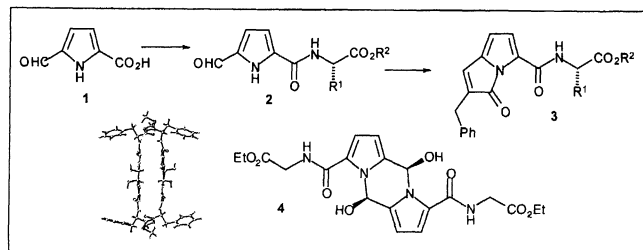


#### AN AMINO ACID PROTECTING GROUP THAT CAN BE COLORED ON DEMAND

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We present a method that releases a characteristic and highly colored red tag from a precursor attached to the N-terminus of an amino acid ester, either in solution or on solid support. This N-protecting group possess a latent ability to be colored (tagged) on demand. To this end, a series of amino acid-based 5-formylpyrrole-2-carboxamides **2** was prepared and their conversion to highly colored pyrrolizin-3-ones **3** was studied. 5-Formylpyrrole-2-carboxylic acid **1** was coupled to an amino acid ester using standard EDCI-peptide coupling methodology to give **2** in high yield. Treatment of these derivatives with hydrocinnamoyl chloride, diisopropylethyl amine and dimethylaminopyridine gave the corresponding orange/red pyrrolizinones, which were fully characterised by NMR, UV spectroscopy and X-ray crystallography. The observed  $\lambda_{max}$  values were found to be characteristic of the constituent amino acid. A similar sequence was successfully carried out using Fmoc-glycine Wang resin to produce characteristically bright red resin beads. The red tags can be removed by treating the pyrrolizinones with sodium methoxide in methanol to give colorless pyrrole acrylic esters.



Attempted acylation of **2** under harsher conditions (NaH and acetyl chloride) gave the cyclic dimers **4** as single stereoisomers that were characterised as H-bonded dimers by X-ray crystallography.

#### SOLID-PHASE SYNTHESIS AND STRUCTURAL STUDIES OF FUNCTIONALIZED $\beta$ - AND $\gamma$ -PEPTIDES

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A major goal of bioorganic chemistry is the synthesis of oligomers with unnatural backbones that combine the structural diversity and functional characteristics of biopolymers like polypeptides and polynucleotides, with the stability of synthetic polymers. Oligomers of  $\beta$ -amino acids ( $\beta$ -peptides) have recently been shown to exhibit residue-controlled secondary structure<sup>1,2,3</sup> (helix, sheet, turn) and have remarkable stability toward proteases.  $\gamma$ -amino acid oligopeptides can also adopt helical structures<sup>4,5</sup>. Our goal is to obtain by solid-phase synthesis new unnatural oligomers ( $\beta$ - and  $\gamma$ -peptides) with a well-defined secondary structure and to use them to present different functional groups in a predetermined arrangement. Selected scaffolds include cyclic  $\beta$ -peptides derived from  $\beta$ -alanine and 2,3-diaminopropionic acid and linear  $\gamma$ -peptides derived from 4-aminoproline and isonipecotic acid with a conformationally restricted backbone.

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#### THE DISCOVERY OF A NON-PEPTIDE THROMBIN RECEPTOR ANTAGONIST IN HUMAN AND ALSO RABBIT PLATELETS: A NOVEL DRUG LEAD

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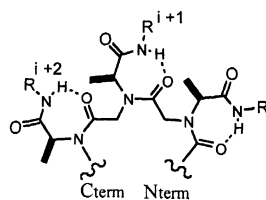
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An antagonist specific for the thrombin receptor would be a remedy for the thrombosis therapeutics. The identification of the proteolytically activated "tethered ligand" thrombin receptor on a variety of cell types has increased our understanding on how thrombin signals are mediated within the cells and stimulated our interest in synthesizing new thrombin receptor antagonists. We have recently demonstrated, through structure activity studies (SAR), that a cluster of the two groups (phenyl, guanidine) together with an adjacent primary amino group is important for expression of maximum biological activity by thrombin receptor activating peptides (TRAPs). In this regard, we have synthesized novel small-molecule non-peptide TRAP analogues. These compounds carrying the essential pharmacophoric groups of Phe, Arg and NH<sub>2</sub> incorporated onto small, bifunctional templates were subsequently tested for biological activity in washed human and rabbit platelet aggregation assays. All compounds were inactive when assayed for human and rabbit platelet aggregation, indicating that they are not agonists. Interestingly, these TRAP mimetics were found to be able to prevent thrombin induced platelet aggregation in both human and rabbit platelets. In the human platelet aggregation assay, the mimetic substance (S) N-(6-guanidohexanoyl)-N'-(2-amino-3-phenylpropionyl)piperazine (**1**) exhibits an IC<sub>50</sub> of 0.09mM and is the most potent non-peptide antagonist for the thrombin receptor reported to date. This compound prevents also thrombin induced platelet aggregation in rabbits at a relatively higher concentrations (IC<sub>50</sub> = 1.81mM). This lower activity is owned to that rabbit platelets do not respond to TRAPs that activate human platelets. Thus, it seems likely that this antagonist activity in rabbit platelets does not involve the moderate affinity PAR-1 receptor, but probably involves an alternative receptor, or receptors. Substance **1** could lead to most potent thrombin receptor antagonists that might be efficacious and potentially safer compared to thrombin active site inhibitors.

#### HYDROGEN BOND: A NEW TOOL IN PEPTOID DESIGN

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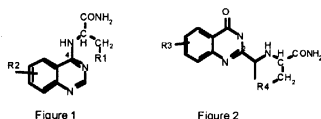
Mimicry of biopolymer structure and function is a field that evolved very quickly in recent years<sup>1</sup>. In all of these approaches, success relies primarily on new efficient synthetic methodologies. In this context, the preparation of water-soluble unnatural oligomers possessing a variety of side chain properties as well as a stable secondary structure is still a difficult task. The peptoid sub-monomer approach has been shown to give access to enantiopure large macromolecules (i.e. > 500 M.W. or 5mer to 36 mer). To further expand the set of monomers that give rise to peptoid secondary structure such as 1-S-phenylethylamine (Nspe)<sup>2</sup>, we initiated the development of new synthetic sub-monomers that are able to create unique peptoid secondary structure in aqueous solution. Using these enantiopure primary amines, we have prepared a series of different peptoid oligomers and evaluated the importance of monomer structural elements in their propensity to help the formation of secondary structures in aqueous solvent. Using CD and NMR spectroscopy, we have been able to find a new family of sub-monomers that use restricted bond rotation, steric effects and hydrogen bonds to create a secondary structure in peptoid oligomers. Furthermore, we have demonstrated that these monomers can be prepared in only two steps, with no column chromatography and be used to display a wide variety of side chains in peptoid oligomers.

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#### SCREENING OF PEPTIDOMIMETIC LIBRARIES FOR ANTITUMOR ACTIVITY

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The aim of our work was to screen peptidomimetic tyrosine kinase inhibitory libraries for antiproliferative and apoptotic or necrotic effect. We also wanted to distinguish between cytostatic and cytotoxic activities.



4-Benzylaminoquinazolines can be potent reversible inhibitors of Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase. It is known that after the benzylic methylation the biological activity depends on the chirality of the carbon atom, therefore we synthesized 4-amino acid quinazoline derivatives (Figure 1) varying the nature and the configuration of the amino acid and/or the substituent of the benzyl ring (R<sub>2</sub>). A similar library was developed when we substituted quinazolinone derivatives in position 2 with different amino acid derivatives (Figure 2). The synthesis of these libraries resulted in more than 100 compounds of each library. We systematically tested the above libraries using three different biological assays. 1. MTT test: fast screening of big number of compounds for the inhibition of human carcinoma cell proliferation. 2. Counting of the cells: screening of the compounds which showed some biological activity in the previous assay. 3. Tyrosine kinase assay. The results of the above assays will be discussed.

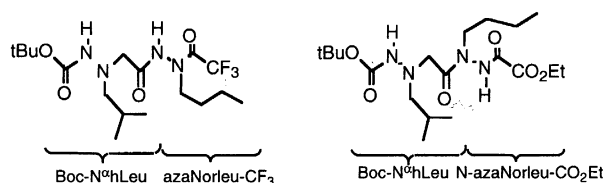
#### HYDRAZINOAZAPEPTOIDES, A NEW CLASS OF PSEUDOPEPTIDES AS POTENTIAL INHIBITORS OF PROTEASOME.

