ever found [1]. The three-dimensional structure of MCoTI-II has been elucidated [2,3].



In this communication, we will present the total synthesis of MCoTI-I following the method using a thio-ester resin as described by Tam & Lu [4]. A comparison with the classical air-oxidation method used for the synthesis of EETI-II will be attempted [5].

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P A163 - Synthesis, mass spectrometry analysis and potency of human parathymosin (101 amino acids)

L. Leondiadis[1], V. Koutrafouri[1], E. Livaniou[1], N. Ferderigos[2], C. Zikos[1], D. S. Ithakissios[3], G. P. Evangelatos[1]

1. IRRP, National Centre for Scientific Research "Demokritos" - Greece
2. Department of Chemistry, University of Athens - Greece
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Parathymosin is an 11.5kDa acidic protein (pI=4.15) containing 101 amino acid residues. It was originally isolated from rat tissues and found to be highly conserved and widely distributed among tissues and cell types of various species. In humans it is coded by a gene consisting of five exons, located on human chromosome 17. Parathymosin shares 40% homology with Prothymosin alpha. Both proteins contain a nuclear localization signal at their carboxy termini, and have been found to bind linker histone H1. The central region of Parathymosin is predominantly composed of aspartic acid and glutamic acid residues and is reported to contain two binding sites specific for Zn^{2+}. Data on the biological function of Parathymosin are limited, but it is believed that this protein is involved in cell cycle progression, proliferation and differentiation. Parathymosin is predominantly localized in the cell nucleus and has been found to be associated with early replication domains, which suggests that it plays a role in the replication of active chromatin. Parathymosin levels are reported to be significantly higher in neoplastic human breast tissue compared to those in normal breast tissue, implying a relationship with tumor growth.
Due to the difficulties of isolating this protein from natural sources, we decided to chemically synthesize this molecule in working quantities, according to the sequence deduced from a cloned human kidney cDNA, and thus to promote the studies on the biological role of Parathymosin. Well established solid phase peptide synthesis protocols were used according to the Fmoc orthogonal methodology. As solid support we used a p-cyanotrityl resin that has proved to be suitable for the synthesis of long peptides due to its stability in acidic conditions. Briefly, Fmoc protected amino acids (5eq) were coupled to the growing polypeptide by equimolar quantities of DIPC/HOBt in DMF. Deprotection was carried out by 20% piperidine in DMF. Additional double or triple coupling was performed when the Kaiser test was positive. At the end of the synthesis the polypeptide was acetylated and removed from the resin with 90% TFA in water and scavengers. The crude product precipitated in cold ether and lyophilized. The purified protein was obtained by preparative HPLC using a C18 reverse phase column. The overall yield of the synthesis was 30%. The RP-HPLC profile of the purified product was homogeneous and the amino acid analysis was in good agreement with the theoretical composition. Verification that the synthetic protein had the right composition without side chain modifications was proved by mass spectrometry analysis using the electrospray ionization technique. Mass spectra revealed only one product having the exact molecular weight at 11,440.3 ± 0.8 (Theoretical molecular weight 11,441). The synthetic protein was evaluated for its biological potency. It was found that synthetic Parathymosin inhibits angiogenesis in the chick chorioallantoic membrane (CAM) model, and 1μg of Parathymosin had the same effect on angiogenesis as 100 μg of Hydrocortisone, a well known antiangiogenic reagent.

P A164 - Strategies for the synthesis and fluorescent labeling of protein a-derived affinity proteins

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Different strategies for the synthesis and fluorescent labeling of small affinity proteins have been evaluated, as part of the development of fluorescence-based methods for the detection of protein-protein interactions. A recent study showed that the interaction between the 58 amino acid B domain from staphylococcal protein A and its high affinity protein ligand could be monitored using a fluorescence assay, in which the B domain was site-specifically labeled by two different fluorophores. The fluorescence emission spectrum of the doubly labeled protein showed a shift in the relative emission of the two fluorophores in the presence of Fc3 [1] fragments, which bind specifically to the B domain, whereas the presence of a control protein did not affect the emission ratio, indicating that the labeled protein could be used as a biosensor to monitor the presence of a specific target protein. In this case, site-specific labeling of recombinant protein was achieved by labeling a unique Cys residue with a thiol-reactive probe and directing an amine-reactive probe to the N-terminus by pH control of the reaction. In the present study, solid phase peptide synthesis and on-resin incorporation of fluorophores has been investigated as an alternative strategy for the preparation of fluorescent-labeled proteins.

