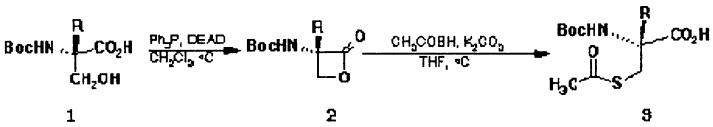
P A105 - Cyclic opioid peptides containing α-alkylcysteines

A. Olma[1], M. Kedzierska[1], A. W. Lipkowski[2], A. Ejchart[3], M. Oleszczuk[3]

1. Institute of Organic Chemistry, Technical University, Lodz - Poland
2. Medical Research Centre, Polish Academy of Sciences - Poland
3. Institute of Biochemistry and Biophysics, Polish Academy of Sciences - Poland

Modified and conformationally constrained peptides are important tools for structure-activity studies. Cyclization of bioactive peptides is generally employed to improve their biological properties such as metabolic stability, activity and receptor selectivity. Cyclization via side chains have produced several potent analogues of opioid peptides, bradykinin and LHRH. To expand the scope of backbone cyclization we have synthesized α-alkylated cysteines. These α-alkylcysteines (α-mercaptomethyl-α-amino acids) **3** were prepared in a two-step reaction. N-Boc-α-hydroxymethyl-α-amino acids **1** were subjected to Mitsunobu reaction conditions to give the β-lactones **2.** Upon treatment with tioacetic acid and K_2CO_3 at room temperature β-lactones **2** undergo cleavage to give α-substituted S-acetyl-cysteine **3**.



1 2 3

Fig. 1 - Synthesis of α-alkyl- S-acetylcysteines from α-hydroxymethylamino acids

Starting α-hydroxymethyl-α-amino acids **1** could be easily obtained via selective α-hydroxymethylation of respective N-benzoyl-amino acid oxazolones. The use of α-alkylated cysteines as peptide building block allows to construct cyclic analogues of biologically active peptides with preserved side chain. The synthesis of cyclic analogues of opioid peptides containing α-substituted cysteines (analogues DPDPE, JOM-13), biological activities and structural studies will be discussed.

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P A106 - Oxygen induced convertion of peptides and peptidomimetics from flax

B. Picur[1]

1. Faculty of Chemistry, University of Wroclaw, 14 F. Joliot-Curie Street, 50383 Wroclaw - Poland

It has been shown that there is present in chosen varietes of flaxes among other cyclic peptides the peptide CLX [1]. The sequence of CLX was determined as cyclo (PPFFILLX) where X is [2S,4R] γ-amino-N-methylproline [2]. The construction of X strongly suggests that motile nonplanar peptidomimetic moiety δ-CH_2-N(CH_3)- might represents transition states for *cis/trans* amide bond isomerisation process realized by rotamases during cellular responce and posttranslational events [3]. Using chemical and spectroscopical methods it was evaluated, that natural as well as synthetic CLX in aerobic conditions converts to new cyclic peptide by exclusion of two phenylalanine residues. The new peptide CLX* has the sequence cyclo (PPILLX*) where the X* is [2S,4R] γ-amine-N-methylglutamic acid. The spontaneous process of oxidative transformation of X to X* (it is going with pyrrolidyne ring disruption) is very similar to known from biochemistry of proline transformation to glutamic acid realized under oxidases presence. There are no doubt of above observation evidenced for synthetic and natural CLX but the mechanism of cyclic octapeptide selfcleavage and new cyclic hexapeptide creation is not fully evaluated yet and need more advanced separate searches.
The new amino acid residue [2S,4R] γ-amino-N-methylglutamic acid (named here as X*) can appear in cyclic peptide in its open form as well as in the form of lactame: [2S,4R] γ-amino-N-methylpiroglutamic acid. The intraresidual N-methylated cis amide bond is not flexible in lactame what is giving for this new natural structure an application for synthesis biologically active peptides with the rigid cis amide bond. The tendency of X* to intermolecular condensation giving insoluble layers (analogical to nylon) was observed. The residues of γ-amino-N-methylproline and γ-amino-N-methylglutamic acid are new, appeared only in flax. The progress in searches of above new objects and processes will be presented.

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P A107 - A new choice for orthogonality: the highly base sensitive BSMOC group for improved peptide synthesis

E. S. Price[1]

1. PolyCarbon Inds., Inc., Leominster, MA 01453 - U.S.A

Louis Carpino of the University of Massachusetts, who developed both Boc and FMOC amino protecting groups, recently developed with his co-workers a new base sensitive group that can be selectively removed in the presence of FMOC or FM esters and vice versa due to it's unique deblocking mechanism. While piperidine can be used for deprotection even at lower concentrations (2-5%), other bases can be selected as well, such as 4-(aminomethyl)piperidine or tris(2-aminoethyl)amine (TAEA). The adducts formed during deprotection, especially with TAEA, are also highly water soluble. The lower concentration of piperidine is advantageous due to the suppression of base sensitive side reactions and also this group does not produce the troublesome dibenzofulvene generated during FMOC removal. While the BSMOC group (1) can be used for solid phase synthesis, the most advantageous uses of this new derivative are for rapid continuous solution phase synthesis (2) and combinatorial chemistry.

P A108 - Assembly of cyclic peptides and pseudopeptides designed for complexation of bivalent transition metal ions by different strategies

S. Reissmann[1], S. Kuenzel[1], S. Nolden[1], R. Reissmann[1], J. Eckstein[1], I. Agricola[1]

1. Institute of Biochemistry and Biophysics, University of Jena - Germany

Using the programme COSMOS™ [1] cyclic hexa- and octapeptides were designed for complexation of transition metal ions. The complexes are expected to act as artificial metallo enzymes. To reexamine the predicted preference of metal complexation we synthesized some linear and cyclic peptides with different sequences. The syntheses of the cyclic peptide and pseudopeptide ligands require the application of four selectively removable types of protecting groups like Z/OBzl, Boc/OBut, Alloc/OAll, ODmab and Fmoc, combined with different imidazole protecting groups and acid labile resins. We studied the assembly of pseudopeptides with N-functionalized glycine residues as well as by formation of the N-alkylated amide bond on the resin and by the use of preformed dipeptide building units. The synthetic approaches were compared regarding to yield, purity and side reactions. The cyclization tendency was studied depending on the resin linker used. One of the unexpected side reactions was the formation of pyroglutamic acid from protected Glu at position 3 in the assembled octapeptide. Especially the synthesis of the cyclic octapeptide ligand for formation of heterodinuclear metal complexes

cyclo[Lys^1-Ala^2-Glu^3-Lys^4-Ala^5-His^6-Gly^7-Glu^8]

requires a highly sophisticated strategy. Complexation of as well as linear and cyclic peptides with the transition metal ions Zn^{+2}, Cu^{+2}, Co^{+2}, Fe^{+2}, Ni^{+2} and Mn^{+2} was estimated by ESI- MS, MALDI-MS, HPCE and CD. Catalytic activities of some of the complexes towards ester hydrolysis [2] and oxidation of catechol will be discussed with respect to used metal ions and peptide ligands.

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P A109 - Top 25 side-reactions and contaminants observed during the solid phase synthesis of linear and cyclic peptides

P. Romanovskis[1], A. F. Spatola[1]

1. Department of Chemistry and the Institute for Molecular Diversity and Drug Design, University of Louisville, Louisville, KY 40292 - U.S.A

During the course of our work on the synthesis of a variety of linear and cyclic peptides and peptide mixtures, (including cyclic, bicyclic AChE inhibitors, and those with C-alpha, alpha-disubstituted glycines) we have used MS, particularly MALDI-TOF MS for confirmation of the presence of the desired peptides in the crude synthesis products as well as for analysis of contaminants. Both Boc- or Fmoc- strategies were used in these solid phase preparations. Our syntheses were performed on a wide variety of acid and amide resins. Additionally, we used both carboxy or amino acid side chain attachments (for head to tail cyclic peptides), t-butyl, benzyl, OFm, ONb, or allyl protecting groups, and typically TFA or anhydrous HF cleavages. Peptide elongations were stepwise, and couplings or cyclizations were performed with either uronium or phosphonium condensing agents, with or without additives. It follows from our analysis that each of the synthesis steps can be a possible source of various contaminants (incomplete deprotection, alkylation of aromatic residues, "wrong" interactions with the condensing reagents, transesterification in the presence of strong bases, oligomerization on cyclization, intramolecular reactions, acylations, amidation, and others). Some of these are undoubtedly sequence specific. But based on the above analysis, and a review of similar literature reports on peptide side reactions, we have compiled a list of what we believe to be the most typical types of mass spectral variances that may be observed. We hope that this listing, while admittedly incomplete, will provide a useful reference point for further analysis and discussion and should stimulate efforts at preventing their occurrence.

Table 1. MALDI-TOF-MS characteristics and identification of side products in crude linear precursors and their cyclic counterparts on cyclization

Signal	Cyclic	Linear	Identification
-48	+	+	Met decomposition
-3	+	-	Met decomposition + C-terminal dimethylamide
-18	+	-	succinimide from Asp
+18	+	-	linear precursor
+22	+	+	sodium
+ 31	+	-	methyl ester from transesterification
+38	+	+	potasium
+45	+	-	C-terminal dimethylamide
+80	+	-	C-terminal piperidinamide
+90	-	+	benzyl-derivative
+96	-	+	N-terminal TFA-acetylation
+114	+	-	N-terminal TFA-acetylation
+116	+	-	N-terminal tetramethylguanidino derivative
+124	+	+	2-chlorobenzyl-derivative
+169	+	-	2-chlorobenzyloxycarbonyl-derivative
+206	-	+	N-terminal dicyclohexylguanidino derivative
+248	+	-	di-(2-chlorobenzyl)-derivative
[2M + H]	+	-	cyclic dimers

P A110 - New antitumoral cyclic astin analogues containing uncoded amino acid residues: synthesis, structural and biological studies.