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The proteasome being an essential element in the regulation of diverse cellular function such as DNA repair signal transduction, or cell cycle progression is widely recognized as the central enzyme complex of non lysosomal protein degradation. A number of modified peptides on the C-terminal position has been already reported to inhibit proteasome. As far as we know, no oligomeric inhibitors such as "peptoids" have been tested. Therefore, we prepared pseudopeptides with enriched nitrogen backbone bearing the side chains and modified C-terminal position. These original molecules which have no chiral center with fixed configuration, combine a C-terminal azaamino acid unit with a N-hydrazino acid unit.

We show that each of these units can be assembled in a twostep procedure, actually a bromoacetylation followed by a substitution involving a pool of N,N'-disubstituted hydrazines.



Our goal is to mimic the biological activity of well-known proteasome inhibitors such as Acetyl-Leucyl-Leucyl-Norleucinal (ALLN) and other dipeptides which have similar activity. We will present the first biological results.

#### SYNTHESIS, SPECTROSCOPIC AND BIOLOGICAL STUDIES OF NEUROPEPTIDE Y ANALOGS CONTAINING 2-AMINO-3-METHOXYCARBONYL-1-CYCLOPROPANE CARBOXYLIC ACID

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β-Turn mimetics are useful tools for studying structure-affinity/activity relationships of biologically interesting peptides. Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the brain and it is involved in many important physiological functions such as vasoconstriction, anxiety, neurotransmitter release, and food intake.<sup>1</sup> So far, five distinct NPY receptors have been cloned and characterized which belong to the superfamily of the G-protein coupled receptors: these are called Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>4</sub>, Y<sub>5</sub> and y<sub>6</sub>. It is well established, that the C-terminus of NPY represents the receptor-binding site, however its bioactive conformation is still unknown. In order to gain a better insight into the structural requirements for strong and selective receptor binding, we synthesized full-length and N-terminally or centrally truncated NPY analogs containing a 2-amino-cyclopropane carboxylic acid (Acc) derivative that has been recently proposed as a turn mimic.<sup>2</sup> The peptides were prepared by solid phase synthesis using the Fmoc-/t-Bu strategy and the turn mimic was introduced as Fmoc-Xxx-Acc-OH dipeptide. Results of the *in vitro* binding affinity studies revealed a Y<sub>5</sub>-receptor selectivity of the full-length analogs, while the shorter peptides proved to be Y<sub>4</sub>-receptor preferring ligands. Circular dichroism spectra indicated the presence of both the helix- and β-structure, and for some peptides the β-structure was the predominant component. These results show that the cyclopropane derivative could be a useful tool to gain selectivity with respect to the multi-receptor system of NPY.

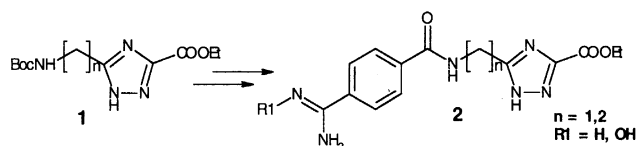
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#### USE OF 3,5-DISUBSTITUTED 1,2,4-TRIAZOLES FOR THE SYNTHESIS OF RGD MIMETICS

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Certain properties of 1,2,4-triazole ring system, such as its geometry, electrostatic potential and ability to act as a hydrogen bond donor or acceptor, make it very interesting for designing peptidomimetics. The synthesis of 3,5-disubstituted 1,2,4-triazoles from Boc-protected amino acids has previously been described [1]. In our case, these compounds were used for the synthesis of fibrinogen GPIIb/IIIa receptor antagonists, which are based on the RGD (Arg-Gly-Asp) bioactive sequence [2]. These agents are inhibitors of platelet aggregation and are used in therapy for preventing thrombosis related to cardiovascular and cerebrovascular diseases. Boc-protected 3-aminoalkyl-5-alkoxyloxycarbonyl-1,2,4-triazoles (**1**) were prepared through the intramolecular condensation of acylamidrazones, obtained from Boc-glycine and Boc- $\alpha$ -alanine. The protection group was removed and the resulting hydrochlorides were acylated with p-cyanobenzoyl chloride. Cyano compounds were then transformed into amidines **2** (R<sub>1</sub>=H), active in micromolar range (*in vitro* platelet aggregation assay). In addition, analogous amidoximes **2** (R<sub>1</sub>=OH) were prepared as an amidoxime group has been demonstrated to act as a prodrug for an amidino group.[3]



#### References:

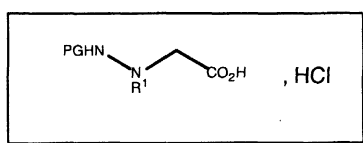
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### SYNTHESIS OF HYDRAZINOPEPTIDES, A NEW CLASS OF PSEUDOPEPTIDES PERSPECTIVES AND BIOLOGICAL EVALUATION.

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Hydrazinopeptoids, a new class of pseudopeptides, which are oligomers formally made up of *N*<sup>α</sup>-substituted hydrazinoglycine units will be described. To enable the synthesis of such analogues using Solid Phase Synthesis (SPS), we undertook to develop accurate *N*<sup>β</sup>-protected-*N*<sup>α</sup>-substituted hydrazinoglycine monomers.



*N*<sup>β</sup>-protected-*N*<sup>α</sup>-hydrazino acide  
PG = Fmoc, Boc

The methodology to obtain such monomers which allows the introduction of various proteogenic or non proteogenic side chains linked to nitrogen atoms will be described. We show that these monomers could be used either for preparation of pseudopeptidic oligomers of the peptoidic family, where all the C<sup>α</sup> are replaced by an isoelectronic nitrogen or to be introduced in some definite positions in modified peptides. We will describe the first results of immunological tests on these modified peptides in comparison with the wild-type sequence.

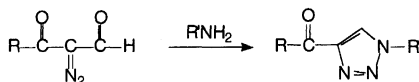
### 1,2,3-TRIAZOLE DERIVED PEPTIDOMIMETICS

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Native peptides are rarely directly usable as drugs, due to inherent limitation. Peptides are modified into mimetics with specific chemical, physical and biological characteristics in efforts to address these limitations. These so-called peptidomimetics are derived from peptides by partly or completely removing the amide bonds while retaining essential amino acid side chains in a defined, spatial relationship [1-3].

This work has been done with the goal of synthesis of new 1,2,3-triazole derived scaffold peptidomimetics. Since triazole compounds have a rigid nucleus which may handle the amino acid side chains in a defined direction and are potentially biologic active compounds, they were chosen for the peptidomimetic templates in this work.



R, R' = Amino acid side chains or their precursors

Peptidomimetics were derived from 1,2,3-triazoles [4] which formed by the reaction of  $\alpha$ -diazo- $\beta$ -oxoaldehyde derivatives [5] with different amine compounds. Substituents of triazole compounds were chosen as amino acid side chains or their precursors.

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### SOLID-PHASE POLYPROLINE DENDRIMER SYNTHESIS

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The synthesis of dendrimers has recently attracted much interest. These new materials have been suggested to have promise in a whole host of important potential applications, ranging from specialized drug delivery systems to catalyst carriers.

Dendrimers, in contrast to linear polymers, are highly branched, fractal-like macromolecules of defined three-dimensional, size, shape and topology which can be prepared with a very narrow molecular weight distribution<sup>1</sup>.

Generally these molecules emanate from a core, and like a tree, they more and more ramify with each subsequent branching unit.

Two main methods exist for the synthesis of dendrimers: a divergent approach, where dendrimer is assembled in a totally linear manner, or a convergent approach where fragments of dendrimer are condensed together. These two methods suffer from major problems when it comes to practical synthesis, in particular, the necessity for repeated and time consuming purification. The solid phase synthesis of dendrimers would have many advantages. Firstly, large excesses of reagents could be used without the problems associated with purification. Secondly, the use of orthogonally protected building blocks would allow more control over the synthesis.

Here we present two examples of polyproline dendrimers, synthesised by solid-phase, using polyproline peptides as building blocks and two different orthogonally protected cores attached to the resin, a 2,5-diketopiperazine Lys-Lys one and a spermidine one.

It is very well known that oligoproline peptides can adopt two different helical conformations in solution. Water, acetic acid, and other acidic solvents favor the formation of proline II helix, which contains only *trans* peptide bonds and has a left handed twist. *n*-Propanol, acetonitrile, and other less acidic solvents generally favor the formation of the proline I helix, which contains only *cis* peptide bond, has a right-handed twist and is more compact than the proline II helix. This particular behaviour will be exploited looking at the applicability of this novel compounds.