Reference
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P A165 - The synthesis of the third transmemebrane segment of proteolipid protein (PLP) using Fmoc chemistry with pseudoproline amino acid building blocks or Boc chemistry: a comparative study

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Proteolipid protein (PLP), the most abundant protein of the central nervous system myelin, is a tetraspan membrane protein. PLP is assumed to have a structural role providing stability and maintaining the multilamellar state required for myclin function. As the 3-D structure determination of such integral membrane protein is very difficult, we have decided to synthesize fragments of PLP in order to investigate their physicochemical properties (secondary structure, interaction with lipids,...). A 34 residue peptide corresponding to the third transmembrane helix of PLP (PLP146-179Ê) was synthesized by solid phase synthesis:

Ac-GHPDKFVGITYALTVVWLLVFASSAVPVYIYFNT-NH_2

Previous results have shown that the Fmoc SPPS was leading, beside PLP146-179, to high yield of deleted (Leu/Ile) and truncated peptide side products (major compounds). due to its hydrophobicity, the transmembrane peptide could only be purified by gel permeation on LH-60 in organic solvents but could not be separated from the deleted peptide (>50%). the different peptides were characterized by ESMS. To improve the synthesis of this «difficult» sequence, we have undertaken a comparative study between the use of pseudoproline building blocks in Fmoc chemistry and classical Boc chemistry.
the use of pseudoproline building blocks (**XX** in the sequence) increased the overall yield of the synthesis, suppressed the formation of the Leu/Ile deleted peptide and reduced greatly the yield of truncated peptides. PLP 146-179 was obtained after gel permeation on LH-60 with a purity > 90% (7.5% of a deleted peptide Ile-Thr). In comparison, the Boc synthesis of PLP146-179 reduced the yield of truncated peptides to less than 15% , but could not avoid the formation of a deleted peptide (35% Val deletion). Furthermore incomplete side chain deprotections occurred during the HF cleavage and PLP 146-179 could not be separated from the side products by gel permeation.

P A166 - Fmoc solid phase synthesis and immunochemical evaluation of thymosin β-4

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Thymosin β-4, a 43 amino acid residue peptide (AcSDKPDMAEIEKFDKSKLKKTET-QEKNPLPSKETIEQEKQAGES) of great phylogenetic, biochemical, diagnostic and maybe therapeutic interest, was synthesized for the first time, to our knowledge, using the Fmoc solid phase peptide synthesis strategy, on an in-house prepared o-Cl-trityl polystyrene resin with excellent swelling characteristics. Thymosin β-4 was synthesized manually following the DIC/HOBt activation approach. Briefly, coupling was performed by dissolving an excessive quantity (5 mol equiv.) of Fmoc-protected amino acid and HOBt in DMF. The solution was cooled on ice and then DIC (5 mol equiv.) was added. The reaction mixture was left for 10 min on ice, for 10 min at 25 °C and was then added to the resin (asparagine, glutamine and glycine were added to the resin immediately after remaining for 10 min on ice) and allowed to react for 3 h. Coupling efficiency was checked using the Kaiser ninhydrin test. Double coupling was necessary for the residues S_1, D_2, K_3, P_4, D_5, I_9, K_{14}, K_{16}, K_{25}, L_{28} and S_{30}. Crude synthetic thymosin β-4 was obtained at a ~90 % yield. After its purification with preparative HPLC, synthetic thymosin β-4 was obtained in a very pure form (analytical HPLC, amino acid analysis, electrospray ionization mass spectrometry). The synthetic peptide was obtained at a very satisfactory final yield (~30 %), much higher than those previously reported (~5 %, ~12 %) for a Boc- solid phase synthesis on a hydroxymethylphenylacetamidomethyl resin or for synthesis by a liquid phase classical synthetic approach, respectively. Due to the methionine residue oxidation, thymosin β-4 sulfoxide appeared as a minor side-peak in the crude product; the percentage of this oxidized peptide could be decreased mainly by suitably modifying the cleavage conditions. The immunoreactivity of the synthetic thymosin β-4 was tested by an ELISA system. As shown, the synthetic peptide was recognized by an anti-thymosin β-4 antiserum, which had been raised against natural thymosin β-4 isolated from bovine lungs, while thymosin β-10 (a thymosin β-4 homologue, which accompanies thymosin β-4 in human tissues), previously synthesized in our laboratory, was hardly recognized by the anti-thymosin β-4 antiserum. In addition to the o-Cl-trityl polystyrene resin, three other trityl-type resins, which have been prepared in our lab., i.e. trityl-polystyrene, o-F-trityl-polystyrene and p-CN-trityl-polystyrene, were applied to the Fmoc solid phase synthesis of thymosin β-4, leading to similar results with an equally high final yield.

