Posters: topic A6

P 109 Chemistry of Amino-acids P 110

ASYMMETRIC SYNTHESIS OF CIS-AND TRANS-3-PROLINOLEUCINE

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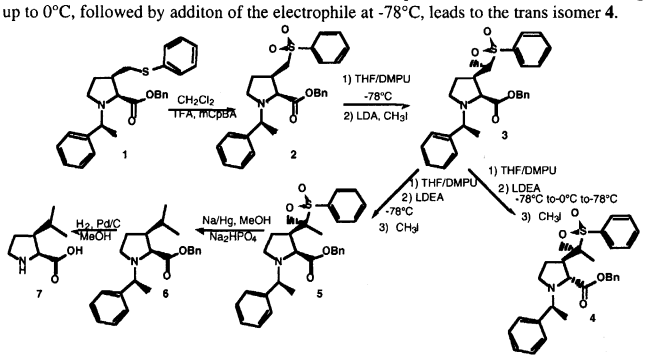
Chimeric prolinoamino acids which combine the ring constraint of a proline and the side chain of an amino acid play an essential role in the discrimination between conformational and steric requirements for binding potency of amino acids and peptides to their receptor. In this regard, we have recently demonstrated the utility of both cis- and trans-3 prolinomethionine in SAR studies of Substance P (SP). The results obtained with these SP analogues pointed out the need of such constrained amino acids to probe subtle interactions in ligand-receptor complexes[1]. To further delineate the interaction between SP and the hNK-1 receptor, we have designed both cis- and trans-3-prolinoleucine (Figure). The asymmetric syntheses of these probes were achieved by dialkylation of the chiral sulfone **2**, obtained from the general methodology we have previously described[2], [3]. The nitrogen protecting group (chiral auxilliary) prevents the deprotonation in position α to the ester group. However the control of the temperature allows the obtention of both isomers : at - 78°C, the second alkylation leads to the cis derivative **5** whereas deprotonation at -78°C and warming up to 0°C, followed by additon of the electrophile at -78°C, leads to the trans isomer **4**.



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AN EASY AND RAPID ACCESS TO ENANTIOMERICALLY PURE POLYFUNCTIONALIZED β-HYDROXY α-AMINO ACIDS

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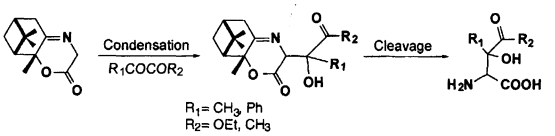
β-hydroxy α-amino-acids have attracted a great deal of synthetic attention during the last decade[1]. This interest stemmed from the biological role played by these compounds[2] and from their dense functionnalization, which makes them useful synthetic precursors for other biologically active molecules[3].

A versatile approach to enantiopure polyfunctionnalized β-hydroxy α-amino-acids is described. The key step involves a diastereoselective condensation of ketones with chiral enolates of the oxazinone.



The flexibility of the methodology has been demonstrated by the synthesis of some new α-amino-acids, which will be used as building blocks in combinatorial chemistry.

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P 111 P 112

SYNTHESIS OF NEW PHOSPHINIC SYNTHONS GENERAL TYPE: Fmoc-Phe-[PO(OAd)CH_2]XaaOH (where Xaa= Arg, Lys, Orn)

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Phosphinic peptides represent an important class of transition state analogues that have been shown to be effective inhibitors for a variety of Zinc-Metalloproteases. Many phosphinic peptides, containing various alkyl or arylalkyl side chains, have been successfully prepared. However, the synthesis of phosphinic synthons bearing functional side chains appears to be more complicated.
Herein, we present the synthesis of three new phosphinic pseudodipeptide synthons having at P_1' position amino–acid analogues of Lysine, Arginine and Ornithine. The 1, 1, 4, 4-tetramethyl-disilylethylene-1, 4-diyl was used as temporary protecting group for the ε- and δ-amino functions of Lysine and Ornithine respectively, while the hydroxyphosphinyl was protected by the 1-adamantyl group which is compatible with Fmoc-solid phase strategy. The synthesis of Arginine analogue was achieved by reaction of the δ-amino group of the Ornithine synthon with N,N'-bis(*tert*-butoxycarbonyl)thiourea and chloro-1-methyl pyridinium iodide (Mukaiyama's reagent).
These new phosphinic synthons could be used as key intermediates for the development of phosphinic peptide inhibitors of various Zinc proteases, which are able to cleave specifically peptide bonds preceding basic residues such as Lysine or Arginine.

SYNTHESIS OF OPTICALLY ACTIVE FLUORINATED ANALOGUES OF GLUTAMIC ACID AND GLUTAMINE WITH POTENTIAL BIOLOGICAL ACTIVITY.

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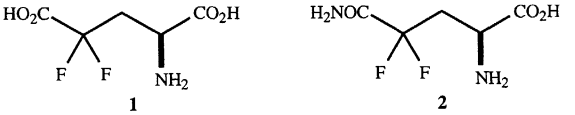
In living cells, glutamine represents one of the main storage forms of nitrogen and is a major physiological source of ammonia for the biosynthesis of aminoacids, aminosugars, purine and pyrimidine nucleotides and coenzymes.
Glutamine-dependent amidotransferases perform nitrogen transfer from the amide group to various electrophiles. This migration results from the cleavage of the amide bond by an active site cysteine residue to give a covalent γ-glutamyl thioester and ammonia. Ammonia is then transferred to the electrophile and hydrolysis of the thioester gives glutamate and regenerates the catalytically competent cysteine.
Fluorinated analogues of glutamic acid and glutamine are expected to interfere with such biological processes due to the strong electron withdrawing effect of fluorine atom (without significant steric consequence), inducing modulation of binding and/or electronic properties. These compounds might therefore behave as reversible or irreversible active site-directed enzyme inhibitors.
Particularly, 4,4-difluoroglutamic acid **1** and 4,4-difluoroglutamine **2** could lead to anticancer agents resulting from the inhibition of purine and pyrimidine biosynthesis or to compounds active against pathogenic fungi by inhibition of cell wall edification.
First results in the synthesis of optically active derivatives of **1** and **2** from serine will be described.



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Posters: topic A6

P 113 Chemistry of Amino-acids P 114

SYNTHESIS OF A NOVEL CLASS OF α,α-DISUBSTITUTED AMINO ACIDS: α-ALKYL-γ-DIHYDROXYPHOSPHORYL GLUTAMIC ACIDS.

