Oral presentations

L1 - Unsaturated non-proteinogenic α-amino acids as versatile synthetic building blocks

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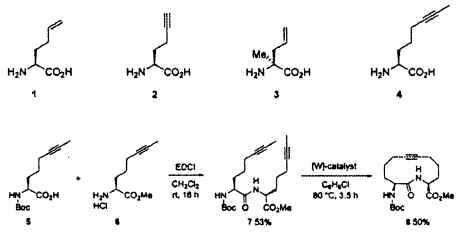
Over the last years DSM Fine Chemicals (DFC), in collaboration with the Universities of Nijmegen and Amsterdam, and more recently with the start-up SynBio, has developed new technology for the synthesis and production of non-proteinogenic amino acids (AAs) of increasing complexity. A large set of non-proteinogenic amino acids containing unsaturated side-chains has become readily available in enantiomerically pure form, using a biocatalytic resolution methodology (*viz.* **1-4**, both L and D).[1] AAs **1** and **2** have been shown to be excellent methionine mimics, and can be incorporated into peptides as such, allowing for functionalization at the peptide stage. Alternatively, the unsaturation in the AA side chain can be used directly as a handle for further functionalization. For example, α-Me-α-AA **3** has been used in the construction of β-turn mimics, via a diolefin ring closing metathesis approach.[2] Similarly,dipeptide **7** -- derived from the acetylenic AA **4** -- was subjected to a ring closing alkyne metathesis reaction, which afforded the potential β-turn mimic **8**. We wish to present some of our recent results in the synthesis of such unsaturated non-proteinogenic amino acids and application as versatile synthetic building blocks, a.o. for the construction of peptidomimetics.



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L2 - Exploring different approaches for the stereoselective synthesis of amino acid-derived 4-alkyl-4-carboxy-2-azetidinones

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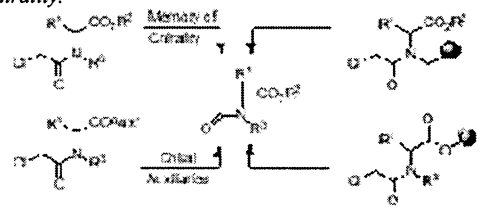
The incorporation of conformationally constrained amino acid derivatives into peptide sequences is a strategy widely used for the optimization of peptide ligand-receptor interactions [1]. Therefore, methods giving access to new non-proteinogenic amino acids with appropriate topographical constraints around χ's, ϕ and ψ torsion angles could be of a great value. In this sense, we have recently developed a three-step synthetic route in solution for the preparation of 1,4,4-trisubstituted 2-azetidinone derivatives, from commercially available amino acid alkyl esters [2]. Surprisingly, modest selectivity was found in the N^{α}-C^{α}-intramolecular cyclization of Trp, Phe and Leu derivatives (ee up to 56%), suggesting that, in the case of these β-ramified amino acids, the cyclization reaction proceeds by way of planar enolate intermediates, which possess axial chirality, according to the recently proposed concept of the *memory of chirality.*



Scheme 1.- Solution and solid phase approaches to amino acid-derived 2-azetidinones

To determine whether the synthesis of these 2-azetidinones could be performed in an enantioselective way, we have respectively explored the enhancement of the memory of chirality and the application of chiral auxiliary-based methodologies commonly used for the asymmetric synthesis of α-alkyl amino acid derivatives. To evaluate the possibility of preparing combinatorial libraries based in this β-lactam privileged structure we have also investigated two alternate procedures for the preparation of these amino acid-derived 2-azetidinones using solid phase protocols (Scheme 1). In this communication we will describe in detail the scope and limitations of all these synthetic pathways.

References
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L3 - Asymmetric synthesis of fully protected (2S, 3R)-N-(1',1'-dimethylally)-3- hydroxytryptophan, a new amino acid found in anti-inflammatory cyclic peptide cyclomarin C

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Cyclomarin A, B and C are three novel cyclic heptapeptides, which were recently found in a marine bacterium (*Streptomyces* sp.) in California [1] by Clardy et al. and show potent anti-inflammatory activities both in vivo and in vitro assays. Because of the potent bioactivities, special structural features and rare nature source, they become more attractive targets of synthetic chemists. Of the seven amino acid of cyclomarin C (**1** in Figure 1), four are the unusual amino acids and two of them were found for the first time. Herein we wish to report our first synthesis of one of the new amino acids, (*2S, 3R*)-*N*-(1',1'-dimethylally)-3- hydroxytryptophan (**2**) with full protection, by a chiron approach starting from the common amino acid tryptophan (**3**). In the synthetic sequence, the N-*tert*-alkylation and stereo-controlled introduction of β-hydroxyl group are the key steps.

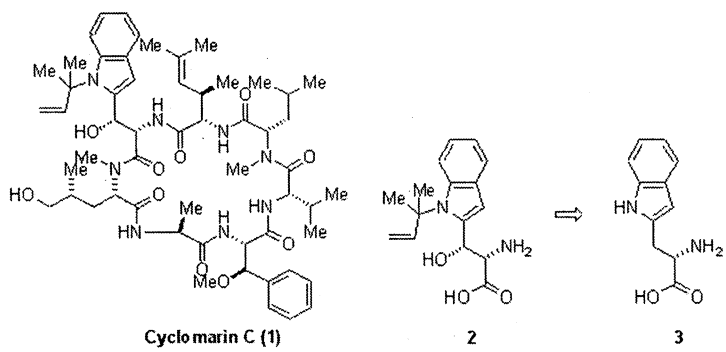

Figure 1. Chemical Structure of Cyclomarin C.

Reference
[1] Renner, M. K., et al. J. Am. Chem. Soc. 1999, 121, 11273-11276.

L4 - Synthesis of non-proteinogenic amino acids from N-(4-toluenesulfonyl)-dehydroalanine derivatives

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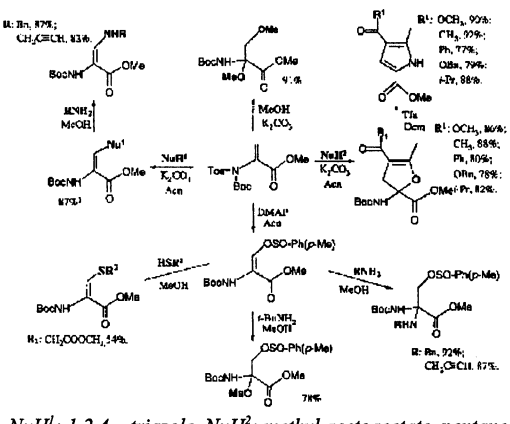
Non-proteinogenic amino acids may find important applications either as biologically active compounds or as components for the synthesis of peptides. We have previously described the use of the methyl ester of *N*-(4-toluenesulphonyl)-*N*-(*tert*-butyloxycarbonyl)-dehydroalanine [Tos-ΔAla(*N*-Boc)-OMe] to obtain β-substituted alanines and various dehydroalanine derivatives.[1,2] Now, we report the preparation of new amino acids from Tos-ΔAla(*N*-Boc)-OMe. (Scheme 1) By treating Tos-ΔAla(*N*-Boc)-OMe with different reactants under different reaction conditions, a variety of new amino acids are obtained (Scheme 1). These include derivatives of (i) furanic amino acids, which can be readily converted into their pyrrole derivatives (dehydroproline derivatives), (ii) α-alcoxy-α-amino acids and α,α-diamino acids, and (iii) novel β-substituted dehydroamino acids.