P A167 - Isolation and characterization of antifreeze protein from sunn pest (*Eurygaster integriceps* Put.)

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Sunn bug pest (*Eurygaster integriceps* PUT.) is serious and key pest of wheat. Sunn bug pest is univoltine and in most part of Iran emigrate from altitude in mountains, to fields from March to late Aprile. In the field, emigrant and nymph of different instar and new adults active nearly for two month. New adults, after about 2 weeks feeding migrate to aestivation and hibernation site in mountains and stay for 9 month. In outbreak period food is key factor and climatic factors especially temperature are secondary. *E.integriceps* with poor nutrition was sensitive to adverse condition and in some reports nearly 60 % died in some year. With considering of life cycle that take place in mountains, study of cold hardiness from biochemical point was necessary. Proteins are essential compounds, that divers group used as antifreeze. These proteins produce a thermal hysteresis whereby the freezing point of an aqueous solution containing the protein is depressed, by noncolligative mechanism, well bellow the melting point of the solution. These compounds protect insects from cold with hysteresis freezing-point depression, supercooling effects and recrystallization inhibition. In this study an antifreeze protein was purified from adults of *E.integriceps* collected from altitudes Ateshgah during late autumn and winter (with minimum temperature -13 °C), and compared with the summer one. Isolation was carried out by using ethanol fractionation procedure, DEAE ion-exchange chromatography and sephadex G-100 gel filtration techniques. The purified protein was also analyzed on SDS polyacrylamide gel electrophoresis. It showed a molecular weight of 19 kDa dalton and N-terminal amino acid was alanine. The thermal hysteresis activity was 4.4 °C at concentration of 50 mg/ml. PI of this protein was determined in range 5-5.5 using isoelctric focusing technique. This protein was absent in summer samples. Therefor it is suggested that antifreeze protein in winter samples has important function in survival with considering cold hardiness strategy of *E. integriceps* at autumn and winter.

P A168 - New chromogenic substrates derived from insulin

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Desoctapeptide insulin (DOI) is a well-known precursor often used in the semisynthesis of analogues of human insulin. Dozens of different insulin analogues with variously substituted amino acids in the B^{23}-B^{30} sequence have been prepared and studied. Trypsin-catalysed condensation of the carboxyl of arginine in position B^{22} with the amino group of the modified peptide has been used rutinely.

B^{23} B^{30}
Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr is the natural B^{23}-B^{30} sequence of human insulin.

Synthetic analogues, composed of three to eight amino acids, have been designed with the aim of studying the binding to receptors and the biological response. We present the results of the condensation of chromogenic substrates for aminopeptidases with the common formula
X-p-NA (where X is an amino acid or a peptide sequence with a free N-terminal amino group and p-NA is p-nitroanilide) with the caboxyl group of arginine in position B^{22} , resulting in DOI-X-pNA. The molar ratio of trypsin to DOI was 1:50 and that of DOI to the amino component 1:10. The condensation reaction was performed in 55% DMF, pH 6.9-7.1 at room temperature for 5-25 hours. The course of the reaction was monitored by analytical RP-HPLC (Column Watrex, Nucleosil 120 C18 5 µm, 250x4 mm, 8-72% ACN gradient in 0.1% TFA). The products were isolated by preparative RP-HPLC on a Vydac 218TP510 column (25x1 cm) and characterized by MS and CZE. The conversion of the newly prepared substrates was followed in several systems containing insulin receptors. The results indicate the combined action of trypsin-like enzymes and aminopeptidases on the substrates.

