## P A69 - Acyl groups as protection for imidazole. Stability studies of N-acylimidazoles and application to solution and solid-phase synthesis of histidine-containing peptides

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We report a novel protection strategy for imidazole functions, which involves hindered aromatic acyl groups. Among a number of acyl groups with increased bulkiness at the carbonyl center, 2,6-dimethylbenzoyl and 2,4,6-trimethylbenzoyl proved to be stable in TFA (from 3 to 95%) in chloroform, in DBU?piperidine mixtures (2%-2%) in DMF, in presence of HOBt, and yet removable in mild ammonolytic conditions. Therefore, these acyl groups are orthogonal with Fmoc and Boc groups, widely used in peptide chemistry, and MMTr and DMTr, employed in oligonucleotide synthesis. Consequently, these acyl groups can be used for the preparation of oligonucleotide-imidazole conjugates and nucleopetides, which are thought to exhibit a variety of biological properties in processes such as membrane permeation and enzymatic activity.
Fmoc-histidine protected with the 2,6-dimethoxybenzoyl group on the imidazole was employed in a synthesis of a dipeptide in solution as well as of a hexapeptide on solid-support with high yields and little racemization of the histidine residue. This protecting group should enable the incorporation of histidine-containing peptides in oligonucleotide structures, either in a stepwise fashion or by post-synthetic conjugation.

## P A70 - Synthesis of conotoxins with simultaneous formation of disulfide bonds.

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Conotoxins are indispensible tools for studying various receptors and ion channels. Synthesis of conotoxins using orthogonal protection groups for cysteine and, as a consequence, the stepwise formation of disulfide bonds does not always give a desirable peptide with a good yield. Earlier, we proposed an approach to the synthesis of conotoxins based on simultaneous formation of disulfide bonds which resulted in the predominant formation of isomers corresponding to the naturally occurring conotoxins [1]. The present work is devoted to the study of different reaction conditions for the disulfide bond closure exemplified with the synthesis of the following conotoxins: AuIB, H-GCCSYPPCFATNPDC-$NH_2$ (disulfide bonds between $Cys^2$/$Cys^8$ and $Cys^3$/$Cys^{15}$) [2] and mr10a, H-NGVCCGYKLCHOC-COOH (disulfide bonds between $Cys^4$/$Cys^{13}$ and $Cys^5$/$Cys^{10}$, O= trans-4-hydroxyproline) [3].
The linear products were synthesized by means of standard Fmoc-chemistry by using Trt protective group for cysteine. The purified peptides were subsequently oxidized by different methods: on air, in the presence potassium ferricyanide , glutathione, by dimethylsulfoxide. Relative yields of isomers were determined by HPLC. As a result, the most suitable method of disulfide bonds formation was found for each conotoxin.

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## P A71 - Peptidomimetics: control of chemical shape and reactivity

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The geometry of a peptide, or protein, defines its biological function. For example, when a bioactive peptide binds to its target enzyme, or receptor, it does so in a well-defined and often extended conformation.[1] Here we present studies on incorporating amino acids and peptides into heterocycles,[2] and other molecular scaffolds, to influence, define and pre-organise their molecular shape, and hence biological function. We also present some new conformationally constrained peptidomimetics that act as enzyme inhibitors and biological probes.[3] The shape of peptidomimetics can, in some cases, be controlled to allow the modulation of activity. For example, we have recently incorporated a molecular switch into some peptidomimetics that is able to change shape on irradiation with light of a defined wavelength.[4] This induced change in shape is transmitted throughout the molecule to give a controlled modulation of chemical reactivity and bio-function. We have also designed and produced aromatic heterocylic-based molecular switches that are activated by other external sources, such as a specific chemical reaction.[5] Non-aromatic heterocycles have also found wide application as a basis of new peptidomimetics and the recent expansion of ring closing metathesis chemistry has opened up new and convenient routes to such compounds.[6] We have employed this method to access a number of new cyclic α- and β-amino acids, examples of which will be presented here. These compounds give rise to important biomarkers and bio-probes some examples of which will be discussed.

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## P A72 - New constrained amino acids for the study of the role of the basic residues in bradykinin antagonists

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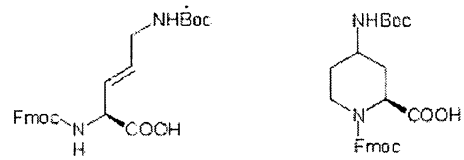
Bradykinin (BK) is a 9-amino acid peptide hormone involved in many physiological processes such as inflammation, contraction of smooth muscle, vasodilatation and transmission of pain. Several antagonists for kinin receptors have been developed. Among these the second-generation peptide HOE140 (H-d-$Arg^1$-$Arg^2$-$Pro^3$-$Hyp^4$-$Gly^5$-$Thi^6$-$Ser^7$-d-$Tic^8$-$Oic^9$-$Arg^{10}$-OH) represents the model for the preparation of highly potent compounds. The basic residues at the *N*- and *C*-terminal positions of BK and of its antagonists play an important role in the binding interactions with the amino acids involved in the B2 receptor.
The *N*-terminal position H-D-$Arg^1$-$Arg^2$, characterized by an amino function and by two guanidino groups, and the *C*- terminal position, with its guanidino and carboxyl functions, are susceptible to a wide range of modifications.To verify the importance of the basic residues in HOE140, we synthesized a series of peptide analogues. The most interesting data were related to the two peptide HOE140 analogues in which $Arg^{10}$ was substituted respectively with Lys or with Orn. The antagonist activities for [$Orn^{10}$]HOE140 ($pA_2$ = 8.9) and [$Lys^{10}$]HOE140 ($pA_2$ = 8.8) were only slightly less potent than for HOE140, while the binding assays (hWI38) displayed surprisingly a greater affinity for the B2 receptor for [$Orn^{10}$]HOE140 ($pK_b$=10.8) compared to HOE140 ($pK_b$=10.6). [$Lys^{10}$]HOE140 showed the same binding affinity of HOE140. These results prompted us to synthesize new constrained ornithine analogues amino acids, Fmoc protected for SPPS to be introduced at the *C*-terminus in HOE140. $N^δ$-Boc-$N^α$-Fmoc-(E)-3,4-didehydro-l-ornithine (**1**) and $N^γ$-Boc-$N^α$-Fmoc-4-amino-l-pipecolic acid (**2**) were synthesized (Fig. 1).




**1** **2**

*Fig. 1 - The β,γ -unsaturated amino acid 1 was chosen because the double bond provides a minimal, but well-defined, conformational constraint [Papini et al., In J. Martinez and J.A. Fehrentz (Eds.), Peptides 2000, EDK, Paris, France, 2001, p. 359-360].The pipecolic derivative 2, obtained by reductive amination of the 4-oxo-pipecolic acid [Brandi et al., Tetrahedron, 2001, 57, 4995-4998], introduces a much higher constraint in the amino acid side chain. The two amino acids 1 and 2 were anchored to a trityl resin and the SPPS strategy of the two new HOE140 analogues was performed in the Advanced&ChemTech batch synthesizer APEX396.*

## P A73 - An improved peptide SPOT synthesis method for CD8 T cell epitope mapping

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The identification of T cell epitopes is a principal step for the understanding of the T cell response against tumors, pathogenic microorganisms and autologous tissues. MHC molecules on the surface of antigen presenting cells (APC) present peptide fragments derived from foreign proteins and display them to lymphocytes. Once a T cell target protein is defined, T cell epitopes can be mapped with synthetic peptide libraries synthesized according to the known sequence of the target protein[1,2]. Although peptides restricted by MHC class I molecules can also be predicted from the relatively strict peptide binding motifs of individual MHC class I molecules, the mapping approach is advantageous since the prediction approach does not detect all CD8 T cell epitopes[3]. The synthesis of large numbers of individual peptides is expedited by applying the SPOT synthesis technique that allows the automated and economic assembly of multiple peptides[4]. The SPOT synthesis methodology is one of the most efficient strategies for the miniaturized synthesis of large numbers of peptides for a variety of screening purposes[5]. The basic principle involves the positionally addressed delivery of small volumes of activated amino acid solutions directly onto a coherent membrane sheet. The resulting droplets can be considered as microreactors and functional groups fixed on the membrane surface react with pipetted reagents. Accordingly, conventional and highly parallel solid phase peptide syntheses can be carried out in a very flexible and economical format. The resulting peptide arrays can either be used for solid phase screening assays performed directly on the membranes or upon cleavage of the spatially addressable peptides for solution based cellular assays[6]. Cleavable, SPOT synthesized peptide libraries have been used for the identification and characterization of CD4 and CD8 T cell epitopes[7,9]. The main disadvantage of the SPOT synthesis technique is that peptides detached from the membrane carrier have an additional linker residue at the C-terminus that could interfere with MHC binding or T cell recognition. Our report describes a modification of the SPOT synthesis method for the generation of peptides with variable C-terminal amino acids and subsequent application of the new technique for the synthesis and testing of several CD8 T cell epitopes with different C-terminal alterations. The evaluation of the antigenicity of four MHC-class I $K^d$-restricted CD8 T cell epitopes derived from *Listeria monocytogenes* showed that the influence of altered C-terminal amino acid has to be considered if synthetic peptide libraries are used for the analysis of CD8 T cell epitopes.