NuH^1: 1,2,4 – triazole. NuH^2: methyl acetoacetate, pentane-2,4-dione, benzoyl acetone, benzyloxycarbonyl acetone, isobutylcarbonyl acetone

Scheme 1

References
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L5 - Preparation and application of new amino acids available for oxime ligation

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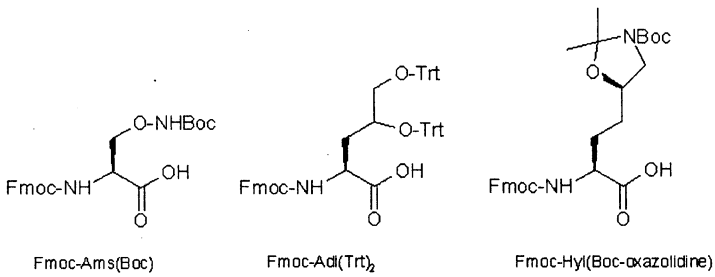
For the preparation of artificial proteins and constructs such as peptide dendrimers and peptide isosters the chemoselective ligation approach is a very powerful tool [1]. Here, we present several strategies for synthesizing peptide dimers and other peptide ligation constructs using the three orthogonally protected amino acid residues, Fmoc-Ams(Boc), Fmoc-Adi(Trt)$_2$ and Fmoc-Hyl(Boc-oxazolidine) (see below). Recently, all three amino acids have been synthesized [2] with side-chains containing either a masked aldehyde (Adi and Hyl) or an oxyamino (Ams) function. The aldehyde and the oxyamino groups form an oxime bond in the presence of other amino acid functionalities. The new amino acids are incorporated into the peptide sequence during standard Fmoc-based solid phase synthesis and deprotected with TFA. For the two residues, Adi and Hyl, their side chain aldehyde group is generated by the subsequent mild periodate oxidation and the presence of reactive aldehyde during the TFA-cleavage is therefore avoided. Overall synthetic strategies for preparing homo- and hetero peptide dimers, cyclic peptides and glycopeptides utilizing the oxime ligation chemistry will be discussed including synthesis of the three new fully protected amino acids.



Fmoc-Ams(Boc) Fmoc-Adi(Trt)$_2$ Fmoc-Hyl(Boc-oxazolidine)

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L6 - First total synthesis of the nematicidal cyclododecapeptide Omphalotin A containing nine N-methyl amino acids

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Omphalotin A cyclo(Trp-MeVal-Ile-MeVal-MeVal-Sar-MeVal-Melle-Sar-Val-Melle-Sar) has been recently isolated as the major component of a family of cyclic dodecapeptides produced by *Omphalotus olearius*. The selective action against plant pathogenic nematodes was reported to be better than that of Ivermectin [1]. Many attempts to synthesize this novel agent failed due to bad coupling yields even with the best reagents including triphosgene [2]. Racemization, diketopiperazine formation, acid lability of the product and further side reactions were observed. However, we found a decisive new modification of the triphosgene coupling method [2] which allowed us to produce Cyclosporin O and Omphalotin A in excellent and reproducible quantities and high purities by solid phase synthesis starting with Fmoc-Sar-OH on tritylchloride resin followed by cyclization of the linear highly methylated polypeptides. Our new procedures are considered as a breakthrough for the automated production of all variations of similar N-alkylated peptides in larger amounts and high purities (patent pending).

References
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L7 - Solid-phase synthesis of dehydropeptides

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1. National Institute of Advanced Industrial Science and Technology - Japan

A number of dehydro amino acids are found from natural sources as a component of bioactive peptides. Among them, AM-toxin or Sch20561 are known as members of this class of molecules, as well as substances possessing various biological activities. The structures of these compounds are that of cyclic depsipeptides containing α,β-dehydroalanine (ΔAla) or amino dehydrobutylate (ΔBut). We designed β-selenated amino acids as an anchoring residue to a resin by side chain tethered strategy. A key feature of this approach, an oxidative cleavage from the solid support, will give the corresponding α,β-dehydro amino acid residues after N,C-bi-directional peptide elongation and cyclization.
Our synthesis started with the preparation of β-selenated amino acids. The allyl ester of N-Fmoc-L-serine or threonine was reacted with an aryl selenocyanide possessing a "hidden carboxylate" and Bu_3P to give the corresponding selenoethers. After an appropriate activation of carboxylate, the protected amino acid was immobilized on a common resin for peptide synthesis. After the cleavage of the protecting groups, the peptide elongation and cyclization was successively achieved. Finally, oxidative cleavage *via* selenoxide from resin was carried out by treatment with a peroxide to give desired cyclic dehydrodepsipeptides with concomitant formation of an unsaturated bond.
The synthesis of AM-toxin II, possessing dehydroalanine residue, was successfully achieved by this synthetic strategy (*Tetrahedron Lett.*, **2001**, *42*, 8337). Synthetic studies toward the natural products possessing amino dehydrobutylate (Sch20561) will be presented, also.

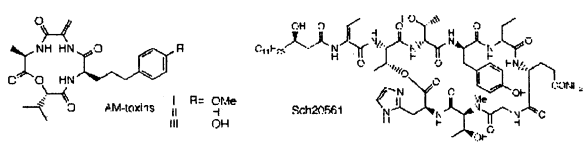

Fig. 1 - Structures of Naturally Occurring Dehydropeptides

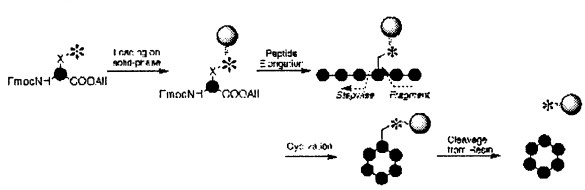

Fig. 2 - Novel Synthetic Strategy of Cyclic Peptides

L8 - Peptide aldehyde reactivity in aqueous solution: influence of the hydrated form studied by Electrospray Mass Spectrometry

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The use of the aldehyde group for site-specific and backbone modification of peptides and proteins has been of paramount importance since the appearance of chemoselective ligation in peptide chemistry. One of the chemoselective methods involves oxime chemistry based on a regiospecific reaction between a C-terminal peptide aldehyde as the electrophile and a N-terminal (aminooxy)acetyl peptide as the nucleophile.[1] In this context, we have recently developped a strategy based on successive generation of two aldehyde functions from an acetal and a 2-amino alcohol leading to an α-methyl-β-aldehyde and α-oxo-β-aldehyde, respectively.[2] Differences have been noted in the reactivity of the two peptide aldehydes handled. They were attributed either to the α-substituent of aldehyde function or to a steric hindrance phenomenon. To document this point, we decided to synthesize two model peptide aldehydes of very close molecular weight which differ by the α-substituent of the aldehyde group, *i.e.* a methyl or a carbonyl. Their reactivity towards a (aminooxy)acetyl peptide was evaluated for the formation of the oxime bond. As previously observed, the methyl aldehyde was more reactive than the ceto-aldehyde yet the more electrophilic aldehyde. As the reaction took place in buffered solution, we reasoned that the hydrated form of the aldehyde could play a role. The equilibrium between the peptide aldehyde and its hydrated form was further investigated by the electrospray mass spectrometry method recently described in our group which takes advantage of in-source fragmentation of fragile bonds.[3] Mass spectrometry experiments show not only that the ceto-aldehyde is nearly totally present under its hydrated form but also that the hydrated form of the ceto-aldehyde is more stable than that of the methyl aldehyde. This stability could explain the worse reactivity of the ceto-aldehyde when compared to the methyl aldehyde. These results may help to design synthetic strategies involving peptide aldehyde-based multi-ligations.

References
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[3] Buré C., Gobert W., Lelièvre D., Delmas A., *J. Mass Spectrom.* 2001, *36:* 1149.

L9 - Effects of substrate mimetics on the flexibility of enzymatic peptide synthesis

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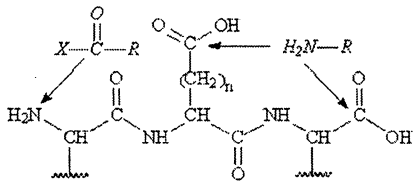
Enzymatic peptide synthesis by proteases is widely considered to proceed in a highly regio- and stereospecific manner avoiding the need of extensive site-chain protection regimes. In principle, it enables the preparation of short peptides as well as proteins simply by coupling of mainly unprotected amino acids or peptide fragments. However, even the most impressive examples published so far are handicapped by the intrinsic drawbacks of the protease catalyst that are mainly: i) the restricted specificities and selectivities of the available proteases and ii) the permanent risk of proteolytic side reactions both of the starting compounds and the products formed. Summarizing these characteristics, proteases appear to be far away from being perfect catalysts for universal and flexible peptide synthesis. Inevitably, this conclusion holds true for classical approaches, but is wrong when considered as a general rule. In fact, the combined use of substrate mimetics,[1] genetically optimized peptide ligases,[2] solid phase peptide synthesis approaches[3] and the expression of protein fragments as reactants enable novel syntheses in a highly flexible manner. This allows the coupling of longer peptide fragments, selective modifications of peptides and proteins at their *N*- as well as *C*-termini, and reactions at the side-chains of gutamic acid and aspartic acid. Besides the coupling of coded amino acid moieties a broad spectrum of synthetic amines and carboxylic acids undergo the coupling approach and further broadens its scope for organic synthesis.[4] Selected original examples including the synthesis of a longer polypeptide, peptide isosteres, isopeptides, structural diverse *N*-linked peptidoclycans, and the selective coupling of non-amino acid derived carboxylic acids to peptides and proteins will be presented. Particular attention will be paid to the synthetic utility of this powerful chemoenzymatic approach and to its unique degree of flexibility. Furthermore, our attempts to design new peptide ligases starting from a pool of more than 60 rationally designed trypsin variants are in the focus of this presentation. The resultant trypsin species was found to act as a substrate mimetics-specific peptide ligase with practically no undesired cleavage activity and was partly used as the biocatalyst for the syntheses mentioned above.