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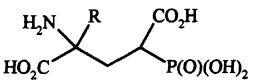
Amino acids possesing phosphono- group in its side-chain are important class of compounds interfering with excitatory amino acid receptors. As an example: both enantiomers of 2-amino-4-phosphonobutyric acid (AP-4) act either as a weak, non-selective NMDA antagonist (D-enantiomer) or selective group III mGlu agonist (L-enantiomer). α-Methylation of this compound leads to the selective group III mGlu antagonist in some systems.

In the present study we disclose a simple procedure providing an access to α-substituted-γ-phosphonoglutamic acids of the general formula:



R= methyl, ethyl, benzyl *i*-propyl, *i*-butyl

The crucial step in our synthetic pathway is Michael addition of 2-phenyl-4-alkyl-5(H)-oxazolone derived from α-amino acid to triethyl-α-phosphono acrylate. The subsequent oxazolone ring opening and stepwise deprotection of all functional groups constitute the remaining part of the synthesis. It must be pointed out that some intermediates bear phosphonodiester and carboxylic ester groups at the γ-carbon. This makes them potential substrates for introducing alkylideno functionality *via* Horner-Wadsworth-Emmons reaction.

This work was supported by Polish State Committee for Scientific Research (KBN - Dz.St.).

POLY(ETHYLENE GLYCOL) AS POLYMERIC SUPPORT IN THE PARALLEL SYNTHESIS OF α-AMINOACIDS

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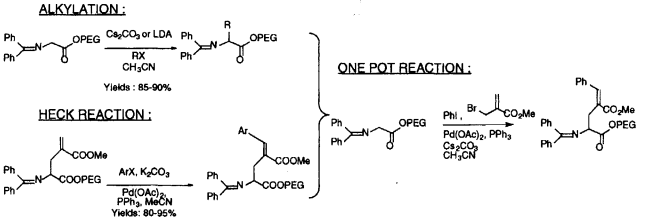
The liquid phase method, known for more than 30 years in peptide synthesis, has been recently applied to the synthesis of small organic molecules and to combinatorial chemistry.[1] Using soluble polymer supports such as polyethylene glycol (PEG), this method combines some advantages of solution and solid phase methods. The synthesis is carried out in homogeneous solution while purification is performed by precipitation of the polymer. Furthermore, molecules anchored to soluble polymers can be readily characterized by NMR spectroscopy and mass spectrometry.[2]

We present herein the synthesis of non-proteinogenic α-amino acids (more specifically excitatory amino acids) using an alkylation reaction on a PEG supported glycine[3] and a Heck reaction on a methyl acrylate moiety.[4] We have demonstrated in both reactions the dual role of the polymer as phase-transfer catalyst and polymeric support.



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P 115 P 116

SYNTHESIS OF NOVEL PEPTIDE ANALOGUES EMBODYING 4-[(PHOSPHONO)2',2'-DIFLUORO-1'-HYDROXYETHYL] PHENYLALANINE. INHIBITION STUDIES TOWARDS PROTEIN TYROSINE PHOSPHATASES.

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The phosphorylation states of strategic tyrosines in a variety of intra and extra cellular proteins is directly or indirectly associated with a great number of physiological processes including cell-cell or cell-matrix interactions, ligand binding and signal transduction. Protein tyrosyl phosphorylation is regulated by protein tyrosine kinases (PTK) and -phosphatases (PTP). Specific inhibitors of PTK and PTP could be useful as biochemical tools or, more importantly, as pharmaceutical agents. Peptidic inhibitors based on the structures of specific PTP substrates that contain non-hydrolysable mimetic of phosphotyrosine are obvious primary targets.

We describe the synthesis of a novel non-hydrolysable phophotyrosyl mimic N-Fmoc-4-[(diethylphosphono)2',2'-difluoro-1'-hydroxyethyl] phenylalanine suitable for utilisation in solid phase peptide synthesis. This analogue has several potential advantages over others previously described. Introduction of fluorine atoms simulates hydrogen bonding interactions similar to the phosphate ester oxygen in phosphotyrosine. The addition of hydroxyl group enabled to mimic a water molecule found in the reactive catalytic complex. Its structure was confirmed by X-ray diffraction analysis.

The new phosphotyrosine analogue was incorporated into a series of peptide sequences belonging to the autophosphorylation sites of epidermal growth factor receptor. These ones have been identified to exhibit good kinetic properties towards several model PTPases. Namely, inhibitory properties of the synthesized peptides have been studied towards *Yersinia enterocolitica* PTPase and *Trypanosoma Brucei* PTPase.

PSEUDODIPEPTIDES SYNTHESIS VIA α-HYDROXY ACIDS TRIFLATES

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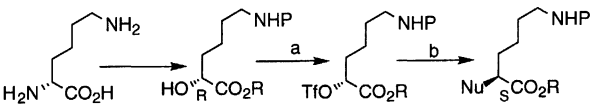
During our structure-activity study of the hematopoietic tetrapeptide NAc-Ser-Asp-Lys-Pro-OH[1], analogues modified on peptide backbone were needed, in particular on Asp-Lys bond cleaved *in vivo* by Angiotensin Converting Enzyme (E.C.A.). We thus prepared pseudodipeptides analogues de Asp-Lys.

The triflate was obtained through a sequence of reactions starting from D-Lysin deamination, appropriate protection of the ε-amino and α-carboxylic group followed by treatment with triflic anhydride [2] (3 equiv.). Substitution of the triflate by diverse N or S nucleophiles was performed with high stereoselectivity. Coupling of the amino compounds with an aspartic acid derivative through the acyl fluoride method using TFFH gave pseudodipeptides with good yields. Triflates of the peptide where R = Pro-OBu[t] were also prepared.



a : $(Tf)_2O$, lutidine, CH_2Cl_2; b : nucleophile, NEt_3 , CH_2Cl_2

R=Bzl or ProOBut, Nucleophile: NH_2CH_3, $NH_2CH_2CH(OEt)_2$, NH_2OBzl, $BocNHNH_2$, $CH_3COS^-K^+$

References:
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2. Effenberger F., Burkard U., Willfahrt J., *Liebigs Ann. Chem.* **1986**, 314-333,
Weber I., Potier P., and Thierry J., *Tetrahedron Lett.* **1999**, *40*, 7083-7086

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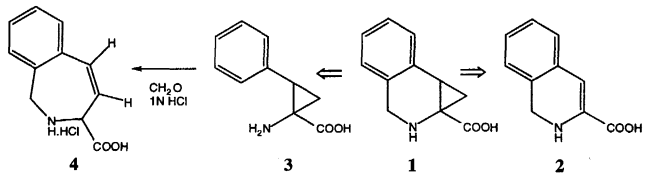
THE SYNTHESIS OF 2,3-DIHYDRO-1H-2-BENZAZEPINE-3-CARBOXYLIC ACID : AN UNEXPECTED FINDING AND A MECHANISTIC STUDY.