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### SOLID-PHASE SYNTHESIS OF ASPARTIC PROTEASE MIMICS

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The design and synthesis of enzyme mimics provides a means for achieving a better understanding of the mechanism of action of the native enzyme in order to replicate their activity. The aspartic proteases are a class of proteolytic enzymes which contain two aspartic acid residues situated in their active site which catalyzes the hydrolysis of amide bonds. We are exploring a model of the active site of an aspartate protease that consists of a cyclic peptide containing rigid dipeptide units that help to orient two carboxylic acid side-chains into close proximity. As the rigid dipeptide units, we have explored the use of (3*S*, 6*S*, 9*S*)-indolizidin-2-one amino acids (I<sup>2</sup>AA's) which have been shown to adopt type II'  $\beta$ -turns when incorporated into model peptides. Introduction of the I<sup>2</sup>AA residues into small cyclic peptides containing aspartic and/or glutamic acid residues has been accomplished using a solid-phase protocol. In particular, we have prepared the cyclic peptides c[Asp-I<sup>2</sup>AA-Asp-I<sup>2</sup>AA] and c[Asp-I<sup>2</sup>AA-Glu-I<sup>2</sup>AA] on Wang resin using an Fmoc protection strategy with allyl and *t*-butyl esters via side-chain carboxylate attachment to the resin. Our presentation will detail the synthesis of these peptides, their conformational analysis in water and results to characterize their ability to act as catalysts.

# Posters: topic C14

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Peptidomimetics

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## DESIGN AND SYNTHESIS OF ANALOGUES OF ANTISTATIN WITH POTENTIAL ANTICOAGULASE ACTIVITY

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Factor Xa is the serine protease that activates prothrombin to yield thrombin. Inhibitors of Factor Xa, which play a crucial role in curtailing thrombin generation are a promising alternative to currently available anticoagulants for treatment and for prevention of thrombotic disorders. The peptide antistatin (ATS), isolated from the salivary gland of the Mexican leech *Haementeria officinalis* is a slow-binding inhibitor of Factor Xa. The basic C-terminal region of ATS /residue 109-119/ is important for the interaction of ATS with Factor Xa ( $IC_{50}=3nM$ ). The shorter analogues 109-117 and 109-116 show considerable activity too ( $IC_{50}=5-20nM$ ). The analogues 109-113 and 109-112 have lower activity ( $IC_{50}=500-740nM$ ). The tripeptides as D-Phe-Pro-Arg, D-Arg-Gly-Arg and Tyr-Ile-Arg inhibit thrombin and Factor Xa too. The design of new peptides allow us to combine both fragment of ATS and some of the active groups. We synthesized the analogues of isoform 2 and isoform 3 of ATS and analogues of rATS: Tyr-Ile-Arg-Pro-Lys-Arg, Tyr-Ile-Arg-Pro-Lys-Arg-Lys, D-Arg-Gly-Arg-Pro-Lys-Arg, D-Arg-Gly-Arg-Pro-Lys-Arg-Lys and D-Arg-Gly-Arg-Pro-Lys-Arg-Lys-Leu-Ile-Pro by adding of Tyr-Ile and D-Arg-Gly, respectively to arginyl residue in position 109 of antistatin. The protected peptides were obtained by azide and HBTU/DIEA condensation of the corresponding fragments (2+4), (2+5) and (2+8). The protected tetrapeptide, pentapeptide and octapeptide were synthesized by consecutive attachment of the corresponding N-protected amino acids to C-terminal residue by DCC/HOBt and HBTU/DIEA methods. The protective groups Z-, Boc-, -NO<sub>2</sub> and Pac- were removed by treatment with boric trifluoroacetat and penicillin amidohydrolase, respectively. The anticoagulant activity of the newly synthesized peptides will be discussed.

## CYCLIC AND CROWN ETHER ANALOGUES OF RGD PEPTIDES

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Proteins containing the tripeptide motif, RGD, as their minimum active sequence are responsible for controlling biological processes such as blood clotting, bone growth and cancer cell proliferation. This motif has been the focus of considerable attention, both in an effort to understand the mechanism of action and in the design and synthesis of mimics which could be used as antagonists of proteins that recognise receptors, such as the GPIIb/IIIa integrins.

Like other research groups we have constrained RGD motifs into cyclopentapeptide analogues, but while others have reported that the aspartyl residue, D, has to be present for activity, one of our most potent cell adhesion analogues has turned out to be an analogue where D is replaced by  $\gamma$ -carboxyglutamic acid (Gla). The present work will report on our synthesis of the diastereoisomers, cyclo (RG(L)GlaSK) and cyclo (RG(D)GlaSK) and the nmr and mass spectral studies we have carried out on these new analogues. Fundamental to the work was the need to have available workable quantities of a Fmoc-diester protected Gla derivative in both D- and L-forms.

We have now also entered a novel area of peptidomimetics, where we have attempted to place relevant amino acid side chains onto a crown ether scaffold, in such a way that the 18-crown-6 ring mimics a cyclopentapeptide or cyclohexapeptide ring. Again the motif we have chosen is the RGD sequence, whose active side-chains have been attached to chiral glycol entities for linking into the crown ether ring system. The asparaginyl residue is incorporated via an ornithinyl-based side chain, while various malic acid derivatives serve as the source of the aspartyl side chain. It is hoped that the cyclic ether mimetic will overcome the sensitivity to enzyme degradation, usually associated with all-peptide mimetics.

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## PHYSICO-CHEMICAL AND IMMUNOCHEMICAL STUDY OF METHYLENAMINO PEPTIDE BOND PSEUDOPEPTIDES MIMICKING A NEUTRALIZING DECAPEPTIDE EPITOPE ON FOOT-AND-MOUTH DISEASE VIRUS.

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The replacement of one or several amide bonds in a peptide chain by other chemical bonds is a useful approach to assess the contribution of each part of the peptide backbone to a particular function. This approach is also highly valuable to identify protease-resistant peptidomimetics able to replace biodegradable natural peptides. In this work, a series of ten peptide mimics incorporating one methylenamino (-CH<sub>2</sub>-NH-) peptide bond at every different position along the peptide chain 147-156 of the capsid protein VP1 from foot-and-mouth disease virus (FMDV) has been synthesized. On the other hand, monoclonal antibodies (mAbs) have been generated against the parent decapeptide 147-156 and have been tested for their physicochemical and immunochemical properties. Culture supernatants have been assayed for titration in enzyme-linked immunosorbent assay (ELISA) and for determining the isotype of the mAbs (all are of the IgG1 isotype). The active antibody concentration was measured using the BIAcore flow method and we also calculated the binding kinetic parameters *i.e.* the kinetic association constant (k<sub>a</sub>), the kinetic dissociation constant (k<sub>d</sub>) and the equilibrium association constant (K<sub>a</sub>). We determined the amount of the free homologous and analogue peptides able to inhibit 50% of the mAb-peptide interaction (IC<sub>50</sub>) in a BIAcore competitive assay and measured the inhibition constant (K<sub>i</sub>) with respect to the parent peptide. The results show that according to the peptides and antibody tested, the mAbs bind to the methylenamino peptide bond analogues with lower, equal, or higher affinity compared to the binding to the corresponding parent peptide. Different patterns of inhibition have been found with the pseudo-peptides and no general rule could be defined with respect to preferred methylenamino modifications that are allowed in the peptide chain for the antibody recognition. These findings highlight that prior to application of methylenamino peptides to biological purpose a thorough screening of their activities is requested. They also show that such strategy of replacement is efficient to identify peptidomimetics showing advantageous properties with respect to a given antibody.

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## DESIGN, SYNTHESIS AND STUDY OF NEW PEPTIDIC AND PEPTIDOMIMETIC INHIBITORS OF THE HUMAN CYCLOPHILIN hCyp-18<sup>1</sup>

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The human cyclophilin hCyp-18 is a ubiquitous cytoplasmic peptidyl-prolyl isomerase (PPIase). It catalyzes the *cis/trans* interconversion of the amino acyl-prolyl amide bond in peptides and proteins. Cyclophilin plays an important role in one of the limiting steps of protein folding. As a consequence hCyp-18 is involved in many biological processes such as cellular multiplication and communication, immunosuppression, as well as in several pathologies including AIDS. In particular, hCyp-18 specifically interacts with a specific site of the HIV-1 pre-protein Gag and several portions of the viral capsid protein, and is therefore implicated in the replication of the virus. Inhibition of the Gag:hCyp-18 interaction irreversibly leads to the release of non-infectious virions.

We investigated the inhibitory potential of tetrapeptides containing Ala-Pro or Gly-Pro surrogates as transition-state analogs of the PPIase activity. The substitution of proline with fluoroproline, which cause a partial deconjugation of the amide bond, did not inhibit hCyp-18. On the other hand, peptides containing mimics of a constrained "keto-amine" moiety, effectively blocked the enzyme.