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## P A74 - A general procedure for the preparative scale synthesis of protected C α (ω fictionalized alkyl) amino acids suitable for solid phase synthesis

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In order to increase the diversity of backbone cyclic peptide libraries and cycloscan [1] we introduced a novel method for backbone cyclization called C-backbone cyclization (C-BC). C-BC allows us to form the cyclization from the peptide skeleton, without side chain manipulations. This overcomes one of the capital drawbacks of the classical cyclization. This new method of cyclization enables to increas the diversity of the secondary structure of backbone cyclic peptides. N-backbone cyclization (N-BC) uses "proline-like" building units and thus might brake helical structures. The novel C-BC method relies on "Aib-like" building units that should induce helical secondary structure.C-BC requires a new set of protected $C^{\alpha}$ functionalyzed building units, suitable for solid phase synthesis. We have developed a novel procedure that enables the large scale synthesis of series of C-BC building units (C-BU's) which have the general structure shown in figure 1. The procedure is general, allowing facile synthesis of C-BU's having all the possible lengths of the alkyl chain (n=1-6) and different R groups (Fig. 1). These new amino acid derivatives are suitable for SPS, namely, the different functional groups are protected with orthogonal protecting groups. This procedure also affords gram quantity of BU in each batch. It requires commercially available and cheap starting materials and reagents. The synthesis allows to obtain both enantiomers, and hence, multiple the diversity of C-backbone libraries. As shown in Fig. 2, C-BC comprise of five new modes of cyclization in addition to classical cyclization (4 modes) and N-backbone cyclization (4 modes). We intend to incorporat the C-BU's into a model backbone cyclic peptide and determine their structure and biological activity compared to classical and N-backbone cyclic peptides.

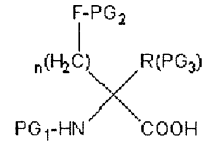


*Fig. 1 - Building units for C-backbone cyclization (R=amino acid side chain; F=functional group (e.g. -NH-; -COO-; -S-); n=1-6; PG1,2,3=orthogonal protecting groups).*

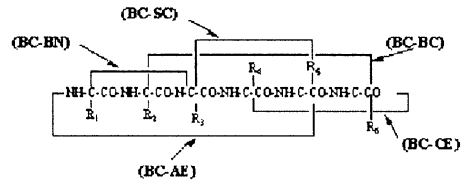


*Fig. 2 - Modes of C-backbone cyclization; BC-BN = backbone C to backbone N; BC-SC = backbone C to side chain; BC-BC = backbone C to backbone C; BC-CE = backbone C to carboxy end; BC-AE = backbone C to amino end.*

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## P A75 - Solid phase synthesis of bioactive fullero-peptides

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The recent progress in the chemistry of fullerene has allowed the use of this allotropic form of carbon as a novel building block in organic synthesis. Among other classes of derivatives, fullerene-based amino acids and peptides are particularly interesting both for structural studies and biological applications [1]. In this context, the solid phase synthesis of peptides containing fullero-amino acids has not been explored and developed properly. This is due in part to synthetic problems, related to the use of [60]fullerene-based amino acids and but also their sensitivity to base and nucleophile treatments. A novel $C_{60}$-functionalized amino acid has been very recently prepared for use in solid phase peptide synthesis [2]. This amino acid was coupled at the N-terminus of a water soluble peptide and the resulting oligomer displayed antimicrobial activity. In this Communication we present the solid phase synthesis and the biological implications of a new fullero-peptide in which the [60]fullerene amino acid is inserted into the peptide sequence. We have chosen to prepare an analogue of the 64-78 peptide derived from the nucleosomal protein, histone H3, in which residue Gln68 has been replaced by the novel fullero-amino acid residue. The parent 64-78 peptide has been recently identified in our laboratory as a dominant T-cell epitope that may be important in the development of the immune response in systemic lupus erythematosus (SLE). Its modification by introduction of non-coded amino acids could allow to obtain new ligands able to restore self-tolerance in the autoimmune response.

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## P A76 - A new racemization mechanism for amino acids

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Mixtures of protein L-amino acids (2.5 mM) were heated at 100°C for 24-96 h together with an excess (278 mM) of glucose (Glc) or fructose (Frc) in aqueous solution of pH 2.5 (AcOH) or pH 7.0 (NaOAc). In the controls saccharides were omitted. The degree of racemization, i.e. formation of D-amino acids from L-amino acids, was determined by chiral capillary gas chromatography on Chirasil-L-Val [1]. On heating at pH 2.5 the saccharides induced racemization of all amino acids whereas at pH 7.0 in particular Asp and Glu underwent racemization. Parts of the amino acids were used up in the course of the reaction. The reaction of reducing sugars with amino acids is know as Maillard reaction[2]. As to the racemization mechanism it is postulated that the reaction of Glc with amino acids begins with the reversible formation of labile Schiff bases and proceeds with the cyclization to the corresponding *N*-glycosyl amino acids that undergo Amadori rearrangement and from relatively stable 1-amino-1-deoxy-2-ketoses (Amadori compounds). It is assumed that D-amino acids are generated and released at various early stages of Maillard reaction. At the advanced stages heterocyclic flavor compounds and brown melanoidins are formed irreversibly with consumption of amino acids. As the early stages of the Maillard reaction proceed already under mild conditions[2] the proposed mechanism provides also a feasible explanation for the nonenzymic generation of D-amino acids in foods and biological systems.

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## P A77 - Synthesis of enantiomerically pure cyclopropane analogs of proteinogenic amino acids using chiral HPLC

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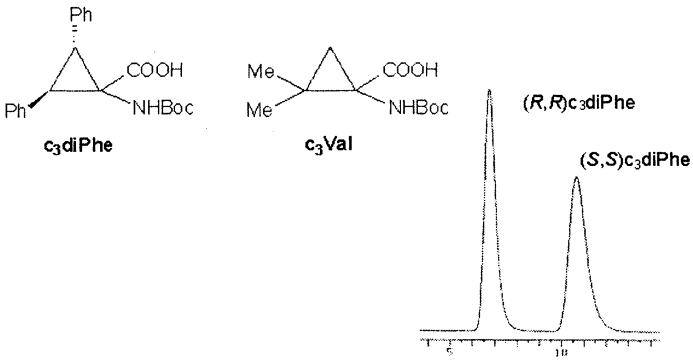
The introduction of α,α-disubstituted amino acids into peptide chains constitutes a powerful means of reducing the conformational freedom of the backbone. Cyclopropane amino acids are particularly attractive since, in addition to α,α-disubstitution, the rigidity of the three-membered ring forces the side substituents to adopt a well-defined orientation with respect to the backbone, an orientation which is different of each stereoisomer. As a consequence, cyclopropane analogues of proteinogenic amino acids are extremely helpful to study the relationship between side chain disposition and backbone conformation. However, progress in this investigation is limited by the availability of enantiomerically pure materials.
We have developed an efficient methodology for the multi-gram scale preparation of two cyclopropane amino acids ($c_3$diPhe and $c_3$Val, analogues of phenylalanine and valine, respectively) in optically pure form and appropriately protected for incorporation into peptides. Starting from readily available substrates and through high-yield transformations, racemic precursors of $c_3$diPhe and $c_3$Val have been prepared and subjected to HPLC resolution on non-commercial polysaccharide-derived chiral stationary phases to afford several grams of enantiomerically pure compounds. It should be mentioned that the high performance of these enantioseparations lies to a great extent on the stability of the HPLC stationary phases used with chlorinated solvents, a distinct advantage over the polysaccharide phases commercially available.




