P A33 - Peptides of 4-methyl-pseudoproline derived from alpha-methylserine: synthetic approach and conformational aspects.

J. Katarzynska[1], S. Jankowski[1], K. Huben[1], M. T. Leplawy[1], J. Zabrocki[1]

1. Institute of Organic Chemistry, Technical University of Lodz - Poland

4-Methyl-pseudporoline (MeSer(Pro)) are reversibly modified derivative of α-methylserine (MeSer), which belongs to the family of α-alkyl-α-hydroxymethyl amino acids. The aim of this work was to evaluate 4-methyl-pseudoproline as peptide building blocks providing an access to peptides of α-alkyl-α-hydroxymethyl amino acids.We have shown previously[1] that 4-methyl-pseudoproline are readily obtained in a good yield by cyclocondensation of α-methylserine with aldehydes or ketones (Fig.1). 4-Methyl-pseudoproline is rather unstable, however after protection of NH-group it can be isolated and stored for a long time. N-protected-C-activated 4-methyl-pseudoproline (Boc-, Z-, Fmoc-) is an efficient acylating component in formation of peptide bond. The acylation of NH-group in 4-methyl-pseudoproline with C-activated amino acids gives the expected dipeptides with C-terminal pseudoproline in very low yield.

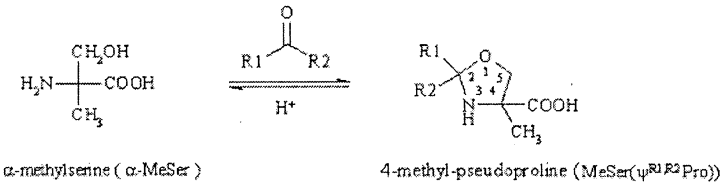


Fig.1. Transformation of α-methylserine to 4-methyl-pseudoproline.

Here we report the synthesis and conformation studies of α-methylserine containing "host-guest" peptides where we applied the pseudoproline concept. All peptides were characterized by mass spectrometry and by two-dimensional NMR spectroscopy. On the basis of experimental results possible *cis-* or *trans-* isomers due to the C-2 substituents in the 4-methyl-pseudoproline unit will be discussed.

References
[1] J. Katarzynska, M.T. Leplawy, J. Zabrocki: "4-Alkyl-pseudoprolines as a new building blocks in peptide chemistry", XVI *Polish Peptide Symposium*, Krakow-Przegorzaly 2001.

Acknowledgements: Supported by The Polish State Committee for Scientific Research (KBN), GRANT 4 T09A 065 22.

P A34 - Solid-phase synthesis of tyrosine-sulfate containing α-conotoxins

K. Kitagawa[1], Y. Sekikawa[1], H. Fujiwara[1]

1. Niigata College of Pharmacy - Japan

Conotoxins are a family of small peptides isolated from cone snails and act on ion channels. They are classified into several groups based on their structures and pharmacological characteristics. Recently three α-conotoxins; EpI, PnIA and IB, which contained the sulfated tyrosine residue [Tyr(SO_3H)], were isolated. These α-conotoxins have 16 amino acid residues and two disulfide bridges. As a part of our program of a versatile synthetic method aiming at the biological studies of Tyr(SO_3H)-containing peptides, we investigated the solid-phase synthesis of the sulfated α-conotoxins.

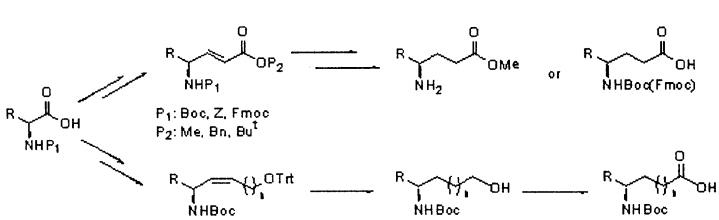
We examined first the disulfide bond formation using oxytocin sulfate as a model and found that the DMSO oxidation and the I_2 oxidation in 50% AcOH were compatible with an acid-labile Tyr(SO_3H) residue. The linear peptides for the sulfated α-conotoxins were constructed on Rink amide resin using Fmoc-based chemistry. Fmoc-Tyr(SO_3Na) was used as a building block to introduce the Tyr(SO_3H) residue. In order to form the two disulfide bonds, both a simultaneous oxidation (approach A) and a selective oxidation (approach B) were examined. In both approaches, cleavage/deprotection of the peptide-resin was conducted with 95% TFA-TIPS (0°C, 8h). Although detachment of the peptide from the Rink resin was not quantitative, loss of the sulfate during the prolonged deprotection was negligible. The Trt groups were adopted for the four Cys residues in approach A, and the thiol-free peptide obtained after the TFA-TIPS treatment was subjected to the DMSO oxidation. Objective sulfated peptide was obtained with the disulfide bond isomers. In approach B, a combination of the Trt and Acm groups was adopted for the pairwise Cys residues. After the TFA-TIPS treatment, a two-step disulfide bond formation was conducted, first with the DMSO oxidation followed by the I_2 oxidation in aqueous AcOH, to afford an objective sulfated peptide. Alternative approach, in which the trityl-based polymer support is used to link with the side-chain carboxyl of Asp residue or with the sulfhydryl of Cys residues, will be also presented at the symposium.