Several short peptides derived from the N- and C-terminal parts of the capsid protein were also studied. As previously reported, a 7-mer derived from the N-terminus of the capsid protein interacted with hCyp-18 with a millimolar affinity and did not inhibit hFKBP-12, another abundant PPIase. Sequential and combinatorial modifications of the heptapeptide led to the design of a 5-mer modified peptide which specifically interacts with hCyp-18 with a better affinity than the capsid protein. Improvement of the potency and selectivity of this inhibitor by combinatorial chemistry is under way.

The design of selective inhibitors of hCyp-18 should lead to the development of a novel class of anti-AIDS agents which could complement the widely used "cocktail-therapies".

<sup>1</sup> This work was supported by SIDACTION, ANRS and CEA.

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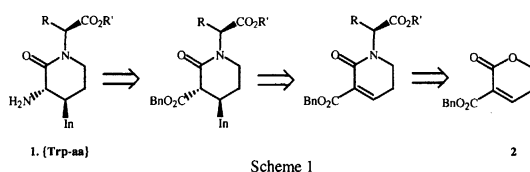
#### SYNTHESIS OF CONFORMATIONALLY RESTRICTED TRYPTOPHAN SURROGATES

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We have recently developed a method for the synthesis of Trp-Gly surrogates, that contain the 3-amino-2-piperidone backbone. The main steps of this synthetic pathway are a conjugate addition of indole and a Curtius rearrangement.<sup>1</sup> 3-Aminolactams are known to mimic  $\beta$ -turn conformations.<sup>2</sup>

Our next goal is to extend this methodology to the preparation of other conformationally restricted pseudopeptides containing tryptophan, according to the retrosynthesis shown in Scheme 1.



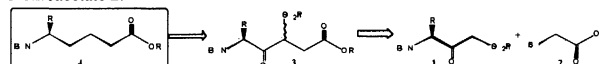
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#### SYNTHESIS OF THE TYR $\Psi$ [CH<sub>2</sub>CH<sub>2</sub>]GLY CARBAPEPTIDE ANALOG OF A MHC CLASS II ANTIGENIC PEPTIDE

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In our ongoing study of the rules governing the association of peptides with Molecular Histo compatibility Complex Class II (MHC-II) molecules and the recognition of the complex by T cell receptors we planned to synthesize the Tyr<sup>52</sup>-Gly<sup>54</sup> carba peptide analog of the antigenic peptide HEL(52-61) so as to compare its activity with several analogs already obtained such as the *E*-alkene one. Recently, we exposed a new application of the Julia olefination for the synthesis of Tyr-Gly *E*-alkene and carba isostere pseudodipeptides.<sup>1</sup> This strategy implied the condensation of an  $\alpha$ -amino acid-derived  $\beta$ -ketosulfone **1** onto protected bromoacetate **2**.



Next,  $\beta$ -ketosulfone ester **3** was transformed into carba peptide **4** by reduction of the  $\gamma$ -ketone, reductive desulfonation of the acetylated  $\beta$ -hydroxysulfone moiety and finally catalytic hydrogenation of the *E*-alkene dipeptide. Introduction of this carba peptide moiety into HEL(52-61) sequence was then carried out by convergent Fmoc-based solid phase peptide synthesis. First, pseudopeptide **4** was deprotected by TFA and then coupled to Fmoc-Asp(*t*Bu)-OH protected amino acid. The target carba peptide analog Tyr<sup>53</sup> $\Psi$ [CH<sub>2</sub>CH<sub>2</sub>]Gly<sup>54</sup>-HEL(52-61) was finally obtained in 25% yield by coupling the pseudotripeptide Fmoc-Asp(*t*Bu)-Tyr $\Psi$ [CH<sub>2</sub>CH<sub>2</sub>]Gly onto HEL(55-61) with HBTU/HOBt/DIPEA in DMF for 15 hrs. Biological activity of this new analog in the context of MHC-II-peptide interactions will be exposed.

#### Reference

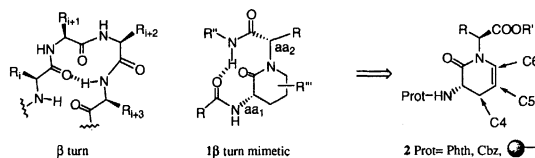
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#### SYNTHESIS OF 3-AMINO-1,2,3,4-TETRAHYDROPYRIDIN-2-ONES AS PEPTIDOMIMETICS

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Our group has been working on the synthesis of conformationally restricted pseudopeptides presenting a 3-amino-2-piperidone backbone as potential  $\beta$ -turn mimetics.<sup>1,2</sup> A 3-amino-1,2,3,4-tetrahydropyridin-2-one (**2**) has been used as a scaffold to obtain a large number of 3-aminolactams by further functionalization of the C4, C5 and C6 positions.<sup>3</sup>



At the present moment we are developing a strategy for the synthesis of enamides **2** in solid phase, in order to increase the diversification, not only of the aa<sub>2</sub> residue but also of the functionalization of the ring.

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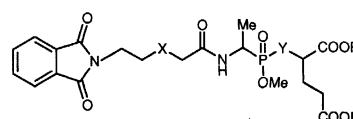
#### SYNTHESIS OF PHTHALOYLATED PHOSPHONATE AND PHOSPHONAMIDATE PSEUDOPEPTIDES AS POTENTIAL INHIBITORS OF THE D-GLUTAMIC ACID-ADDING ENZYME

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Peptidoglycan is a network of alternating *N*-acetyl muramic acid and *N*-acetyl glucosamine units that are cross-linked by D-amino acid-containing tetrapeptides. It provides rigidity for the cell wall and protects bacteria from osmotic lysis. Peptidoglycan biosynthesis is therefore an important target for antibiotic research. Among numerous steps of this pathway, the D-glutamic acid-adding enzyme (MurD), which catalyzes the formation of the peptide bond between UDP-*N*-acetylmuramoyl-L-alanine and D-glutamic acid, has recently become important in the search for new antibiotics. Phosphonic and phosphinic analogs of its tetrahedral intermediate have been synthesized and evaluated.

Recently we found that some simple *N*-acyl phosphonoalanine derivatives like *N*-(5-phthalimidopentanoyl)- or *N*-(2-(2-phthalimidoethoxy)acetyl)phosphonoalanine, are weak inhibitors of MurD. We concluded that the 5-phthalimidopentanoyl and 2-(2-phthalimidoethoxy)acetyl groups impart to phosphonoalanine a certain affinity for MurD. In order to improve the activity of these phthalimido derivatives, the glutamic acid fragment has been added. We wish to present the synthesis of a series of phosphapeptides designed to mimic the tetrahedral intermediate that occurs during the peptide bond formation catalyzed by MurD. Phosphonamidate (comp. 1-4) and phosphonate (comp. 5-6) pseudopeptides shown below have been synthesized.



Comp.	X	Y	R
1	CH <sub>2</sub>	NH	Bzl
2	CH <sub>2</sub>	NH	H
3	O	NH	Bzl
4	O	NH	H
5	CH <sub>2</sub>	O	Me
6	O	O	Me

# Posters: topic C14

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Peptidomimetics

## STABILIZATION OF TYPE VIa $\beta$ -TURN IN TETRAPEPTIDES

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Mimicry of the structural elements of a type VIa  $\beta$ -turn was previously achieved by employing the steric interactions of (2*S*, 5*R*)-5-*tert*-butylproline at the C-terminal of a series of *N*-(acetyl)dipeptide *N*-methylamides (*J. Org. Chem.* 1999, 64, 3312-3321). Both the prolyl amide *cis*-isomer as well as the intramolecular hydrogen bond between the *N*-methylamide proton and the acetamide carbonyl were observed in these model peptides of the type Ac-L-Xaa-5-*t*-BuPro-NHMe using NMR and CD spectroscopy as well as X-ray crystallographic analysis. To study further the factors that stabilize type VIa  $\beta$ -turn conformations in peptides, we have recently incorporated (2*S*, 5*R*)-5-*tert*-butylproline into a series of tetrapeptides possessing the general structure of Ac-Ala-Xaa-5-*t*-BuPro-Yaa-NHMe. In this model peptide, we have examined the influence on peptide folding of the nature of the amino acids at the *N*- and *C*-termini of the 5-*tert*-butylproline residue. Furthermore, replacement of the *N*-methylamide with its methyl ester counterpart was studied to minimize hydrogen bonding that may disrupt the type VIa structure. Measurements of the amide *cis*- and *trans*-isomer populations using NMR spectroscopy indicated that the *cis*-isomer population varied from 40% up to 73% in water. Particular elements have now been elucidated for stabilizing type VIa geometry in longer peptides in water.