## P A78 - Synthesis and *in vivo* anti-inflammatory activity of long chain 2-amino-alcohols

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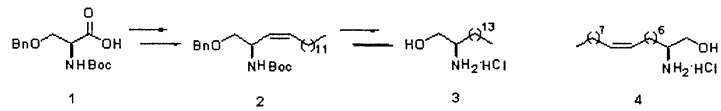
*N*-Protected 2-amino alcohols, which are easily obtained from amino acids [1], are useful chiral intermediates for the synthesis of a variety of compounds, such as á-amino-aldehydes, 1,2- and 1,3-diamines, 2-substituted taurines, etc. Long chain 2-amino alcohols have reported to present interesting *in vitro* cytotoxicity and immunosuppressive activity. Enantiopure long chain 2-amino alcohols may be prepared by reduction of chiral lipidic α-amino acids [2]. We present here a convenient method for the synthesis of saturated long chain 2-amino alcohols starting from serine, the synthesis of 2-amino-oleyl alcohol and the *in vivo* study of their anti-inflammatory activity. Boc-L-Ser(Bn)-OH (**1**) was converted into corresponding alcohol as described by Kokotos [1a]. The alcohol was oxidized to aldehyde by NaClO in the presence of a catalytic amount of acetamido-TEMPO and used directly for the next step. Wittig olefination of the aldehyde with the ylide generated from *n*-tridecene-triphenylphoshonium bromide with KHMDS in toluene at 0° C produced the protected unsaturated alcohol **2**. Catalytic hydrogenation of **2**, followed by removal of Boc group produced (*R*)-2-amino-hexadecanol (**3**). The (*S*)-enantiomer of **3** was prepared from Boc-D-Ser(Bn)-OH. We have recently presented a synthetic route to (*S*)-2-amino-oleic acid [3]. Boc protected 2-amino-oleic acid was converted into its corresponding alcohol and after deprotection to unsaturated alcohol **4**. The *in vivo* activity of (*S*)- and (*R*)-2-amino-hexadecanol and (*S*)-2-amino-oleyl alcohol was studied using the rat carrageenin-induced paw edema assay as a model for acute inflammation. Both enantiomers of 2-amino-hexadecanol exhibited the same anti-inflammatory activity ($ED_{50}$ 0.017 mmol/kg), while (*S*)-2-amino-oleyl alcohol presented even better anti-inflammatory activity ($ED_{50}$ 0.010 mmol/kg).

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## P A79 - Racemization and coupling efficiency studies on novel Cl-HOBt-based coupling reagents

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The key-step in peptide synthesis is the coupling reaction between two amino acids. In this concern the important aspects are the efficiency, in terms of peptidic yield, and the stereoisomeric purity which depend largely upon the coupling reagents chosen to elongate the peptide chain.
In the past years, many new peptide coupling reagents have been designed, synthesized and commonly used in peptide synthesis. Among these reagents, HOBt- and HOAt-based uronium, phosphonium and immonium salts have been proven to be very efficient and are broadly used.
In stepwise solid-phase peptide synthesis (SPPS) the problem of racemization is generally assumed to be less dramatic than for other strategies. Moreover there is the assumption that racemization is unlikely to occur, or that it need not to be examined. However, reports indicate that racemization of internal cysteine and serine can be a serious concern in Fmoc SPPS.[1,2]
Novel Cl-HOBt-based coupling reagents (kindly provided by Luxemburg Industries (Pamol) Ltd., Tel-Aviv, Israel) have been evaluated for coupling efficiency and racemization extent, setting up a model assay based on the solid-phase assembly of the tripeptide H-Gly-Ser-Phe-$NH_2$.

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## P A80 - 3-Aminopiperidin-2-ones as constrained pseudopeptides

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Over the last few years, our group has developed a pool of diversely functionalized 3-aminopiperidin-2-one pseudodipeptides. We have studied their synthesis, their conformational behaviour, and their application as surrogates for diverse dipeptides. For instance, spirolactam 1 (figure 1) was introduced as a surrogate for the Gly-Leu dipeptide to build a constrained model of the C-terminal Gly[9]-Leu[10]-Met[11]-$NH_2$ tripeptide of Substance P. Another example is aminohydroxylactam 2, which has been used as a surrogate for the Ser-Leu dipeptide in the natural cycloheptapeptide Stylostatin 1. The results obtained in these two cases will be presented.

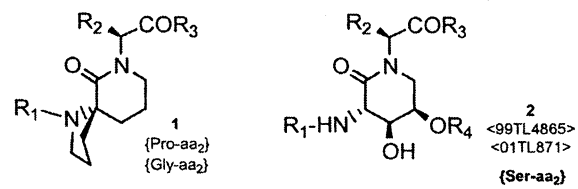


*Figure 1*

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## P A81 - Effect of increasing albumin concentration during extrusion of semolina mixed with glucose

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The present work was designed to explore the changes that might take place in wheat semolina admixed with 5% glucose and increasing amounts of albumin (5, 10, 20 and 30%) during extrusion. The desired mixture was processed in a single screw extruder at the maximum temperature of 140°C and the processing time of 30 second. The results indicated that, the nonenzymatic browning was only moderate, but it was substantially more intensive in mixtures with glucose and albumin than in wheat semolina (control) or its mixture with glucose alone. However, the increase in the added concentrates of albumin by 10, 20 and 30% w/w resulted in gradual increase in the colour intensity. The odour acceptability was affected by the presence of glucose and albumin in different concentrations as almost negligible, but the intensities were different, higher in extruded mixtures with glucose and albumin than in wheat semolina or its mixture with glucose only. The increase in albumin concentration was accomplished by a gradual increase in odour intensity. Differences in the sensory profile were observed especially for fatty and roasted notes. Roasted, burnt, and caramel notes were increased after addition of glucose to the wheat semolina, however marked decrease in such attributes was observed after addition of albumin with different concentrations. In contrast, gradual increase in the fatty, oily and sulphuryl attributes was observed due to albumin addition. Volatiles were extracted by Solid Phase Micro-Extraction using a Carbowax - divinylbenzene fiber, and analyzed by gas chromatography with mass spectrometric detection. Extrusion of semolina with glucose and albumin produced more volatiles (80-85 identified compounds) than in extruded semolina (48 compounds) or its mixture with glucose alone (72 compounds). Pyrazines, furans and pyrans were the most important sensory active compounds identified in all extruded samples. It seems that, pyrazines and furans formation was enhanced quantitatively as well as qualitatively by addition of either glucose or glucose with 5% albumin to wheat semolina. Most pyrazines detected were methyl, ethyl, vinyl and acetyl derivatives. It is obvious that, the addition of albumin over 5% level suppressed the formation of pyrazines and furans; however it increased greatly pyrans generation e.g. maltol and butyrolactone which is in agreement with the sensory evaluation of the extruded products.

## P A82 - Efficient solid phase synthesis of complex fluorescent peptides

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Biological assay systems employing fluorescence-based detection techniques such as fluorescence plate readers, Fluorescent-Activated Cell Sorting (FACS), Fluorescence Correlation Spectroscopy (FCS) or Laser Scanning Microscopy (LSM) require the incorporation of fluorescent tags into either one or several assay components. In addition to measurement modalities using only one single fluorophore, modalities are gaining significance in which molecular interactions, conformational changes or molecular breakdown are derived from a spectrally resolved detection of photons emitted from a fluorophore, spectrally interacting with a second chromophore (Fluorescence Resonance Energy Transfer, FRET) or the temporally and spectrally resolved detection of light emitted from two spectrally distinct fluorophores, as is the case in cross-correlation Fluorescence Correlation Spectroscopy (FCS). Herein we introduce new protocols that enable the routine solid phase synthesis of fluorescent peptides, carrying one or more fluorophores. Our efforts were focussed on the implementation of orthogonal labelling strategies employing carboxy-fluorescein as a tag for labelling the N-terminus or lysine side chain e-amino-groups in solid phase peptide synthesis. For many applications, this fluorophore may still be considered a "dye-of-choice" due to its low tendency to aggregate and to interfere with the biological function of labelled molecules as well as its low price. Instead of using preactivated 5(6)-carboxy-fluorescein such as the isothiocyanate or the N-succinimidylester, the free carboxylic acids were used and activated in situ affording the use of a molar excess of this compound for a complete turn-over of the reactive groups. Resin-bound carboxy-fluorescein, however, engages in a number of side reactions in the labelling reaction itself as well as during further modifications of the labelled peptide on the resin. These side reactions include e.g. the formation of esters with further activated carboxylic acids. Other side products derive from the treatment of carboxy-fluorescein with hyrdrazine, required for orthogonal side chain deprotection or from treatment with alkylating reagents, required for the activation of a "Safety-Catch" Linker.
To circumvent these problems a number of different protecting group strategies were developed for carboxy-fluorescein. The Boc/Trt protecting groups which are introduced on resin enable the efficient generation of fluorescein-labelled thioester building blocks for native chemical ligation and of peptides labelled with two different fluorophores.