F. Rossi[1], G. Zanotti[2], R. Iacovino[1],M. Saviano[1], P. Palladino[1], G. Saviano[3], P. Amodeo[4], T. Tancredi[4], P. Laccetti[1], D. Spalletti[5], E. Benedetti[1]

1. Dipartimento di Chimica Biologica e Ist. di Biostrutture e Bioimmagini - C.N.R. Via Mezzocannone, 6 - Napoli - Italy
2. ICB, Università di Roma - La Sapienza - Italy
3. Dipartimento STAT - Isernia - Italy
4. ICB, Pozzuoli (NA) - Italy
5. Dipartimento di Biologia e Patologia cellulare e molecolare "L.Califano"- Via Campi Flegrei, 43 - Napoli - Italy

Astins are a family of natural antitumor cyclopentapeptides characterized by a 16-membered ring and by the presence of several uncoded aminoacid residues. Conformational analysis of the bioactive chlorine-containing cyclic astins A,B and C was carried out by a combination of X-ray, NMR and computational techniques [1]. A *cis* proline amide bond is considered to play an important role in the antitumor acitivities.
With the aim to improve our knowledge of the structural and conformational properties influencing the bioactivity in this class of compound, we synthetized some astins related cyclopeptides differing from the natural products by the presence of some uncoded β^2 and β^3 amino acid residues. The conformational properties of the analogues have been investigated in solution by NMR tecniques and in the solid state by x-ray diffraction analysis. The analogues: c(-Pro-Thr-Aib-β^2-Phe-Abu-) (**I**), c(Pro-Thr-Aib-β^3-HPhe-Abu) (**II**) , c[Pro-Abu-Ser-β^3-Hpheψ(CH_2-SO_2-NH)-Abu] (**III**), c[Pro-Thr-Aib-β^2-Pheψ(CH_2-SO_2-NH)- Abu] (**IV**) were obtained by the classical method in solution. NMR and x-ray solid state conformational investigations are compared with the biological data of these compounds. The antitumor activity tested *"in vitro"* with NPA human cell lines shows LD_{50} value for cyclopeptide **III** comparable with that natural astins A and B.

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P A111 - Enantioselective synthesis of β^2-amino acids by 1,4-radical addition followed by hydrogen atom transfer

M. Roumestant, J. Huck, J. Martinez

1. Laboratoire des Aminoacides, Peptides et Protéines (LAPP) CNRS - UMR 5810, CC 22 - Universités Montpellier I & II Place Eugène Bataillon 34095 Montpellier Cedex 5- France

Recently considerable attention has been directed towards understanding the biological activity of β-amino acids and their derivatives. Naturally occurring β-amino acids are compounds with an interesting pharmacological profile. They are also found as components in a wide variety of biologically active compounds, including peptides, with antibiotic, antifungal, and cytotoxic properties. Oligomers of β-amino acids (β-peptides) are subjects of increasing attention.
During the last decade, radical chemistry and particularly stereochemical aspects have been intensely studied. Some progress has been extremely rapid with the use of Lewis acids.In our previous work, we have achieved the synthesis of new racemic β^2-amino-acids using radical 1-4 addition [1].
Thus, the intent of this investigation was to synthesise enantiopure β^2-amino acids by asymmetric 1,4 radical addition. To control the new asymmetric center, different methods were studied, diastereoselective reactions using chiral auxiliaries or enantioselecive reactions using chiral Lewis acids.

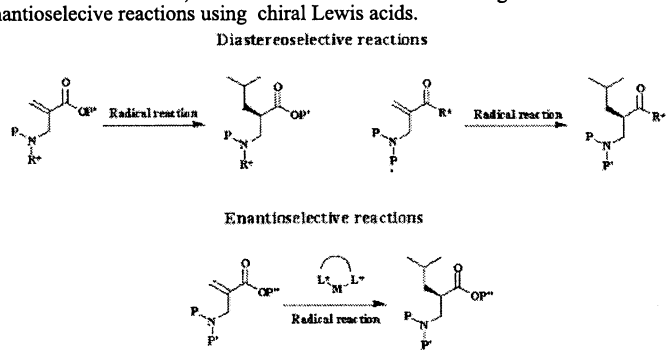

Fig. 1 - Radical Reactions P, P', P'': Protections - R: Chiral Auxiliaries - L*ML*: Chiral Lewis Acids*

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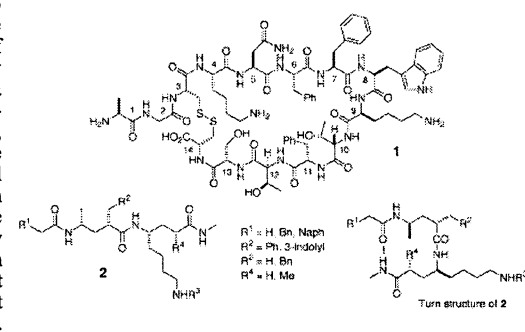
P A112 - Investigation of N-acyl-γ-dipeptide amides as somatostatin analogs: synthesis and receptor-affinity measurement

L. Schaeffer[1], D. Hoyer[2], D. Seebach[1]

1. Laboratorium für Organische Chemie der Eidgenössische Technischen Hochschule, ETH-Hönggerberg, CH-8093 Zürich - Switzerland
2. Novartis Pharma AG, Nervous System Research, S-386-745, CH-4002 Basel - Switzerland

While oligomers consisting of 2,4-disubstituted γ-amino acid residues (γ-peptides) with relative configuration *like* form stable 2.6_{14} helices in solution [1], oligomers built of two residues of *unlike* configuration form a 14-membered H-bonded ring, *i.e.*, a γ-peptidic turn [2]. Interestingly, this type II' β-turn is present in a large variety of naturally occurring oligopeptides. In order to prove the relevance of the use of *N*-acyl-γ-peptides amides as peptidomimetics, we synthesized and measured the affinity of appropriately substituted $\gamma^{2,4}$-dipeptide derivatives for the human somatostatin receptors.
Somatostatin, **1**, is a hypothalamic hormone possessing a number of important biological functions [3]. The pharmacophore of this cyclic α-tetradecapeptide is known to involve the sequence Phe[7]-Trp[8]-Lys[9]-Thr[10] and presents a type II' β-turn spanning the Trp-Lys fragment. In a previous study, linear β^2/ β^3-di and α/ β^3-tetrapeptides derivatives have been found to be good mimetics for this motif [4]. In order to extend this observation to γ-peptides and to verify that they can be used as analogs of somatostatin, we now have synthesized a variety of *N*-acyl- $\gamma^{2,4}$-dipeptides amides **2**, with different side chains. The biological studies are currently in progress, and we hope this work will open new avenues for the design of low molecular weight and peptidase-resistant peptidomimetic drugs.



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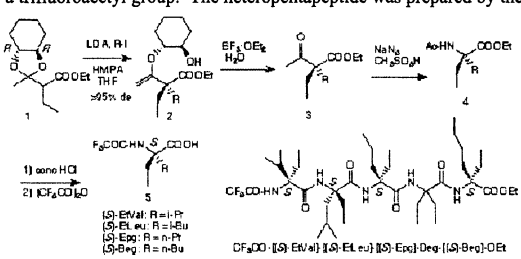
P A113 - Conformational study of heteropeptide prepared from chiral α-ethylated α,α-disubstituted α-amino acids

M. Tanaka[(1)], S. Nishimura[(1)], M. Oba[(1)], Y. Demizu[(1)], M. Kurihara[(2)], H. Suemune[(1)]

1. Graduate School of Pharmaceutical Sciences, Kyushu University - Japan
2. Division of Organic Chemistry, National Institute of Health Sciences - Japan

Peptide-foldamers, which have interesting well-defined conformational properties, have been focused among peptide, organic, and medicinal chemists. As a folding pattern, an extended planar conformation, which has torsion angles of $\phi = 180°$, $\psi = 180°$, was first found as a conformation of glycine homotripeptide, and then was discovered as the preferred conformation of homopeptides prepared from achiral α,α-disubstituted α-amino acids, such as diethylglycine (Deg), dipropylglycine, and diphenylglycine. Recently, we reported that the homopeptides prepared from a chiral α-ethylated α,α-disubstituted α-amino acid (αEtAA), (*S*)-butylethylglycine [(*S*)-Beg] formed the fully planar C_5-conformation in solution and in the solid state, whereas those of homopeptides prepared from chiral α-methylated α,α-disubstituted amino acids have been known to be 3_{10}-helical structures [1-3]. Here we wish to report the conformation of heteropeptides prepared from chiral αEtAAs. As a heteropeptide, we designed pentapeptide CF_3CO-[(*S*)-EtVal]-[(*S*)-EtLeu]-[(*S*)-Epg]-Deg-[(*S*)-Beg]-OEt because each of amino acid residues in the pentapeptide is different, and no conformational property of αEtAA heteropeptide has been reported. We synthesized the optically active αEtAAs by an asymmetric alkylation of β-keto ester using (*R*,*R*)-cyclohexane-1,2-diol, and subsequent Schmidt rearrangement. That is to say, the chiral acetal **1**, which consists of (*R*,*R*)-cyclohexane-1,2-diol and ethyl 2-ethylacetoacetate, was alkylated with LDA (5 eq.), R-I (5 eq.) and HMPA (5 eq.) in THF to give enol ethers **2**. The cyclohexane-1,2-diol moiety in **2** was removed by treatment with BF_3-OEt_2 to afford β-keto esters **3** (>95% ee). The obtained β-keto esters **3** could be converted into αEtAAs **4** by Schmidt rearrangement. The protecting groups in **4** were removed by hydrolysis with concentrated HCl, and the N-terminus was protected as a trifluoroacetyl group. The heteropentapeptide was prepared by the solution-phase method using EDC. We determined the crystal structures of the tri-, tetra-, and pentapeptides by X-ray analysis, and also studied the conformation of pentapeptide in solution by ^{1}H NMR and IR spectra. The results of conformational analyses will be presented.