Targets of enzymatic modifications

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L10 - Rigid oligopeptides mimicking the polyproline II helix

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Peptide sequences assuming a left-handed polyproline-II helix are found frequently at protein-protein interfaces. We describe the synthesis and the structural analysis of an oligomeric peptide surrogate which displays essential features of the ppII helix: (1) extended left-handed helix, (2) carbonyl groups oriented perpendicular to the helix axis, (3) polar side chain functionalities, and (4, Fig.1) a hydrogen bond between a side chain hydrogen donor tethering the subsequent amide.
The monomeric building blocks are derived from commercial γ-glucuronolactone. The hydroxy groups form the basis of further synthetic modifications to mimic side chains of encoded amino acids, for example. Structurally diverse oligopeptides will be presented which are assembled from various dipeptide building blocks and amino acids. The solution structures of the oligoamides were characterized by NMR and CD spectroscopy. Some pseudo-peptides were available for x-ray analyses. Rigid peptide surrogates possessing heteroatomic side chains are interesting synthetic targets because of their potential for probing recognition events in protein chemistry. Data from biological binding assays will be discussed, too.

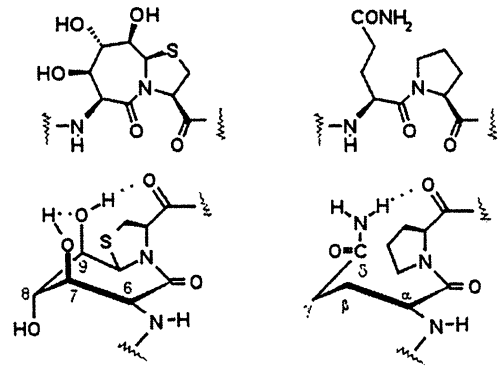

Fig. 1 – A bicyclic polyhydroxylated dipeptide (left) tethers the following amide group within a hydrogen bond. A similar hydrogen bond can be found in many polyproline II helices with polar amino acids as shown for a Gln-Pro dipeptide sequence (right).

L11 - Chromatin boundaries and the developmental regulation of gene expression

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It has been clear for some time that regulation of gene expression in eukaryotes is strongly dependent on mechanisms that modify chromatin structure. For some years we have used the chicken beta-globin locus and its surrounding genes (~55 kb long) as a system to examine the interaction between chromatin structure and gene expression. The globin locus is surrounded by other genes with strong positive regulatory elements, and on its 5' side by a 16 kb segment of condensed chromatin. This raises the question: how is globin gene expression shielded from the influence of nearby regions that might inappropriately stimulate or silence gene expression? We have identified DNA segments that flank the beta globin locus and have the characteristic properties of 'insulators', which may afford such protection. The 5' flanking insulator may function both to prevent the action of a strong upstream enhancer belonging to another nearby gene (enhancer blocking activity), and also to prevent encroachment of the upstream condensed chromatin (barrier activity). We have dissected this element and studied the properties of its individual components. The enhancer-blocking activity can be ascribed to binding of a single protein factor, CTCF, and it is used for that purpose at other loci as well - notably at the imprinted Igf2/H19 locus in mice and humans. Our studies of the barrier activity suggest that it protects downstream genes from being shut off by interfering with propagation of histone modification signals. Such functions are likely to be essential at many locations in the genome.

L12 - PNA-DNA chimeras as decoy molecules against the transcription factor Sp1.

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PNA are DNA analogues composed by a peptide-like backbone to which eterocyclic bases are connected. The interest toward these molecules is due to their high stability "*in vitro*' and "*in vivo*" and to their resistance to chemical agents such as strong acids and bases. PNAs form very tight complexes with complementary PNA, DNA and RNA following the Watson Crick hydrogen bonding rules; the conformation of these molecules has been extensively studied by NMR, X-Ray. PNA/PNA, PNA/DNA and PNA/RNA have a very different conformation when compared to analogues DNA/DNA and DNA/RNA duplexes showing a wider and deeper major groove and a narrower minor groove. For this and other reasons, such as the lack of charge on the backbone, PNA/PNA or PNA/DNA hybrids are not good DNA mimics for protein interactions studies.
In this work we focused on the study of PNA-DNA conjugates, in which a DNA core is flanked at the 5' and at the 3' end by PNA tails. Double stranded PNA-DNA-PNA conjugates have been investigated in their ability to interact with transcription factors involved in the regulation of the expression of a variety of genes relevant in human pathologies, including those encoding for the Vascular Endothelial Growth Factor (VEGF), and the HIV-1. These molecules have been designed in order to keep at least in the core a conformation as close as possible to a DNA duplex. The PNA tails prevent the 5' and 3' terminal to be attacked by exonucleases, providing at the same time further interactions with proteins by the bases. In a previous work it was shown that PNA-DNA conjugates were able to suppress molecular interaction between the HIV-1 LTR and the transcription factor NF-kB, competing with the LTR for the protein binding site [1].
In the present work we investigated by gel shift analysis whether PNA-DNA conjugates could act as decoy against Sp1. Furthermore we investigated the biological activity on K562 erythroid differentiation of PNA-DNA chimeras mimicking Sp1 binding sites. We found that hybrid molecules based on PNA-DNA chimeras are very effective decoy molecules. In addition, their effects on K562 erythroid differentiation are very similar to those of Sp1 DNA/DNA hybrids. The finding that DNA-PNA chimeras stably interact with Sp1 transcription factors, encourages further experiments focused on the possible use of these molecules for the development of potential agents for a decoy approach in gene therapy.

Reference
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L13 - Ability of bis-intercalators peptide libraries functionalized with 9-amino acridine to block an aggregation of HuPrP106-126 (difficult sequence) in the presence of gc-rich DNA

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Prion diseases are characterized by the accumulation of abnormal forms of the cellular prion protein (PrP^C), termed PrP^{Sc}, in the brain. In contrast with the former, the PrP^{Sc} is partly resistant to digestion with protease due to enhanced content of â-sheet conformation forming insoluble aggregates and amyloid fibrils. A peptide which includes residues 106-126 of human PrP (HuPrP106-126) has been shown to be highly amyloidogenic and is toxic to neurons *in vitro* [1,2]. Therefore, this peptide could represent a suitable model compound with properties of the infectious PrP^{Sc}. An active role of nucleic acids was found necessary for experimental transfer of BSE infection to mice and for aggregation of HuPrP106-126 in solution [3]. gc-DNA which is known to bind 9-aminoacridines was found to induce polymeration of mouse PrP^C more rapidly than other DNAs [4]. In the first part of our study, the heneikosapeptide HuPrP106-126 was prepared on Wang resin using Fmoc/tBu chemistry. Up to the heptapeptide sequence, the Fmoc cleavage with piperidine-Triton-X100 mixture in DMF and couplings using DIC-HOBt in the same solvent mixture were performed, however, due to an apparent aggregation of the growing peptide, NMP had to be added latter to the solvent mixture. In the recoupling steps, PyBOP-HOBt-DIEA reagents were used in the former solvent mixture, however, some difficult steps were performed also in a DMSO-DMF. Finally, a very efficient coupling reagent TOTU-DIEA was successfully used in some critical steps of this "difficult sequence" synthesis. After deprotection and detachment from the resin with a TFA-H_2O-EDT-TIS mixture, the heneikosapeptide was purified by HPLC and was characterized by ÂAA and MALDI-TOF mass spectrometry. In the second part of our study, the synthesis of library of bis-intercalators based on peptides functionalized with 9-aminoacridine (Acr-NH_2) was carried out. We have chosen a structural skeleton Acr-Gly-X-Y-Z-Glyψ[CH_2NH]NH-Acr which was synthesized using the chemistry based on modified SPPS methodology. Fluorescence spectroscopy and competitive binding of peptides library with DNA in the presence of HuPrP 106-126 were used to monitor the ability of library prepared to block an aggregation of HuPrP 106-126 in the presence of gc-rich DNA. The results from fluorescence spectroscopy competitive assay will be presented.