Karolien Van Rompaey[a], Dirk Tourwé[a], José C. Martins[b], Geza Toth[c], Csaba Tömböly[c], Guyla Wittmann[c], Norbert De Kimpe[d]; [a]Department of Organic Chemistry (ORGC) and [b]HNMR Center, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium; [c]Biological Research Center, Hungarian Academy of Sciences, Isotope Laboratory, H-6701 Szeged, Hungary; [d]Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, University of Gent, Coupure Links 653, B-9000 Gent, Belgium

The amino acid 3,4-methano-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid **1** was designed to combine the conformational constraints of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) with those of a 2,3-methano-amino acid.



4 **3** **1** **2**

The synthesis of **1** can be performed in two ways, either by the cyclopropanation of α,β-dehydro-Tic **2**, a successful method followed by Czombos and De Kimpe[1]; or by the Pictet-Spengler cyclisation of α,β-cyclopropyl-Phe **3**.

The result of this reaction, which required very mild conditions (1N HCl and r.t.), was an unstable compound, which was not the expected **1**, but it was identified by NMR-analysis as 2,3-dihydro-1H-2-benzazepine-3-carboxylic acid **4**.

A mechanistic study was performed by means of NMR-experiments and selective deuterium labelling. The results of the experiments using a pentadeuterophenyl label suggest an initial [3,3] sigmatropic rearrangement, followed by a [1,5] hydrogen shift to yield α-deuterated **4**.

[1] J. Czombos et al., J. Org. Chem., in press

APPLICATION OF RING CLOSING METATHESIS TO THE SOLUBLE POLYMER SUPPORTED SYNTHESIS OF ANALOGUES OF PIPECOLIC ACID.

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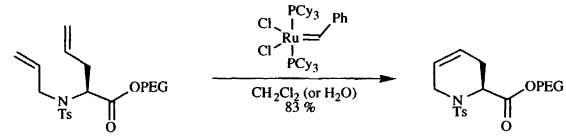
Ring closing metathesis (RCM) is a powerful method for the construction of functionalized carbocycles and heterocycles.[1] The discovery of well-defined ruthenium alkylidene catalysts has greatly expanded the scope and versatility of this reaction.

In an ongoing research project dealing with the synthesis of small molecules using the soluble polymer support techniques,[2] we have thought of applying the RCM on a substrate anchored to a poly(ethylene glycol) (PEG).

We present herein the synthesis of a pipecolic acid analogue perfomed in an organic solvent or in water without any racemization at the α-carbon center as checked by chiral HPLC analysis after cleavage.



We have demonstrated that RCM is a good tool for the synthesis of small molecules on a soluble polymer.

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Posters: topic A7

P 119 Ligation Chemistry/Protein Modification P 120

CHEMICAL SYNTHESIS OF PROTEIN TARGETS USING EXTENDED CHEMICAL LIGATION

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The use of native chemical ligation[1] reactions has facilitated the total chemical synthesis of many protein targets of pharmaceutical and biochemical importance. Native chemical ligations exploit a chemoselective reaction that requires the presence of a cysteine residue in the target sequence. In order to broaden the applicablity of the native ligation concept to *all* protein sequences, we have developed a generalized methodology called extended chemical ligation (ECL). Using the ECL concept, protein sequences lacking cysteine residues can be readily prepared. We first established the utility of the ECL reaction by preparing a series of model peptides up to ~ 70 residues in length. ECL reactions were then used to prepare a family of native proteins based on cytochrome b562, a 106 residue four helix bundle. The synthetic cytochromes were reconstituted with their heme active sites and fully characterized by biophysical methods.

1 Dawson,P.E.; Muir,T.W.; Clark-Lewis, I; Kent, S.B.H. *Science* **1994**, 266 776-779

ENGINEERED MUTANTS OF RANTES : NANOMOLAR INHIBITORS OF HIV-1 CORECEPTOR ACTIVITY WITH REDUCED HEPARIN AFFINITY

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Consideration of circulatory half-life is of great importance in the development of a therapeutic agent. The potency of a drug *in vivo* may depend more on its ability to evade systemic clearance than its precise efficacy in an *in vitro* test. Our laboratory has produced potent *in vitro* inhibitors of R5 HIV-1 envelope fusion and viral infection using N-terminally modified derivatives of the CC chemokine RANTES, notably Aminooxypentane (AOP) RANTES and Nonanoyl (NNY) RANTES. In a SCID mouse model of HIV-1 infection these compounds successfully inhibit infection but their circulatory concentrations after treatment suggest rapid systemic clearance. Chemokines such as RANTES bind to cell surface glycosaminoglycans (GAG) as well as their specific seven transmembrane receptors. GAG binding may increase the local concentrations of chemokines at cell surfaces influencing their role in cellular activation. It may also act as a sink to a therapeutically administered chemokine, reducing its circulatory concentration and efficacy. Previously we identified groups of basic amino acids in the RANTES molecule that contribute to GAG binding. Here we describe the synthesis and characterisation of 21 novel RANTES variants. Seven basic residues have been mutated to alanine and each protein synthesised with the natural amino acid or an AOP group or an NNY group at the amino terminus. We measured heparin affinity as a model of GAG for each variant and also ability to inhibit R5 HIV-1 envelope coreceptor usage. Preliminary data suggests a correlation between heparin affinity and inhibition of coreceptor mediated cell to cell fusion. Several of these mutants are variants with reduced heparin affinity that maintain sub-nanomolar fusion inhibition. They may have improved pharmacokinetics and efficacy *in vivo* compared to the parent molecules.