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P A169 - Fluorescent monitoring of kinase activity in real time: development of a robust fluorescence-based assay for Abl tyrosine kinase activity

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We have as our long-term goal the engineeering of proteins containing biophysical probes for studying biological processes in living cells. Here we describe our current progress on the development of fluorescent protein biosensors. Using a solid-phase version of protein semi-synthesis, we incorporated two fluorophores at specific sites within a truncated version of the c-Crk-II protein. The resulting fluorescent protein biosensor permits the real-time monitoring of Abl kinase activity and provides a robust and rapid method for assaying Abl kinase inhibitors.
Activation of the Abl tyrosine kinase causes chronic myeloid leukemia (CML). Here we have a developed a sensitive, fluorescence-based assay for monitoring Abl kinase activity in real time. We used this assay to test a number of analogs of an Abl kinase inhibitor (STI-571,Novartis) that has recently been approved by the FDA for the treatment of CML. The basis for the assay is that the Abl kinase phosphorylates the CrkII adapter protein on a specific tyrosine residue (Y222). Phosphorylation of the CrkII protein is thought to induce a conformational change which in turn regulates its protein-protein interactions in signal transduction. We have engineered a truncated CrkII protein (amino acids 1-228) to biosense this phosphorylation-dependent conformational change. We used expressed protein ligation to join synthetic peptides, one containing a rhodamine and another containing a fluorescein, to the recombinant CrkII protein at its amino and carboxyl terminus respectively. Excitation of fluorescein in this dual labeled CrkII protein (Rh-CrkII-Fl) leads to emission of both fluorescein and rhodamine through fluorescence resonance energy transfer (FRET). Upon phosphorylation of the CrkII protein by the Abl kinase, we see greater than a 20% change in FRET. As expected, this change in FRET, which likely reflects the phosphorylation-induced conformational change in the CrkII protein, correlates with phosphorylation of CrkII on Y222. Inhibition of Abl kinase activity by STI-571 results in a dose-dependent decrease in the change in FRET with an EC50 of ~ 640nM.
We also report the inhibitory activity of a number of analogs of STI-571. Thus, we have developed a rapid, non-radioactive screening procedure that is useful for identifying other novel Abl kinase inhibitors for the treatment of CML.

P A170 - The mechanism of protein splicing: variations on a theme

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The autocatalytic protein splicing mechanism is mediated by the intein plus the first downstream extein residue. Conserved intein residues include a Ser or Cys at the N-terminus and the dipeptide, His-Asn, at the C-terminus, followed by a Ser, Thr or Cys at the beginning of the C-extein. As more inteins have been sequenced, especially in thermophilic archaea, polymorphisms in nucleophiles and conserved residues have become more evident (see InBase, the on-line intein database: http://www.neb.com/neb/inteins.html). For example, Gln has been observed at the C-terminus of several inteins.
Approximately 10% of inteins lack a penultimate His. The conserved intein penultimate His is believed to assist in Asn cyclization, which results in branch resolution and C-terminal splice junction cleavage. *Methanococcus jannaschii* (Mja) phosphoenolpyruvate (PEP) synthase and RNA polymerase subunit A' inteins contain a Phe or Gly, respectively, at the intein penultimate position. However both inteins are still capable of splicing in *E. coli*. "Reversion" of the PEP synthase intein penultimate Phe to His improved splicing whilst "reversion" of the Mja RNA polymerase subunit A' intein penultimate Gly to His inhibited splicing, branch resolution and Asn cyclization. We propose that the Mja RNA polymerase subunit A' intein has better evolved to use an alternate residue to assist Asn cyclization than the PEP synthase intein, which may not be unexpected for such a highly expressed, essential protein. Previous substitutions of an intein N-terminal Ser or Cys with Ala have rendered the intein incapable of splicing by the canonical protein splicing mechanism and therefore the Mja KlbA intein, containing an Ala at the intein N-terminus, was initially postulated to be inactive. We demonstrate that the Mja KlbA intein splices efficiently in the MIP (Maltose binding protein::Intein::Paramyosin) context, despite the presence of this N-terminal Ala. Essential amino acids required for protein splicing were found at both splice junctions and in the conserved intein Block B motif. The splicing of the Mja KlbA intein also proceeds via the branched intermediate present in the standard protein splicing pathway. All experimental data are consistent with a protein splicing mechanism that is identical to the canonical mechanism with the exception that the C-extein nucleophile directly attacks a peptide bond at the N-terminal splice junction, rather than a (thio)ester bond. This suggests that the KlbA intein has overcome the barrier to direct nucleophilic attack on the peptide bond that is present in inteins that naturally begin with Ser or Cys. Packing at the KlbA intein active site may allow the downstream nucleophile access to the carbonyl carbon of the scissile peptide bond or conformational changes may have occurred in the intein that allow proper alignment of the downstream nucleophile without first forming an ester at the N-terminal splice junction. Structural information is needed to resolve this issue.