P A35 - From α-amino acids to enantiopure γ- and ω-amino acids with proteinogenic side chains

G. Kokotos[1], V. Loukas[1], C. Noula[1]

1. Laboratory of Organic Chemistry, Department of Chemistry, University of Athens - Greece

Unnatural amino acids play an important role in the design and synthesis of pharmacologically relevant molecules, analogues of bioactive peptides, peptide mimetics and enzyme inhibitors. Peptides constructed from β- and γ-amino acids can adopt helix, sheet or reverse turn conformations in solution or solid state as evidenced by NMR, CD, X-ray or modeling studies. The complete stability of β- and γ-peptides against common proteases suggests that they may suitable for pharmaceutical applications. We present here a general methodology for the synthesis of enantiopure γ- and ω-amino acids with proteinogenic side chains starting from the corresponding natural α-amino acids. The synthesis of γ-amino acids was based on a Wittig olefination reaction of *N*-protected α-amino aldehydes, obtained from amino alcohols by oxidation with NaOCl in the presence of AcNH-TEMPO. The use of a variety of *N*-protective groups (Boc, Z, Fmoc) and *C*-protective groups (Me, Bn, Bu^t) of phosphoranylidene acetate was studied. Various *N*-protected amino aldehydes reacted with methyl, benzyl and *tert*-butyl phosphoranylidene acetate to produce ,α-unsaturated γ-amino esters. Simultaneous hydrogenation of the double bond and removal of either benzyl or benzyloxycarbonyl group led, in high yield, to *N*- or *C*-protected γ-amino acids. *N*-Protected ω-amino alcohols were synthesized by the reaction of protected amino aldehydes with the ylides generated from $TrtO(CH_2)_nCH_2P^+Ph_3I^-$, and subsequent hydrogenation. *N*-protected ω-amino acids were obtained in high yields by the oxidation of these alcohols using NaOCl in the presence of a catalytic amount of AcNH-TEMPO and $Bu_4N^+HSO_4^-$. The present route to ω-amino acids permits the insertion of any chain length between the amino and carboxy functionalities depending on the chain length of the starting ylide used for the Wittig olefination reaction.

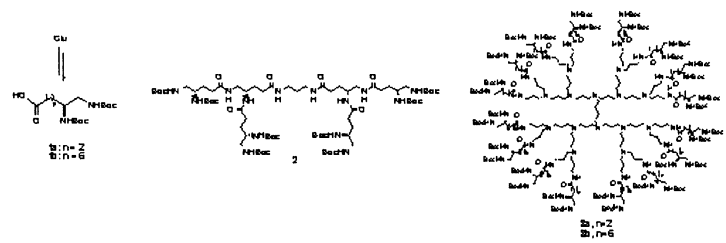


P A36 - Synthesis of dendrimers based on chiral n,n+1-diamino acids

G. Kokotos[1], E. Bellis[1], T. Markidis[1]

1. Laboratory of Organic Chemistry, Department of Chemistry, University of Athens - Greece

Dendrimers are highly branched macromolecules, synthesized stepwise from a central core and leading to a well-defined number of generations and end groups. The number, size and function of peripheral groups of dendrimers determine many of the dendrimers properties, such as dense-shell packing, overall shape, solubility, physical state (semi-crystalline, glass, liquid crystalline, liquid). Chiral, enantiomerically enriched dendrimers are of special relevance, because they would potentially allow enantioselective catalysis and molecular recognition. The aim of this work was to synthesize chiral diamino acids, which were applied for (a) the construction of a novel class of amino acid based dendrimers and (b) the functionalization of already existing dendrimers. Starting from L-glutamic acid we have synthesized Boc-protected 4,5-diamino- pentanoic acid (**1a**) and 8,9-diamino-nonanoic acid (**1b**). The divergent approach was applied to prepare the second generation dendrimer **2** using 1,3-propanediamine as the core and compound **1a** as the branching unit. The commercially available diaminobutane poly(propyleneimine) dendrimers of third generation (16 free amino groups) were coupled with compounds **1a,b** to produce the modified dendrimers **3a,b**.



P A37 - Synthesis of spatially addressed library of alanine dipeptides from rac-Z-Ala-OH by means of sub-library of chiral triazine condensing reagent immobilized on cellulose

B. Kolesińska[1], Z. J. Kamiński[1]

1. Institute of Organic Chemistry, Technical University of Łódź, 90-924 Łódź - Poland

An application of enantiomereselective coupling reagents offers numerous synthetic opportunities and should be evaluated as one of the most promising tools facilitating an access to the broad range of new optically active products [1] directly from racemic substrates. In case of the peptide synthesis their value is, however, seriously limited due to i) the unknown structure of coupling reagent best suited to the given synthetic goal; ii) unpredictable results of condensations involving complex diastereomeric substrates.The efforts to eliminate the ambiguity of condensations bring us to developing the novel generation of enantiomereselective coupling reagents [2]. According to the concept, reagents consist a binary system obtained *in situ* from the two readily accessible components – an already known triazine coupling reagent, and the chiral auxiliary – appropriate tertiary amine. Thus, departure of chiral auxiliary after activation of carboxylic function leads to the active acylating species identical with already known triazine acylating agent.