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P A114 - Strategies for the solid phase synthesis of chiral PNAs with a high optical purity

T. Tedeschi[(1)], S. Sforza[(1)], R. Corradini[(1)], A. Dossena[(1)], R. Marchelli[(1)]

1. Dep.Organic Chemistry University of Parma - Italy

Peptide Nucleic Acids (PNAs) are oligonucleotide analogues with a polyamidic backbone based on aminoethyl-glycine. They are able to interact with complementary sequences of DNA and RNA with high affinity and selectivity. Many PNA analogues have been described in recent years with the aim of improving the binding properties [1]. One of the most interesting modification is the introduction of one or more chiral center in the PNA backbone [2]. With the aim of evaluating the role of chirality in PNA:DNA and PNA:RNA interactions, we have studied the performances of PNAs based on D- and L- amino acids. It has been proposed that PNAs containing D-amino acids promote the formation of right-handed structures [3]. In particular, the presence of three monomers based on D-Lys in the middle of the sequence ("chiral box") gives high stability to the PNA:DNA complexes and improves the selectivity (direction control and mismatch recognition) [4]. Although the methods for the synthesis of chiral PNA monomers and oligomers with SPPS have been optimized, [5, 6], the introduction of chirality brings about several additional problems that deserve to be studied in detail, first of all "racemization". To this purpose we have recently developed a chromatographic method by GC for measureming the optical purity of chiral PNAs [7]. In the present comunication we describe a systematic study of the experimental conditions for the synthesis of PNAs (in particular the coupling reaction) with the aim of reducing the epimerization of the monomers and the racemization of the oligomers. High optical purity was obtained using a DIC/HOBt coupling protocol. Moreover we devised a new approach in the synthesis of chiral PNAs by using a submonomer strategy on solid phase which allows us to obtain chiral PNA oligomers with higher optical purity compared with the same oligomers made with the classical protocols. The influence of optical purity on the binding properties of PNAs towards nucleic acids will be also discussed.

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P A115 - Protease-catalysed isopeptide synthesis via substrate mimetic strategy

S. Thust[(1)], N. Wehofsky[(2)], M. Alisch[(1)], K. Burger[(1)], F. Bordusa[(2)], B. Koksch[(1)]

1. University of Leipzig, Institute of Organic Chemistry, Johannisallee 29, D-04103 Leipzig - Germany
2. Max-Planck-Society, Research Unit "Enzymology of Protein Folding", Weinbergweg 22, D-06120 Halle - Germany

The trifunctional structure of Asp and Glu allows both amino acids to be incorporated into peptides via the side-chain and the *C*-carboxyl moiety as well. While the latter results in the formation of normal linear peptides, the involvement of the side-chain carboxyl group in the peptide backbone leads to the corresponding isopeptides. Isopeptides are ubiquitously found in nature and are considered to be one of the most common forms of non-enzymatic degradation of linear polypeptides under physiological conditions.[1] Recent findings have demonstrated that wide-spread accumulation of isopeptide linkages in cellular proteins greatly increases the immunogenicity and disrupts a wide range of important biochemical pathways by competitively displacing normal proteins in protein-protein or protein-ligand interactions.[2] Because of this function, isopeptides were found to be highly useful to study complex biological phenomena and for the development of therapeutic agents, respectively, resulting in a great interest in their synthesis.A few enzymes, i.e. protein carboxyl methyltransferases, isopeptidases, and transglutaminases, are known to be active on the side-chain of Glu and Asp and, therefore, may be interesting biocatalysts for the isopeptide synthesis. However, due to the highly restricted substrate and reaction specificity, none of these enzymes is capable of catalysing the formation of isoAsp-Xaa or isoGlu-Xaa bonds. For this purpose, the use of normal proteases, such as the Glu-specific endopeptidase from *Staphylococcus aureus* strain V8 (V8 protease), trypsin, and -chymotrypsin, was investigated. The key feature of this approach is the combination of enzymes with a novel type of substrate mimetics that directs the synthesis activity of the protease to the side-chain carboxyl moiety of Asp and Glu (Fig. 1). Similar to classical linear substrate mimetics, the newly developed acyl donors bear a site-specific ester leaving group (4-guanidinophenyl ester or carboxymethyl thioester functionality) that mediates the acceptance of non-specific acyl moieties by proteases.[3]
The successful application of these artificial substrates for formations of isopeptide bonds catalysed by the Arg-specific trypsin, the Glu-specific V8 protease, and -chymotrypsin, which is specific for aromatic amino acid residues, could be demonstrated. The influence of the length of the donor esters on the synthesis reaction was studied using isosubstrate mimetics derived from single amino acids up to pentapeptides

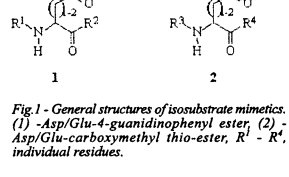

Fig.1 - General structures of isosubstrate mimetics. (1) -Asp/Glu-4-guanidinophenyl ester, (2) -Asp/Glu-carboxymethyl thio-ester, R^1 - R^4, individual residues.

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P A116 - Pro[10]-Tyr[11] substitutions provide potent or selective NT(8-13) analogs

D. Tourwé[(1)], K. Iterbeke[(1)], G. Török[(1)], G. Laus[(1)], F. Fülöp[(2)], A. Péter[(3)], P. Kitabgi[(4)]

1. Vrije Universiteit Brussel, Department of Organic Chemistry - Belgium
2. University of Szeged, Institute of Pharmaceutical Chemistry - Hungary
3. University of Szeged, Department of Inorganic and Analytical Chemistry - Hungary
4. CNRS UMR 6097 - France

Neurotensin (NT) is a 13-amino acid peptide which acts as a neuromodulator. Three NT receptors (NTS1-3) have been characterized. In contrast to the NTS1, which has been studied extensively, little is known about the biological role of NTS2. One of us recently reported that NT(8-13) analogs modified at position 11 with bulky amino acids, displayed moderate affinity and selectivity for the hNTS2 and behaved as partial inverse agonists in transfected COS cells [1]. Consequently, we have made further amino acid substitutions at positions 10 and 11 in the biologically active NT(8-13) sequence: Arg-Arg-Pro-Tyr-Ile-Leu-OH and we have determined the affinity of these new analogs for the human NTS1 and NTS2 receptors expressed in COS cells. Thus, potent but non-selective analogs were obtained using β-methyl-Tyr, 2',6'-dimethyl-Tyr, 2'-Br-Tyr or m-Tyr substitutions in the native NT(8-13) sequence. The 5-HO-2-aminoindane-2-carboxylic acid (5-HO-Aic), the L-Tcc and the cis-2-amino-cyclopentanecarboxylic acid [10] (ACPC) analogs showed moderate potency and selectivity for the NTS2 receptor. Several of these substitutions were combined with the replacement of the Arg-Arg peptide bond by LysΨ(CH_2NH)Arg, and Tle[12] resulting in metabolically stable compounds as evaluated in plasma, liver and kidney homogenates using HPLC/MS detection.
Furthermore, the analogs Arg-Arg-Pro-D-Tcc-Ile-Leu-OH and LysΨ(CH_2NH)Arg-Pro-6-HO-Tic-Tle-Leu-OH were shown to be partial inverse agonists for the constitutively active hNTS2 receptor.

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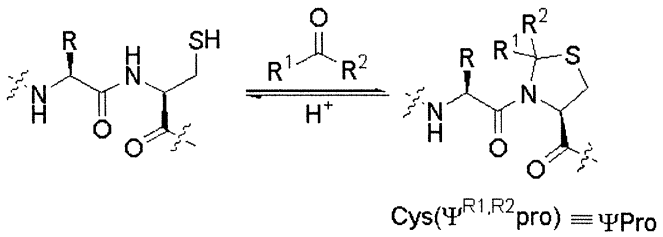
P A117 - Direct insertion of pseudo-proline (ΨPro) systems into cysteine containing peptides

G. Tuchscherer[1], J-F. Guichou[1], C. Boyat[1], O. Turpin[1], M. Mutter[1], L. Patiny[1]

1. Institute of Molecular and Biomolecular Chemistry (ICMB), EPFL, CH-1015 Lausanne - Switzerland

We have shown previously that serine or threonine containing peptides can be reversibly converted to oxazolidine (pseudoproline, ΨPro) containing analogues featuring novel chemical and structural properties[1]. According to this approach, serine or threonine containing peptides are converted into the corresponding five membered ring systems (oxazolidines, ΨPro) by intraresidual N,O-acetalisation)[2]. Due to substantial differences in the formation and physico-chemical properties of thiazolidines and oxazolidines, the incorporation of Cys-derived ΨPro-systems into native peptides was only feasible so far by total chemical synthesis via its N-unprotected derivative Cys($\Psi^{R1,R2}$pro) in stepwise peptide synthesis[3]. With the goal of extending the ΨPro concept as tool in biomolecular recognition studies, we elaborate here the direct insertion of ΨPro-systems into cysteine containing peptides (Figure). The experimental conditions for this direct insertion and the effect upon the conformational and functional properties of a series of Cys-containing peptides including a cyclosporine A analogue will be presented.