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L14 - *Cis* peptide bonds in proteins and *cis/trans* isomerases in intracellular parasites

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Peptide bonds in proteins normally occur in the *trans* conformation. The only exception is the peptidyl prolyl bond which adopts the *cis* conformation in 5% of the cases. Non-prolyl *cis* peptide bonds are extremely rare in proteins. Interestingly, they are, in the majority of the cases, found in active sites of enzymes or other functionally important sites [1-3]. A detailed analysis reveals that the energetically unfavorable *cis* conformation is often stabilized by C-H...π hydrogen bonds [4,5]. The isomerisation of peptide bonds is often a rate-limiting step in protein folding. Three classes of peptidyl-prolyl cis/trans isomerases have been characterized: the cyclophilins, the FK506-binding proteins (FKBPs), and the parvulins. Recently, enzymes belonging to the FKBP class have been characterized as virulence factors in intracellular parasites such as *Legionella pneumophila* [6], *Chlamydia spp.*, and *Trypanosoma cruzi* [7]. Crystal structures for these proteins will be presented and their possible function as chaperones will be discussed.

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L15 - Structure and dynamics of membrane-bound hormones as studied by NMR

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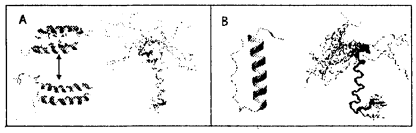
For binding of neuropeptide Y (NPY) to its receptor we propose a three-step model that includes membrane association as an important step preceding receptor recognition according to the model developed by Schwyzer[1] and Moroder. We have previously structurally and dynamically characterized NPY bound to a model membrane[2] and determined the binding interface by using micelle-integrating spin labels and H,D exchange data. Recently, a key motif to derive agonists that selectively bind to the Y_5-receptor-subtype of NPY[3] was developed. We subsequently determined the structure and dynamics from ^{15}N relaxation data of one such mutant both in solution and when bound to the membrane and identified residues from the membrane-hormone interface[4]. The data indicated that the part of the molecule containing the message is differently oriented with respect to the membrane. From these data we have now developed a structural model for the initial event during receptor recognition thereby possibly explaining receptor-subtype specificity. Moreover, we have elucidated the structure and internal dynamics of bovine pancreatic polypeptide (bPP) when bound to the membrane and compared it to the fold of PP published for both the solution[5] and the solid-state[6]. Characteristic structural differences are encountered which are reflected in differences in the internal dynamics and which can be attributed to binding to the membrane. In contrast to the solution structure of bPP, which displays the PP fold (the N-terminus is back-folded onto the C-terminal α-helix), the membrane-bound form possesses a fully flexible N-terminus.



Structures of NPY (A) or bPP (B) in solution (ribbon drawings) or when bound to DPC micelles (bundle of conformers).

To conclude, the hormones from the NPY family investigated by us so far exhibit a common membrane-binding motif with the hydrophobic side associated with the membrane surface in a parallel fashion and pointing towards it. In solution, in contrast, this hydrophobic side is either forming the dimerization interface (NPY) or is covered by a back-folded N-terminus (bPP).

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L16 - Binding interactions of derived HPK1 Pro-rich peptides to HS1-SH3 domain suggest a possible adapter function of HS1 protein

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Hematopoietic lineage cell-specific protein HS1, a presumed adapter, contains an SH3 domain and becomes Tyr phosphorylated following antigen receptor cross-linking. HPK1 protein, a member of Ser/Thr protein kinases has been implicated in the regulation of MAP kinase and in particular is a potent stimulator of the stress-activated JNK/SAPK protein kinase cascade. HPK1 consists of an N-terminal Ser/Thr kinase domain, followed by a central region harbouring four proline-rich motifs and a presumably regulatory C-terminal tail. The Pro-rich domains play an important role in the interactions of this kinase with different adapter proteins.
The interactions of the four peptides derived from HPK1 [P1 (PELPPAIPRRIR), P2 (PPPLPPKPKF), P3 (PPPNSPRPGPPP) and P4 (KPPLLPPKKE)] with the C-terminal SH3 domain of HS1 protein were analysed by non-immobilized ligand interaction assay by circular dichroism (NILIA-CD). Upon peptide addition, the binding was monitored by the CD changes of the Trp side-chains of the conjugate GST-$SH3_{HS1}$ (302aa) and the SH3HS1 domain (84aa) respectively. The dissociation constant Kd was determined analysing the CD data at 291nm using a non-linear regression method. The results show that the four HPK1 Pro-rich regions are not equivalent. P2 appears to have the highest affinity (Kd=1.4μM+0.2μM) than P1 (Kd=12.5μM+2.4μM) and P4 (Kd=130.4μM+7.6μM) whilst P3 does not interact at all. The CD titration of P2 with the SH3HS1 domain revealed a weaker binding (Kd=5.8μM+0.5μM) that could be ascribed to a higher stability of the Pro-rich binding site of the GST-$SH3_{HS1}$ conjugate. The finding of SH3 conformational changes supports this. The observed CD spectrum of the conjugate GST-$SH3_{HS1}$ was different than that simulated by adding the CD spectrum of GST to that of $SH3_{HS1}$.

L17 - Interaction of β-amyloids with cell membrane proteins and signalization

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The interaction of β-amyloid peptides with cell membrane proteins plays pivotal role in the neurotoxicity of these peptides. We have studied different β-amyloids using SH-SY5Y human neuroblastoma cell line and MTT (formazan)-test for investigation of neurotoxicity. Aβ 1-42, Aβ 1-40, Aβ 25-35 and also the short peptide Aβ 31-35 were highly toxic, if aggregated. Also the all-D Aβ 1-42 was as toxic as the parent compound. After pretreatment of the cells with trypsin (10 min) Aβ-peptides showed no toxicity, proving that integrated membrane proteins are necessarry for the neurotoxic effect. Revers amiloid sequences (e.g. Aβ 42-1, Aβ 35-25) showed almost no neurotoxicity. These peptides do not show β-conformation. Reversing the amino acid sequence causes a gross conformational change in the peptide structure [1]. (Aggregated amyloid peptides can bind to membrane proteins like integrins and APP. The RHDS tetrapeptide sequence can not play important role in this binding, because short C-terminal peptides having no RGD-like sequence (Aβ25-35, Aβ31-35, Aβ37-42) are neurotoxic. According to our hypothesis all the aggregated Aβ sequences are neurotoxic and the primary event is the binding of these aggregated peptides to APP or integrins. Activation of focal adhesion proteins by integrins might be the next step of downstream signaling which starts apoptosis and cell death

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L18 - New linkers for directionally organized assemblies of helical peptides on surfaces

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We have prepared two new linkers - sulfur-functionalized adamantanes **2** and **3** - which bind as monolayers on polycrystalline gold. From these surface anchors, both (L) and (D) isomers of alanine can be grown as thin films of α-helical polypeptides directed from the gold surface using the appropriate N-carbonic anhydride. FTIR studies show that these layers are roughly 1000 Å thick and that, under the same growth conditions the (L) polypeptide layers grow at a rate approximately 30% greater than that from the non-natural (D) amino acid. X-ray photoelectron spectroscopy studies show that, upon equilibration, all three of the thiol ethers of **2** are bound to the gold surface and that on average three of the four thiols of **3** are chemoadsorbed.