Herein the further improvement of our approach to enantiomereselective condensation is presented. This is based on the application of immobilized triazine condensing reagents [3]. Treatment of 2,4-dichloro-6-methoxy-1,3,5-triazine immobilized on cellulose membrane with a pool of chiral amines afforded *sub*-library of chiral condensing reagents. Coupling of *rac*-Z-Ala-OH with H-Aaa-OR yielded the spatially addressed library of Z-Ala-Aaa-OR dipeptides with altered configuration and variable enantiomeric purity of alanine residue. Dipeptides were isolated from the support by extraction and their enantiomeric purity were determined by HPLC and/or GC on chiral stationary phase.

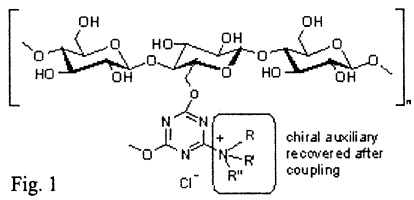


Fig. 1

References:
[1] Fu, G.C. *Acc. Chem. Res.* 33, 412-420 (2000); b) Vedejs, E.; Dauglis, O.; MacKay, J.A.; Rozners, E. *Synlett*, 10, 1499-1505 (2001); c) Copeland, G.T.; Miller, S.J. *J. Am. Chem. Soc.* 123, 6496-6502 (2001).
[2] Kamiński; Z.J.; Kolesińska; B.; Kamińska; J.E.; Góra, J., *J. Org. Chem.* 63, 4248-4255 (2001).
[3] Kamiński, Z.J.; Kolesińska, B., Cierpucha, M. *Peptides 2000. Proc. 26th EPS*, Ed. Martinez, J.; Fehrentz, J-A, EDK-Paris, 965-966 (2001).

Acknowledgements: The study was supported by the Polish State Committee for Scientific Research under the Project 3 T09A 029 16.

P A38 - High throughput peptide synthesis

O. J. Kreuzer[1], M. Birringer[2], K. A. Zarse[1], R. T. Roeder[2], J. Henkel[3], F. Bier[3], W. Zinsser[4]

1. Peptides & Elephants GmbH - Germany
2. German Institute of Human Nutrition - Germany
3. Fraunhofer Institute of Biomedical Engineering - Germany
4. Zinsser Analytic GmbH - Germany

In the beginning 1990 combinatorial chemistry starts its triumphant advance in modern drug discovery strategies. Especially the solid phase synthesis become a powerful tool to create a high diversity of molecules in one attempt. Libraries with millions of different molecules could easily be generated and the bottle neck of the drug discovery process shifted from the compound synthesis to the rapid identification of biological active substances. Ultra high throughput screening systems were build up to test these millions of molecules in a minimum of time [1,2]. Nowadays the task is rapid identification and isolation of the hits from such fast screening assays. But even modern analytical techniques are less sensitive then a biological assay system [3]. Therefore the library sizes came down to about hundred thousand of different molecules to yield higher amount of product. Parallel synthesis techniques were developed to create this diversity for defined substances in one step, no identification was needed. The light-directed parallel synthesis [4] and the spot-synthesis [5] were the first elegant methods to generate peptide arrays in parallel. For most of the cell based assay system especially for the screening of G-Protein coupled receptor agonist or antagonist soluble compounds are needed . As the commercially available automatic peptide synthesizers were just able to synthesis about hundred different peptides in parallel [6], we build a system which is able to handle ten microtiter plates to synthesis 960 peptide amides per run. Up to 52 different amino acid building blocks can be used in one set up. Each building block is stored and delivered via an individual cartridge to prevent cross contamination and to reduce wash steps and volume. Deprotection and cleavage of the peptide will take part in the same set up and a novel valve block allows to collect the free peptides at the end of the synthesis. Via intelligent software the robot movement will be optimized for each step to reduce run time. Using barcodes for plates and cartridges mix ups are eliminated. Furthermore large plate libraries can easily be organized (handled). Our system is the first fully automated system that generates up to 960 deprotected, dissolved peptides amides (about 1mg each, 10mer) per day.

References
[1] Gallop et al, *J. of Med Chem*, 1994, Vol. 37, No.9 pp 1233-1251
[2] Gordon et al *J. of Med Chem*, 1994, Vol. 37, No.10 pp 1385-1401
[3] Nothacker et al, *Nature cell biology*, 1999, Vol. 1,pp 383-385
[4] Fodor et al, *Science*, 1991, Vol. 251, pp 767-773
[5] Frank et al, *Tetrahedron*, 1992, Vol. 48,No. 42, pp 9217-9232
[6] Gausepohl et al,1992, *Peptide Chemistry* 1992, N. Yanaihara (Ed.), *Proc.2.JSPC.* (pp. 107-109). ESCOM, Leiden

P A39 - Selective microscale 3H-labelling of peptides in solution by using photolabile protecting groups