## P A83 - Serendipitous discovery of a reaction leading to stable peptide dialkyl peroxides

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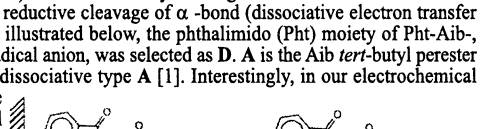
In the last few years the study of intramolecular electron transfer (ET) reactions in donor(**D**)-spacer-acceptor(**A**) molecules has provided relevant information on how electrons are transferred through bonds and space (solvent). We have recently investigated intramolecular ETs within systems in which **A** undergoes reductive cleavage of α -bond (dissociative electron transfer or DET). More specifically, as illustrated below, the phthalimido (Pht) moiety of Pht-Aib-, which is easily reduced to its radical anion, was selected as **D**. **A** is the Aib *tert*-butyl perester function, a well-characterized dissociative type **A** [1]. Interestingly, in our electrochemical study we found that the DET rate of Pht-Aib-OO*t*Bu is several orders of magnitude lower than the adiabatic limit.



In the second step of our investigation we decided to focus on the distance dependence of intramolecular DET and the exploitation of rigid, $3_{10}$-helical peptide spacers to control the electronic interaction between the **D** and **A** redox sites. Accordingly, we planned the synthesis of the homologous peptide perester series Pht-$(Aib)_n$-OO*t*Bu (**1**, $n$ = 2-5, 8). Unexpectedly, upon treatment of the 5(4*H*)-oxazolones from Pht-$(Aib)_n$-OH ($n$ = 2-5, 8) with 4-(dimethylamino)pyridine and *tert*-butyl hydroperoxide in methylene chloride under reflux, the compounds isolated proved not to be the expected peresters **1**, but rather the peptide dialkyl peroxides (**2**) ($n$ = 1-4, 7). These new peptide derivatives were characterized by IR absorption, $^1$H and $^{13}$C NMR, mass spectrometry, X-ray diffraction, and cyclic voltammetry. A mechanistic study of this novel reaction, by use of Aib- and L-(αMe)Val-based peptide spacers, implies perester formation, followed by loss of tert-butyl alkoxide and $CO_2$, and subsequent alkylation of the planar, iminium intermediate, eventually leading to the dialkyl peroxide.

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## P A84 - Synthesis and structural investigations of an α-hydroxylated β-peptide

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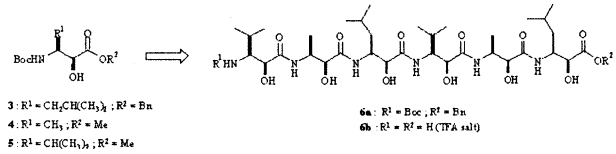
Over the last decade considerable attention has been directed toward the synthesis of β-peptides since it has been demonstrated that they can fold in stable secondary structures [1] similar to those of α-peptides and also because of their biological stability [2]. The introduction of substituents such as OH, capable of forming H-bonds, directly on the peptidic backbone, may result in changes of the secondary structure, as compared to the known helices, sheets and turns. On continuation of our investigations in the field of β-peptides, we have therefore synthesized the α-hydroxylated β-hexapeptide **6**, in order to be able to compare its secondary structure with that of the known $\beta^3$-hexapeptide **1** [3] and $\beta^{2,3}$-hexapeptide **2** [4] which form $3_{14}$ helices in MeOH solution [cf. 5,6]. We will report on the synthesis of α-hydroxylated $\beta^3$-HVal-, $\beta^3$-HAla- and $\beta^3$-HLeu-amino-acid derivatives **3**-**5** which we used as building blocks for the synthesis of the novel β-hexapeptides **6**. Furthermore, we describe investigations aimed at the elucidation of the structure of these β-peptides.



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## P A85 - Sulfonamido β-peptides. Synthesis and activity of For-Met-Leu-Phe-OMe analogues containing β-substituted taurine residues

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Several specific substances termed chemotactic factors stimulate the migrations of phagocytic cells (e.g. human neutrophils) against invading microorganisms. The tripeptide For-Met-Leu-Phe-OMe is among the most potent and structurally simple of these agents; its action on neutrophils is mediated by the interaction with specific membrane receptors and includes, in addition to the directed migration, other relevant biochemical events such as release of lysosomal enzymes and superoxide anion production. Thus, the activity of neutrophils, stimulated by the interaction with chemoattractant factors, is crucial in the defence system against bacterial infections [1].
The fMLF is the most extensively studied member of the family of the chemotactic peptides and represents the prototypical ligand for studying formylpeptide receptors. Several synthetic analogues of this tripeptide have been designed and more potent and selective ligands have been obtained [2].
As a part of a research program aimed at studying β-peptides containing the $SO_2NH$ junction in place of the usual CONH [3], we report here synthesis and bioactivity of the first fMLF-OMe analogues **1a,b** and **2a,b** obtained by adopting this bioisosteric modification.
The new ligands have been fully characterized and examined for their agonistic and antagonistic activity towards human neutrophils.
First results indicate that replacement at the *N*-terminal position is not compatible with the activity whereas modification at the central position leads to a new ligand which maintains the activity with improvement of the selectivity.

**1)** R-Met-Leu-ψ($CH_2SO_2$)-Phe-OMe **2)** R-Nle-ψ($CH_2SO_2$)-Phe-OMe
**a)** R = Boc, **b)** R = For

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## P A86 - Bivalent transition metal complexes of pseudopeptides useful for the hydrolysis of phosphoric acid esters

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To investigate the enzymatic mechanism in the active site of metalloenzymes it is essential to study the mode of interaction between metal atoms and the side chains of amino acids. Therefore we have synthesized small peptides with optimized side chains for the complexation of transition metal ions. Recently, we could demonstrate that the **His-N($CH_2$-$CH_2$-$NH_2$)Gly-His** moiety, designed as an model peptide of the catalytic center of the carboanhydrase, has a similar hydrolytic activity towards phosphoric acid esters like the well known 1,4,7,10-tetraazacyclododecane (cyclen) zinc complexes [1,2]. Because it is known that complexes with two metal atoms have a higher hydrolytic activity than those with only one, we have now synthesized new peptide ligands with His-N($CH_2$-$CH_2$-$NH_2$)Gly-His units containing two metal binding sites. To investigate the ability of these peptide ligands to form homo nuclear as well as hetero nuclear metal complexes we have used 2 types of ligands: (I) symmetric peptides with two His-Aaa-His motives and (II) asymmetric ligands containing a cyclen subunit (II).

**I** **Bz-His-N($CH_2$-$CH_2$-$NH_2$)Gly-His—linker—His-N($CH_2$-$CH_2$-$NH_2$)Gly-His-$NH_2$**
**II** **1N-(carboxymethyl)—linker—His-N($CH_2$-$CH_2$-$NH_2$)Gly-His-$NH_2$**
**1,4,7,10-tetraazacyclododecane**

linker: Pro, Pro-Pro, ε-aminohexanoic acid

The synthesis of the ligands was performed by the Fmoc SPPS on Rink amide resin. The incorporation of the N-alkyl chains (cylen, aminoethyl) into the backbone was carried out by N-alkylation of bromoacetic acid residues on the solid support or via synthesis of the corresponding N-alkyl building units in solution. The complexation tendency of the ligands for bivalent transition metal ions, like $Zn^{2+}$, $Cu^{2+}$, $Co^{2+}$ and $Ni^{2+}$ was investigated by circular dichroism spectroscopy, capillary electrophoresis and mass spectrometry and was compared with the complexation behaviour of the monomer ligand **Bz-His-N($CH_2$-$CH_2$-$NH_2$)Gly-His-$NH_2$** with only one metal binding site. The different hydolytic activities of the metal complexes towards phosphoric acid esters will be discussed.

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## P A87 - Application of non-proteinogenic amino acids

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Non-proteinogenic amino acids with heterocyclic and aromatic structures are of potential interest for the design of peptidomimetics. They have been used as non-natural building blocks for the synthesis of HIV- [1] and Matrix Metallo Protease Inhibitors [2] as well as for inhibition of proliferation states in cell cycles [3]. Nevertheless, little has been published about the experimental details in their application.

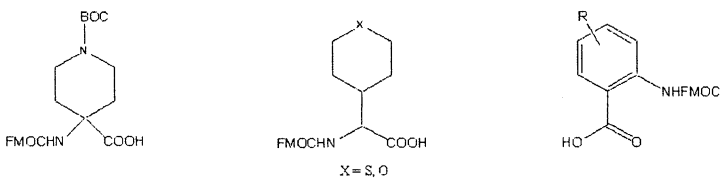


Fig. 1

In this poster, we compare different activation procedures as well as different coupling methods leading to optimized coupling protocols. In addition, we describe the application of the (orthogonal) protected non-proteinogenic building blocks by incorporation into short peptide sequences by SPPS. Finally, we will discuss the advantages/disadvantages of FMOC-protected anthranilic acid derivatives.