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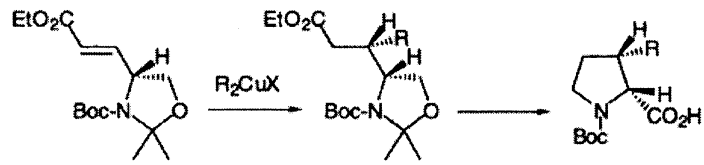
P A118 - Preparation of 3-alkylproline derivatives with *cis*-selectivity

M. Ueki[1], H. Suzuki[1], T. Ozeki[1]

1. Department of Applied Chemistry, Science University of Tokyo, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601 - Japan

Prolines with proper substituents have been used for studying biologically active conformations of peptides by increasing conformational constraints. Especially, 3-substituted prolines having ring structure corresponding to natural α-amino acids are important in studying effects of side chain orientation. Various methods for preparation of 3-substituted prolines have been reported, however most of them give stable trans isomers. In this study we were able to establish a new method for preparation of 3-alkyl (methyl, ethyl and propyl) prolines with cis-selectivity. A key step is a stereoselective addition of dialkylcopper reagents to (*E*)-4-(2-ethoxycarbonylethenyl)oxazoline derivative, which were derived from L-serine in 5 steps. For cyclization our previously reported method using nucleophilic attack by α-amino N was used [1].



The corresponding Z-isomer was less reactive and less selective.

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P A119 - Combined 2'6'-dimethyltyrosine and β-methylphenylalanine substitutions in TIPP provide potent δ-opioid ligands.

I. Van den Eynde[1], D. Tourwé[1], G. Carlens[1], J. Piron[1], G. Toth[2], M. Ceusters[3], M. Jurzak[3], L. Heylen[3], T. Meert[3]

1. Organic chemistry department, Vrije Universiteit Brussel - Belgium
2. Biological Research Center, Szeged - Hungary
3. Janssen Research Foundation, Beerse - Belgium

The tetrapeptide Tyr-Tic-Phe-Phe-OH (TIPP-OH) represents the prototype of a class of potent and selective δ-opioid antagonists. We previously reported that the agonist/antagonist properties of this tetrapeptide was strongly influenced by β-methyl substitution. [1] The use of 2',6'-dimethylTyr[1] (Dmt) substitution in TIPP-NH_2 has been shown by Schiller et al. to provide mixed μ agonist/ δantagonists with a potent analgesic effect in rats.[2] We report the results of combined substitutions in TIPP peptides with Dmt[1] and the different stereoisomers of β-methylPhe. The biological activity profile of the new analogs depends greatly on the configuration of the β-methyl substituted carbon. Very potent analogs were obtained having the Dmt-Tic-(2R,3S)β-MePhe-Phe-OH or its corresponding C-terminal amide structure. These compounds show low nM affinity for both the μ- and δ-opioid receptors and behave as mixed μ-agonists/δ-antagonists in the GTPS assay. After intrathecal administration Dmt-Tic-(2R,3S)-MePhe-Phe-OH induces long lasting analgesia in rats.

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P A120 - A general synthesis of 2-substituted 4-amino-1,2,4,5-tetrahydro-2-benzazepine-3-ones, conformationally constrained phenylalanine mimetics

K. Van Rompaey[1], I. Van den Eynde[1], D. Tourwé[1]

1. Vrije Universiteit Brussel / Organic Chemistry Department - Belgium

A mild and versatile synthesis for 2-substituted 4-amino-1,2,4,5-tetrahydro-2-benzazepine-3-ones has been developed. This heterocycle has been used before as a constrained Phe-Gly analog [1,2].
There are two possible disconnections for this type of benzazepinones (fig. 1). In path a the bicyclic product is formed by an intramolecular electrophilic aromatic substitution on the N-acyliminium ion of **1** or **3** [3]. Both cases require harsh acidic conditions. We chose for alternative b: formation of the amide bond from **4**. Compounds **4** could not be obtained by nucleofilic substitution of **6** with RBr, due to immediate di- and tri- alkylation. However, reductive amination reactions of aldehyde **5** with a variety of amines (Phe-OBn, Gly-OBn, Val-OMe, Ava-OMe, $cHxNH_2$, $iPrNH_2$) or of amine **6** with a variety of carbonyl compounds (Fmoc-Leu-H, Fmoc-D-Phe-H, Fmoc-Ser(tBu)-H, isobutyraldehyde, benzaldehyde) lead to compounds **4**, which can be cyclised to the corresponding benzazepinones (fig. 2) by intramolecular amide bond formation. Both aldehyde **5** and amine **6** were prepared by reduction of the corresponding nitrile, using RaNi and 10% Pd/C respectively. N-methylation in aldehyde **5** was necessary to avoid intramolecular condensation.

Fig. 1 -

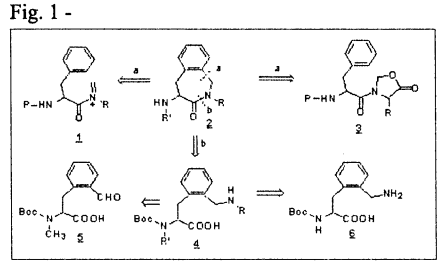
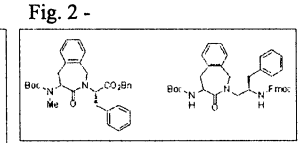


Fig. 2 -



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P A121 - Synthesis of peptide containing extremely hindered amino acid residues: N-methylaminoisobutyric acid peptides

M. Beyermann[(1)], P. Henklein[(1)], L. A. Carpino[(2)], M. Bienert[(1)]

1. Forschungsinstitut für Molekulare Pharmakologie, Peptide Synthesis Group - Germany
2. University of Massachusetts, Department of Chemistry - U.S.A.

Short peptides may be conformationally stabilized by the presence of additional alkyl substituents either on the -nitrogen or -carbon which restrict the values of the torsional variables and as compared with those available to proteinogenic amino acids. Peptides containing a high portion of C-methyl-alanine (-aminoisobutyric acid (Aib)), such as the peptaibols, form helical secondary structures (3_{10}- or -helix) as well as peptides consisting of C-methyl- or ethyl-valine. The investigation of the effect of N-,C-dialkyl-substituted amino acids on peptide conformations have not been possible yet, because of the lacking methods for their incorporation into peptides. Although Fmoc-amino acid fluorides are excellent reagents for coupling of Aib-to-Aib, they are not suited for significantly more hindered systems (e.g. Aib-to-MeAib). While urethane-protected amino acid chlorides are inherently more reactive than the fluorides they are also ineffective for the coupling of very hindered amino acids due to competing oxazolone formation. Benzenesulfonyl-protected amino acid chlorides are not able to form corresponding oxazolones. Recently, Fukuyama, T. et al. (Tetrahedron Lett. 38, 5831 (1997) reported on the use of the *o,p*-dinitrobenzenesulfonyl (DNBS) amino protecting group which can be easily removed under very mild conditions. This mild deprotection ($HSCH_2COOH/Et_3N$ in CH_2Cl_2 within 5 min, rt) makes the DNBS group in conjunction with the acid chloride technique very useful for the coupling of very hindered amino acids. We describe here the synthesis of MeAib-containing peptides such as MeAib-Lys-MeAib-Lys-Ala-NHR and MeAib-Aib-MeAib-Aib-Ala-NHR. Using DNBS-amino acid chlorides the synthesis of peptides bearing even adjacent MeAib residues (MeAib-MeAib-MeAib-Lys-Ala-NHR) was accomplished.

P A122 - Backbone isomerization at aspartic-, glutamic acid –cysteine ligation site during native chemical ligation.

M. Villain[(1)], P. Botti[(1)], H. Gaertner[(1)]

1. Protein Synthesis, Geneva Branch, Geneprot - Switzerland

We report a side reaction that occurs during Native Chemical Ligation (NCL) at Asp-Cys and Glu-Cys ligation sites. To get a better insight into this problem two thioester peptides, LYRAD-sR and LYRAE-sR, were prepared by standard Boc chemistry. Both completely unprotected peptides were used in a ligation reaction with an unprotected peptide containing an N-terminal Cys, CSYRFL. In each case the ligation reaction resulted in a major peak corresponding to the expected ligation product and a minor one present in a 1:3 ratio, both with the correct final masses, but with different retention times. A possible explanation for the formation of this side product is a migration of the thioester moiety during the ligation reaction from the α carbonyl of the C-terminal Asp or Glu to the side chain carbonyl, with formation of the corresponding isopeptide. In order to confirm this hypothesis both the peptides with the normal alpha backbone and the peptides with β/γ isopeptide bond at the ligation site were prepared by total SPPS. The unnatural peptides have exactly the same chromatographic retention time as the two minor compounds obtained from the ligation reactions. This result was further confirmed by enzymatic digestion with the Asp, Glu specific V8 protease. The two minor impurities, as well as the fully synthetic β and γ peptides were resistant to Asp V8 digestion, thus definitively confirming the nature of the side reaction. A possible solution to overcome this side reaction is to employ a transient side chain protection for the Asp and Glu when they are involved n the ligation reaction. Ideally this protection should be resistant to HF, and removed after ligation using relatively mild conditions, to generate only the correct product. The different approaches that have been explored in our team to minimize this side reaction will be discussed.