M. Lang[1], N. Koglin[1], R. Rennert[1], A. G. Beck-Sickinger[1]

1. University of Leipzig, Institute of Biochemistry - Germany

For many bioactivity studies there is an increasing demand for new and selectively radiolabelled peptides. Mainly binding studies of peptides to membrane bound proteins are still most efficiently made by radiolabelled tracer molecules in contrast to fluorescence labelled compounds. By far the most frequent tracer used is ^{125}iodine, mainly because of its easy post-synthesis introduction by oxidation of tyrosine residues. However, ^{125}iodine has a short half-life and because of its size it may not be useful in many cases. In our hands ^{3}H-labelled tracers are more suitable, both with respect to handling and activity. For the synthesis of ^{3}H-labelled peptides certain aspects have to be considered: the introduction of the tracer should be the last step and the work-up has to be easy and effective. We furthermore wanted to use the advantages of solid phase synthesis. Unfortunately most of the standard-techniques of peptide synthesis are optimized for milligram to gram scale. This means that even for a few milligrams a large quantity of radioactivity is necessary for the labelling of resin-bound peptides and great efforts during work-up and purification are required. However for many high-affinity ligands only a few micrograms are sufficient for a lot of investigations. Therefore we developed a new synthesis strategy to circumvent this disproportion and were able to synthesize selectively ^{3}H-labelled peptides in the µg-scale. For the introduction of the radiolabel we used the tritiated propionyl-group of N-hydroxysuccinimidyl (NHS)-propionate. With this active-ester only free amino groups are affected. However, without any protection a fully deprotected peptide with more than one amino group will not be labelled selectively.
In order to selectively protect amino groups, which should not be modified later, we used the photolabile protecting group 6-nitroveratryloxycarbonyl (Nvoc). After standard peptide synthesis the Nvoc-group can be introduced at defined positions directly on the resin. During the cleavage process and further purification steps this photolabile protecting group is stable.
Highly pure and selectively amino-protected peptides can be obtained by the method using standard peptide synthesis techniques. In those peptides only the amino group that is to be labelled is accessible for the labelling reagent. Small amounts such as some µg's from this peptide blocked with photolabile protecting groups can then be labelled in solution at a defined position. After the labelling procedure the Nvoc-group can be removed by irradiation with UV-light. Accordingly selectively radiolabelled peptides can be obtained with only one HPLC purification step after labelling and without any loss of the labelled peptide during work-up and purification after the cleavage procedure. The highly flexible usage of this photolabile protecting group and especially its application for the µg-scale labelling of peptides may open new ways for the facile synthesis of radioligands.

P A40 - Study on efficient synthesis of tryptophan and arginine rich peptide sequences using Nsc-amino acids in SPPS

H. J. Lee[1], W. Chweh[1], H. J. Kim[1]

1. Research Institute of A&PEP Inc., Sosabon-1-dong, Bucheon - South Korea

The synthesis of peptides containing both tryptophan and arginine based on the aryl (arene) sulfonyl group occur a major side reactions in the solid-phase peptide synthesis. It was resulted from modification of the Trp indole by reactive carbonium ion species released during acidolytic cleavage and sulfonation during the deprotection of the sulfonyl group. For example, the hexapeptide sequence H-Trp-Arg-Arg-Arg-Arg-Val-OH[1], a sequence which is very susceptible to Trp and Arg modification, was chosen as a model peptide to synthesize the effectiveness of various methods proposed to minimize Trp and Arg modification during the solid-phase peptide synthesis. Thus, we focused on the potential for improving the yield and purity of peptides containing both rich Trp and Arg by utilizing 2-(4-Nitrophenyl)sulfonylethoxycarbonyl (Nsc)-amino acids derivatives during the Nsc solid-phase peptide synthesis.

References
[1] Riniker, B., Hartmann, A. In *Peptide: Chemistry, Structure and Biology*, Proceedings of the 11th APS ., 950(1990).

P A41 - Gelsolin and gelsolin peptides binding to their conformation regulators

I. Liepina[1], P. Janmey[2], C. Czaplewski[3], A. Liwo[3]

1. Latvian Institute of Organic Synthesis, Aizkraukles str. 21, Riga, LV1006 - Latvia
2. Institute of Medicine and Engineering, University of Pennsylvania, Philadelphia, PA 19104 - U.S.A.
3. Faculty of Chemistry, University of Gdansk, 80-952 Gdansk - Poland

The action of gelsolin (an actin-binding-and-severing six-domain protein) is regulated by the Ca^{2+} ions and by the phosphoinsitides, which bind to this protein changing its conformation. The binding of the calcium ions changes the conformation of gelsolin from an inactive to the active actin-binding state. The binding of phosphoinositide lipids embedded in the intracellular side of the membrane occurs when gelsolin is bound to actin and probably changes the conformation of the fragments comprised by residues 160-169 and 135-142 of the S2 domain from extended strands (which occur in apo-gelsolin) to α-helical. This results in moving the S1 domain away and, consequently, uncapping gelsolin from actin. In our earlier studies we found that the low-energy structures of the two gelsolin peptides consisting of the sequences from the two sequences of the S2 domain mentioned above: G150-169 and G135-142 are α-helical and their binding to the PIP2 lipid has a hydrophobically-electrostatic nature. Electrostatic interactions took place between the lysine residues of the peptides G150-169 and G135-142 and the phosphate groups of the PIP2 molecules, while hydrophobic interactions occurred later between the hydrophobic residues of the peptides and the fatty-acid tails of PIP2, resulting in partial disruption of the lipid bilayer near the PIP2 cluster. Our present study is aimed at investigating the conformational changes of gelsolin induced by calcium cations and phosphoinositides. To determine the effect of calcium on the conformation of gelsolin, a molecular dynamics (MD) study of the gelsolin-calcium system was carried out. The simulated system consisted of the crystal structure of horse gelsolin (PDB code: 1DON), calcium cations, as well as sodium and chloride counterions to neutralize the charge in a periodic water box. The system was energy-minimized with the AMBER 5.0 force field and subsequently subjected to a 1000 ps molecular dynamics simulation at 298 K. Domain movement was monitored during the simulation. An MD simulation of gelsolin without calcium cations was carried out for comparison. To investigate the effect of PIP2 lipid on the conformation of the S2 domain of gelsolin, we carried out MD simulations of the S2 domain with calcium cations in a periodic water box. The system was first energy-minimized with the AMBER 5.0 force field and subsequently subjected to a 1000 ps MD simulation at T=298K, to investigate if that domain keeps its stability in water. The S2 domain was subsequently docked near residues 150-169 and 135-142 (which comprise the sequences of the two gelsolin peptides studied in our earlier work) to the phosphatidylinositol 4,5-bisphosphate (PIP2) cluster inserted in the periodic dimyristoyl-phosphatidylcholine (DMPC) lipid-water box (PIP2 lipid) and MD simulations were continued to determine conformational changes.