Funded by NIH (OC) GWER (RF), the Swiss National Research Foundation (OH)

P 121 P 122

Minimizing the pharmacophore of a potent HIV-1 inhibitor

Chantal Devin-Chaloin, Oliver Hartley, Keith Rose and Robin Offord.
Correspondence: tél: 41-22-702-54-73
E-mail: chaloic8@etu.unige.ch

NNY RANTES (n-nonanoyl-des-ser1-RANTES) is an extremely potent inhibitor of CCR5-mediated entry of HIV into T-lymphocytes, having an IC50 value more than an order of magnitude lower than our original lead compound, AOP RANTES (the n-pentane oxime of [glyoxylyl] RANTES). Both compounds are believed to act by causing profound and sustained downmodulation of CCR5, and as such could be considered as members of a novel class of receptor inhibitor.
The combination of automated peptide synthesis and native chemical ligation techniques to join unprotected peptides via amino-terminal cysteine residues greatly facilitates the synthesis of chemokine sized proteins, giving rapid access to novel analogues. We used NNY-RANTES as a lead compound in an attempt to define which parts of the molecule are necessary for its activity. Our strategy involved removing stretches of amino acids from NNY-RANTES and replacing them with chemical linkers of appropriate length.
Since N-terminal residues are important for binding and receptor signalling, and residues towards the C-terminus are required for biologically important interactions with cell surface glycosaminoglycans, we decided to begin by truncating residues 12 to 32 of NNY RANTES. We used two types of linker to replace part or all of this region: "poly Gly" or "polyamide". One of the compounds made in this way retained significant inhibitory activity, and we are currently investigating truncations at the C-terminus of NNY-RANTES. We hope that this type of information will help identify the physico-chemical properties and the spatial location of the regions of NNY-RANTES that are responsible for its activity. Such information could then inform the rational design of small, non-protein molecules with a similar potency and mechanism of activity.

EVALUATION AND OPTIMIZATION OF A NEW LINKER FOR THE SYNTHESIS OF C-TERMINAL PEPTIDE α-OXO-ALDEHYDES.

Jean-Sébastien Fruchart, Cyrille Grandjean, Hélène Gras-Masse, and Oleg Melnyk, from UMR CNRS 8525, IPL/IBL 59021 Lille, France

This communication will focus on the synthesis, evaluation and optimization of a new tartaric acid based linker, which allows the efficient synthesis of C-terminal peptide α-oxo-aldehydes[1].
In first time, (+)-dimethyl-2,3-O-isopropylidene–D-tartrate (**IPT**) has been anchored to an amino PEGA or amino PEG-PS resins (Argogel, Novagel) through an amide bond. The second ester moiety was reacted with a symetrical diamine to give a derivatized amino PEGA support ready for Fmoc/*tert*-butyl SPPS. Then, after deprotection of the peptide and the 1,2-diol on the solid phase following peptide elongation, the periodic oxidation of the peptidyl-resin permitted the simultaneous cleavage of the product from the solid support and the formation of the α-oxo-aldehyde moiety. In water/acetic acid mixtures, reaction times as short as 30s were enough to generate the α-oxo-aldehyde. Met-containing α-oxo-aldehydes were better obtained at pH 6.0 using dimethylsulfide as cosolvent. Various structures were synthesized using this methodology (4 to 30 AA peptides, Cys(S*t*Bu)-containing peptides, **IPT**-linker prepared with differents diamines). Moreover, the **IPT**-linker allowed the elaboration of lysyl-dendrimers bearing chloroacetic groups at the periphery and a C-terminal α-oxo-aldehyde. C-terminal peptide aldehydes thus generated were found to be useful partners in hydrazone, oxime and thiazolidine chemical ligations. Polyfunctional lysyl-dendrimers allowed the one-pot synthesis of glycodendrimers linked to antigenic peptides using thioether and hydrazone chemical ligations.
In a second time, to prevent the destruction of the amide bond between the tartrate and amino moiety of resins during treatments with diamines or repeated piperidine treatments, shown up by HR-MAS NMR, the synthesis of tartared linker has been optimised in order to obtain resins more funtionnalized and to allow more efficient synthesis of long peptides.

[1] J.-S. Fruchart; H. Gras-Masse; O.Melnyk; *Tetrahedron Lett.*, **1999**, 40, 225-6228. Melnyk, O. ; Fruchart, J.-S. ; Bourel, L. ; Gras-Masse, H. Support solide pour synthèses organiques et ses applications, FR990524 (04/21/1999).

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CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF LINEAR PSEUDOPEPTIDES

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Metal complexes are often components in the active sites of enzymes, e.g. carboanhydrase and play an important role in catalytic mechanisms. To investigate the metal ligand interactions we have synthesized linear tripode ligands of the general structure **Bz-His-X-His-NH_2** with a substituted N-alkyl glycine residue (X) in the core position. The incorporation of a pseudopeptide unit allows the variation of the chain length and the character of the donor atoms. The synthesized metal complexes are obtained as amorphous powders and can therefore not be analyzed by X-ray crystallography. Thus, NMR, potentiometry, spectrophotometry and MS were used for the investigation of the metal complexes in solution. ESI-MS was used to estimate semiquantitatively the complexation tendency of the peptide ligands towards transition metals (Zn, Cu, Co, Ni, Fe). Most of the metal complexes are monomeric. The species distribution at different pH values and the complex stability constants were determined by potentiometry. NMR investigation of the Zn complex of Bz-His-N($CH_2CH_2NH_2$)Gly-His-NH_2 shows different structures of the metal complex in water and DMSO. We found that the character of the metal anion has an influence on the ligand substitution reaction and is therefore important for the formation of the metal complexes as was investigated by VIS-spectrophotometry. To check the catalytic properties of the synthesized metal complexes in comparison to natural metalloenzymes, like proteases, phosphatases or superoxide dismutases, the hydrolysis of ester bonds as well as the activation of oxygen have been examined.

CHARACTERISATION OF THE NEUROPEPTIDE Y RECEPTORS BY PHOTOAFFINITY LABELLING AND SUBTYPE SELECTIVE ANTIBODIES

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Five receptors, named Y_1-, Y_2-, Y_4-, Y_5- and y_6-subtypes have been cloned, which belong to the rhodopsin-like G-protein coupled, 7 transmembrane helix-spanning receptors and bind either the 36-mer neuromodulator Neuropeptide Y (NPY) with nanomolar affinity or pancreatic polypeptide (PP). In this study, the Y_2-receptor subtype expressed in a human neuroblastoma cell line (SMS-KAN) and in transfected Chinese hamster ovary cells (CHO-hY_2) was characterized on the protein level by using photoaffinity labelling and anti-receptor antibodies. Two photoactivatable analogues of NPY were synthesized, in which a Tyr residue was substituted by the photoreactive amino acid 4-(3-trifluoromethyl)-3*H*-diazirin-3-yl-phenylalanine ((Tmd)Phe): [N_α-biotinyl-Ahx_2, (Tmd)Phe^{36}] NPY (Tmd36) and the Y_2-receptor subtype selective [N_α-biotinyl-Ahx_2, Ahx^{5-24}, (Tmd)Phe^{27}] NPY (Tmd27). Both analogues were labelled with ^{3}H-succinimidyl-propionate at Lys^4 and bind to the Y_2-receptor with affinity similar to the native ligand. A synthetic fragment of the second (E2) extracellular loop was used to generate subtype selective anti-receptor antibodies against the Y_2-receptor. Photoaffinity labelling of the receptor followed by SDS-PAGE and detection of bound radioactivity and SDS-PAGE of solubilized receptors and subsequent Western blotting revealed the same molecular masses. Two proteins correspondingly have been detected for each cell line with molecular masses of 58 ± 4 kDa and 50 ± 4 kDa, respectively[1]. Similar studies were performed for the Y_1- and the Y_5 receptor, that demonstrate that both other subtypes were also highly glycosylated transmembrane proteins.