P A123 - Solution and solid phase diversification of phosphinic peptide analogues

A. Yiotakis[(1)], M. Matziari[(1)], D. Georgiadis[(1)], A. Makaritis[(1)], V. Dive[(2)]

1. Department of Chemistry, Laboratory of Organic Chemistry, University of Athens, Panepistimiopolis Zografou, 15771, Athens - Greece
2. CEA, Département d'Ingénierie et d'Etudes des Protéines, 91191, Gif/Yvette Cedex - France

Phosphinic peptides represent an important class of transition state analogues, that have been shown to be effective inhibitors for a variety of zinc metalloproteases, in which the scissile peptide bond has been replaced by the phosphinic bond. The family of proteins called matrix metalloproteinases (MMPs) is a class of structurally related proteins, which are collectively responsible for the metabolism of extracellular matrix proteins. Through this action, they play an important role in both normal and pathological tissue remodelling. 3-D structure analysis of MMP-inhibitor complexes has shown that the side chain in the P_1' position of the inhibitor fills a particular cavity of the MMP active site, the S_1' subsite. This observation has provided an obvious approach for designing specific MMP inhibitors by incorporating in the P_1' position of the inhibitor unusual long side chains of broad chemical diversity. Systematic investigation of the influence of different side chains of phosphinic inhibitors on selectivity should elucidate structure-activity relationships. During the course of this study, concerning the development of selective MMP inhibitors, we became interested in a strategy that would allow the easy diversification of the P_1' position of a suitably functionalised pseudopeptidic precursor. This would lead to the combinatorial assemble of P_1' diversified phosphinopeptidic libraries, avoiding the use of cumbersome parallel synthetic routes which constitute the only access to such molecules reported to date. Thus, a new method has been developed, which allows the diversification of the P_1' position of phosphinic peptides. The synthesis of phosphinic pseudopeptides containing a dehydroalanine residue in the P_1' position is described, using both solution and solid phase methodologies. The application of this kind of molecules as templates for the introduction of various side chains in the P_1' position *via* conjugate additions was also investigated, using sulfur anions. In addition, elongation of the basic pseudodipeptidic unit from both amino and carboxylic termini was achieved, as well as diversification of the resulting pseudopeptides, using both solution and solid phase methodologies.

P A124 - Peptide-derived nonpeptidic $\alpha_4\beta_7$ integrin antagonists

T. Arndt[(1)], J. Boer[(1)], D. Gottschling[(1)], H. Kessler[(1)]

1. Institut für Organische Chemie und Biochemie, Technische Universität München - Germany

Integrins are heterodimeric transmembrane glycoproteins consisting of one α and one β subunit. They play a crucial role in cell-cell and cell-matrix interactions. The $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins – recognizing the Leu-Asp-Thr (LDT) and Leu-Asp-Val (LDV) motif- play an important role in numerous inflammatory and autoimmune disorders. Utilizing the previously developed "spatial screening" technique, we obtained highly selective $\alpha_4\beta_7$ integrin antagonists out of stereoisomeric constrained penta- and hexacyclic peptide libraries. The structural data thus obtained was used to define the optimal conformation required for binding to the receptor. Subsequent modifications were undertaken to optimize the rigidified structure (Figure 1)[1]. Based on the findings out of this initial work we focused our further research on the development of peptidomimetica with enhanced bioavailability, metabolic stability and biological absorption profiles. Starting from the linear recognition sequence, specific modifications of the *N*-terminal residue were made, applying metal-catalyzed C-C-couplings (Heck- and Sonogashira-reactions) on solid support as well as synthesis of heterocyclic scaffolds. Rational and combinatorial modifications of the C-terminal residue lead to more potent compounds omitting the C-terminal amino acid residue of the native recognition sequence. Furthermore the backbone was modified by reductive amination and introducing aza-amino acids. These results finally lead to the discovery of an active and selective dipeptidic compound [2], which fulfills the "Pfizers rule of five", and now serves as a lead structure for further optimization.

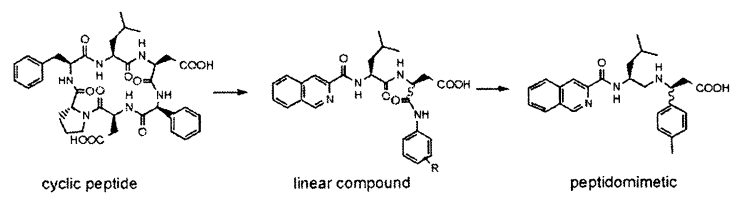

Fig. 1: Deriving a non-peptidic $\alpha_4\beta_7$ integrin antagonist

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P A125 - Crystallographic study of the collagen-like polypeptide [(Pro-Hyp-Gly)$_{10}$]$_3$: implications for collagen assembly

R. Berisio[1], L. Vitagliano[1], L. Mazzarella[2], A. Zagari[3]

1. Istituto di Biostrutture e Bioimmagini C.N.R. Napoli - Italy
2. Dipartimento di Chimica, Via Cinzia - Napoli - Italy
3. Dipartimento di Chimica Biologica, Via Mezzocannone, 6 - Napoli - Italy

Type I collagen is the most abundant animal protein, as it constitutes bones, skin and several other tissues. The structure of such collagen has a distinct motif: a triple helix formed by three poly-proline II-like chains, wrapped around a common triple helical axis. The triple helical structure restricts the amino acid sequence to contain glycine at every third residue. Indeed, the close packing of the chains near the central axis does not leave room for larger residues. In the repeating sequence motif, X-Y-Gly, X and Y residues are very frequently proline and 4-(R)-hydroxyproline (Hyp), respectively. Several experiments have shown that the proline hydroxylation occurring in Y position, strongly contributes to the triple helix stability [1]. Furthermore, defective proline hydroxylation in collagen have been associated to a variety of diseases, like scurvy. In the last decade, the contribution from the use of collagen-like polypeptides has been illuminating [2]. Indeed, such polypeptides form single crystals and, as such, they allow a detailed description of the triple helix structure. In addition, the crystal packing, which is an unwanted complication in globular proteins may provide, for these polypeptides, valuable information regarding the assembly of collagen molecules in the natural fibres. With this in mind, we have solved the full-length structure of the imino acid rich collagen-like polypeptide [(Pro-Hyp-Gly)$_{10}$]$_3$ using sinchrotron x-ray data.The structure of a similar collagen model, named as [(Pro-Pro-Gly)$_{10}$]$_3$, has already been obtained in our laboratory at 1.3 Å resolution [3,4]. Based on this structure and on statistical analyses, a model for the stabilisation of the collagen triple helix by imino-acids has been proposed[5,6]. Unlike [(Pro-Pro-Gly)$_{10}$]$_3$, the crystal structure of [(Pro-Hyp-Gly)$_{10}$]$_3$ exhibits a crystal packing which closely resembles that of natural collagen [7,8]. The analysis of the crystal packing in relation to collagen assembly into fibres is here analysed and discussed.

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P A126 - Antitumor screening of peptidomimetic compounds

G. Bökönyi[1], L. Őrfi[2], R. E. Schwab[3], E. z. Szabó[4], F. Hollósy[4], G. Kéri[4]

1. Semmelweis Univ./Medical Chemistry - Hungary
2. Semmelweis Univ. - Hungary
3. Cooperative Research Centre - Hungary
4. Semmelweis Univ - Hungary

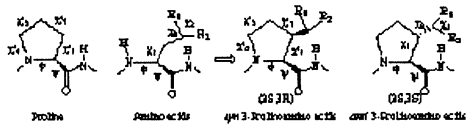
Tyrosine kinase (TK) inhibitors can be similar to peptide ligand when binding the ligand- binding site then can be ATP-like heterocycle when acting at the ATP- binding site or then can show both kind of interactions (like tyrphostins). First we have selected a series of small heterocyclic inhibitors of TK enzymes already published. In order to increase both the activity and bioavailability of heterocyclic inhibitors published, we have synthesized a series of combined structures.We have developed simple solid phase synthetic methods for the preparation of new types of quinazoline derivatives using reactive quinazoline-halogenides as reagents in the alkylation reactions of various resin-bound amino acid derivatives. The desired products were cleaved from resin by TFA and analyzed by LC-MS. Compounds with a purity over 80% were subjected to biological assays.
As a nex step, we have screened the antiproliferative, apoptotic or necrotic activities of heterocyclic compounds which were synthesized by using two different human tumor cell lines: A431 colon adenocarcinoma and Panc-1 ductal pancreas human carcinoma . Furthermore, we wanted to distinguish between cytostatic and cytotoxic activities, too. The biological activity of the compounds was measured in various concentrations against TT- 232 as a positive control. Finally, the best compounds were tested in a non-radioactive TK assay. The result of the above assays will be discussed in this paper.