L19 - Protein-detection systems using structure-based peptide libraries and peptide microarrays

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In the postgenome era, construction of easy and effective detection systems for proteins, "protein chips", is one of the most promising technologies in proteomics. One such system is the method based on the binding abilities of proteins. In this study, we have attempted to construct protein-detection systems using the change in fluorescence intensity of structure-based designed peptide libraries with fluorescent probes, which have the capabilities of forming secondary structures such as α-helix, β-strand, or β-loop.
At first, we selected model peptides known to interact with a structured protein to establish the synthetic and detection methods. One example is an α-helix peptide which is a cationic amphiphilic peptide and binds to calmodulin. Another peptide has a β-loop structure derived from the active site of tendamistat, an inhibitor of α-amylase. We examined the changes in the fluorescence intensities of these peptides with various fluorophores upon addition of target proteins in solution and on multi-titer plates or glass plates. Furthermore, we have constructed peptide libraries with different charges and/or hydrophobicity based on secondary structures and have detected various proteins using peptide microarrays of these libraries.

L20 - Large-scale protein-protein-interaction mapping with synthetic peptide arrays: Epitope-targeted proteome analysis

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Function is tantamount to interaction. Thus, global functional genome and proteome research aims at a complete description of the network of protein interactions within a cell or organ(ism) that is diagnostic for a specific cellular state such as fetal or adult, brain or liver, healthy or oncogenic/pathogenic etc. Proteins interact via surface accessible interaction sites which involve amino acid residues and backbone contacts along (but not necessarily contiguous!) a linear segment of the protein chain (linear epitopes) or involve amino acid residues from two or more segments of the protein chain brought together by the folded conformation (conformational epitopes). Note that the term epitope is used here in its broadest sense and far beyond the mere immunological meaning. Linear epitopes can be effectively represented by small peptide fragments which are readily available through simultaneous and parallel chemical synthesis. This is also partly true for conformational interaction sites that can specifically recognize mimotope peptides. Many proteins, most prominently those of regulatory function, are built from smaller domains which are stably folded structural modules still displaying their specific functional property. The catalogue of such domains that recognize linear epitopes is rapidly growing (kringel, SH2, SH3, PH, EVH1, PDZ, WW, etc.) indicating a more general principle utilized by nature. These domains are found to be involved in divers molecular organization and regulation phenomena.
Complementary to other, molecular biology approaches such as the yeast-two-hybrid method, a global peptide screening approach directly addresses functional protein interaction sites, leading to a detailed insight into the discovered molecular recognition events, placing them in the context of the whole genome and even allows to rapidly decipher the chemical nature of these interactions. This information can then be transferred into powerful small peptide tools that interfere with these interactions in vivo and help to link targets with phenotypes. One important aspect of functional genomics/proteomics is to gain direct access to new targets for drug discovery. Thus, it is logical to set up a genome wide search for all "drugable" proteins and validate these as relevant pharmaceutical targets by modern proteome analysis. It can be concluded that these "drugable" targets primarily belong to that repertoire of proteins which can bind small molecule ligands. Synthetic peptides are practical tools readily at hand to address this property. Although peptides themselves have lost attractiveness as pharmaceutical drugs, they are perfect molecular probes for the search of new pharmaceutical targets.
The above considerations consequently led us to develop an automated system for genome-wide systematic mapping of protein-domain to peptide-ligand interactions which is entirely based on high-throughput parallel micro-array technology. Peptide arrays are being manufactured by SPOT synthesis (Frank, 1992,*Tetrahedron 48*, 9217) and these are screened against a library of randomly cloned human cDNA fragments expressed and displayed as fusion proteins on T7 phage particles.

L21 - α-Oxo semicarbazone polypeptide microarrays for the sensitive and specific serodetection of antibodies

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2. Sedac - France
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Today, the detection of a serie of pathogens for each blood donor is obligatory for transfusional security and require the utilization of a large number of tests, some of them being laborious, costly and time consuming. Consequently, there is a need for an alternative serodiagnostic format allowing, in one step, the simultaneous detection of multiple pathogens using very small blood samples, and which may be easily upgraded to allow the detection of new pathogens with minimal additional delays and costs. The data presented in this paper demonstrate that a novel generation of peptide arrays, assembled by α-oxo semicarbazone site-specific ligation of peptides to glass slides and imaged using a DNA microarray fluorescence scanner, allows the highly sensitive and specific detection of various antigens (HCV core and NS4, EBV), and are thus promising tools for the development of a rapid, simple and low cost multiple serodiagnostic test.[1]

Reference
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L22 - Peptide micro-arrays for kinase substrate profiling

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3. Pharmaceuticals/ Jerini AG - Germany

Peptide micro-arrays for accelerating crucial steps in kinase drug discovery have been developed. This was achieved by applying a combination of the SPOT technology [1,2] for high throughput peptide synthesis and subsequent chemo-selective printing of compounds onto glass slides. Kinases are key enzymes for the control of a variety of cellular functions. They are potential targets for the treatment of cancer or Alzheimer's disease. However, only a small fraction of the approximately 500 kinases predicted from the human genome is characterized so far.
For profiling novel kinases different types of substrate micro-arrays have been designed: (i) peptide scans through histone, myelin basic protein and G-coupled receptors, (ii) peptides containing all annotated human phosphorylation sites and (iii) random peptide libraries. Phopshorylation of substrates upon incubation of the arrays with kinase was detected either by incorporation of ^{32}P or with phosphotyrosine specific antibodies. Applications of these novel types of micro-arryas include: (i) identification of novel specific substrates, (ii) design of substrate binding site inhibitors and (iii) anti-target profiling with kinase inhibitors. The results of profiling more than 35 kinases will be reported.

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L23 - Assembly of membrane-targeted heterotrimers

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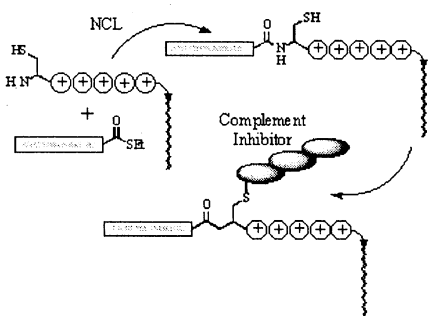
The native chemical ligation process is used widely to allow the joining together of two components containing a C-terminal thioester and an N-terminal aminothiol respectively.[1] This methodology generates typically a sidechain thiol at the newly-formed junction site that is frequently regarded as a 'necessary evil'. We have used this thiol moiety to direct the assembly of branched heterotrimeric entities containing three or more modalities with interesting biological properties.



This process is exemplified by the assembly of entities containing a membrane directed antithrombotic and at least one other function. A thioester-containing version of the thrombin-inhibiting peptide hirulog[2] is ligated to a aminothiol-containing peptide known to home to the mammalian vasculature. This Prodaptin peptide is amphiphilic, comprising a membrane insertive fatty acyl portion, and a membrane associative polycationic sequence. The binding energy associated with the two parts is additive resulting in strong membrane binding. This membrane targeted antithrombotic is then linked, *via* a short PEG linker, to a recombinant protein comprising the first three short consensus repeats of human complement receptor 1 (CR1), solubly expressed in a prokaryotic host. This recombinant protein has been shown to prevent complement-mediated damage *in vitro* and *in vivo*.[3] The combined effects of a membrane directed antithrombotic and complement inhibitor will be discussed in the context of several *in vitro* assays and *in vivo* applications.
The general concept of heterotrimer assembly is not limited to the molecules described but rather limited only by the imagination of the experimenter. Other examples incorporating haptens, fluoro-phores, antibiotics, peptides, and recombinant proteins will be discussed, and their properties described.