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## P A88 - Diastereoselective hydroxylation of piperidin-2-ones. An expedient synthesis of (2S,5R)-5-hydroxylysine

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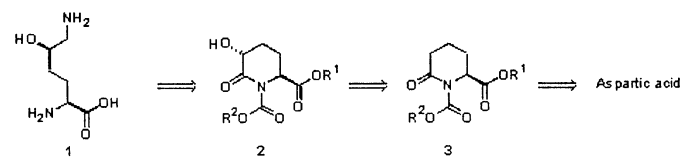
(2*S*,5*R*)-5-Hydroxylysine is one of the amino acids unique to collagen and collagen-like proteins. It is formed by post-translational hydroxylation of lysine and can be glycosylated with either a β-D-galactopyrannosyl- or an α-D-glucopyrannosyl-(1→2)-β-D-galactopyrannosyl- residue. Although (2*S*,5*R*)-5-hydroxylysine is commercially available it is particularly expensive and it requires lengthy procedure for protection prior to use in peptide synthesis. The few approaches[1,4] recently investigated suffer from limitations including lengthy synthetic procedures and/or separation of diastereoisomers. As part of a program dedicated to the study of glycopeptides T cell epitopes derived from collagen II, we developed an expedient stereoselective route to (2*S*,5*R*)-5-hydroxylysine. It is based on asymmetric oxidation of enolates generated from *N*-protected-6-substituted piperidin-2-ones **3** to the corresponding α-hydroxy carbonyl compounds **2**. The piperidin-2-ones are prepared from aspartic acid as an inexpensive chiral adduct;[5] the α-stereogenic center serving for asymmetric induction at C-5. The complete study of the hydroxylation step allowed us to obtain **2** with excellent yields and high d.e.




The high level of 1,4-asymmetric induction achieved during hydroxylation is a consequence of the allylic A(1,3)[6] strain that force the ring substituent at the C-5 position to adopt an axial conformation. The 1,4-asymmetric induction is confirmed by the X-ray crystal structures obtained for the compound **2a** ($R^1$=$R^2$=$^t$Bu) and the corresponding piperidin-2-one **3a**.

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## P A89 - Synthesis of the first highly potent inhibitor of the three vasopeptidases: endothelin converting enzyme (ECE), angiotensin converting enzyme (ACE) and neprilysin (NEP).

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Fluid homeostasis and blood pressure are regulated by two antagonist peptidergic systems. Angiotensin II and endothelin are two powerfull vasoconstrictor peptides, which are processed respectively by angiotensin converting enzyme (ACE, EC 3.4.15.1) and endothelin converting enzyme (ECE, EC 3.4.24.71). Bradykinin (Bk) is a vasorelaxant peptide inactivated by neutral endopeptidase (NEP, EC 3.4.24.11, neprilysin) and ACE. The atrial natriuretic peptide (APN), which induces diuresis and natriuresis is inactivated by NEP. Therefore, inhibition of these three enzymes with a single inhibitor could be of therapeutic interest in the treatment of cardiovascular diseases[1,2]. The joint inhibition of these enzymes is an exciting challenge requesting the design of a molecule able to recognize efficiently at least the S'1 subsite of the three peptidases. Futhermore this inhibitor should be nearly equipotent on the three targetted enzymes. With this goal we have synthesized various mercaptoacyl amino acids of general formula HS-CH2-CH(R'1)CO-Trp-OH possessing more or less aromatic constrained R'$_1$ side chains in order to test the occupation of the S'$_1$ subsite. The mercaptoacyl synthons contain one or two asymetric centers leading to the corresponding inhibitors as mixtures of two or four diastereoisomers. In a first rapid screening, the synthetized compounds were tested without separation for their ability to inhibit ECE-1, NEP and ACE activities in vitro. Four compounds with $10^{-9}$, $10^{-8}$ and $10^{-7}$ M affinities on NEP, ACE and ECE respectively emerged from this study. The separation of the four stereoisomers was performed using semipreparative HPLC or resolution by chiral amine allowing the determination of the inhibitory potency of each stereoisomer. Their absolute configuration was determined using NMR and x-ray cristallography allowing the stereochemical preference of each enzyme to be determined. A guideline for the joint inhibition of the three peptidases was obtained with compound 1. Even though these peptidases differ in their substrate specificity, the P'$_1$ side chain of the inhibitor could adopt the different conformations required for optimal recognition of the S'$_1$ binding site of each targeted enzyme. However

| | | Ki (nM) | | |
|---|---|---|---|---|
| Cmpds | Conf. | NEP | ACE | ECE |
| 1 | Racemic | 1.8 ± 0.2 | 20 ± 2 | 104 ± 8 |
| 1a | 2S 3S | 10 ± 1 | 41 ± 3 | 1140 ± 60 |
| 1b | 2R 3R | 0.7 ± 0.3 | 43 ± 2 | 26 ± 3 |
| 1c | 2S 3R | 2.1 ± 0.1 | 10 ± 1 | 290 ± 20 |
| 1d | 2R 3S | 27 ± 1 | 70 ± 1 | 680 ± 9 |

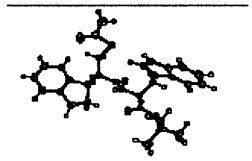

Fig. 1 - Ortep view of compound 1c

compound 1 remains to be optimized essentially for ECE inhibition. This has been achieved by introduction of various substituents on the indanyl moiety of the P'$_1$ residue, leading to the first highly potent triple inhibitor of NEP, ACE and ECE[3]. In conclusion, new potent triple inhibitors of NEP, ACE and ECE generated after optimization of the lead compound 1 fulfill the requirements to strongly reduce blood pressure. Pharmacological studies have demonstrated that the three targeted enzymes are inhibited.

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## P A90 - Synthesis and theoretical studies of cage amino acids in non-natural peptides

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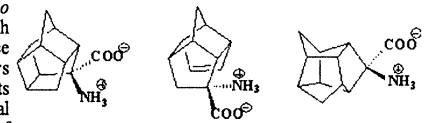
The synthesis of the novel (R)-(-)-8-amino-pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$] undecane-8-carboxylic acid 1 (PCU cage α-amino acid) and two analogous (2 and 3) will be presented. The effect of the rigid "cage" amino acid on the secondary structures of peptides could be useful in the design of the receptor site specificity of target peptides. An exploration of the conformational space of this molecule by means of computational methods at the molecular mechanics and quantum mechanical levels will be discussed.
For this purpose, the amino acid residues, glycine (Gly), alanine (Ala), acetylisobutyric acid (Aib) and proline (Pro) were selected due to their varied conformational characteristics. Consequently, the conformational preferences of the PCU cage peptides with the N-terminal and C-terminal blocking groups of the following series of peptides, Ac-X-NHMe (where X= Gly, Ala, Aib and PCU cage); Ac-X-Y-NHMe (where X=Ala, PCU Cage, Pro and Y=Ala, PCU cage); Ac-X-Y-Z-NHMe (where X, Y and Z=Ala, PCU cage) and Ac-3X-Y-3Z-NHMe (where X, Y and Z=Ala, Aib and PCU cage) were studied using the AMBER force fields. The validity of the AMBER force field parameters used in the characterization of the PCU cage structure was determined by a conformational analysis at the *ab initio* level (HF/6-31G*) and compared with those computed using the AMBER force fields. The low-energy conformers characterized by the Ramachandran plots of the (ϕ,ψ) backbone torsion angles, reveal the helical-conformational preferences of the PCU cage dipeptide. The



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conformational space of the series of peptides, Ac-X-Y-NHMe, Ac-X-Y-Z-NHMe and Ac-3X-Y-3Z-NHMe was extensive explored using molecular dynamics (MD) and iterative simulated annealing (SA) was performed (AMBER). Initial results indicate that the PCU cage residue restricts the conformational freedom considerably in comparison with the other conformationally hindered residues used. Furthermore, no significant differences in the results were observed using both computational techniques. In all cases the low energy conformers have a tendency to form helical, β-turn and bent structures, which are desired as lead compounds in the development of new drugs. Since the PCU cage amino acid displays a tendency to limit the conformational freedom of peptides, the results of this investigation suggest that it has the potential to be incorporated in the design of constrained cyclic peptide analogues, with a view to improving the structure-function relationship in biologically active compounds. Lastly our first attempts to incorporate these Cage amino acids into non-natural peptides using Solid Phase Peptide Synthesis will be presented.