P A127 - Prolinoaminoacids as probes for the determination of bioactive conformations of peptides

G. Chassaing[1], J. Quancard[1], P. Karoyan[1], O. Convert[1], S. Sagan[1], S. Lavielle[1], O. Lequin[1]

1. UMR 7613 CNRS-Université Paris VI - France

The ideal building block to probe the bioactive conformation of peptides should be a molecule capable to induce a unique conformation for both the side chain and backbone. These last few years we have focused our efforts on prolinoamino acids which are viewed as chimeric amino acids between a natural amino acid and proline. The conformational behaviours of these prolinoamino acids have been first analysed by energy calculations on model dipeptides. All conformational states of model dipeptides (*syn* and *anti* N-acetyl-L-prolinoamino acid-methylamide of Val, Leu and Met) were generated by molecular dynamic calculation using CFF91 force field in Discover. The interdependence between the puckering of the pyrrolidine ring, side chain and backbone conformations have been analysed using the (χ_1,ψ) and (χ_2,ψ) isoenergetic contour maps of these model peptides. The five dihedral angles of the pyrrolidine ring are interdependent and their values are correlated to Φ_M maximum angles of puckering (Φ_M = 58°) and to the Δ phase angle of pseudorotation which can assume any value. The envelope conformations (χ'_1= ± 36°± 10) of pyrrolidine ring are found in all low energy regions. The $_1$ torsion angle of side chains is related to the χ'_1 of pyrrolidine and to the chirality of the C-β carbons (*anti* $_1$ = -120 ± χ'_1 ; *syn* $_1$ = 120 ± χ'_1). The observed limit values χ_1 = -90°, 90° and 160°± 5 are assimilated to gauche (-), gauche (+) and trans rotamers of natural amino acids, respectively. During molecular dynamic calculations, the proline moiety of *syn* and *anti* 3-prolinoamino can visit the (P_{II}), (γ) and (α) regions. The (ψ, χ_1) conformational isoenergetic contour map for weak dielectric constant (ε = 1r) present six low energy regions for the *anti*-prolinovaline corresponding to (P_{II}/ g$^-$), (P_{II} / t), (γ/ g$^-$), (γ/ t), (α / g$^-$) and (α/ t) structures. These minima were observed in all *anti*-prolinoamino acids. For the *syn*-prolinoamino acids, the number of conformational regions is limited to four, the (γ/ g$^+$) and (P_{II} / t) structures are destabilised by steric interactions between the substituents in position 3 and the oxygen from proline. The increase of hindrance from methyl (Val), methylsulfur (Met) to isopropyl (Leu) restrains strongly the fluctuation domains of the (P_{II}), (γ) and (α) structures. For weak dielectric constant (ε = 1r), the (γ/t)-turn is the most stable structure, whereas at higher dielectric constant (ε = 4r) P_{II} structure is the most stable one. The energy differences between an helix and the (P_{II}) / (γ) more extended structures increase from proline, prolinovaline, prolinomethionine to prolinoleucine. However, these differences remain weak (2 kcal/mol^{-1}) and thus do not exclude an conformation when bound into the receptor. On the other hand, systematic mutual superposition between the different conformers of the *anti* and *syn* isomers reveal a good overlap of heavy atoms of the trans rotamers. From this study, it can be concluded that comparison of the binding potencies of peptides substituted with either *syn* or *anti* 3-prolinoamino acids must allow to define the local bioactive conformation. Consequently, 3-prolinomethionine and 3-prolinoleucine have been incorporated instead of Met and Leu in the sequence of Substance P (RPKPQQFFGLM-NH$_2$:SP). The biological potencies for the NK-1 receptor of these SP prolino-analogs have been determined and the three-dimensional structures of these peptides have been analysed by NMR analysis.



R_1=R_2=H Prolinovaline ; R_1=R_2=CH_3 Prolinoleucine ; R_1=H and R_2= S-CH_3 Prolinomethionine

P A128 - New synthetic routes to dipeptide mimetics

A. N. Chulin[1], I. L. Rodionov[1], V. T. Ivanov[1]

1. Branch of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry / Laboratory of Peptide Chemistry - Russian Federation

Aminoacyl incorporation reaction known since the early sixties was evaluated as a general synthetic route to various dipeptide mimetics. Aminoacyl incorporation reaction is effectively a ring expansion *via* intramolecular acylation of amino group (or another nucleophilic functions like HS-, HO-) by the activated cyclic diacylamino moiety (I) with intermediate formation of bicylic azacyclols (II).

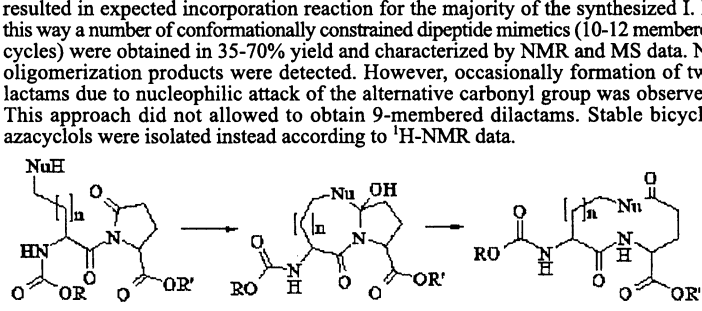
In the present study the above reaction was extended to aminoacylated pyroglutamic acids

R-Xaa-pGlu-OR' (I),

derived from diaminoacids Lys, Orn, Dab and Dpr, monoaminoacids 2-aminobenzoic acid, 3-aminopropionic acid, a class of unusual peptides that remains almost unexplored yet. 3 different synthetic approaches to I were evaluated:

1. direct acylation of sodium derivative of pGluOR";
2. pyrrolidone ring closure in the linear dipeptides R-Xaa-Glu(OX)-OR" promoted by bases and/or by activation of side chain carboxyl of Glu;
3. selective oxidation of the related proline dipeptides Boc-Xaa-Pro-OR' by RuO_4-$NaIO_4$.

Scope and limitation of the above approaches will be discussed and exemplified by the synthesis of more than 30 protected dipeptides I. Deprotection of amino or hydroxy functions followed by exposure to potassium carbonate buffer (pH 9.5, H_2O-CH_3CN) resulted in expected incorporation reaction for the majority of the synthesized I. In this way a number of conformationally constrained dipeptide mimetics (10-12 membered cycles) were obtained in 35-70% yield and characterized by NMR and MS data. No oligomerization products were detected. However, occasionally formation of two lactams due to nucleophilic attack of the alternative carbonyl group was observed. This approach did not allowed to obtain 9-membered dilactams. Stable bicyclic azacyclols were isolated instead according to ^{1}H-NMR data.



I II III

Aminoacyl Incorporation Reaction

P A129 - Cross-antigenic and cross-immunogenic properties of topological derivatives of amyloid β-peptide 1-40

M. Della Selva[1], M. Rossi[1], A. Verdoliva[1], A. Scarallo[1], M. Colombo[1]

1. TECNOGEN S.C.p.A. 81015 Piana di Monte Verna (CE) - Italy

Recent works on topological related peptides showed a generalized cross-antigenicity between the normal peptides and all their topological variants obtained by inversion of side chain chirality (inverso derivatives) or by inversion of backbone polarity (retro derivatives) or by both modifications (retro inverso derivatives). Polyclonal antibodies raised against 6, 13, and 15 residues antigens were able to recognize, with similar affinity and specificity, all topological derivatives [1,2,3]. To investigate this molecular recognition phenomenon in more complex structures, we have studied the antigenic properties of a longer peptide, the Amyloid β 1-40 peptide (Aβ 1-40) that plays a relevant role in the Alzheimer's disease.
A full set of data, deriving from direct and competitive ELISA assays, showed that commercial rabbit antibodies raised against the Aβ 1-40 were able to cross-react, in a dose dependent manner, with the complete series of topological related peptides retro Aβ 1-40, inverso Aβ 1-40, retro-inverso Aβ 1-40. In addition, all Aβ 1-40 derivatives were used to immunize Balb/c mice. These peptides showed a similar immunogenicity and the obtained polyclonal antibodies were found able to cross-react with the topologically related variants in linear binding ELISA assays. These data show the possibility of redesigning complex antigenic peptides maintaining immunological and antigenic properties.

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P A130 - Monomeric and dimeric cyclic peptides as potent mimetics and inhibitors of BDNF

J. M. Fletcher[1], R. A. Hughes[1]

1. Department of Pharmacology, University of Melbourne, Victoria 3010 - Australia

Brain-derived neurotrophic factor (BDNF) is a potent survival-promoting factor for variety of central and peripheral neurons. In a number of animal models, BDNF - or compounds which mimic its action - has shown tremendous potential for the treatment of neurodegenerative disorders. Inhibitors of BDNF, on the other hand, may be of benefit in the treatment of poor-prognosis, highly metastatic, BDNF-dependant neuroblastoma [1]. As a step to obtaining low molecular weight mimetics and inhibitors of BDNF for therapeutic use, we have designed and synthesised novel, monomeric-monocyclic and dimeric-bicyclic peptides based on the solvent-exposed loops of BDNF: L1, L2, and L4, which are known to mediate receptor binding. Our laboratory has previously demonstrated that monomeric peptides based on L2 and L4 of BDNF act as incomplete BDNF inhibitors, while homodimeric L2-L2 peptide mimetics are BDNF-like partial agonists [2]. To further our understanding of the role of the BDNF loops, we designed and synthesised (a) additional monomeric-monocyclic peptides, including an analogue of L1, and (b) novel dimeric-bicyclic analogues consisting of combinations of BDNF loop mimetics: the heterodimers L1-L2 and L2-L4, and the homodimer L4-L4.
Following synthesis, compounds were assayed in primary cultures of embryonic chick dorsal root ganglion sensory neurons for their ability to either inhibit BDNF-mediated neuronal survival, or to promote survival intrinsically. In competition with BDNF all monomeric-monocyclic and dimeric-bicyclic peptides produced concentration dependent inhibition of BDNF-mediated neuronal survival with the heterodimeric-bicyclic peptide L1-L2 the most effective (64±5% inhibition, $p<0.001$, $n=3$) at 10^{-9}M. Neither the monomeric-monocyclic nor the heterodimeric-bicyclic peptides exhibit intrinsic neuronal survival promoting effects. However a homodimeric-bicyclic peptide L4-L4 possesses weak BDNF-like agonist activity, promoting the survival of sensory neurons at 10^{-5}M (24±9% of BDNF mediated survival, $p<0.01$, $n=4$). The data suggest that all three solvent-exposed loops of BDNF are involved in mediating neuronal survival. This information will be invaluable in the development of more efficacious and potent BDNF inhibitors and mimetics.