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L24 - Design and synthesis of dendrimers based on poly(Pro) sequences. Exploration of their use as drug-delivery agents

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There is an increasing interest in the synthesis of peptide-based dendrimers owing to their structural resemblance to globular proteins. Such biomimetic dendrimers represent potencially novel drug-delivery agents or bio-drugs with enhanced biocompatibilities. We are interested in using synthetic dendritic systems based on poly(Pro) sequences as drug-delivery agents. Proline rich sequences have biological relevance in different proteins and specific features useful for drug-delivery systems: high resistance to proteases, ability to cross cell membranes and two well defined secondary structures (PPI and PPII) that are stable in different solvents. We have explored the conformational change between the two conformations in poly(Pro) based dendrimers as a new mechanism to encapsulate drugs. The synthesis of such peptide-based compounds also presents a synthetic challenge either by on solid-phase methods or in solution. In the present work we discuss different synthetic strategies to prepare dendrimers based on poly(Pro) chains. Circular dichroism studies have been used to show that these dendrimers present the typical poly(Pro) conformational plasticity. We have also demonstrated that a poly-proline dendrimer is actively internalized by rat kidney cells and we report the first results on the use of these dendrimers as drug-delivery agents.

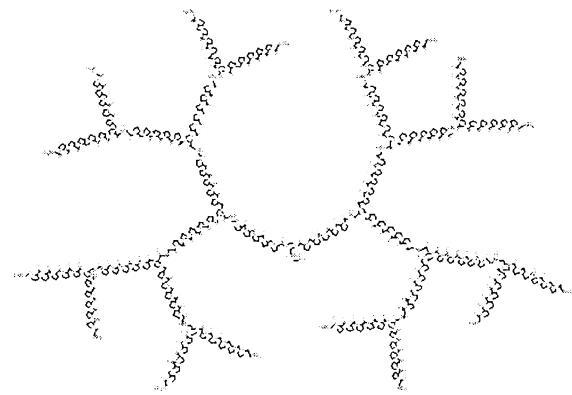

Fig.1 - Representation of dendrimers based on poly(Pro) sequences

L25 - De novo design and synthesis of quinoproteins for light-induced electron transfer

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De novo design of redox proteins is an attractive approach to investigate the factors involved in electron transfer and to create proteins with novel functions. Peptide synthesis and chemical ligation methods enable us to construct new proteins for electron transfer (H. K. Rau, et al. (1998) PNAS 95, 11256). In this contribution we report our attempts to include natural quinones into TASP-proteins constructed from four amphipathic helices with antiparallel orientation. Quinones play an important role as electron acceptor in respiration and photosynthesis, and quinoenzymes use covalently bound quinones as cofactors for electron transfer and catalysis. The crystal structure of one quinoprotein-amine dehydrogenase has disclosed a novel cysteinyl tryptophanquinone cofactor (CTQ) (S. Datta, et al. (2001) PNAS 98, 14268). In a similar way as CTQ we have bound ubiquinone or menaquinone by covalently attaching it to a cysteine by formation of thioether. It is positioned within the hydrophobic core of the four-helix bundle protein, which is suitable for electron transfer. Then another cofactor Zn-protoporphyrin IX (ZnPP) with photoactivatable activity is placed also in the hydrophobic core of the protein by the coordination with histidine. These molecules (See Fig.1) are stably assembled in aqueous solution after incorporation of quinones and ZnPP, and they mimic the light-induced electron transfer in the photosynthetic reaction centre. In a second approach, we have introduced a synthetic amino acid carrying flavin and also coupled ubiquinone to the four-helix bundle protein. The final incorporation of heme by bis-histidine coordination was successful in this novel protein with three native-like cofactors: flavin, heme, and ubiquinone (See Fig. 2). The flash-activatable flavin (c. f. R. E. Sharp, et al. (1998) PNAS 95, 10465) and the suitable redox properties of these cofactors along the protein hydrophobic core will allow the intramolecular electron transfer from flavin to heme then to ubiquinone after excitation of the flavin. All of these quinoproteins have been successfully synthesized for the first time and characterized by the UV-visible and fluorescence spectroscopy, ESI-MS, CD, ^{1}H-NMR, FT-IR and EPR.

Fig. 1 - Molecular model of quinoprotein with ZnPP

Fig. 2 - Molecular model of quinoprotein with flavin and heme

L26 - A 16.6 kDa disulfide-linked dimeric 4-helix bundle carboprotein: synthesis, CD spectroscopy, adsorption to Au(111) electrodes, and in situ STM and XPS studies

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Carbopeptides and carboproteins are a novel class of peptide-carbohydrate chimeras [1]. We have previously reported the preparation of *de novo* designed 4-helix bundle carboproteins by chemoselective oxime ligation of *C*-terminal peptide aldehydes to tetra-aminooxyacetyl functionalized monosaccharides [2, 3]. This synthetic strategy has now been extended to the preparation of a 16.6 kDa 4-helix bundle dimer by ligation of 8 peptide aldehydes to a dimeric disulfide-linked d-galactopyranoside template. The dimeric carboprotein and its monomer were characterized by ESI-MS, SEC, and CD spectroscopy. Further, the disulfide functionalized template allowed specific adsorption of the carboprotein on single-crystal Au(111) electrodes, and hence application of electrochemical techniques, *in situ* scanning tunneling microscopy (STM), and X-ray photoelectron spectroscopy (XPS) to study features of the adsorped carboprotein.

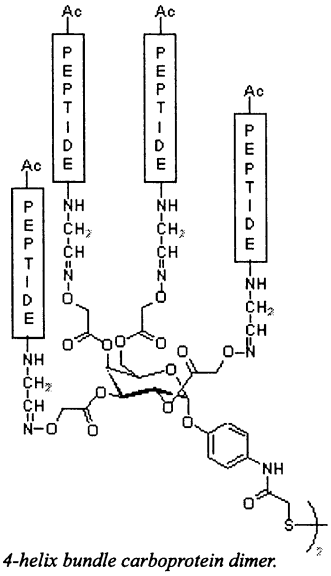

Fig. 1 - The 4-helix bundle carboprotein dimer.

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L27 - Common structural principles in protein-protein and protein-DNA recognition

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The interfaces in 75 protein-protein and 65 protein-DNA complexes were examined for common geometric, conformational and chemical features. The geometric features were the interface area, which measures the size of the contact region, and the Voronoi volume, which evaluates close-packing of interface atoms; conformational features, changes within the proteins or the DNA. Chemical features were the atomic and the amino acid or base compositions of the interfaces, and the presence of solvent molecules. Interfaces were also dissected into recognition patches as defined by clustering interface atoms on the surface of the protein or DNA. Most interfaces with areas in the range 1200-2000 Å^2 were observed to comprise a single recognition patch on the protein surface and their formation to be compatible with rigid-body association. Larger interfaces (2000-5000 Å^2) could be split into several recognition patches and their presence correlated with major conformation changes in the proteins or the DNA components. With very few exceptions, the interfaces were close-packed. This was shown by measuring the Voronoi volumes of interface atoms and comparing their sum to a standard set of atomic volumes. Crystallographic solvent positions contributed to the packing at the interface and had to be taken into account in this evaluation. Solvent positions were often seen to surround a 'dry' core region from which water was excluded, surrounded by a rim of atoms in contact with water. In protein-protein interfaces, the core had a distinctive amino acid composition, whereas the rim could not be distinguished from the rest of the protein surface. In protein-DNA interfaces, the protein side was highly enriched in basic residues making DNA recognition patches much easier to identify on the protein surfaces than protein recognition patches.