P A171 - Substrate mimetics-based approaches to the chemoenzymatic synthesis of proteins

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In vitro preparation of large polypeptides still represents a highly challenging task of modern peptide chemistry that seriously suffers from the lack of a universal synthesis method. Step by step synthesis on solid supports, routinely used to the preparation of peptides, is still limited to targets of about 50-60 amino acids in length and, hence, only provides access to fragments of the final protein targets. For their chemical condensation, the 'native chemical ligation' approach is the most popular choice presently. Although efficient and relatively easy to apply, it essentially needs special amino acids at the site of ligation to make the reaction selective. To achieve the synthesis of proteins lacking those special amino acid moieties we are active in developing protease-based ligation methods for several years. Especially the utilization of substrate mimetics [1,2] combined with the use of protease variants [3] designed for peptide ligation rather than their cleavage efficiently circumvent the limitation well-known for the classical protease-based peptide synthesis approach.
In the present contribution, we describe a straightforward method that combines the use of substrate mimetics and designed peptide ligases with chemical solid-phase peptide synthesis and the expression of peptide fragments. The function of this novel chemoenzymatic approach to protein synthesis is verified on the example of the full length peptidyl-prolyl-*cis/trans*-isomerase parvulin 10 from *E. coli* as the target of synthesis. Attention will be paid on the genetic as well as chemical preparation of the peptide fragments as substrate mimetics, on the designed peptide ligase used as the ligation catalyst, and the outcome of synthesis. Finally, the characterization of the intermediates and the final synthesis product will be presented.

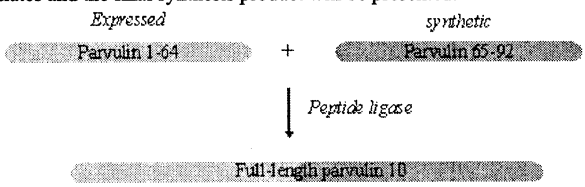

Fig. 1 Strategy for the chemo-enzymatic synthesis of full length parvulin 10 from E. coli.

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P A172 - Enzymatic synthesis of N-linked peptidoglycans mediated by substrate mimetics

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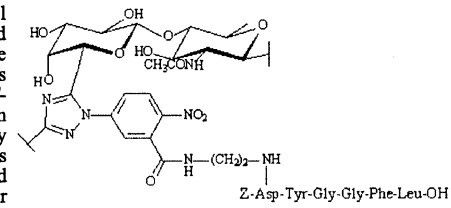
Glycosylations represent one of the most widespread 'post-synthesis' modification of proteins that modulate the structure and stability of proteins as well as regulate their biological activities. The involvement in adhesion, differentiation, and growth of cells are only a few examples of the prominent role of glycoconjugates finally resulting in a great biological and pharmaceutical interest in these compounds.[1] The isolation of glycosylated polypeptides from natural sources or their production by recombinant DNA techniques, however, usually lead to derivatives with inhomogeneous or altered glycosylation patterns. Up to now only chemical and chemoenzymatic strategies provide access of well-defined glycopeptides and analogues as well. Despite the large number of methods developed for their synthesis, the high complexity, hydrophilicity, and acid- and base-lability of the carbohydrate part, however, make coupling of glycans with peptides to a vast challenge being far from any routine.[1] Here we demonstrate an efficient one-step strategy to the synthesis of a broad variety of *N*-linked peptidoglycans. The key feature of this approach is the use of the cysteine peptidase clostripain from *Clostridium histolyticum* as the biocatalyst combined with classical linear and novel type substrate mimetics as the amino acid and peptide precursors that mediate the acceptance of non-specific acyl residues by the original Arg-specific protease.[2] This novel enzyme/substrate mimetics-based methodology allows regioselective coupling of carboxylate moieties derived from Asp, Glu, and the *C*-terminus of peptides with both simple monomeric and highly complex carbohydrates, such as the amino-functionalised moenomycin A analogue **1**, under extraordinary mild reaction conditions.



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