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P A131 - Synthesis of γ-turn mimetics utilising fast microwave-assisted palladium-couplings

J. Georgsson[1], M. Larhed[1], A. Hallberg[1]

1. Organic Pharmaceutical Chemistry, Uppsala University, Box 574, SE-75123 Uppsala - Sweden

In recent years a large number of endogenous ligands have been identified. Furthermore many peptide receptors identified are now well established as targets for drug intervention. Peptides though are not suitable as lead-structures due to lack of oral bioavalability and insufficient metabolicstability.
To convert peptides by an iterative displacement of peptide fragments for organic moieties, and thereby achieve organic drug-like molecules, is a tremendous challenge. We are addressing this important issue by utilising Angiotensin II (Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-Phe^8) as a model peptide.
A turn structure centred around the Tyr^4 in Angiotensin II has been suggested. The target benzene scaffold of this study was designed to partly fill the criteria of an inverse γ-turn. The aim is to develop an efficient synthetic route, synthesise the turn mimetic and introduce it in Angiotensin II.
In the investigation of a fast and efficient route to the γ-turn mimetic, the use of cheap, commercially available starting materials was a demand. In the synthesis, fast microwave-assisted palladium-catalysed couplings were used in combination with more traditional synthesis. Importantly we found the use of gaseous carbon monoxide less convenient in the carbonylation coupling and developed therefore a reaction relying on *in situ* liberation of CO from the cheap, solid $Mo(CO)_6$.

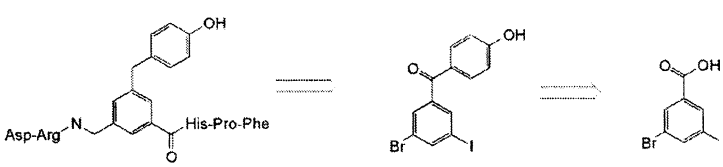

Fig. 1 - Retrosynthesis of a gamma-turn mimetic

P A132 - Urotensin-II (UT-II) analogues containing modifications at disulfide bridge: synthesis, biological and conformational studies

P. Grieco[1], P. Campiglia[1], E. Novellino[1], A. Carotenuto[2], P. Rovero[2], E. Zampelli[2], R. Patacchini[3], C. A. Maggi[3]

1. Dep. Chimica Farmaceutica e Toss., University of Naples "Federico II" - Italy
2. Dep. Scienze Farmaceutiche, University of Salerno - Italy
3. Menarini Ricerche, S.p.A., Firenze - Italy

Human Urotensin II (hU-II) is a disulfide bridged undecapeptide recently identified as the ligand of an orphan G protein-coupled receptor. hU-II (H-Glu-Thr-Pro-Asp-cyclo[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) has been described as the most potent vasoconstrictor compound identified to date. We have recently synthesized and tested a series of lactam analogues of hU-II minimum active fragment, i.e. hU-II(4-11). We found that the partial loss of activity observed in these synthetic analogues as compared to the native peptide may be due to either an important role of the disulfide bridge or to the different orientation of one of the key amino acid side chains. With the aim of highlight the importance of the disulfide bridge we have synthesized some analogues where Cys was replaced by hCys and by conformationally constrained Pen residues. By this approach we have explored the importance of ring dimension on activity and constrained the side-chain conformation of Cys residues involved in disulfide bridge without modify the overall backbone conformation.
In this work we report the synthesis, biological results and a preliminary structural analysis performed by 2D ^{1}H-NMR of these analogues compared to hU-II.

H-Asp-[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH hU-II(4-11) X_{aa}=*hCys, Pen or DPen*
H-Asp-[X_{aa}-Phe-Trp-Lys-Tyr-X_{aa}]-Val-OH

P A133 - Morphiceptin analogues containing a new β-turn thiazolidine mimetic

P. Grieco[1], P. Campiglia[1], I. Gomez-Monterrey[1], A. Carotenuto[2], L. Giusti[3], E. Novellino[1]

1. Dep. Chimica Farmaceutica e Toss., University of Naples - Italy
2. Dep. Scienze Farmaceutiche, University of Salerno - Italy
3. Dep. Farmacologia, University of Pisa - Italy

Morphiceptin (H-Tyr-Pro-Phe-Pro-NH_2) is an opiod agonist with high selectivity for μ opioid receptor. This peptide contain a Pro residue in the 2- and 4- positions of the peptide sequence. Consequently, it is possible to have a *cis/trans* isomerization at both respective peptide bonds. *Cis/trans* isomerization around the peptide bond preceding a Pro residue in a peptide is a dynamic process, and the two isomers cannot be isolated at room temperature. It is thought that *cis* conformer relate to Tyr-Pro bound, might bind to the receptor but direct experimental determinations of the conformation of a receptor-bond peptide are not yet feasible. Previously [1,2] studies on morphiceptin using 2-C-dimethylated pseudoproline or proline analogues have permitted to induce a *cis* peptide-bond demonstrating that the *cis* conformation around the Tyr-Pro amide bond is required for the the opiod activity. Here we describe the synthesis and biological results of morphiceptin analogues in which the Pro residues in position 2- and 4- were replaced with a thiazolidine moiety. This thiazolidine scaffold, previously synthesized in our lab, is a β-turn motif and favour quantitatively a *cis* conformation at peptide bond in our peptides. We have performed a preliminary 2D ^{1}H NMR spectroscopy study (COSY, NOESY and TOCSY) to correlate the structure of these new morphiceptin analogues with lead compound.

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P A134 - Modification of Phe^1-Gly^2 peptide bond in nociceptin/orphanin FQ(1-13)-NH_2: synthesis and characterization in the mouse vas deferens.

R. Guerrini[1], R. Cotugno[1], M. Zucchini[1], D. Rizzi[2], G. Calò[2], D. Regoli[2], S. Salvadori[1]

1. Department of Pharmaceutical Sciences and Biotechnology Center Section of Pharmacology, University of Ferrara, via Fossato di Mortara 17, 44100 Ferrara - Italy
2. Department of Experimental and Clinical Medicine, Section of Pharmacology, University of Ferrara, via Fossato di Mortara 17, 44100 Ferrara - Italy

The nociceptin/orphanin FQ receptor (NOP) is a G protein coupled receptor that modulates several peripheral and central functions [1]. The endogenous ligand of the NOP receptor is a heptadecapeptide (H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH) named nociceptin or orphanin FQ (N/OFQ), whose primary sequence is closely related to that of Dynorphin A. Structure-activity relationship studies [2] on N/OFQ have established that a) N/OFQ residues 1-13 are sufficient for high affinity binding and receptor activation, b) the basic residues in the C-terminal sequence of the peptide (address domain), especially Arg^8, appear to be instrumental for NOP receptor recognition, and, in combination with the N-terminal Phe^1 residue, for excluding N/OFQ interaction with classical opioid receptors, c) contrary to opioid peptides which strictly require Tyr in position 1, the active core that activates the NOP receptor is the Phe^4 pharmacophore, d) subtle changes of the N-terminal sequence, especially at Phe^1, led to reduction of efficacy, generating partial agonists ([Phe^{1}ψ(CH_2NH)Gly^2]-N/OFQ(1-13)NH_2) or pure antagonists ([Nphe^1]-N/OFQ(1-13)NH_2, Nphe = N(Benzyl)Glycine). In the present study, the amide bond between Phe^1-Gly^2 of N/OFQ(1-13)NH_2, has been replaced with methyleneoxy [ψ(CH_2O)], thioether [ψ(CH_2S)] and ketomethylene [ψ(COCH_2)] bonds. We considered also the retro-inverso [ψ(NHCO)] peptide bond and the nitrogen methylation of the reduced peptide bond [ψ(CH_2(CH_3)N)]. These modifications have been designed to investigate the importance a) of the hydrogen bond donor/acceptor properties of the Phe^1-Gly^2 amide bond for NOP receptor occupation and activation and b) of the basicity of the reduced peptide in the partial agonist activity of [Phe^{1}ψ(CH_2NH)Gly^2]-N/OFQ(1-13)NH_2.

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P A135 - Evaluation of primary amines for peptoid synthesis and identification of by-products using LC-MS.

P. R. Hansen[1],T. S. Neerup[1], X. Doisy[1],

1. Chemistry Department, Royal Veterinary and Agricultural University - Denmark

Peptoids are oligomers of *N*-substituted glycine residues and are structurally similar to α-amino peptides, stable to proteolysis, easy to synthesize, and have potent biological activity. Recently, it has been shown that some peptoids display antibacterial activity. Here, we wish to report the synthesis and analysis of a collection of peptoids using 50 primary amines in a model system.
Most of the primary amines are commercially available and were chosen from the following classes: aliphatic linear, aliphatic β-branched, positively charged, aliphatic hydroxylic, heterocyclic, negatively charged, small volatile, bulky, aromatic, and aromatic hydoxylic.

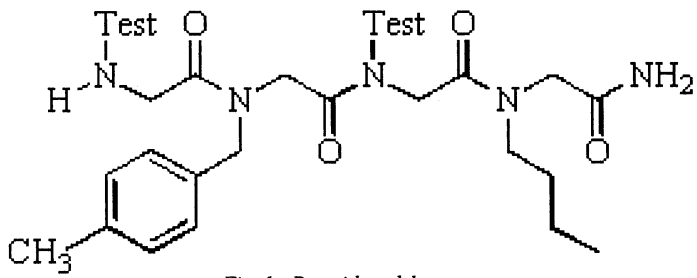

Fig. 1 - Peptoid model system

The model peptoids were synthesized on a Tenta Gel S RAM resin with the submonomer approach: bromoacetic acid, DIC, a primary amine, and NMP as solvent. Following synthesis, the product was cleaved from the resin with TFA/H_2O (95:5), lyophilised and analysed by LC-MS. Most of the tested primary amines performed well in the syntheses, yielding clean peptoids.
The successful building blocks are currently used in peptoid libraries suitably designed to display antimicrobial activity.