Acknowledgements: Supported by NATO Collaborative Linkage Grant LST.CLG.976647. Inta Liepina is a fellow of the Józef Mianowski Foundation, Poland.

P A42 - Peptide synthesis mediated by lipases: further studies and new perspectives

C. W. Liria[1], F. Bordusa[2], M. T. M. Miranda[1]

1. Biochemistry Department, Chemistry Institute, University of São Paulo, P.O.Box 26077, 05513-970, São Paulo - Brazil
2. Max-Planck Society, Research Unit "Enzymology of Protein Folding", Weinbergweg 22, D-06120 Halle/Saale - Germany

Due to their esterase activity lipases can be used for enzymatic peptide bond formation when the carboxyl donor is a C-esterified amino acid or peptide and the amine donor is an amino acid or peptide [1]. In previous work we confirmed such synthetic possibility and reported the optimal reaction condition to synthesize Ac-Tyr-Gly-NH_2 using Ac-Tyr-OEt as carboxyl component, Gly-NH_2 as amine component and crude porcine pancreatic lipase (cPPL) as catalyst. In that condition the coupling yield found in 5 minutes of reaction was 90%, although some secondary hydrolysis of the product was observed in much longer times (30-40% in 24h) [2]. Because of our current interest in condensing peptide segments with the help of enzymes we tried to study the specificity of cPPL for amino acids. At first, cPPL-mediated hydrolyses of methyl esters of Z-Gln, Z-His, Z-Asp, Z-Glu, Z-Phe, Z-Tyr, Z-Leu, Z-Lys and Z-Arg were carried-out. A high preference for Lys, Arg and His, moderate for Gln, Tyr and Phe and low preference for Asp, Glu and Leu became evident, which led us to wonder if such data exposed the real specificity of lipases for amino acids or resulted from a possible contamination of it by -chymotrypsin and trypsin. The evaluation of the tryptic and -chymotryptic activities of cPPL on Bz-Arg-pNA and Ac-Tyr-OEt, respectively, before and after its treatment with irreversible inhibitors of -chymotrypsin (TPCK) or trypsin (TLCK) revealed that both proteases could indeed be interfering in the esterase activity of cPPL. Conversely, cPPL-TPCK showed reduction of the ester hydrolysis rate when tested on Z-Phe-OMe, Z-Tyr-OMe and Z-Leu-OMe while no diminution was observed when cPPL-TLCK was tested on Z-Lys-OMe and Z-Arg-OMe. The data confirmed the interference of -chymotrypsin in the cPPL esterase activity and indicated that this enzyme preparation displays not identical, but trypsin-like specificity. SDS/PAGE analysis of cPPL and synthesis of Ac-Tyr-Gly-NH_2 catalyzed by cPPL-TPCK without undesirable secondary hydrolysis at 24h sustained the data cited above. More significant, however, was the occurrence of the Z-group removal during all the ester hydrolyses performed. Since proteases are inactive in this respect, such activity is fully attributed to lipase. Although this side reaction disable us to calculate the ester hydrolysis initial rates for the amino acid derivatives as desired, it exposed a new possibility for the application of enzymes in peptide chemistry. Experiments directed to conclude the cPPL amino acid specificity study and to the investigation of the Z-group removal by this enzyme are on progress in our laboratories.

References
[1] Matos, R. J. et al *Biotechnol. Lett.* 9 (1987) 233.
[2] Liria, C.W. & Miranda, M.T.M., *Peptides 2000* (2001) 331.

Acknowledgements: Financial Support: FAPESP and CNPq.

P A43 - Strategies for the preparation of oligopeptides tethered to luminescent and non-luminescent lanthanide(III) chelates

K. Loman[1], J. Hermonen[1]

1. PerkinElmer Life Sciences, Wallac Oy, Research Reagents, FIN-20101 Turku - Finland

Due to their unique luminescence properties lanthanide(III) chelates are often used as non-radioactive markers in a wide variety of routine and research applications.[1] Since lanthanide(III) chelates give strong, long decay-time luminescence, they are ideal labels for assays where high sensitivity is required. Time-resolved fluorometric assays based on lanthanide chelates have found increasing applications in diagnostics, research and high throughput screening. The heterogeneous DELFIA® technique is applied in assays requiring exceptional sensitivity, robustness and multi-label approach.[1] Development of highly luminescent stable chelates extends the use of time resolution to homogeneous assays based on energy transfer (TR-FRET) quenching, or changes in the chelate's luminescence properties during a binding reaction.[2] Introduction of lanthanide(III) chelates to oligopeptides and proteins in solution can be performed between an amino or mercapto group of a bioactive molecule and isothiocyanato, haloacetyl or 3,5-dichloro-2,4,6-triazinyl derivatives of lanthanide(III) chelates. *N*-terminal threonine or serine residues can be labeled with aminooxychelates after periodate oxidation. An alternative labeling strategy includes introduction of chelate precursors to the oligopeptide structure as blocks during machine assisted oligopeptide synthesis (see J.Peuralahti et al, Abstract in this Conference). After completion of the oligopeptide synthesis the oligopeptides are released from the resin and deprotected. Treatment of the deblocked oligomers with lanthanide(III) citrate converts the oligopeptide conjugates to the corresponding lanthanide chelates. We describe here our strategies for the preparation of oligopeptide conjugates. Also application data is included.