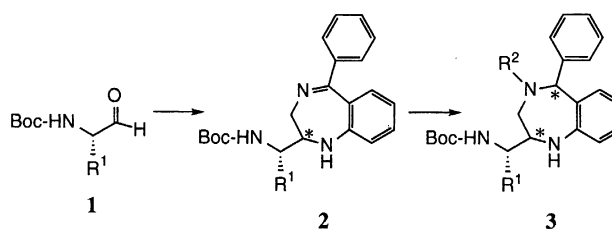
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## SYNTHESIS OF CHIRAL 2,3-DIHYDRO- AND 2,3,4,5-TETRAHYDRO-1H-1,4-BENZODIAZEPINE SCAFFOLDS FOR PEPTIDOMIMETIC BUILDING

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It is apparent that certain core structures are frequently found in ligands for multiple G-protein coupled receptors. Among these structures, the benzodiazepine template has proved to be a privileged scaffold for a surprisingly diverse collection of receptors, such as opioids, cholecystokinin, bradykinin, fibrinogen, vasopressin or somatostatin receptors<sup>1,2</sup>. In this communication we will present an easy and versatile synthesis of chiral 1,4-benzodiazepine derivatives **2** and **3** useful for elaboration of diverse peptidomimetics.



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## STEPWISE PEPTID-PEPTOID-TRANSFORMATIONS VIA SPOT-SYNTHESIS

Berit Hoffmann<sup>a</sup>, Thomas Ast<sup>a</sup>, Ulrich Reineke<sup>b</sup>, Jens Schneider-Mergener<sup>a,b</sup> and Rudolf Volkmer-Engert<sup>a</sup>.

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Bioactive peptides are of great interest for the development of novel drugs. However, peptides itself have many drawbacks such as degradation by proteases and low bioavailability for their direct use as therapeutics. Therefore, the transformation of bioactive peptides into peptidomimetics with improved properties is of great interest. In this communication the stepwise transformation of peptide ligands into peptoids via peptomers under preservation of the binding specificity is described for two model systems: a) the peptide epitope VVSHFND recognized by the monoclonal anti-transforming growth factor  $\alpha$  (TGF  $\alpha$ ) antibody Tab2 and b) the peptide PPPPY which is the ligand of the YAP- (Yes kinase-associated protein) WW domain. This was achieved using the SPOT-synthesis technique<sup>1</sup> which is a rapid and easy method for the spatially addressed generation of large numbers of peptides, peptide-peptoid hybrids (peptomers)<sup>2</sup>, peptoids and other small organic compounds on planar surfaces such as cellulose or polypropylene membranes. Substitutional analyses of both peptides were carried out in which each position – but only one at the time – was exchanged by a set of different peptoid building blocks resulting in a peptomer array. After binding assays with the respective ligands directly on the solid support best binding peptomer spots were selected and subjected to an iterative transformation to complete peptoids. Chosen compounds of each transformation step were resynthesized and binding constants were determined by competitive ELISA and BIACORE techniques. The binding constants revealed a specific binding of each peptoid and transition peptomer to the corresponding protein.

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## SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL RGD-PEPTIDOMIMETICS AS POTENTIAL FIBRINOGEN RECEPTOR ANTAGONISTS

Vladimir M. Kabanov, Sergei A. Andronati, Andrei A. Krysko, Tamara L. Karaseva, Tatyana A. Kabanova, Viktor I. Pavlovsky.

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Arterial thrombosis is dependent upon platelet adhesion, activation and aggregation. The final and obligatory step in the formation of platelet aggregates involves the binding of fibrinogen (recognition sequence Arg-Gly-Asp, RGD) to glycoprotein IIb/IIIa (GP IIb/IIIa) on the surface of activated platelets.

We have synthesized the series of linear non-peptide RGD-mimetics on the base of 4-oxo-4-(piperazine-1-yl)butyric acid. In these compounds guanidine function of Arg residue have been substituted by piperazine fragment and C-terminal moiety – by  $\beta$ -aryl- $\beta$ -alanine residues. The synthesized peptidomimetics have been evaluated for their antiaggregatory potency using *in vitro* bioassays on inhibition of ADP-induced aggregation of platelets in human platelet rich plasma. These studies have demonstrated the high antiaggregatory activity of investigated compounds (IC<sub>50</sub> of 10<sup>-6</sup> – 10<sup>-8</sup> M). The most active peptidomimetics have been selected for further study in experiments on inhibition of binding of fluorescein-labeled fibrinogen to GP IIb/IIIa, its receptor on activated platelets. Binding experiments have been carried out by bound-phase separation assay on human washed platelet suspension. Investigated compounds have inhibited the binding of fibrinogen to its receptor with IC<sub>50</sub> of 10<sup>-7</sup> – 10<sup>-9</sup> M. In order to obtain the potent compounds with longer duration of antiaggregatory activity upon oral administration we have synthesized the pro-drugs on the base of most active peptidomimetics by masking of their zwitterion nature.

#### CYCLOLINOPEPTIDE A ANALOGUE CONTAINING (2*S*,4*R*)-4-AMINOPYROGLUTAMIC ACID (4tAPy) RESIDUE AS A *CIS*-PEPTIDE BOND MOTIF

Krzysztof Kaczmarek<sup>a</sup>, Janusz Zabrocki<sup>a</sup>, Jerzy Sillbering<sup>b</sup>, Marek Smoluch<sup>b</sup>, Marek L. Kowalski<sup>c</sup>, Barbara Szkudlińska<sup>c</sup>, Ewa Grochulska<sup>c</sup>, <sup>a</sup>Institute of Organic Chemistry, Technical University of Łódź, 90-543 Łódź, Poland, <sup>b</sup>Faculty of Chemistry, Jagiellonian University, 30-060 Kraków, Poland, <sup>c</sup>Department of Immunology, Medical University of Łódź, 92-215 Łódź, Poland

Cyclolinopeptide A (CLA), a cyclic nonapeptide with the sequence cyclo(-Pro<sup>1</sup>-Pro<sup>2</sup>-Phe<sup>3</sup>-Phe<sup>4</sup>-Leu<sup>5</sup>-Ile<sup>6</sup>-Ile<sup>7</sup>-Leu<sup>8</sup>-Val<sup>9</sup>-) was isolated from linseeds and was shown to possess strong immunosuppressive activity in respect to both – humoral and cellular immune response. A peculiarity of CLA structure is the presence of *cis*-amide bond situated between both proline residues. Such structure of CLA was postulated by Siemion et al. [*Rocz. Chemii* 1977, **21**, 1489]. X-ray, NMR and computational study confirmed this hypothesis [*J. Am. Chem. Soc.* 1989, **111**, 9089; *Biopolymers* 1989, **28**, 573; *Int. J. Peptide Protein Res.* 1991, **37**, 81]. In the aim to answer the question, if the *cis*-geometry of Pro-Pro amide bond is important for CLA biological activity, we have synthesized analogs of CLA containing tetrazole ring as a *cis*-peptide bond surrogate. Both cyclic peptides showed biological activity comparable to those of parent compound [*16<sup>th</sup> American Peptide Symposium*, 1999, Abstract Book P537]. To check, if  $\beta$ -turn type VIb, generated by (2*S*,4*R*)-4-aminopyroglutamic acid residue is accepted by the biological system as well as a *cis*-peptide bond conformation, we have synthesized two analogues (linear and cyclic) of CLA, in which Pro-Pro segment has been replaced by 4tAPy. The strategy of synthesis as well as biological activity of synthesized peptides will be discussed.

This work was supported by Polish State Committee for Scientific Research (KBN), Grant 4 PO5F 004 13

#### OPIOID RELATED PEPTIDE ALCOHOLS: STRUCTURE-ACTIVITY STUDIES

László Kocsis<sup>a</sup>, Sándor Benyhe<sup>b</sup>, Anna Borsodi<sup>b</sup>, András Z. Rónai<sup>c</sup>, Anna Magyar<sup>d</sup> and György Oroscz<sup>a</sup>  
<sup>a</sup>Department of Organic Chemistry, Eötvös University, <sup>b</sup>Biological Research Center of Hung. Acad. Sci., Szeged, <sup>c</sup>Semmelweis Medical University, Budapest, <sup>d</sup>Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Budapest, Hungary

Enzymatic degradation of peptide derivatives can be suppressed by the application of C-terminal peptide alcohols. In our studies, the alcohol derivatives of the recently discovered endomorphin 1, 2 (Tyr-Pro-Trp-Phe-NH<sub>2</sub>, Tyr-Pro-Phe-Phe-NH<sub>2</sub>)  $\mu$  receptor and nociceptin receptor ligands were studied.