P A136 - Oostatic peptides with 3H-labeled proline: synthesis, biological activity, binding and distribution studies in the flesh fly *Neobellieria bullata*

J. Hlaváček[1], B. Bennettová[2], J. Slaninová[1], B. Černý[3], J. Holík[3], R. Tykva[1]

1. Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6 - Czech Republic
2. Institute of Entomology, Academy of Sciences of the Czech Republic, 370 05 České Budějovice - Czech Republic
3. Institute of Experimental Botany, Academy of Sciences of the Czech Republic, 142 00 Prague 4, Czech Republic - Czech Republic

Oostatic peptides derived from decapeptide H-Tyr-Asp-Pro-Ala-Pro$_6$-OH [1] inhibit development of egg in flesh fly *Neobellieria bullata* and can be potentially used in a control of insect reproduction. A shortening of the decapeptide sequence from the C-terminus results in acceleration and enhancement of this effect [2-4]. Our interest was further focused on elucidation of the mechanism of the action. For this purpose analogues **1-6** containing 3,4-dehydroproline (ΔPro) in the C- terminus or inside of the peptide chain were synthesized. In the case of decapeptide **1** and pentapeptide **2**, protected N- terminal nona- and tetra-peptides were prepared using 2-chlorotrityl chloride resin. After detachment from the resin both the fragments were coupled to H-ΔPro-OMe hydrochloride using TPTU reagent in the presence of HOBt and DIEA in DMF solution. The Boc-Tyr(BrZ) and Asp(OtBu) protecting groups were removed simultaneously by treatment with a TFA-TFMSA mixture containing thioanisole and EDT. In the case of peptides 3-6, an efficient reagent TOTU with HOBt and DIEA in DMF had to be used for the acylation of ΔPro imino group with Boc-Asn(Bzl)-OH. Corresponding peptide chains were prolonged by reaction with Boc-Tyr-OSu in DMF (peptides **3** and **4**), followed by deprotection with TFA-water-TIS mixture. The same deprotection was used with dipeptide **5** and tripeptide **6**.
The deprotection was followed by saponification and ionex chromatography and HPLC purification. The catalytic hydrogenation

H-Tyr-Asp-Pro-Ala-X-Y-OH

1, X = Pro$_5$, Y = ΔPro	**3**, H-Tyr-Asp-ΔPro-OH	**5**, H-Asp-ΔPro-Ala-OH
2, X = ΔPro, Y = 0	**4**, H-Tyr-Asp-ΔPro-Ala-OH	**6**, H-Asp-ΔPro-OH

of the ΔPro containing peptides with gaseous tritium afforded ^{3}H-labeled peptides (specific activity of about 1.7 TBq/mmol). These tritiated peptides were used for binding studies using fractions from homogenates of the flesh fly ovaries and heads and for distribution studies into different parts of the flesh fly body after bolus injection application. According to preliminary results, the radioactivity is very quickly distributed and the peptides degraded till the dipeptide **6**, the accumulation of the radioactivity being faster in the ovaries than in the head.

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P A137 - Estimation of hydrophobicity in peptidomimetic libraries

F. Hollósy[1], T. Lóránd[2], D. Erös[3], G. Kéri[1]

1. Peptide Biochemistry Research Group of the Hungarian Academy of Sciences in Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry. Puskin u. 9, 1088 Budapest - Hungary
2. Department of Medical Chemistry, Faculty of Medicine, University Pécs Szigeti út 12, 7624 Pécs - Hungary
3. Institute of Pharmaceutical Chemistry, Semmelweis University, Högyes u. 9, 1088 Budapest - Hungary

Hydrophobicity plays an important role in the biological and physicochemical behavior of numerous classes of organic compounds including peptides and peptidomimetics as well. Among the descriptors used in QSAR correlations, the hydrophobic parameter, logP, ususally expressed as the partition coefficient in the water-octanol system, is one of the most popular, because the characterization of this parameter may help to predict toxicity, reactivity or transport parameters in molecule libraries and it offers a help to compose a more rational library. Various methods have been developed for the characterisation of hydrophobicity including the reversed-phase high performance liquid chromatography (RP-HPLC) measurement and the software calculation method. In our experiments both methods were used to study the relative importance of hydrophobicity in a peptidomimetic library.
Based on Mannich ketone structure, a series of peptidomimetics were synthesized as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor and substitutions were carried out with morpholinyl, pirrolidinyl, piperidyl and tetrahydro-isoquinolyl groups in various position on three different skeletons. Hydophobicity of Mannich ketones was characterized by RP-HPLC as logk' and by software-calculated parameters as clogP data. Furthermore, compounds were tested their ability to inhibit EGFR activity of A431 cell by MTT assay. Our results showed that the determination of hydrophobicity by measuring the logk' or by calculating the clogP values of the compounds may help to predict the biological activity of the elements of peptidomimetic libraries.

P A138 - Synthesis and inhibition properties of diastereoisomeric hydroxyethylamine pseudopeptides

M. Hradilek[1], J. Prejdova[1], T. Uhlikova[1], J. Weber[1], M. Soucek[1], J. Konvalinka[1]

1. Institute of Organic Chemistry and Biochemistry, Prague - Czech Republic

Potent transition state analogue inhibitors for aspartyl protease are obtained by replacing the scissile peptide bond by the hydroxyethylamine isostere. In the continuation of our systematic study of protease inhibitors a series of pseudopeptides in which the scissile amide bond was replaced by hydroxyethylamine isostere were synthesized. The inhibitors were prepared by alkylation of N-terminal amino group of a peptide by diasteroisomerically pure N-protected 3-amino-1,2-epoxy-4-phenyl butanes. The alkylation was performed at elevated temperature in a protic solvent. The course of the reaction was strongly dependent on thermal stability of diastereoisomeric pairs of epoxides that was governed both by stereochemistry of the epoxide and by nature of its N-protecting group. The structure of the product formed in unsuccesful reaction was identified and a plausible mechanism for its formation was suggested. Synthesis was performed by in solution method or by solid phase method in dependence on sequence of peptide chain. The set of prepared compounds was tested for in vitro activity with HIV-1 protease and its mutants resistant towards clinically used protease inhibitors. Experiments showed that synthesized pseudopeptides are tight binding inhibitors with Ki in subnanomolar or nanomolar region. Potent inhibition of viral polyprotein processing was found in tests on mammalian cell tissue cultures.

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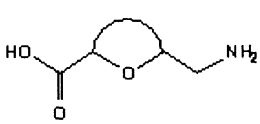
P A139 - An unsaturated peptidomimetic assembly derived from a carbohydrate

J. H. Jones[1], Y-K. Chung[1], T. D. W. Claridge[1], G. W. J. Fleet[1], S. W. Johnson[1], A. V. Stachulski[2]

1. Department of Chemistry, University of Oxford - United Kingdom
2. Department of Chemistry, University of Liverpool - United Kingdom

Peptidomimetics derived from carbohydrates are of particular current interest, because of the potential for precise stereochemical design which carbohydrate building blocks offer. Recent studies at Oxford, which are ongoing, have focused on the synthesis and conformational predilections of diverse oligopeptidomimetics of this class, especially structures in which there is a repeating pseudodipeptide unit derived from a saturated carbohydrate amino acid of type **1**. It seemed worth exploring the extension of these studies to oligopeptidomimetics derived from αβ-unsaturated carbohydrate amino acids of type **2**, because such oligomers would have additional conformational restraints. Our investigations of the synthesis and properties of the series **3** will be reported.



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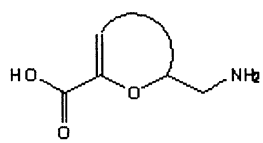

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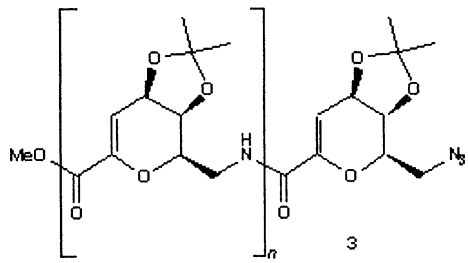


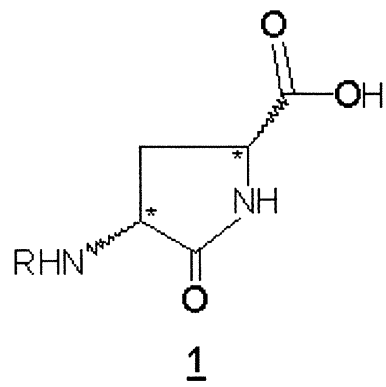
3

P A140 - A novel *cis*-peptide bond motif inducing type VI β-turn. synthesis and biological evaluation of conformationally restricted enkephalin and morphiceptin analogues

K. Kaczmarek[1], N. N. Chung[2], P. W. Schiller[2], J. Zabrocki[1]

1. Institute of Organic Chemistry, Technical University of Łódź - Poland
2. Laboratory of Chemical Biology, Clinical Research Institute of Montreal - Canada

We have recently published synthesis and biological evaluation[1,2] of analogues of [Leu^5]-enkephalin amide, having incorporated four stereoisomers of 4-amino-pyroglutamic acid (**1**, R=H) residues in positions 2-3. Our *cis*-peptide bond motif is a hybrid of glycine and alanine, which limits its utilization as a replacement for dipeptide sequences not possessing longer alkyl, aromatic and/or containing heteroatom(s) side chains. In order to convert compound **1** (R=H) into more convenient tool for probing existence of particular peptide bond in *cis*-conformation, we have synthesized N-monoalkylated (on NH_2 group) derivatives of **1** (R=benzyl, p-benzyloxybenzyl) through reductive alkylation reaction performed on solid support [3].
Synthetic strategy as well as biological activity of analogues will be discussed.



1

(2S,4R), (2R,4S) - trans
(2S,4S), (2R,4R) - cis

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