[1]*N. Ingenhoven, C. Eckard, D. Gehlert, A. G. Beck-Sickinger (1999) Molecular characterisation of the human Y_2 receptor, Biochemistry 38, 6897-6902.*

P 125 P 126

NATIVE CHEMICAL LIGATION BY FMOC CHEMISTRY

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Native chemical ligation is rapidly establishing itself as a method for reproducible, practical total chemical synthesis of small-to-medium size proteins [1]. According to this method, two unprotected peptides, the first one with a C-terminal thioester and the second one with an N-terminal cysteine, are joined together by a chemoselective reaction in aqueous solution at neutral pH. The key intermediate for this reaction, the C^α-thioester, has so far been prepared only by the use of the least prevalent Boc solid phase method. We [2], as well as others [3], have recently described a novel method for the solid phase synthesis of thioesters by the most prevalent Fmoc/t-Bu method. Our method is based on the use of a 3-carboxypropanesulfonamide safety-catch linker, which is fully stable to repetitive exposure to the basic conditions needed for Fmoc cleavage. Activation with diazomethane or iodoacetonitrile followed by displacement with a suitable thiol produces the thioester in good to excellent yields. The conditions giving rise to the highest yield of the target thioester are activation with TMS-diazomethane followed by displacement catalysed by thiophenoxide. In situ thiol exchange ensures formation of the more stable thioester derived from the excess thiol.
The new method will be illustrated by several examples of Native Ligation reactions where all the components are assembled by Fmoc/t-Bu chemistry, performed on all the most commonly used SPPS resin supports; these include the synthesis of a transmembrane protein [4] and synthetic vectors for non-viral gene delivery.
Although emphasis is given to Fmoc chemistry, our method is also compatible with Boc chemistry, and allows for a combination of the two (for example to prepare cyclic or branched peptide thioesters). Moreover, all the necessary reagents are commercially available.

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2. Ingenito, R., Bianchi, E., Fattori, D. and Pessi, A., *J. Am. Chem. Soc.* **121** (1999) 11369.
3. Shin, Y., Winans, K. A., Backes, B. J., Kent, S. B. H., Ellman, J. A. and Bertozzi, C. R., J. Am. Chem. Soc. **121** (1999) 11684.
4. Bianchi, E., Ingenito, R., Simon, R. J., and Pessi, A., *J. Am. Chem. Soc.* **121** (1999) 7698.

The development and application of a novel hydrazine linker

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Abstract: A novel hydrazine linker was developed which is very easy to make and stable enough for long storage at RT. This linker differentiates the reactivity of the two nitrogen centres and, moreover, allows facile monitoring of the resin loading. The linker also affords intermediates for the chemical coupling of peptide fragments and C-terminal modifications.

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CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITY OF INTERLEUKIN 8 ANALOGS

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Earlier, having determined epitope specificity of neutralizing monoclonal antibodies directed to human interleukin 8 (IL-8) and biological activity of a panel of synthetic peptides overlapping its sequence, we describe regions 1-12, 23-33 and 53-72 as important for biological activity manifestation [1]. In an effort to obtain an antagonist of IL-8, we have synthesized and studied activity of analogs that have multiple amino acid substitutions within these regions resulting in analogs with point mutations in a decrease in Kd and an increase in EC30 [2].
Using method of native chemical ligation [3] we have synthesized [1-72, Ala^{33}]IL-8 (1); [4-72, $Ala^{4,5,33}$]IL-8 (2); [3-72, $Ala^{3,4,5,6,33}$,Leu^{6},Arg^{9},Phe^{11},Leu^{17},Glu^{23},Lys^{24}]IL-8 (3) and [3-72, $Ala^{3,4,5,6,33}$,Leu^{6},Arg^{9},Phe^{11},Leu^{17},Glu^{23},Lys^{24},Ala^{55},Ser^{68}]IL-8 (4). The peptides were tested for functional activity by measuring induction of hemotaxis and exocytosis of human neutrophils. The interaction of these analogs with human CXCR1 and CXCR2 receptors expressed in stably transformed murine fibroblasts were analyzed. The experiments demonstrated that polypeptide 1 had biological activity and binding properties identical to recombinant wild type IL-8. Analogs 2, 3, 4 lacked IL-8 activity. However, according to the binding data Kd's of the analogs were at least on two order of magnitude higher than Kd of wild type IL-8. Obtained results confirm significance of sequence 4-6 (ELR) both for biological activity and primary binding with specific receptors.

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SOLID-PHASE SYNTHESIS OF UNPROTECTED PEPTIDE BEARING TWO ALDEHYDE FOR ORTHOGONAL PEPTIDE LIGATION BY OXIME BOND.

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The development of new vaccine strategies requires the production of small artificial proteins, either linear or branched constructs, consisted of covalently attached T-helper (Ta), T- cytotoxic (Tc) and B-cell (B) epitopes. To obtain these constructs in a modular way, we have taken advantage of the chimioselective ligation *via* an oxime bond between an aldehyde- and an aminoxy- peptide. This note deals with the introduction of two aldehydes on the same peptide in order to permit two chemical ligations in an orthogonal way.
We have recently elaborated a very simple solid-phase synthesis of a C-terminal peptide aldehyde to obtain the aldehyde function masked as an acetal (1). The key step was the cleavage of the peptide-PAM-resine by aminolysis with aminoacetaldehyde-dimethylacetal. We present here that this original strategy is compatible with the generation of a second function aldehyde by oxidation of a serine fixed on the ε-NH2 of a lysine. The validation of the strategy of double ligation was conducted on model sequences.
This methodology was further applied to natural epitopic sequences. The peptide bearing two aldehyde functions was of 32 amino acid long, corresponding to two copies of the Ta-epitope; the TaTa sequence exhibited a better activity than the Ta sequence alone (2). The synthesis of the 32-mer peptide aldehyde needed to be optimized. With that in mind, we tested the influence of the peptide length, the temperature, solvent and the support on the reaction of aminolysis. During these experiences, several side reactions were characterized by HPLC and electrospray mass spectrometry.