## P A91 - Computational study on conformation of oligopeptides containing chiral α,α-disubstituted amino acids

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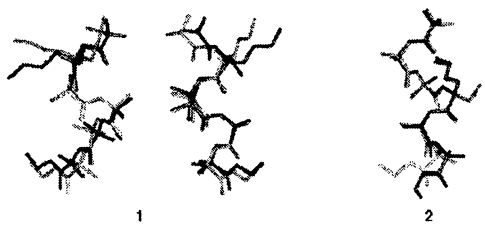
Prediction of the conformation of peptides using computational methods presents an interesting challenge for the design of functionalized molecules. α,α-Disubstituted amino acids have two alkyl substituents at the α-position of α-amino acids and are conformationally restricted. Peptides comprising α,α-disubstituted amino acids are currently attracting attention mainly because of their stable secondary structures. Recently, we have shown[1,2] computational simulation was in agreement with their conformational properties in the solid state determined by X-ray crystallographic analysis of oligopeptides. Here we report conformational analysis of oligopeptides containing chiral α, α-disubstituted amino acids using conformational search calculations by *MacroModel* and semi-empirical molecular orbital calculation by SPARTAN. Conformational energy computations on heterooligopeptides constituted by Aib and (*S*)-butylethylglycine (Beg) were performed using molecular mechanics. Conformational search calculations were carried out by the Monte Carlo method of *MacroModel* (ver. 6.5, Schrodinger, Inc.) on SGI $O_2$ workstation. When AMBER* was used as the force field, the global minimum energy conformations were found to be a right-handed $3_{10}$-helix. In the case of **2**, the right-handed helix was more stable than the left-handed one. The difference in energies is 2.3 kcal/mol. But there is a small difference in energies between the right-handed helix and the left-handed one in the case of **1**. These results are in agreement with their conformational properties in the solid state determined by X-ray crystallographic analysis of peptides.



1 2

**Figure 1**. Global minimum energy conformations (light) of peptides superimposed on structures (dark) determined by X-ray crystallographic analysis (**1**: $CF_3CO$-((*S*)-Beg)-$(Aib)_4$-OEt **2**: $CF_3CO$-$(Aib)_2$-((*S*)-Beg)-$(Aib)_2$-OEt )

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## P A92 - "SLAM", alkoxyamine/maleimide water soluble heterobifunctionnal crosslinker, application and derivates

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We are involved in the development of an immunoconjugate between HIV recombinant gp160 and Alkaline Phosphatase as a revelation enzyme. A periodate oxidation leads to an aldehyde activated form of gp160 and the enzyme is functionalized in its free thiol(s) derivate using Traut's iminothiolane reagent.
In order to conjugate specifically the two preactivated proteins, we needed a bifunctionnal hydrazide (or alkoxyamine) / maleimide (or alkyl halide) linker. So, we have tried various commercial crosslinking reagents, including Pierce's EMCH. But due to our need for long spacing chain as well as better water solubility , we have synthesized a linker based on the use of alkoxyamine and maleimide groups [1,2]. The reactive groups of the clinker are separated by several polyethyleneglycol-like aminoacids, namely: 1-amino-3,6-dioxa-octanoyl residues or Ado [3]. We used N-maleoyl-ß-alanyl as the maleimide function and Nß-aminooxyacetyl-diaminopropionyl or Dpr(Aoa) residue to bring alkoxyamine group.

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## P A93 - New diamino acids derivatives containing anthraquinone moiety

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1. University of Gdañsk, Faculty of Chemistry - Poland

Oxidation and reduction occurring in living organisms via an appropriate enzyme action are quite complex processes. To find simpler and effective model for studies of the redox reaction we obtained some lysine homologues containing anthraquinone moiety (Aqn) on ω-amino group:
X-NH-CH[(CH2)nNH-Aqn]-COOH , where: X = H, Boc; n = 1 ÷ 4.

The compounds were prepared by reaction between appropriate Nε-protected lysine homologues and 1-fluoroanthraquinone. Boc-protected amino acid derivatives have been prepared in order of their further use for peptide synthesis by SPPS methodology. Optimisation of the synthesis, based on "low and high dilution procedures", allowed to improve yield of the preparation. Structures of the obtained compounds were confirmed by means of 1H-NMR, MS and IR. The compounds were subjected to potentiometric and complexation studies in order to find their redox properties and stability constants of their metal complexes, respectively. Additionally, we recorded their absorption spectra in different solvents, since these compounds could serve as effective acceptors of energy.
It was found that there were no significant changes in the first reduction potential value (E1) of anthraquinone group in the series of studied compounds in comparison to the model compound (ethylaminoanthraquinone). On the other hand, we found that the second reduction potential value (E2) of Aqn group depended on a length of amino acid side chain. Discussion of an influence of α-amino and α-carboxyl group on physicochemical properties of anthraquinone moiety will be presented.

Acknowledgements: Work supported by Polish Scientific Research Committee (KBN).

## P A94 - A practical synthesis of α,α-disubstituted α-amino acids with a cyclic six-membered side chain

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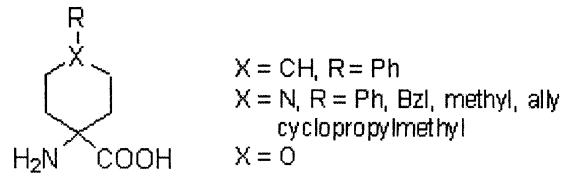
Conformationally constrained amino acids can provide valuable tools in developing highly useful peptide ligands with specific structural features. α,α–Disubstituted amino acids are among the most important constrained amino acids because they have strong tendency to induce secondary structures such as α-helix, β-turn and γ–turn. We have developed practical methods suitable for large scale synthesis of α,α–disubstituted amino acids with a cyclic six-membered side chain; 1-aminocyclohexane-1-carboxylic acids (X = CH, $Ac_6c$), 4-aminopiperidine-4-carboxylic acids (X = N, Apc) and 4-aminotetrahydropyran-4-carboxylic acids (X = O, Atpc). For synthetic simplicity and efficiency we chose a route via hydantoin formation. The corresponding ketones were prepared using various methods and converted to hydantoins by Bucherer-Berg reaction. Diastereomers of cyclohexyl-spiro-5'-hydantoin were separated very efficiently by fractional crystallization. Hydrolysis of hydantoins in severe or mild condition gave desired products. Detailed synthetic procedures and analytical data of intermediates as well as final products will be presented.




## P A95 - Solid phase synthesis of peptidic aspartyl aldehydes

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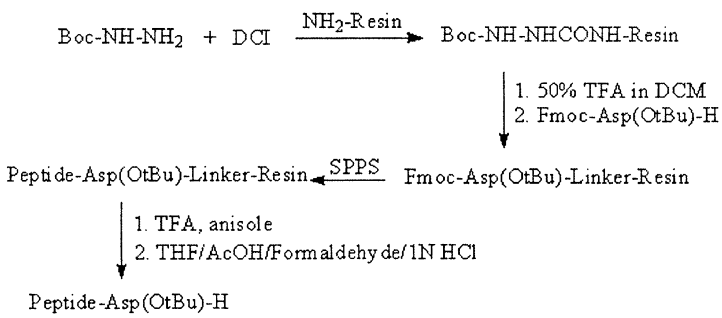
Aspartyl aldehydes have been shown to be potent, reversible inhibitor of interleukin-1 converting enzyme (ICE). [1] In the literature [2], a number of general strategies for peptide C-terminal aldehyde synthesis have been described. As part of our on-going work on the combinatorial synthesis of ICE inhibitors, we are interested in developing a general method for automated synthesis of aspartyl aldehydes. After some preliminary investigation, we decided to use semicarbazone as the protecting group [3] and linker for attaching FmocAsp(OtBu)-H onto resin. The peptide were assembled on aminomethyl resin via Fmoc chemistry. All the protecting groups were removed by TFA treatment before the peptide was cleaved from resin by a mixture of THF/AcOH/Formaldehyde/1N HCl, as shown below.

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## P A96 - Synthesis of peptides derived from $C^{\alpha}$-hydroxymethylserine (HmS): increased risk of side reactions.

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$C^{\alpha}$-Hydroxymethylserine HmS (Figure 1), a hydrophilic analogue of $C^{\alpha}$-methylalanine (MeA or Aib) remains neglected by peptide chemists, despite its potential as an interesting peptide building block. We reported methods for peptide bond formation with this sterically hindered amino acid as the carboxyl or amino component and also for the synthesis of homosequences [1]. The most successful methodology is based on the use of the intermediate O,O-protected HmS in form of an isopropylidene derivative HmS(Ipr), (Figure 1), however the increased danger of side reactions is a serious concern.