The C-terminal Fmoc-amino alcohols were anchored to 2-chlorotrityl resin in the presence of base. Among the conditions probed, the highest loading was obtained in the presence of pyridine. Detailed data are presented on the optimal conditions for loading. Elongation of the peptide chain was performed by standard Fmoc protocol with HBTU/diisopropylethylamine as coupling reagents. The peptide-resin cleavage conditions were optimized to obtain Boc-protected peptide alcohols.

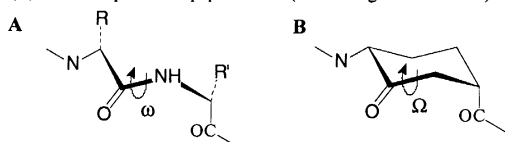
To further increase the enzyme resistance, N-methylated derivatives were synthesized as well.

Beside of the synthetic details, the biological characteristics of the synthesized endomorphin and nociceptin-related sequences will be presented.

#### MIMETICS OF THE DIPEPTIDE MOIETY WITH A NONPLANAR *CIS*-AMIDE BOND

Krzysztof Krajewski, Zbigniew Ciunik, Ignacy Z. Siemion  
 Faculty of Chemistry, University of Wrocław, Poland.

During the *cis/trans* isomerization of the Xaa-Pro amide bond (catalyzed *in vivo* by peptidyl-prolyl *cis/trans* isomerase (PPIase)) the transition state with a twisted amide bond appears (A). In crystal structures of complexes of PPIases with peptide substrates the ligand's Xaa-Pro peptide bond adopts a nonplanar *cis*-conformation ( $\omega \sim 0^\circ - 45^\circ$ ).<sup>1</sup> The X-ray structures of cyclophilin A (CypA) - cyclosporin A (CsA, CypA inhibitor and immunosuppressive drug) complex showed that CsA mimic this transition state geometry. The incorporation of the mimetic of a twisted *cis*-amide bond into peptide should increase its affinity to CypA. We designed a new mimetic - 4-amino-3-oxo-1-cyclohexanecarboxylic acid - which is a conformationally restricted analogue (B) of the nonplanar *cis*-peptide bond (torsion angle  $\Omega$  about  $50^\circ$ ).



Stereoisomers of this system were obtained and its structures were examined (NMR, X-ray).<sup>2</sup> Precursors of four stereoisomeric forms of this mimetic suitable for peptide synthesis ((1*R*, 3*R*, 4*R*)-, (1*S*, 3*S*, 4*S*)-, (1*R*, 3*S*, 4*S*)-, and (1*S*, 3*R*, 4*R*)-4-amino-3-hydroxy-1-cyclohexanecarboxylic acid esters) were synthesized and oxidized after incorporation into cyclolinopeptide A (c-(Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu-Val), CLA) – the cyclic nonapeptide isolated from linseed – whose mechanism of the immunosuppressive activity is the same as CsA,<sup>3</sup> in the place of two proline residues (crucial for interaction of CLA with CypA).

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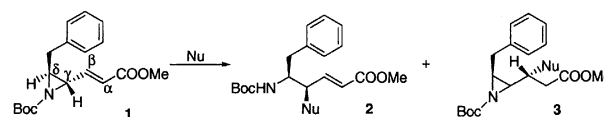
<sup>2</sup> Krajewski K., Ciunik Z., Siemion I. Z. *Tetrahedron: Asymmetry* 1999, 10, 4591.

<sup>3</sup> Gaymes T. J., Cebrat M., Siemion I. Z. *FEBS Lett.* 1997, 418, 224.

#### STEREOSELECTIVE SYNTHESIS OF DIPEPTIDOMIMETICS FROM CHIRAL ALLYLIC AZIRIDINES USING MICROWAVE IRRADIATION.

Erika Larsson,<sup>a</sup> Wei Berts,<sup>a</sup> and Kristina Luthman,<sup>a,b</sup> from <sup>a</sup>Department of Organic Pharmaceutical Chemistry, Box 574, BMC, Uppsala University, S-751 23 Uppsala, Sweden, <sup>b</sup>Department of Medicinal Chemistry, Institute of Pharmacy, University of Tromsø, N-9037 Tromsø, Norway.

$\gamma,\delta$ -Epimino- $\alpha,\beta$ -unsaturated esters (e.g. 1) are useful starting materials for the synthesis of dipeptidomimetics. They contain four possible positions for attack by nucleophiles: the  $\alpha$ -position ( $S_N2'$ -reaction), the  $\beta$ -position (1,4-addition) and aziridine ring opening via an  $S_N2$ -reaction in the  $\gamma$ - or  $\delta$ -positions. We have reacted nitrogen and sulfur nucleophiles with the *trans* and *cis* isomer of aziridine 1. Two major competitive pathways were observed: a 1,4-addition reaction at the  $\beta$ -position to form 2 and/or a  $S_N2$  reaction at the  $\gamma$ -position to form 3.



The nitrogen nucleophiles gave a mixture of products 2 and 3. The configuration at the  $\beta$ -position of the 1,4-addition product 3 appears to be controlled by directing effects of the Boc group. Aromatic sulfur nucleophiles gave exclusively the stereoselective ring opening product 2, both with thermal heating at  $50^\circ\text{C}$  for 10h and with microwave irradiation at 30W for 4 min. Aliphatic sulfur nucleophiles only reacted in the presence of base and gave exclusively the 1,4-addition product 3 at  $0^\circ\text{C}$  for 5h.

We have shown that novel dipeptidomimetics can be synthesized from allylic aziridines in a regio and in some cases stereoselective fashion.

[Supported by The Swedish Natural Science Research Council].

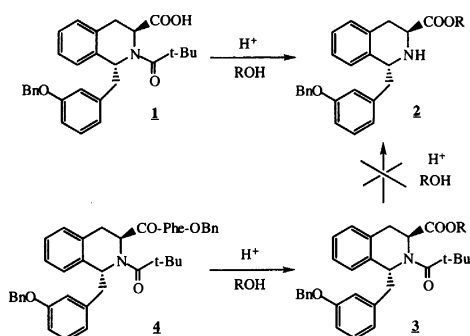
#### AMIDE BOND CLEAVAGES UNDER UNUSUAL MILD CONDITIONS IN N-PIVALOYL-TIC COMPOUNDS: A MECHANISTIC STUDY.

Els Mannekens<sup>a</sup>, Dirk Tourwé<sup>a</sup>, Marco Crisma<sup>b</sup>, <sup>a</sup>Vrije Universiteit Brussel, Laboratorium voor Organische Chemie, Brussel, Belgium; <sup>b</sup>Centro di Studio Sui Biopolimeri, Dipartimento di Chimica Organica, Padova, Italy.

During the synthesis of a new peptidomimetic based on the  $\delta$ -opioid receptor antagonist TIP (Tyr-Tic-Phe-OH) 1-(benzyloxy)benzyl-substituted 1,2,3,4-tetrahydroquinoline-3-carboxylic acid analogues having a N-pivaloyl protection were prepared.

Whereas the pivaloyl group was removed under very mild conditions (0 °C, HCl, MeOH) in **1**, it was stable in ester **3**. In contrast, in **4** PheOBn cleaves under these conditions to ester **3** and the pivaloyl group remains. An X-ray diffraction structure of **4** shows only a slightly twisted tertiary amide bond.

The observed cleavages can be rationalized by assuming oxazolinium/oxazolidinium ion intermediates, as already proposed by M. Goodman for NMeAib containing peptides.<sup>1</sup>



<sup>1</sup> 16<sup>th</sup> American Peptide Symposium, 1999, Minneapolis, Minnesota, USA. Poster P044, C.J. Creighton, T.T. Romoff, J. Bu and M. Goodman.

#### STEREOSELECTIVE SYNTHESIS OF H-Phe $\psi$ [CH<sub>2</sub>O]Phe-OH AND TWO HIV-1 PROTEASE INHIBITORS

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Methylene-oxy isosteric replacement  $\psi$ [CH<sub>2</sub>O] of the peptide bond provides flexible, lipophilic isosteres with enhanced metabolic stability. Moreover, the geometry of the methylene-oxy surrogates resembles the geometry of the peptide bond in extended conformation.[1, 2] We decided to prepare H-Phe $\psi$ [CH<sub>2</sub>O]Phe-OH and incorporate this pseudodipeptide unit into the two HIV-1 protease inhibitors.