References
[1] Hemmilä, I. Ståhlberg, T. and Mottram, P. (Eds.) *Bioanalytical Applications of Labelling Technologies*, Wallac Oy, 1994 ; Hemmilä, I. and Mukkala, V.-M. *Crit. Rev. Clin. Lab. Sci.* 2001, *38*(6), 441
[2] Hemmilä, I. *J. Biomol. Screening*, 1999, *6*, 303; Pope, A.J. ibid, 1999, *6*, 301; Mathis, G. ibid, 1999, *6*, 309; Mathis, G. Clin. Chem. 1993, *39*, 1953

P A44 - Synthesis of the conformationally constrained head-to-tail cyclic gomesin

A. Machado[1], A. Miranda[2], S. Daffre[3], M. A. Fázio[2], M. T. M. Miranda[1]

1. Biochemistry Department, Chemistry Institute, University of São Paulo, P.O. Box 26077, 05513-970, São Paulo - Brazil
2. Biophysics Department, Federal University of São Paulo, 04044-20, São Paulo - Brazil
3. Parasitology Department, University of São Paulo, 05508-900, São Paulo - Brazil

Some antimicrobial peptides, which are effective against Gram-positive and Gram-negative bacteria and fungi, are hemolytic at mM concentration. Gomesin, isolated from hemocytes of the tarantula spider *Acanthoscurria gomesiana*, is an example. This C-terminal amidated peptide has 18 amino acids (a pyroglutamic acid as the N-terminus) and two intramolecular disulfide bridges (Cys^{2-15} and Cys^{6-11}) [1]. In a recent systematic study it was shown that both disulfide bridges are important for the maintenance of the antimicrobial activity of this peptide [2]. We have worked on the synthesis of a conformationally constrained head-to-tail cyclic gomesin so as to increase its stability to enzymatic degradation and, luckily, dissociate its hemolytic and antimicrobial activities [3]. Three different approaches were explored: 1) head-to-tail cyclization prior to selective oxidations of the cysteine residues (Cys^6 and Cys^{11} protected with Meb; Cys^2 and Cys^{15} with Acm); 2) Cys^6 and Cys^{11} oxidation prior to head-to-tail cyclization followed by oxidation of the two other sulfhydryl groups (Cys^2 and Cys^{15}); 3) two subsequent selective oxidations of the cysteine residues prior to cyclization head-to-tail. In all cases the linear precursors were built-up on PAM resin by the solid-phase t-Boc chemistry. Full deprotection and final cleavage were carried-out in the presence of HF and scavangers, the free sulfhydryl groups were air-oxidized, the *S*-Acm-cysteins were oxidized with Tl(III) and the head-to-tail cyclizations were achieved using BOP as the coupling reagent. All the reactions in solution were monitored by RP-HPLC or LC-ESIMS. The second approach gave the best crude peptide. Thus, this was fractionated by RP-HPLC with the help LC-ESIMS. The purified cyclized analogue was characterized by amino acid analysis and LC-ESIMS. Due to the very poor yields obtained we are now exploring approaches involving head-to-tail cyclizations on-resin.

References
[1] Silva Jr., P. I.; Daffre, S.; Bulet, P. *J. Biol. Chem.* 2000, *275*, 33464;
[2] Fázio, M. A.; Daffre, S.; Miranda, M. T. M.; Bulet, P.; Miranda, A. *"Peptides: The Wave of the Future"* Eds. Michal Lebl and Richard A. Houghten, Klumer Academic Publishers, The Netherlands 2001, 495;
[3] Tam, J. P.; Wu, C. W.; Yang, J. L. *Eur. J. Biochem.* 2000, *267*, 3289.

Acknowledgments: Financial Support: FAPESP, CNPq

P A45 - Application of an alternative amphoteric solvent polarity scale for examining polymer solvation