L28 - The EGF receptor and Erb B2 crystal structures: a basis for novel drug design for the treatment of cancer

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The biology and biochemistry of the epidermal growth factor receptor (EGFR) system has intrigued both scientists and clinicians for more than twenty five years. Four members of the EGFR family have been identified in vertebrates, namely EGFR (also known as ErbB1 or Her 1), ErbB2 (also known as Neu) ErbB3 (Her 3) and ErbB4 (or Her 4). These receptors can form homo or heterodimers following activation by more than a dozen potential ligands of the EGF family giving rise to a signalling network whose potential for diversification is enormous [1]. ErbB2 has no known ligand, but seems to be the preferred partner for heterodimerisation. ErbB3 has no intracellular kinase domain, and hence appears to act as a regulatory receptor. Aberrant signalling by the EGF/EGFR family has been shown to be associated with a number of cancers [2], and thus this pathway represents an attractive therapeutic target. Using information obtained from the 3D structure of the first three domains of the extracellular region of the structurally related IGF-I receptor [3], we have generated a truncated form of the EGFR (sEGFR 1-501) [4]. Biosensor studies showed sEGFR 1-501 bound ligand with higher affinity (approximately 20nM) compared to full length EGFR ectodomain (400nM) [5]. Additionally, sEGFR 1-501 was a competitive inhibitor of EGF-stimulated mitogenesis, being almost ten-fold more effective than the full length EGFR ectodomain and three times more potent than the neutralising anti-EGFR monoclonal antibody Mab528. Analytical ultracentrifugation showed that the primary EGF binding sites on sEGFR 1-501 were saturated at an equimolar ratio of ligand and receptor, leading to the formation of 2:2 dimers [4]. We have crystallised ligand-bound fragments of sEGFR501 and the corresponding form of ErbB2 (sErbB2 1 – 509) and, using X-ray diffraction, determined the structures of the 2:2 EGFR:TGF alpha complex and unligated ErbB2. Surprisingly, the interactions of TGF alpha with EGFR differ from interactions seen in other characterised cell-surface receptor complexes. Previous structures have shown ligand binding to two or more receptor molecules, causing dimerisation. In the EGFR structure each ligand is clamped between domains of only one receptor molecule, the ligands do not make contact and dimerisation occurs at a separate receptor:receptor interface. Interestingly, the structure of erbB-2 shows features of what an unligated receptor might look like and suggests how this receptor can hetero-dimerise without binding ligand. These structures indicate why previous attempts to generate ligand-based antagonists have been unsuccessful and offer new possibilities for structure-based drug design.

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L29 - Properties of ordered and disordered H-bonds in complex crystalline networks of biological interest from low temperature Infrared Spectroscopy

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IR spectra (4000-400 cm^{-1}) of polycrystalline carbohydrates and nucleosides are studied at temperatures down to 20K and isotopic concentration less 10%; at these conditions the resolution of bands increases in spectra of H-bonded crystals. The observed narrow intensive OH(NH) bands in stretching region are assigned to the ordered H-bound OH(NH) groups, which form a periodic crystal structure. Their number is equal to the number of mostly medium strength hydrogen bonds, which are given by structural methods, and they can be related to known H-bond distances. The number of narrow OH(NH)-bands, which is found with isotopic exchange in the low frequency out of plane vibration mode region (<1000 cm^{-1}), is two-three time more than the number of bands corresponding to that in stretching region. Scarce literature data show the same tendency in spectra of amino acid, purine and pyrimidine crystal structures. Seven bands in this spectral region are affected by D-exchange in IINS (10K) and IR spectra of crystal of L-threonine [1], where only four H-bonds revealed by X-ray (12K). Six bands disappear at D-exchange in spectra of thymine and cytosine at 12K as compared with two and three H-bonds, expected respectively in these crystals. The extra bands are assigned to disordered very strong and very weak H-bonds of OH(NH) groups, which cannot be seen with structural methods and as well are not seen clearly in the stretching vibration region; their energy and abundance are estimated with the empirical correlations between H-bond energy and spectral parameter changes in IR spectra [2]. It is suggested that application of the same method to crystalline amino acids and small peptides deliver analogous new information about H-bond network structure. The very accurate peak frequencies of narrow stretching vibration mode bands of isotopically diluted AH(AD) nucleosides studied confirm the previously found correlation between the H-bond length r(H…B) in the range 0.12 -0.3 nm and the red shift ($\Delta\nu$) of the stretching A-H vibration in the range 20 - 3000 cm^{-1} ($\Delta\nu \sim r^{-6}$) [3]. The combination of the correlation found $\Delta\nu \sim$ rH…B in crystals with known relationships for H-bonds in the liquid phase: $(\Delta\nu)^{1/2} \sim \Delta H$ ($\equiv De$) leads to unique possibility - to reveal empirically the interdependence of energy of H-bond and its length, r(H…B) as $De \sim r^{-3}$ [3].

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L30 - New chemical tools for mechanism-based discovery and profiling of protein families in functional proteomics

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Proteomics, the differential analysis of the protein entirety expressed by a cell under certain conditions, basically relies on the two-dimensional separation of the proteins. However, as usually more than 10000 different proteins are expressed by a cell at the same time, resolution often remains incomplete. Moreover, sensitive detection and quantification represent further problems.
A new general concept for mechanism-based detection and activity-profiling of proteins and protein families utilizes novel engineered chemical probes containing a ligand (peptide or organic molecule) specific for the particular protein family and a reporter group (fluorescent label, radioactive tag, or biotin). The method does not require the application of ligands binding irreversibly (suicide inhibitors), because the probe is covalently linked to the corresponding protein in a highly selective manner by photoaffinity labelling. Consequently, the labelled proteins can be detected easily after separation by 1D and 2D gel electrophoresis. This technique is applicable to a broad variety of proteins and will facilitate the discovery of new, previously undiscovered members of a protein family.
The feasibility of this approach has been proven for two examples:
i) protein kinases of plants and
ii) matrixmetalloproteases.
Protein kinases have been tagged using a chemically engineered probe that comprises a reversibly binding isoquinolinesulfonamide type inhibitor (H-9), a photoreactive group (4-benzoylphenylalanine), and a fluorescent reporter group (carboxyfluorescein). The specificity of photoaffinity labelling of a particular kinase is highlighted by the fact that in several cases only the catalytic units are tagged, whereas the regulatory units remain unlabelled.
The probes for matrixmetalloprotease tagging utilize peptidic hydroxamate type inhibitors.

L31 - Extraterrestrial C^{α}-tetrasubstituted α-amino acids as inducers of homochirality on earth

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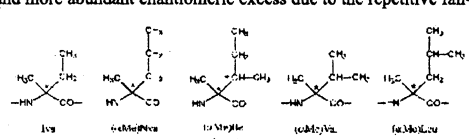
A recent analysis of the aminoacid content of the Murchison meteorite, fallen over Australia in 1969, has revealed the presence of four C^{α}-tetrasubstituted α-amino acids, namely isovaline (Iva), C^{α}-methyl norvaline [(αMe)Nva], C^{α}-methyl isoleucine [(αMe)Ile], and C^{α}-methyl *allo*isoleucine [(αMe)*allo*Ile] with a small, but significant, L(S) enantiomeric excess (up to 9%). Similar findings in the protein amino acid fraction were considered of minor significance in view of a possible terrestrial contamination. On the contrary, this possibility is quite remote for C^{α}-tetrasubstituted α-amino acids, whose occurrence on terrestrial compounds has been reported only for Iva. Moreover, C^{α}-tetrasubstitution protects amino acids against racemization. These results suggest that C^{α}-tetrasubstituted α-amino acids of extraterrestrial origin could have been the homochirality seeds for life in our planet. Nevertheless, the small detected L enantiomeric excess requires an amplification procedure. To check this hypothesis we have planned a detailed study aimed at determining if and how derivatives or peptides based on C^{α}-tetrasubstituted α-amino acids could have reacted with protein amino acids and favoured one of their enantiomers over the other. In this Communication we report on the synthesis and conformational studies of the Iva, (αMe)Nva, (αMe)Val, and (αMe)Leu homochiral homopeptide sequences, used as Ac-(AA)$_n$-OXL [Ac, acetyl; OXL, 5(4*H*)-oxazolone] in competitive experiments with racemic mixtures of a protein amino acid. These C^{α}-tetrasubstituted residues bear either a linear [Iva and (αMe)Nva] or a branched [(αMe)Val and (αMe)Leu] side chain. However, side-chain branching is at the C^{β} atom for (αMe)Val, while at the C^{γ} atom for (αMe)Leu. Solution (FT-IR absorption, CD, ^{1}H NMR) and crystal-state (X-ray diffraction) conformational analyses indicate that all of these homo-peptides tend to fold in β-turn and 3_{10}-helical structures (depending upon main-chain length), the handedness of which is dictated by C^{α} atom chirality and side-chain branching position. We present a discussion of the analytical methods suitable for the quantitative analysis of peptide diastereomeric mixtures and our results on reactions involving the competitive formation of diastereoisomers. The effects of temperature, reaction time, and competition with an additional protein amino acid racemate were also examined. We have found variable amounts of diastereomeric excesses [up to 58% for the longest (αMe)Val homo-peptides]. Our results clearly show that two combined factors are operative in influencing the stereoselection: the nature of the C-terminal chiral residue and the screw sense of the stable secondary structure element. Thus, it is reasonable to imagine a racemic primordial soup of protein amino acids which in the long run is converted to a soup with a more and more abundant enantiomeric excess due to the repetitive fall-out of extraterrestrial C^{α}-tetrasubstituted α-amino acids. At this stage, various possible mechanisms of peptide bond formation may have further amplified this phenomenon, eventually producing the first bioactive proteins.