The synthesis of the H-Phe $\psi$ [CH<sub>2</sub>O]Phe-OH begins with phenylalaninol prepared from natural amino acid. This compound is coupled with 2-chloroacetic acid to form (5S)-5-benzylmorpholin-3-one in the first step. Nitrogen atom of morpholinone ring is then protected with *tert*-butyloxycarbonyl group. The reaction of the enolate generated from (5S)-5-benzyl-4-*tert*-butyloxycarbonylmorpholin-3-one with benzaldehyde followed by elimination of the hydroxyl group provides (2Z,5E)-5-benzyl-2-benzyliden-4-*tert*-butyloxycarbonylmorpholin-3-one. The catalytic hydrogenation of this compound gives (2S,5S)-2,5-dibenzyl-4-*tert*-butyloxycarbonylmorpholin-3-one in de greater than 99%. Acidic hydrolysis of the morpholinone ring provides H-Phe $\psi$ [CH<sub>2</sub>O]Phe-OH. This pseudodipeptide was used for the synthesis of the two possible HIV-1 protease inhibitors Boc-Phe $\psi$ [CH<sub>2</sub>O]Phe-Gln-Phe-NH<sub>2</sub> and Boc-Phe $\psi$ [CH<sub>2</sub>O]Phe-Glu-Phe-NH<sub>2</sub> and biological activity was tested.

[1] Rubini, E.; Laufer, R.; Gilon, C.; Selinger, Z.; Roques, B. P.; Chorev, M. *J. Med. Chem.* **1991**, *34*, 2430.

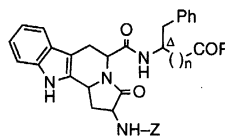
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#### $\beta$ -TURNED CONFORMATIONALLY RESTRICTED DIPEPTOIDS AS POTENT AND SELECTIVE CCK<sub>1</sub> RECEPTOR ANTAGONISTS

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Recently, we have described that replacement of the  $\alpha$ -MeTrp residue of dipeptoids PD-140548 and PD-140547 –CCK<sub>1</sub>- and CCK<sub>2</sub>-selective receptor ligands, respectively– with the highly constrained (2S,5S,11bR)-2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate skeleton [(2S,5S,11bR)-IBTM], a probed type II'  $\beta$ -turn mimic<sup>1</sup>, leads to compounds that behave as potent and selective ligands for CCK<sub>1</sub> receptors<sup>2</sup>. In order to optimize the structural requirements of these IBTM-containing dipeptoid analogues, we have now prepared compounds **1a-1h**, incorporating L- and D-Phe residues instead of the corresponding  $\beta$ -homophenylalanine ( $\beta$ Hph) in **1a** and **1b**. Additionally, to ascertain whether the  $\beta$  II' is the type of  $\beta$ -turn preferred for interacting with the CCK<sub>1</sub> receptor, compounds **1i-1p**, containing the enantiomeric (2R,5R,11bS)-IBTM skeleton, able to stabilize a type II  $\beta$ -turn, have been prepared. Concerning binding affinity, the most remarkable result is that dipeptoids incorporating the type II' mimic showed higher CCK<sub>1</sub>/CCK<sub>2</sub> selectivity than those incorporating the type II analogue. The critical role of the absolute configuration of Phe or  $\beta$ Hph residue and the importance of a free C-terminal carboxylate group on binding affinity and selectivity will also be discussed. Compounds with nanomolar affinity for CCK<sub>1</sub> receptors are able to inhibit the amylase release induced by CCK<sub>8</sub> in pancreatic acinar cells, indicating that these  $\beta$ -turned dipeptoid analogues behave as CCK<sub>1</sub> receptor antagonists.



**1a,b**: n=1; R= OH; (2S,5S,11bR),  $\Delta=S,R$

**1c-h**: n= 0; R= OMe, OH, NH<sub>2</sub>;

(2S,5S,11bR),  $\Delta=S,R$

**1i-p**: n= 0, 1; R= OMe, OH;

(2R,5R,11bS),  $\Delta=S,R$

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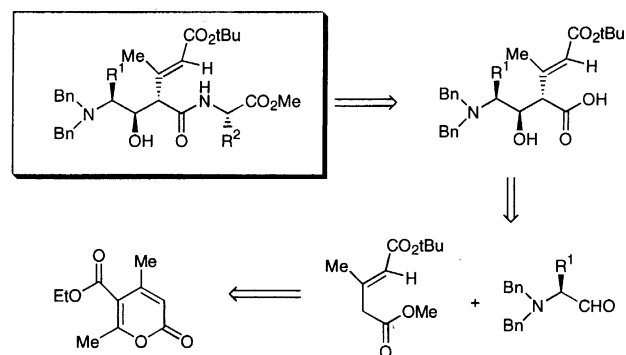
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#### SYNTHESIS OF DIPEPTIDES CONTAINING NEW 2-SUBSTITUTED STATINES AS POTENTIAL PROTEASE INHIBITORS

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In an attempt to design new protease inhibitors, we have been looking for a general method of preparation of new 2-substituted statines. The preparation of these compounds was performed via a directed aldol type condensation between



dimethyl-3-methylglutaconate and chiral  $\alpha$ -aminoaldehydes.<sup>1</sup>

In order to prepare peptidomimetics containing the statine moiety, we describe a methodology allowing the preparation of methylglutaconate bearing two different protecting groups. After selective removal of the methylester, the 2-substituted statines are condensed by means of EDCI reagent to obtain the target dipeptides.

Reference

<sup>1</sup>Nathalie PIVETEAU, Patrick AUDIN, Joëlle PARIS, *Synlett* **1997**, 1269-1270.



#### DESIGN AND SYNTHESIS OF A NEW $\gamma$ -AMINO ACID AS A NOVEL TURN-MIMIC

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School of Chemistry, Seoul National University, Seoul 151-742, South Korea E-mail: [kimbm@snu.ac.kr](mailto:kimbm@snu.ac.kr)

Design and synthesis of unnatural amino acids as building blocks for the construction of biologically active compounds such as enzyme inhibitors and receptor agonists/antagonists are of keen interest in the medicinal chemistry research. Particularly, novel peptide turn mimetic structures have attracted much attention due to the fact that such turn structures play an important role in the enzyme-substrate or receptor-ligand interactions involving biologically active peptides. Herein we disclose a novel, optically active, and conformationally-constrained structure equipped with an unnatural  $\gamma$ -amino acid arrangement in a bicyclic norbornene structure. Synthesis of the amino acid involves as a key step a highly diastereo- and enantioselective Diels-Alder reaction between cyclopentadiene and an acrylate ester employing a chiral Lewis acid catalyst to furnish an optically active endo-norbornenecarboxylic acid derivative. Iodolactamization followed by the removal of the iodide furnished a tricyclic lactam, which is equipped with the required  $\gamma$ -amino acid component. Detailed synthetic pathway and conformation studies including molecular modeling will be presented.

#### PEPTIDYL PRIVILEGED STRUCTURES AS POTENT SOMATOSTATIN RECEPTOR AGONISTS

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<sup>a</sup>Department of Medicinal Chemistry, <sup>b</sup>Department of Biochemistry and Physiology, Merck & Co., Inc., Rahway, NJ 07065, U.S.A.

At Merck we have explored ligand design for G-protein coupled receptors through peptidyl privileged structures, wherein a dipeptide corresponding to amino acid residues in the proposed substrate binding turn are coupled with aromatic moieties of a type widely found in known ligands for G-protein coupled receptors (privileged structures). This strategy was employed in our search for low molecular weight somatostatin agonists, where we observed that D-TrpLys coupled via a urea linker to 4-aryl piperidines afforded potent somatostatin receptor (SSTR) agonists. This presentation will describe our general strategy, then focus on the broad derivatization of D-TrpLys and closely related dipeptides, illustrating the wide diversity in steric and stereoelectronic characteristics tolerated in the privileged structure moiety while still generating potent agonists. Furthermore, the influence of the privileged structure and linker on the properties of the resulting agonists, such as oral activity and receptor subtype selectivity will be discussed.

#### SYNTHESIS OF $\beta$ -KETO ESTERS DERIVED FROM DIPEPTIDES. SEARCH FOR A LOW-RACEMIZING METHOD

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*Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain.*

As a part of our program directed to lactam-type frameworks as useful templates for the design of peptidomimetics<sup>1</sup>, we were interested in the synthesis of  $\beta$ -keto esters derived from dipeptides as starting materials. The synthesis of these compounds was initially performed by application of the most common methodologies employed for the preparation of amino acid-derived  $\beta$ -keto esters. Thus, while the addition of the lithium enolates of alkyl acetates to imidazolides works very well for amino acid derivatives<sup>2</sup>, in the dipeptide series this reaction afforded the corresponding  $\beta$ -keto esters in low yield. Better results, in terms of yield, were obtained by reaction of dipeptide-derived imidazolides with the magnesium salt of malonic half esters<sup>3</sup>. However, the main limitation of these two methods was the epimerization occurring at the C-terminal amino acid residue. This epimerization, which is dependent on the C-terminal amino acid side chain, can be explained by a combination of two factors: the CDI activation reaction and the chiral instability of the intermediate imidazolides in the reaction media. In order to avoid this undesired epimerization, we decided to investigate other less used methods for the synthesis of amino acid-derived  $\beta$ -keto esters<sup>4,5</sup>. Despite of epimerization due to CDI activation was not avoided, the reaction of dipeptide-derived imidazolides with the potassium salt of malonic half esters, in the presence of  $MgCl_2$ , resulted to be the method of choice for the preparation of  $\beta$ -keto esters derived from dipeptides and, probably, for not urethane-protected amino acid derivatives.