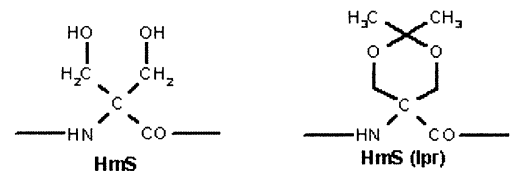


*Figure 1. $C^{\alpha}$-Hydroxymethylserine and O,O-Isopropylidene-$C^{\alpha}$-Hydroxymethylserine.*

To illustrate the problem we give representative examples. Exposure of HmS(Ipr) peptides to acidic conditions, e.g. 95 % aq. TFA used for peptide cleavage from Wang resin causes excessive NO acyl shift leading to depsipeptide side products. The reagent HATU proves the highest efficiency in the joining of HmS(Ipr) residues, however the use of HATU in 2 + HmS(Ipr) condensation results in 45% epimerization of acylating peptide. Spontaneous cyclization to diketopiperazine occurs during Fmoc-removal from dipeptides with C-terminal HmS(Ipr) or HmS residue thus precluding their elongation. Easy formation of hydantoin affects hydrolytic deprotection of dipeptide methyl esters derived from Z-HmS(Ipr). Further examples will be presented and the ways avoiding or minimizing these side reactions will be discussed.

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## P A97 - Incorporation of β-amino acids in bioactive peptides: a β-casomorphine case study

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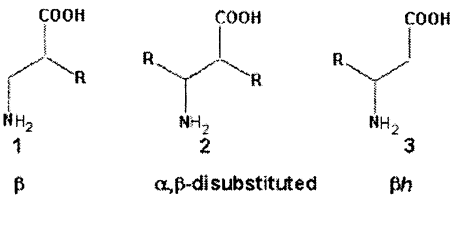
β-amino acids have opened new pathways for the design and the synthesis of peptidic drugs, as described in recent literature[1]. The insertions of these homologated residues in bioactive peptides produces significant stability towards protease, and in some case, this result is accomplished by an increase of biological activity [2]. These two proprieties, together, represent a promising therapeutics application for mixed α/β-peptides derivatives. The initial structural study also revealed the presence of a well-defined secondary structure, like turns and helix in β-peptides[3,4]. However, no data regarding the conformational preferences occurring in bioactive mixed α/β-peptides are available today, and in particular on those containing β-amino acids **1, 2, 3** and the proteinogenic α-amino acids:




If we consider the proteinogenic side chain R only, we could get 1520 possible β-amino acids. Fmoc-β-*homo* amino acids **3** are prepared on a lab-scale by direct homologation of α-amino acids[5], as an alternative to the diazomethane consuming Arndt-Eister procedure, and are introduced into peptides backbone using the normal solid phase synthesis protocol. Mixed α/β-peptides can be rapidly assembled using uronium salts as activating agents. The application reported here concerns the synthesis and the opioid receptors affinities observed in a set of β-Casomorphin, - YPFPGPI - analogues, a bioactive fragment derived from bovine β-casein. Details on the synthetic procedure used will be illustrated, focusing on a synthetic methodology for the preparation of homologues of α-amino acids as **1** in racemic form, and **2** and **3** as pure enantiomers.

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## P A98 - Solid phase synthesis of thiomethylenpseudopeptides

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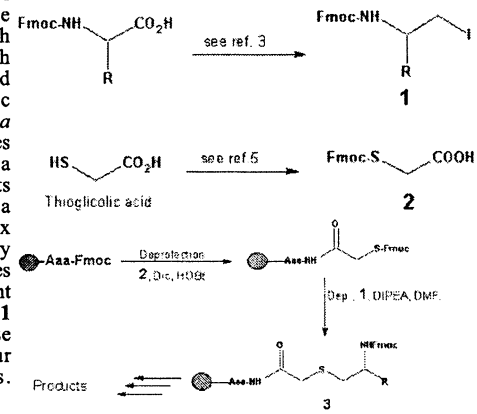
Thiomethylene amide bond replacement [-CONH- → -$CH_2$S-] confers to peptide backbone more flexibility and reduces the polarity respect to natural peptides [1]. The pseudopeptides polarity can be easily modulated by smooth sulphur oxidation [2]. We have now developed a new protocol for solid phase assembly of thiomethylenpseudopeptides, using - as "amine components"- **1**, the enantiopure *N*-Fmoc-β-Aminoiodides [3], usefully intermediates in the homologation of α-amino acids [4]. As acid component we used Fmoc-S-$CH_2CO_2H$ **2**, obtained - as recently reported for cysteine [5] - by simple protection of thiol function of thioglicolic acid with Fmoc-Cl in presence of DIPEA: The solid phase synthesis was performed with a peptide coupling of **2** with a deprotected α-amino acid linked to Wang resin. Fmoc removal and "coupling" - *via* $SN_2$ reaction - with **1** produces the formation of a thiomethylene bond. Results will be presented showing a combinatorial approach (mix and split method) to a library of thiomethylenpseudopeptides obtained using four different *N*-Fmoc-β-Aminoiodides **1** and a successive solid phase alkylation of **3** with four different alkyl halides.

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## P A99 - Novel approaches for synthesizing pyrrole amino acids for peptide mimicry

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Constituents of cytotoxic drugs, such as netropsin and distamycin, 4-aminopyrrole 2-carboxylates 1 have served as principle components for constructing a diverse series of DNA-binding ligands exhibiting antibiotic, antiviral and oncolytic properties. Heteroatomic variants of constrained arylglycines, prolines 2 are novel amino acids that offer potential for restraining both peptide back-bone and side-chain geometry. With the interest in employing pyrrole amino acids 1 and 2 in different approaches for combinatorial library synthesis and peptide mimicry, we have recently developed novel entries into these heterocyclic amino acid systems. Our presentation will illustrate the syntheses of 1 and 2 as well as describe their potential applications in peptide science.

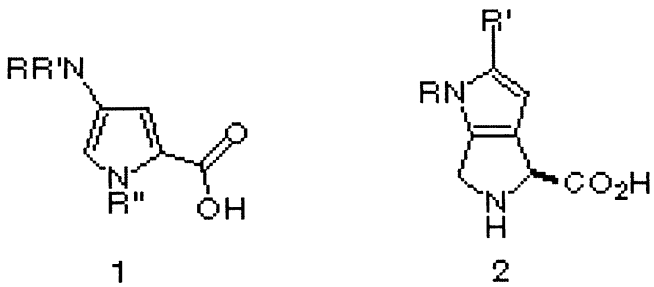


*Figure 1.*

## P A100 - Arginine containing β-peptides and derivatives thereof

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Arginine residues are present in various proteins as well as in natural substrates and synthetic inhibitors. The guanidino group of arginine is capable of forming strong ionic interactions with the carboxylate of aspartic or glutamic acid residues, salt-bridges, stabilizing, albeit to a smaller extent, the protein structure. Such interactions also help the binding of substrates or inhibitors to proteins.
In our continuing effort on examining the stability of the $3_{14}$ helix of β-peptides we have now investigated the effect of salt-bridge formation by the arginine-glutamic acid side chains. We have synthesized the heptapeptide H-(*R*)-β $^3$-HVal-(*S*)-β $^3$-HGlu-(*S*)-β $^3$-HArg-(*R*)-β $^3$-HVal-(*S*)-β $^3$-HArg-(*S*)-β $^3$-HGlu-(*R*)-β $^3$-HVal-OH by standard solid phase methods on a Rink amide resin. NMR and CD spectroscopic investigations of this peptide are in progress.
The stabiliy of β-peptides against proteolytic degradation *in vitro* and *in vivo* has attracted many to develop peptidomimetics. Introduction of β-peptidic spacers in a natural ligand for HLA-B*2705 enhanced the binding of the altered peptide ligands [1]. The arginine residue at P2 is one of the key players in binding of these peptides. Taking a step further, in analogy with the modified ligands and with the help of molecular modelling studies, we designed some β-peptidic "ligands", all containing an arginine residue. These β-peptides with variations in the substitution pattern, in the central part, have now been synthesized. The testing of these peptides for their binding affinities to HLA-B27 is in progress. Also, β-HArg oligomers have been shown to penetrate through cell membrane within 5 min, at 1μM concentration [2].