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2.Partidos C.D., Delmas, A., and Steward, M.W. (1996) Molec. Immunol. 33, 1223-1229.

P 129 P 130

PRO-NPY AND TRUNCATED ANALOGUES ARE SUB-STRATES FOR PROHORMONE CONVERTASE PC1/3

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The biosynthesis of the biologically active 36-mer peptide amide neuropeptide Y (NPY) is accomplished by a three step procedure. The 97-mer pre-pro-NPY consists of a 28-mer leader sequence and the 69-mer pro-NPY, which is enzymatically cleaved at a single dibasic Lys38-Arg39 site. This cleavage results in the formation of the C-terminal peptide on NPY, the so-called CPON with so far unknown function, and pro-NPY 1-39 which is further processed by carboxypeptidase B-like and peptidylglycine α-amidating monooxygenase enzymes. A prohormone convertase (PC) enzyme family has been identified to cleave prohormones at basic amino acid pairs to produce bioactive peptides. Known members include PC1/3, PC2, Furin, PACE4, PC4, PC5/6 and PC7. We recently could demonstrate *in vivo* with a vaccinia virus derived expression system in Neuro2A and NIH T3 and *in vitro*[1] that especially PC1/3 can cleave pro-NPY at the dibasic site. I n addition cleavage *in vitro* of shortened pro-NPY substrates, (20-49)-pro-NPY, (28-43)pro-NPY and analogues, that contain the dibasic cleavage site suggest that substrate length discriminates PC1/3 and PC2 processing activity. We now investigated the relevance of selected positions for cleavage. The peptides were obtained by solid phase peptide synthesis and a combination of native and expressed chemical ligation. For native chemical ligation both segments are synthesized by solid phase peptide synthesis using Fmoc/tBu-strategy. Expressed chemical ligation allows the chemoselective addition of a synthetic peptide to a recombinant protein. An alanine-scan at several positions of the peptide sequence did not influence the conformation as measured by circular dichroism but significantly varied the enzymatic cleavage activity. Arg residues within the sequence of NPY were found to be most important for efficient cleavage by PC 1/3 and demonstrate that specific interaction of enzyme and prohormone plays a crucial role.

[1]*N. Brakch et al. (1997), Biochemistry 36, 16309-16320.*

COMPLEXATION OF METAL IONS BY CYCLIC PSEUDOPEPTIDES

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The structures of two different cyclic pseudopeptides were designed by COSMOS™ and TRIPOS force field with an enlarged set of atom types - calculations for complexation of metal ions. The cyclic pseudohexapeptide (**1**)

cyclo[His-Lys-N(CH_2-CH_2-NH_2)Gly-ßAla-N(CH_2-CH_2-NH_2)Gly-Gly] (1)

designed for Ni^{++} complexation, was assembled on 2-chlorotrityl chloride resin by the Fmoc-strategy using preformed N-functionalized glycine derivatives. The second structure, a cyclic tetrapeptide (**2**)

cyclo[His-Ser-His-Ahc] (2)

designed for complexation of Co^{++} and containing amino hexanoic acid (Ahc) was also synthesized on this resin and cyclized in solution. For both structures the complexation of Zn^{++}, Ni^{++}, Fe^{++}, Fe^{+++}, Co^{++} and Cu^{++} was estimated qualitatively and semiquantitatively by MALDI- and ESI-MS. The conformations of the formed stable complexes were calculated from NMR- and CD-measurements. We additionally investigated the binding of CO_2 and O_2 as well as the catalytic activities as hydrolases of our metal complexes formed from both cyclic peptides. Thus, we used p-nitrophenyl esters, lipids or nucleic acids as substrates to estimate K_M and molecular activity.

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BIOLOGICALLY AND IMMUNOLOGICALLY ACTIVE PEPTIDES DESIGNED ACCORDING TO THE SEQUENCE OF A NEW ONCOPROTEIN MN/CA IX

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A novel tumor-associated protein termed MN/CA IX (briefly MN) is a cell surface protein, which is ectopically expressed in certain human carcinomas (cervical, renal, lung, colorectal etc), in some of these in almost 100%. Normally, MN is present only in stomach and bile duct mucosa. MN has properties which make it a promising diagnostic marker and a target molecule for rational therapy. In the present work we demonstrate that MN protein is a cell adhesion molecule: cells attach to immobilized MN. Monoclonal antibody M75 abrogates cell attachment to MN. Thus the binding site of MN is identical to, or overlapping with the epitope of M75. It is located in the proteoglycan (PG) domain, encompassing a tandem repeat of the motif GEEDLP (4 identical +2 modified repeats). We synthesized a series of peptides designed according to the sequence of PG region. The minimum peptidic sequence competing in binding between MN and M75 was PGEEDLP. This peptide therefore represents the M75 epitope. Using a phage display library of random heptapeptides (Ph.D.-7, NEB) we identified the peptide sequence ITFNAQY with affinity to M75 epitope. This peptide was synthesized with alanine added on both ends, it competes with M75 for the epitope and inhibits adhesion of cells to MN protein. Like many other tumor cells lines, HT29 cells (from human colorectal carcinoma) can grow as colonies in soft agar, manifesting their anchorage independence. This can be abrogated by M75 antibody as well as by peptide AITFNAQYA; neither of them inhibits the growth of HT29 anchored on solid substrate. These data support the view that binding of MN protein to its receptor is a step relevant in oncogenesis.

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COMPUTATIONAL CHEMISTRY AND OPIOIDMIMETICS: RECEPTOR-LIGAND INTERACTIONS OF THREE CLASSES OF BIOLOGICALLY POTENT PEPTIDES.