L32 - Three-dimensional structure of thermolysin-linearized microcin J25: evidence for an essential role of the 11-16 loop in microcin J25 structure and antimicrobial activity.

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Microcin J25, *cyclo*(-V^{1}GIGTPISFY^{10}GGGAGHVPEY^{20}F-) [1], is the unique macrocyclic member among the known microcin antimicrobial peptides produced by *Enterobacteriaceae*. It shows potent activity on various *Salmonella* and *Escherichia* strains. The major structural feature of microcin J25 is a compact structure consisting of a distorted antiparallel β-sheet, twisted and folded back on itself, forming thus the Phe^{21}-Pro^6 and Gly^{11}-His^{16} loops [2]. A cavity only confining the side chains of a valine and a serine, and delimited by two crab pincer-like regions is generated in the structure. Thermolysin cleavage of microcin J25 at the Val^1-Phe^{21} peptide bond located in the Phe^{21}-Pro^6 loop led to an open-chain analog of microcin J25 that keeps a good activity on *Salmonella* strains. The three-dimensional structure of this linear form of the microcin was determined in methanol solution, using two-dimensional NMR spectroscopy and molecular modeling. The structure is characterized by a well-defined region encompassing residues 8-19 and a flexible N-terminal part. Stabilized by several hydrogen bonds, the structure consists of a short antiparallel β-sheet perpendicular to a loop, resulting in a boot-like shaped global fold. The cavity described in the cyclic microcin is not conserved in this structure, as a result of the disruption of the Phe^{21}-Pro^6 loop. By contrast, the well-defined structure of the 8-19 region is remarkably similar in both the thermolysin-cleaved linear variant and the cyclic microcin J25. We discuss the respective roles of the two loops and the ?-sheet on microcin J25 mechanism of action.

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L33 - Conformational investigations of tri and tetrapeptides containing dehydroalanine and dehydrophenylalanine and their influence on Cathepsin C activity

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Dehydroamino acids play a catalytic role in the active sites of some yeast and bacterial enzymes [1] also occur in a variety of peptide antibiotics of bacterial origin, including the lantibiotics [2] (nisin, subtilin, epidermin) and more highly modified peptides.
Dehydroamino acid residues in peptides have been found to influence the main-chain and side-chain dramatically, due to the presence of $C^{\alpha}=C^{\beta}$ double bond [3]. Therefor their presence in a peptide chain produces remarkable conformational consequences. Dehydroalanine adopted a roughly planar conformation with trans orientation for the ψ and ϕ torsions and induced an inverse γ-turn in the preceding residue [1]. Dehydrophenylalanine exerts a β-turn conformation in tetrapeptides and 3_{10}-helical conformation in the case of peptides with longer main-chain [4]. It suggests, that dehydroamino acid residues exert a powerful conformational influence, independent on other constraints. We undertook synthesis and conformational investigations of tri and tetrapeptides containing ΔAla and ΔPhe residues. Since dehydroamino acids are quite reactive and various thiol nucleophiles are known to add to their double bonds [5,6], we hoped that these compounds might act as alkylating inhibitors of cathepsin C (dipeptidyl-peptidase I). Its main function is protein degradation in lysosymes, but it is also found to participate in the activation of neuraminidase and proenzymes of serine proteinases (leukocyte elastase, cathepsin G, granzyme A) [7,8]. Peptides containing dehydroalanine and Δ^{Z}Phe acted only as substrates of the enzyme, with activity comparable with their classic counterparts. Surprisingly, these observations are in good agreement with results of spectroscopic (NMR, CD) and theoretical investigations, which suggested that majority of our compounds adopt extended conformation in solution. The results of investigations for (E)-dehydrophenylalnine derivatives also will be presented.

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L34 - The crystal structure of a peptaibol antibiotic Trichotoxin A50E

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Trichotoxin A50E is a 18-residue peptaibol, isolated from the fungus, *Trichoderma viride*. It is a peptide antibiotic and like a majority of long-chain peptaibols it is capable of forming multimeric ion-channel assemblies in lipid bilayer membranes. The crystal structure of the synthetically prepared peptaibol trichotoxin has been determined at 0.9Å resolution. The structure is predominantly -helix due to nine residues being Aib, a helix-promoting di-substituted amino acid (-methylalanine). Two molecules of trichotoxin, five water and two acetonitrile molecules are present in the unit cell. Bend angles ranging between 8-10 are observed in the structure created by the conserved proline residue in position 13. Two glutamine residues are present in positions 6 and 17 and these contribute to forming the polar face of these amphipathic helices. Unlike the other peptaibols whose crystal structures have been determined, trichotoxin contains only -helix and no 3_{10}-helix. The helix is nearly straight which has important implications for pore formation. A model for an octameric pore has been constructed by assembly of crystallographic monomers. Glutamine residues line the pore, the narrowest diameter of which is 7Å. The glutamine residues are proposed to form a hydrogen bond network between neighbouring helices, for the transport of ions along the length of the pore.

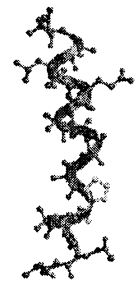

Fig. 1 - The Structure of Trichotoxin

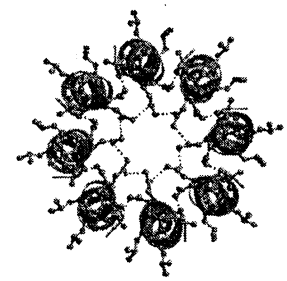

Fig. 2 - The Octameric Pore of Trichotoxin

L35 - The evolution of a simple idea

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Some 50 years after Emil Fischer founded our field of peptide chemistry, an idea occurred to me that might simplify and accelerate the synthesis of peptides and even give higher yields. If a solid support could be used to hold the peptide at one end while it was growing at the other end by stepwise addition of further amino acids I might have a new approach to the problem. The immobilized, but solvated, peptide could be filtered and washed to effect purification at each step without isolation or crystallization. This was the origin of the simple idea of Solid Phase Peptide Synthesis. Over the years the idea has gradually evolved in many ways. In the beginning the problem was simply to find reactants and conditions that would lead to small peptides and demonstrate the feasibility of the new principle. The next stage was to improve each of the parameters, especially the composition and properties of the solid support. This was followed by automation of the process. It was soon shown that nucleic acids and carbohydrates could be synthesized by similar methods. Further evolution of solid phase synthesis included studies on the size range that was accessible; first to hormones of up to 20 or 30 residues and finally to the small protein ribonuclease. Others have extended the upper limit. The remarkable development of ligation methods has further improved the synthesis of proteins, even in aqueous solution without the need for any protecting groups and this approach has been extended to the semisynthesis of proteins of almost unlimited size by ligation of synthetic peptides with natural or recombinant proteins. The extension of the solid phase technique to simultaneous multiple synthesis was discussed as early as 1965, but took another 20 years to develop. This culminated in its application to synthetic combinatorial peptide libraries. We and many other laboratories have turned to this powerful approach. The potential benefit of solid phase synthesis for traditional organic chemistry was also expressed many years ago and recently has been adopted with success by many of the best organic laboratories. They also make use of the combinatorial approach. Further stages in the evolution of the solid phase idea can be expected.

L36 - Fmoc-based solid phase synthesis. An assessment

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The Fmoc protecting group came into routine use in the MRC Laboratory of Molecular Biology in Cambridge in about 1977. It was part of a larger system of solid phase peptide synthesis which included specially designed polar polyamide resins, individual peptide-resin linkage agents, internal reference amino-acids, anhydride and activated ester acylating agents, and later, physically supported resins, continuous flow technology, real time reaction monitoring and complete automation. Not all the features of this system have survived the following twenty five years. The present popularity of the method will be assessed from both scientific and commercial viewpoints.