L. Malavolta[1], E. Oliveira[2], G. Jubilut[1], E. M. Cilli[1], C. R. Nakaie[1]

1. Departament of Biophysics - Brazil
2. Departament of Organic Chemistry - Spain

The correct understanding of polymer solvation phenomenon has been considered crucial for many modern methods. However its dependence to the polarity of the medium is still not entirely established. To improve our knowledge about this physicochemical issue, the swelling degrees of resins with a great variety of characteristics, taken as solute-models, were measured in 28 solvent systems (microscopic measurement of beads) which broadly encompass entirely the polarity scale. Following our preliminarily report [J. Org. Chem. (1996) 61, 8992], the present work aimed at demonstrating the validity of the proposed sum of solvent electron acceptor (AN) and donor (DN) numbers, in 1:1 proportion, as an alternative polarity scale. Other existing parameters such as the Dimroth-Reichardt's $E_T(30)$, the Hildebrand's solubility δ, dielectric constant ε, Kamlet-Taft's α(acidic) and β(basic), etc, were also comparatively evaluate with the (AN+DN) number towards solvation properties of polymers. Figure 1 shows, amongst several other resins here examined, the solvation profiles of the But(DADP)$_4$-BHAR (1.4 mmol/g; 68% peptide-content) and where, its swelling data were correlated with the solvent (AN+DN), ε, $E_T(30)$ and δ parameters. The best fit in this correlation was clearly seen with the amphoteric (AN+DN) term (lesser dispersion of points) than with any others parameters mentioned. The also amphoteric (α+β) term showed good correlation but slightly poorer than the (AN+DN) parameter. The improved relationship with the swelling of resins always occurred with the (AN+DN) scale, thus evincing the enhanced sensitivity of this solvent property to monitor solute (polymer) solvation phenomenon. These findings confirmed the presupposition that the Lewis acidity (AN) and Lewis basicity (DN) terms, when added in 1:1 proportion, may be considered as a novel, dimensionless and more accurate polarity parameter. This polarity scale ranges from zero (toluene) to a maximum of 129 (trifluoromethanesulfonic acid). As a second but also relevant goal of the present approach, a clear differentiation on the solvation of each type of resin is observed depending upon their structural features. This is due to the fact that each resin has its own polarity value and when this value is similar to that of the solvent, improved solvation occurs. The following rule for the occurrence of the maximum solvation region in terms of the (AN+DN) values was observed : lower than 20 for resins such as the apolar PS-DVB and 0.2 mmol/g BHAR and above 40-50 for the ionized 2.4 mmolg BHAR-NH_3^+ and hydrophilic polyacrylamide-based SPAR-50 resin (Advanced ChemTech). An intermediary position is occupied by Bu(DADP)$_4$-BHAR (figure 1) and (NANP)$_4$-BHAR (70% peptide-content), which display enhanced solvation in solvents characterized by (AN+DN) values centered near 40 and 50, respectively. A PEG-grafted polymer (PAC-PEG-PS) may be also included in this class as its maximum solvation region occurred at near 40. The large amount of data herein collected may, in association with those derived for instance, from spectroscopic approaches provide new insights for improvement of polymer-supported methodologies.

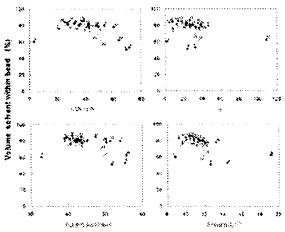


Fig. 1 - Swelling of But(DADP)4-BHAR, 1.4 mmol/g as a function of solvent (AN+DN), ε, ET(30) and δ parameters.

P A46 - Flexibility of the central portion of human parathyroid hormone (1-34) detected by ^{15}N relaxation measurements

M. Marin[1], M. Scian[1], M. Bellanda[1], T. Liqiang[2], J. M. Alexander[2], M. Rosenblatt[2], M. Chorev[2], E. Peggion[1], S. Mammi[1]

1. Biopolymer Research Center, CNR, University of Padova, Department of Organic Chemistry, Via Marzolo 1, 35131 Padova - Italy
2. Bone and Mineral Metabolism Unit, Charles A. Dana and Thorndike Laboratories, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, 02215 - U.S.A.

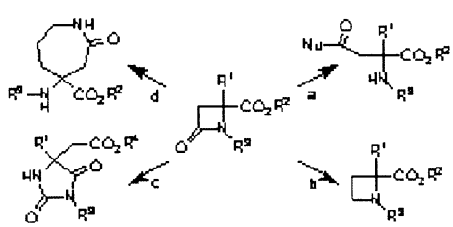
Previous studies on human parathyroid hormone (1-34) [hPTH(1-34)] and many of its analogs indicated the presence of two helices located at the N- and C-terminus, respectively, separated by a region of undefined conformation. It is still unknown whether the undefined structure in the central portion of many analogs studied so far is due to actual mobility or to lack of sufficient experimental constraints. We decided to investigate this problem with an independent NMR technique, *i.e.*, utilizing ^{15}N relaxation measurements to characterize the internal motions of hPTH(1-34). Here, we describe the results of proton-detected ^{15}N-^1H 2D NMR experiments and their interpretation in terms of backbone dynamics of hPTH(1-34). The uniformly ^{15}N enriched peptide was produced by entrokinase cleavage of the overexpressed GST/PTH(1-34)-fusion protein.
Spin-lattice relaxation rate constants, spin-spin relaxation rate constants, and steady-state ^1H-^{15}N nuclear Overhauser effects were determined for all the 33 backbone amide ^{15}N nuclei in two different experimental conditions, *i.e.*, in water at high salt concentrations and in the presence of DPC micelles. The relaxation parameters were analyzed using the model-free formalism of Lipari and Szabo [*J. Am. Chem. Soc.* 104, 4546 (1982)] to yield a generalized order parameter (S^2) and an effective internal correlation time for each amide group. The overall rotational correlation time of the molecule was calculated from the set of R_2/R_1 values.
Results of the present study indicate that in water the central region possesses a high degree of mobility centered at position 12. Under these conditions, the C-terminal helix is less flexible than the N-terminal one. In the presence of DPC micelles, the internal motion of all residues is lower, although relative higher mobility at residue 12 is maintained. The presence of micelles causes an increased stability of the N-terminal helix relative to the C-terminal one and the presence of a new point of local mobility at residues 16-17.
These results support the hypothesis that the relative orientation of the two N- and C-terminal helices is not defined until a positive interaction with the receptor is established. The correct disposition of the two helices is enabled by the flexibility in the central part of the molecule.