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Attempts to evaluate the three-dimensional structure of opioid receptors (δ, μ, κ) using x-ray crystallography or nuclear magnetic resonance spectroscopy have been unsuccessful due to the lipophilic properties of G-protein coupled receptors. However, since the molecular cloning of the opioid receptor subtypes, development of chimeras and site-specific mutagenesis studies identified general regions and specific amino acids involved in ligand association. This information enabled the development of computational models proposing mechanisms of interaction for agonists and antagonists. We developed receptor-ligand models for three classes of biologically potent peptides. One class involved two amphibian skin peptides; the δ-receptor agonist Y-*m*-F-H-L-M-D-NH_2 (DLT), and the δ-receptor antagonist, Y-*m*-F-H-*t*-L-M-D-NH_2 ([*t*-Leu^5]DLT). R291 or R292 of the δ-opioid receptor provided counterions for negatively charged D^7 in both peptides. V295 and V296 formed hydrophobic interactions with L^5 and *t*-L^5. F^3 was inserted into the aromatic pocket of the receptor. The hydroxyl of Y^1 formed a hydrogen bond with H278 and an electronic interaction was modeled between the N-terminal amine of each peptide and W284. X-ray crystal structures of two potent peptides containing 2',6'-dimethyltyrosine (Dmt) and 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) were docked into proposed binding sites of the δ- and μ-opioid receptors. The binding interaction of the δ-selective antagonist, *N,N*-$(Me)_2$-Dmt-Tic-OH (K_i^{μ}/K_i^{δ} = 20,636) was similar to DLT except the N-terminal amine formed a hydrogen bond with T213. E229 in the μ-receptor provided a counterion for N-terminal amine of the μ-agonist H-Dmt-Tic-NH-1-adamantane while the hydroxyl of Dmt formed a hydrogen bond with H297 and the adamantyl interacted with hydrophobic residues V316 and W318. A similar model was proposed for the μ-agonist endomorphin derivative, H-Dmt-Pro-Phe-isoquinolyl except Phe^3 was inserted into an aromatic pocket. These models will be useful as pharmacophores for design of novel opioidmimetics to test the proposed binding mechanisms.

MAG: A HELICOGENIC α-AMINO ACID WITH A SIDE-CHAIN CARBON-CARBON DOUBLE BOND

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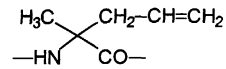
By a chemo-enzymatic approach, developed at DSM Research, we synthesized the optically pure C^{α}-methylated α-amino acid L-Mag (C^{α}-methyl, C^{α}-allylglycine), characterized by a side-chain C^{γ}=C^{δ} bond. By solution methods we prepared a series of model peptides to the pentamer level containing L-Mag in combination with either Aib or Ala. The peptides were fully characterized and their conformational preference was determined in solution by FT-IR absorption and ^{1}H NMR techniques. X-Ray diffraction analyses of $^{\oplus}H_2$-L-Mag-$O^{\ominus}$, Piv-L-Mag-NH*t*Bu, and three peptides (Boc-L-Mag-D-Ala-OMe, Boc-Aib-L-Mag-Aib-OMe, and Boc-L-Mag-L-Ala-L-Ala-L-Mag-D-Ala-OMe) were also carried out.



Mag

The results of the solution conformational analysis, combined with those extracted from the X-ray diffraction study, strongly favour the conclusion that N^{α}-acylated Mag-based tripeptide esters have a great tendency to fold in a β-turn conformation, while the most populated structures adopted by tetra- and pentapeptides are two consecutive β-turns (incipient 3_{10}-helix) and the 3_{10}-helix, respectively. These conclusions are in excellent agreement with those already reported for other C^{α}-methylated α-amino acids. As for the relationship between Mag chirality and peptide helix handedness, the available X-ray diffraction data indicate that L-Mag can easily be accomodated into a right-handed helix.
We expect that Mag, with its remarkable conformational bias towards β-turns and the 3_{10}-helix and the concomitant chemical reactivity of its allyl side chain, would become an important component in the arsenal of peptide chemists.

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INFLUENCE OF A ROTAMER POPULATION ON FLUORESCENCE DECAY OF THE TYROSINE DERIVATIVES STUDIED BY FLUORESCENCE, ^{1}H NMR SPECTROSCOPY AND THEORETICAL CALCULATION

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Tyrosine fluorescence decay in water solution is mono-exponential, whereas that of tyrosine derivatives is multi-exponential. Such heterogeneity in fluorescence decay of tyrosine derivatives and tyrosine incorporated into peptide chains was explained based on the existence of several rotamers in the ground state. These rotamers have longer lifetimes, compared to that of excited state of tyrosine, so their population remains effectively the same also in the excited state. In this communication we discuss the correlation between rotamer population determined by ^{1}H NMR and molecular dynamics and contribution of particular rptamer to fluorescence decay.

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HYDRAZINOPEPTIDES AS FOLDAMERS

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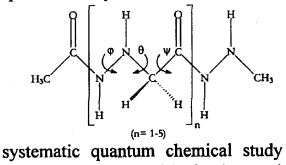
Improving the pharmacological properties of natural peptides is one of the main goals in peptide and protein design. One approach is the substitution of unnatural amino acids for the proteinogenic ones. Such modified peptides have to realize well-defined three-dimensional structures mimicking the essential features of their natural counterparts. In this context, oligomers of β-amino acids, called β-peptides, have gained much attention due to their capability to form characteristic secondary structures, e.g. sheets, turns, and helices. Extensive studies on the conformational space of these foldamers employing quantum chemical and empirical force field methods revealed the variety of secondary structure possibilities and their peculiarities in relations to α-peptides.[1,2]
On the basis of the knowledge of the folding propensities of β-peptides, it is tempting to modify the β-amino acids in order to extent the β-peptide concept. Such a structural variation might be the nitrogen substitution for the C^{β}-atom leading to hydrazinopeptides composed of α-hydrazino acids.



Here, we present a systematic quantum chemical study on the conformational properties of monomers and oligomers of hydrazinopeptides employing *ab initio* MO theory including solvent continuum models. Besides the typical secondary structure elements known for β-peptides, oligomers of α-hydrazino acids form several novel secondary structure elements by incorporating the $N^{\alpha}H$ hydrazino functionality. The obtained results suggest hydrazinopeptides as an interesting novel class of foldamers.

[1] Wu, Y.-D. and Wang, D.-P. (1998).Theoretical Studies of β-Peptide Models. *J. Am. Chem. Soc. 120*, 13485-13493.
[2] Möhle, K., Günther, R., Thormann, M., Sewald, N. and Hofmann, H.-J. (1999) Basic Conformers in β-Peptides. *Biopolymers 50*, 167-184.