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#### PEPTIDE TRANSPORTER FOR DRUG DELIVERY INTO LIVING CELLS.

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Recent progress in biotechnology and peptide synthetic chemistry have resulted in a large number of different oligonucleotides, proteins and peptides which are of outstanding interest for structural-functional studies in basic molecular biology and may hopefully gain importance as potential therapeutics in future. Unfortunately, non-efficient translocation through the plasma membrane resulting in poor cellular import of biomolecules is the major limiting factor so far. Hence, physiological procedures for the delivery of compounds to cells are urgently needed.

Here we describe the design and the synthesis of a biomolecular shuttle consisting of a cellular membrane transit peptide (pAntp-derivative) and an address protein (SV 40 T antigen) with an additional N-terminal activated cysteine to form intracellularly cleavable disulphide bonds between both molecules. Transport and cellular localization of a chromophore-conjugated random peptide either by the complete shuttle or by single modules were evaluated by confocal laser scanning microscopy and fluorescence correlation spectroscopy (FCS) in living cells.

# Posters: topic C14

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Peptidomimetics

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## COMBINATORIAL BIOMIMETIC CHEMISTRY. PARALLEL SYNTHESIS OF SMALL LIBRARIES OF $\beta$ -HAIRPIN MIMETICS

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(http://www.unizh.ch/oci  
http://www.polyphor.com)

Small molecule peptidomimetics of surface epitopes on proteins may constitute novel lead compounds in the search for inhibitors of protein-protein interactions, or components of conformationally defined synthetic peptide vaccines. Here, an approach is described for the parallel synthesis of combinatorial libraries of conformationally defined  $\beta$ -hairpin protein epitope mimetics. The approach involves transplanting the hairpin loop from the protein to a D-Pro-L-Pro template, to afford cyclic peptidomimetics. NMR and MD studies in aqueous solution show that such mimetics may populate conformations which closely mimic  $\beta$ -hairpin structures found in the crystal structures of native proteins. Using the hairpin as a conformational scaffold, small libraries have been synthesized in which individual residues around the hairpin are varied. The libraries are prepared by parallel synthesis in a two stage solid-phase assembly/solution phase cyclization process. The products have been analyzed by MS, NMR and CD. In one library of hairpin mimetics based on a protruding loop in platelet derived growth factor (PDGF) 2D NOESY spectra revealed for many library members characteristic long range NOEs which show that the hairpin conformation is stably maintained. Some mimetics containing Ala residues adjacent to the template, however, lacked the stable hairpin structure, and showed appreciable *cis-trans* peptide bond isomerism. Upon screening the library for inhibitors of <sup>125</sup>I-labelled PDGF-BB binding to its receptor, mimetics were found with >10 fold higher potency than the wild-type loop. The results suggest that this approach may be developed into a robust method for the synthesis of larger libraries of peptide and protein mimetics based on a  $\beta$ -hairpin scaffold. Such libraries may prove to be a valuable source of novel ligands for chemical biology, and drug and vaccine discovery.

**Reference.** Luyong Jiang, Kerstin Moehle, Michael Pech, Boopathy Dhanapal, Daniel Obrecht and John A. Robinson\*. 1999, submitted.

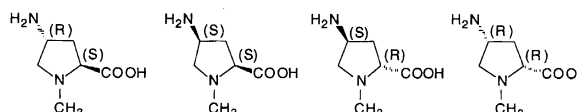
## 4-AMINO-N-METHYL-PROLINE ISOMERS AS THE MIMETICS OF DIPEPTIDE MOIETY WITH CIS-AMIDE BOND

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<sup>2</sup>Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Poland

The cyclic peptide CLX, isolated from linseed, which has a sequence cyclo(PFFILLX)<sup>1</sup>, contains in his structure non-proteinaceous aminoacid 4-amino-1-methyl-proline (X). This amino acid is natural mimetic and can substitute the *cis*-dipeptide moiety in the peptide back bone. Literature data<sup>2</sup> suggest that 4-amino-1-methyl-proline can represent the transition state of a *cis*-amide bond for the *cis/trans* isomerization process catalyzed by peptidyl-prolyl *cis/trans* isomerase (PPIase). Recent investigations proved that CLX possesses the immunosuppressive activity similar to that of cyclosporin A (CsA). 4-amino-1-methyl-proline possesses two asymmetry centres, therefore can occur in four configurational forms :



Using the literature data<sup>3</sup>, a sequence of reactions was elaborated, which enables to obtain all stereoisomers of X. Stereoisomers of 4-hydroxy-proline (2*S*,4*R*) and (2*R*,4*R*) were used as a main substrates. Amine group was introduced via Mitsunobu reaction. All stereoisomers of 4-amino-1-methyl-proline suitable for peptide formation were obtained and used for the synthesis of four CLX analogues, (with (2*S*,4*R*)-, (2*S*,4*S*)-, (2*R*,4*S*)- and (2*R*,4*R*)-4-amino-1-methyl-proline residue), respectively.

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<sup>2</sup> Fisher G. *Angew. Chem. Int. Eng. Ed.* **1994**, 33, 1415-1436

<sup>3</sup> Lowe G., Vilaivan T. *J. Chem. Soc. Perkin Trans.* **1997**, 1, 547-560

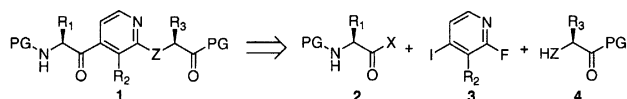
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## PYRIDINE AS A TRIPEPTIDOMIMETIC SCAFFOLD

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Molecular mechanics based conformational analysis and semi-empirical calculations (AM1) of electrostatics has led to our suggestion of pyridine as a tripeptidomimetic scaffold (1). The synthetic strategy was outlined with the aim of providing a general synthetic methodology for any tripeptide to mimic:



R<sub>1</sub>-R<sub>3</sub> = amino acid side-chains  
PG = protecting group  
Z = NH or O  
X = H or N(OMe)Me

The functionalized scaffold (3) is synthesized in two steps starting from 2-fluoropyridine<sup>1</sup>. The attachment of the first amino acid (2) is achieved by a carbonylative coupling between the lithiated scaffold and the aldehyde or Weinreb's amide derivative of the amino acid. The third amino acid (4) is attached to the scaffold by a nucleophilic substitution reaction.

The results from these reactions will be presented.

### References:

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## SYNTHESIS, ANALYSIS AND BIOLOGICAL STUDIES OF HIGHLY HYDROPHOBIC PEPTIDOMIMETICS AGAINST CANCER MULTI DRUG RESISTANCE

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The hydrophobic compounds can abolish the effect of MDR1, that is the multidrug transporter which is responsible for the multidrug resistance (MDR) of the drug resistant cancer cells observed in the chemotherapy of human tumours. The physiological significance of the MDR1 protein is also the extrusion of hydrophobic toxic materials from the cells and formation of an active blood-brain barrier. Almost all of the hydrophobic small peptide derivatives we have synthesised so far contain BOC, Z, OMe, OtBu and/or OBzl groups on their chain termini have different biological effects on the MDR1 ATPase activity when they are administered together with potent anticancer drugs. Because of the extremely high hydrophobicity of the most active peptides e.g. Reversin 121 and 205 as well as most of the cytostatic drugs, our search for new peptidomimetics against drug resistance so far resulted in compounds with very poor solubility in polar solvents which is undesirable both *in vitro* or *in vivo* circumstances. We found an excellent nontoxic soluble carrier molecule with two tertiary amine moieties which did not alter the MDR1-ATPase activity of amino acid derivatives. Our recent results indicate that highly hydrophobic amino acid derivatives dimerized with this soluble carrier resulted in fairly soluble peptidomimetics which specifically act on MDR1 and an other membrane transporter MRP1. We developed reversed phase HPLC and micellar electrokinetic chromatography (MEKC) methods to determine the hydrophobicity of the peptides to find the possible correlation between their hydrophobicity data and their capacity to reverse MDR.

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