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# A2 - Chemistry of amino acids, peptides and pseudopeptides

## P A101 - Solid phase synthesis of new heterogenic nucleopeptides based on an ornithine backbone.

**V. A. Mandrugin**[1], N. V. Sumbatyan[1], K. Hyun[1], G. A. Korshunova[2]

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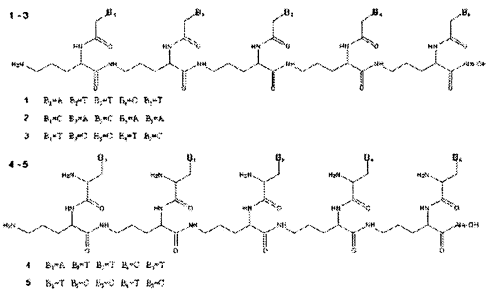
2. Belozersky Institute of Physico-Chemical Biology of Moscow State University - Russian Federation

As a part of our studies on preparation of nucleopeptides and their use for inhibition of oncogene expression and retrovirus proliferation, we report the synthesis of five different structure nucleopeptides (1-5) complementary to the env AUG codon mRNA region of the Friend murine leukemia virus and the GFP mRNA region. The nucleopeptides (NPs) are structural analogues of oligonucleotides in which phosphodiester backbone is substituted by a peptide chain. NPs were desined on the base of d-ornithine peptides where adenine, thymine and cytosine are linked to an a-amino function of ornithine residues. Nucleopeptides were prepared of 5 nucleic bases. NPs were synthesized by solid phase procedure on the hydroxyethyl photolinker Nova Syn TG or Merrifield resins. The use of photolinker allows both base and acid labile protecting groups to be removed during peptide synthesis as well as to keep the protecting groups after the cleavage of peptide from the resin. In the NPs synthesized nucleic bases are coupled with ornithine residues via acylation of their a-amino groups with pyrimidyl-1- or purinyl-9-acetic acids as well as by modification with nucleoamino acids: 3-(thyminyl-1)alanine, 3-(cytosinyl-1)alanine, 3-(adeninyl-9)alanine). In the NPs 4 and 5 trifluoroacetic groups were used to protect a-amino functions of nucleoamino acids. The synthesis was performed using Boc/Fmoc-strategy and O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate as coupling reagent. Cleavage of the peptides from the resin was performed upon irradiation with 365 nm UV light or by treatment with trifluorometanesulfonic acid. Purification of NPs was achieved by reversed phase HPLC. The purity of the target compounds was checked by HPLC, amino acid and mass-spectrometry analyses.



*Structures of nucleopeptides synthesized.*

## P A102 - Design, structure and biological effect of chymotrypsin inhibitor models

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SGCI (*Schistocerca gregaria* chymotrypsin inhibitor) is a 35-residue peptide with a fold consisting of three antiparallel -sheets and three disulfide bridges. The binding loop containing the scissile peptide bond between residues P1-P1' (Leu30-Lys31) is located at the C-terminal end of the molecule and according to NMR studies seems to be less well defined than the core of molecule. The emerging question is that why a relatively flexible loop with a sequence resembling that of good substrates behaves as an inhibitor. The assumption was that the scaffold provided by the molecule should be necessary for the inhibitory effect. Our approach was to design cyclopeptides to mimic build the 'minimal' scaffold responsible for the biological activity of the peptide. Three models were designed to include all known features of the inhibitor: the structurally necessary -sheet and the fragment containing the P1–P1' environment. During the design two non-trivial modifications were made. The first model peptide synthesized had no inhibiting effect and the model was cleaved at two positions by chymotrypsin. Because peptides containing Phe are good substrates for chymotrypsin, in the modified, second model molecule Phe was replaced to Thr. In contrast to the expectations, this molecule exhibited no inhibitory effect on chymotrypsin. (Previously described: Pept., 2000, 132, P145.) NMR investigations of the structure of the first inhibitor revealed that the model has poorly defined β-sheet structure. Therefore a third, bigger model peptide was designed by extending the size of the three β-sheets and retaining two disulfide bonds. The amino acid sequence synthesized was checked by molecular dynamics studies. The latter model peptide in accordance with the molecular dynamics simulations has strong inhibiting effect on chymotrypsin (still less efficient than the original SGCI). The three-dimensional NMR structure of third model peptide shows that the molecule contains well-defined structural regions in contrast to the earlier model peptides.

**Table 1.** *Equilibrium $K_i$ values of SGCI, SGTI and the two model peptides versus bovine chymotrypsin at pH 8.0, 25 °C*[2].

| $Ki_{SGCI}$ | Ki(Compound 1)$_{(Phe)}$ | Ki(Compound 1)$_{(Thr)}$ | Ki(Compound 3) |
|---|---|---|---|
| $6{,}2x10^{-12}$ mol/dm$^3$ | $2.24x10^{-4}$ mol/dm$^3$ | $3.70x10^{-2}$ mol/dm$^3$ | $3.\ x10^{-7}$ mol/dm$^3$ |

*Fig. 1 - NMR structure of first model peptide*

*Fig. 2 - NMR structure of third model peptide*

## P A103 - Synthesis of new Fmoc-protected iminosugars to study the role of the glycosyl moiety in autoantibody recognition in autoimmune diseases

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The importance of carbohydrate recognition in biological events is well established on many experimental findings. The configuration and the spatial arrangement of the involved carbohydrates is, therefore, a subject of ongoing research. We were able to observe that the conjugation of a glycosyl moiety to an Asn (*N*-glycosydic linkage) in the glycopeptide CSF114, allowed the detection of specific antibodies (Abs) in sera of patients affected by Multiple Sclerosis (MS). No Abs were identified with the corresponding non glycosylated peptide sequence. These data demonstrate, for the first time, the relevance of the presence of a sugar moiety, favorably presented by a β-hairpin structure to a glycosylated epitope, for auto-Ab recognition in MS. Interestingly, the detected auto-Abs showed a correlation with disease activity. Diagnosis and therapeutic treatments of autoimmune diseases is a great challenge of our Millennium. There are more and more evidences that glycoproteins play an important role in the pathogenesis of autoimmune diseases. Auto-Abs might be responsible of damages due to the disease onset. In this connection, glycosidase inhibitors can be regarded as immunomodulatory agents. Starting from the observation that iminosugars, like lentiginosine [(1*S*,2*S*,8a*S*)-1,2-dihydroxyindolizidine], and 2-deoxynojirimycin, have shown a remarkable inhibition activity toward glycosidases, we undertook the synthesis of new Fmoc-protected amino acids bearing polyhydroxlated indolizidines and monocyclic piperidine azasugars orthogonally protected for SPPS, following the Fmoc/*t*Bu strategy. The indolizidine iminosugars can be synthesized conveniently in both enantiomeric forms starting from D- and L-tartaric acid. 2-Deoxynojirimycin is available, albeit expensive, also commercially. Several strategies for the conjugation of iminosugars to the side chain of Fmoc-Asp-OPfp will be followed and illustrated in the communication. The new modified amino acids will be introduced in β-hairpin peptide structures, for the detection by ELISA of auto-Abs specific for different autoimmune diseases.

## P A104 - Conformational study of peptides containing (S)-ethylleucine as a chiral α,α-disubstituted amino acid

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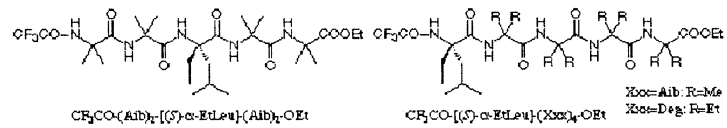
α,α-Disubstituted amino acids are non-proteinogenic amino acids, in which the hydrogen atom at the α-position of natural amino acids is replaced with an alkyl substituent. Introduction of α,α-disubstituted amino acids into peptides changes the conformational freedom and stabilizes the secondary structure of peptides. The conformational studies of peptides containing α,α-disubstituted amino acids have been concentrated on achiral amino acids because they could be easily synthesized. The property of 2-aminoisobutyric acid (Aib) and cyclic α,α-disubstituted amino acids is known to be a $3_{10}$-helical structure, and that of diethylglycine (Deg) and dipropylglycine is to be a fully planar $C_5$-conformation. Although various chiral α,α-disubstituted amino acids were enantioselectively synthesized, only a few papers concerning the conformation of peptides containing the chiral α,α-disubstituted amino acids were published, except for α-methylated α,α-disubstituted amino acids. Recently, peptides containing the chiral α-methylated α,α-disubstituted amino acids were prepared, and the property of α-methylated α,α-disubstituted amino acids has been known to be the $3_{10}$-helical structure.

We prepared the chiral α-ethylated α,α-disubstituted amino acid; (*S*)-ethylleucine [(*S*)-αEtLeu] using (*R*,*R*)-cyclohexane-1,2-diol as a chiral auxiliary and also prepared the heteropentapeptides containing (*S*)-αEtLeu as a guest molecule in the Aib sequence; $CF_3CO$-[(*S*)-αEtLeu]-(Aib)$_4$-OEt, $CF_3CO$-(Aib)$_2$-[(*S*)-αEtLeu]-(Aib)$_2$-OEt and in the Deg sequence; $CF_3CO$-[(*S*)-αEtLeu]-(Deg)$_4$-OEt by the solution-phase methods. The conformation of peptides in the solid state was studied using X-ray crystallographic analysis, and those in solution were studied using IR, $^1$H NMR, and CD spectra. The preferred conformation of Aib heteropeptides containing an (*S*)-αEtLeu was the $3_{10}$-helical structure both in the solid state and in solution, while that of Deg heteropeptide containing an (*S*)-αEtLeu was the $3_{10}$-helical structure in the solid state and that in solution was the planar $C_5$-conformation.




Reference

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