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ENGINEERING POTENTIAL INHIBITORS OF THE INTERACTION BETWEEN THE HIV-1 NEF PROTEIN AND KINASE SH3 DOMAINS

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Negative factor (Nef) is one of the first HIV proteins to be produced at high levels in infected cells. This protein was reported to bind to the Src homology 3 (SH3) domain of various members of the Src family of protein tyrosine kinases (Hck, Lyn, Lck and Fyn). A proline rich motif of Nef is implicated in the complex formation. In addition, an interaction between Nef and the RT loop of SH3 domains has been observed in the X-ray structure of the complex, and shown to form a major part of the binding interface between SH3 and Nef. In this context, molecules which would block Nef-SH3 interactions could prove to be useful therapeutic agents.

A promising approach in designing new biologically active molecules consists in grafting active or recognition sites onto small stable protein scaffolds. One such scaffold, the Cystine-Stabilized β-sheet (CSB) motif is made of a triple-stranded-β-sheet stabilized by two disulfide bridges. We have recently designed a 23-residue peptide which probably constitutes the smallest autonomous folding unit containing the CSB motif. Using this peptide as scaffold, we have synthesized a chimeric molecule with the CSB motif as core and an extension made of the RT loop of the Fyn SH3 domain. The NMR study has shown that this peptide folds as expected. The CSB motif is well conserved and the 3D structure of the RT loop is very close to its conformation in the X-ray structure of Fyn. This observation shows that the CSB motif can be used as a scaffold for design purposes.

A 3D model of the complex between HIV-1 Nef and the chimeric peptide has been built. The model suggests that the main part of the Nef-kinase interaction could be correctly mimicked and that the CSB motif itself might not interfere in the interaction. The interaction of this chimeric molecule with Nef is under experimental investigation. However, the affinity of SH3 domains for Nef is known to depend on the amino acid composition of the RT loop. Therefore, we have undertaken the synthesis of various analogs of the chimeric molecule with RT loop sequences corresponding to different SH3 domains.

AB INITIO CONFORMATIONAL ANALYSIS OF PROLYL-PROLINE PEPTIDES AND THEIR POTENTIAL TO MODIFY THE MAIN-CHAIN FOLD OF PEPTIDES

Ilona Hudáky and András Perczel

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For a number of reasons proline residues significantly effect the conformation of the peptide moiety. First, the nitrogen, lacking a hydrogen attached to it, cannot form a hydrogen bond. Second, torsion angle _ has to accept a value around –60°. Third, the pyrrolidine ring may be puckered in two ways: *up* or *down*. Fourth, the proline residue is found to adopt *cis* peptide bonds more frequently than other amino acid residues. For-L-Pro-NH_2 is the simplest model to calculate (H. A. Baldoni et al, J. of Molecular Structure (Theochem) **1999**, 465, 79-91). A fruitful and logical continuation of such an *ab initio* calculation is the elongation of the polypeptide chain by a suitable amino acid residue (H-J. Böhm, J. Am. Chem. Soc. **1993**, 115, 6152-6158). Therefore, a systematic investigation of the conformational hyperspace of For-L-Pro-L-Pro-NH_2 was carried out at the RHF/3-21G and theRHF/6-31+G* levels of theory. Our study was extended to investigate the entire conformational space, including *cis/trans* isomers, as well as ring puckering. All *ab initio* results (conformational properties, relative population, *etc.*) are compared to relevant X-ray data pulled out from proteins. An attempt is made to use these data for a better understanding of the role of proline residues in peptides and proteins.

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AN *AB INITIO* STUDY ON THE EFFECT OF THE MOLECULAR CHARGE OF HISTIDINE CONFORMERS

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Histidine plays a crucial role in numerous biological reactions such as the catalytic function of serine proteases, where the proton transfer ability and the different protonated states of histidine is essential. Beside other factors, the precise *pKa* value of histidine depends on the actual conformation of the molecule. The structural preferences of this amino acid show a significant correlation with its state of protonation. In order to study the interdependence of pKa and backbone/side-chain conformation, we have conducted a systematic research incorporating all minima at all three different states of protonation. The construction of such conformational library using *ab-initio* methods (e.g. RHF/6-31G*) compiles all possible backbone conformers at all possible side-chain rotamers at all protonation states. Comparing more than 200 structures of For-L-His-NH_2, we concluded, that several conformational minima disappear with the change of protonation state, while other minima show unexpectedly large shift in total energy. Our results focusing on molecular structure of histidine derivatives were checked against PDB. Such comprehensive analysis gives hope for a better understanding of histidine molecular properties in proteins.

DESIGN OF AAB-TYPE HETEROTRIMERIC α-HELICAL BUNDLE BY HYDROPHOBIC CORE ENGINEERING

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The coiled coil has the representative amino acid sequence of $(defgabc)_n$ heptad repeats. The a and d positions are usually occupied by hydrophobic residues and form the hydrophobic core. The amino acids in the hydrophobic core should have more influence on the structure than those at the other positions. To construct an AAB-type heterotrimer, we used a side-chain with a complementary size to engineer the specificity in the packing of the hydrophobic core of the coiled coil.
A peptide, IZ, has an amino acid sequence of YGG(IEKKIEA)$_4$ (defgabc), and forms a parallel triple-stranded coiled coil[1]. We replaced the Ile residue at the a position in the second heptad repeat with either an Ala or a Trp residue, IZ-2A and IZ-2W, respectively. At the peptide concentration of 20 μM and 20 °C, IZ-2A had a random structure by the CD spectrum analyses, while IZ-2W showed the α-helical structure. A mixture of IZ-2A/IZ-2W (2:1) exhibited more helical property than IZ-2W. In this mixture, it is found that $(IZ\text{-}2A)_2$-IZ-2W complex was predominantly formed in solution as determined by Sephadex G-50 gel filtration chromatography followed by HPLC analysis. The melting temperature of $(IZ\text{-}2W)_3$ and the $(IZ\text{-}2A)_2$-IZ-2W complex were 51 and 60 °C, respectively. To unstabilize the IZ-2W homotrimerization without much effect on the formation of the heterotrimer, the f position of IZ-2W was changed to Ala to disrupt the Glu-Lys ion pair between the b and f positions, IZ-2W-A. IZ-2W-A formed α-helical structure with a Tm of 40 °C, which is 11° lower than that of $(IZ\text{-}2W)_3$. On the other hand, the $(IZ\text{-}2A)_2$-IZ-2W-A complex had a Tm of 58°, only 2° decreased. Thus, without a severe change of the thermal stability of the heterotrimer, we could construct the AAB-type helical bundle.

1) K. Suzuki et al., Protein Engineering, 11, 1051-1055 (1998).

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