P A47 - Amino acid-derived 2-azetidinones: versatile synthetic intermediates for the generation of molecular diversity

M. Martín-Martínez[1], M. A. Bonache[1], G. Gerona-Navarro[1], C. García-Aparicio[1], M. T. García-López[1], R. González-Muñiz[1]

1. Instituto de Química Médica (CSIC), E-28006, Madrid - Spain

In connection with our current concern in conformationally constrained amino acids, we recently reported the first preparation of 3-unsubstituted 4-alkyl-4-carboxy-2-azetidinone derivatives, that either by themselves or after transformation into the corresponding azetidines, can be considered as new conformationally constrained amino acids with fixed ϕ dihedral angles [1]. In addition to the known pharmacological properties of monocyclic β-lactams, there is a remarkable interest in azetidinone derivatives as structural units for incorporating conformational restraints into peptides and as precursors of β-amino acids and of different types of heterocyclic compounds. Taking into account the unique structure of our 4-alkyl-4-carboxy-2-azetidinones, chemical manipulations of these compounds could be attractive. In this communication we will describe the different synthetic transformations we have performed, to give an idea of the possibilities of these β-lactams as synthetic intermediates for the generation of molecular diversity. These transformations include: (a) the controlled opening to different aspartic acid and asparagine derivatives, (b) the chemoselective reduction to the corresponding azetidines, (c) the ring opening and closure to novel 3,5,5-trisubstituted hydantoins, and (d) the preparation of azepinone-based amino acids.



Scheme 1.- Generation of molecular diversity from amino acid-derived 2-azetidinones

Reference
[1] Gerona-Navarro, G.; Bonache, M.A.; Herranz, R.; García-López, M.T.; González-Muñiz, R. *J. Org. Chem.* 2001, *66*, 3538.

P A48 - Solid-phase synthesis of peptide nucleic acids from N-tetrachlorophthaloyl protected monomers

X. Mejias[1], M. Planas[1], E. Cros[1], E. Bardají[1]

1. Chemistry Department - University of Girona - Spain

Peptide nucleic acids are DNA analogues of interest as diagnostic and potential therapeutic tools. Their solid-phase synthesis has been described using Fmoc or Boc protected monomers, and standard solid-phase peptide synthesis methodologies have been applied for the synthesis of PNA oligomers. We have recently described the synthesis of use tetrachlorophthaloyl protected aminoacids and its application to the synthesis of peptide amides. Coupling of N-TCP-amino acids can be done by conventional methods (DIC-HOBt; HBTU-DIEA; etc). N-TCP deprotection should be done with hydrazine-DMF (3:17) for 2h at 40°. The application of this TCP/tBu strategy to the synthesis of peptide amides starting with a PS-PEG-PAL resin gave good results. Now we report on the synthesis of new tetrachlorophthaloyl protected PNA monomers and their application to the synthesis of PNA oligomers. Protection of TCP protected amino acids was described using either MWI or solution (reflux) methodologies. For the protection of PNA monomers a modification of this methodology has been developed since such treatments produced degradation of the PNA monomers. Thus, protection started from commercial Boc-protected monomers. Deprotection with TFA-dichloromethane (1:1) for 1h and solvent evaporation gave a residue that was treated with tetrachlorophthalic anhydride (1 eq) in pyridine for 5 h at room temperature. Next, excess of acetic anhydride was added and the reaction mixture was stirred for 2h. Finally, addition of HCl 1N produced the precipitation of the desired tetrachlorophthaloyl protected monomers, with yields between 70-90% and good purities (Fig 1).

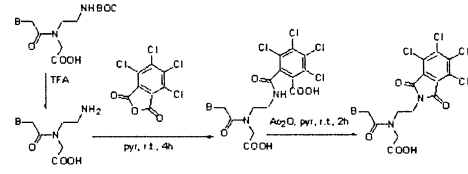


Fig. 1 - Synthesis of N-TCP protected PNA monomers.

The use of these tetrachlorophthaloyl protected monomers for the synthesis of PNA oligomers is shown in Figure 2 and it is interesting to remark that the protected TCP fragments showed a specific maximum absorption band at 335 nm, allowing its specific detection at this wavelength.

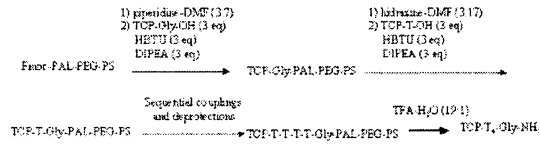


Fig. 2 - Solid-phase synthesis of TCP-T-T-T-T-Gly-NH2.

P A49 - Subtilisin-catalyzed peptide synthesis using carbamoylmethyl esters as acyl donors

T. Miyazawa[(1)], M. Hiramatsu[(1)], T. Yamada[(1)]

1. Department of Chemistry, Faculty of Science and Engineering, Konan University - Japan

We have recently reported that the carbamoylmethyl (Cam) ester acts as an excellent acyl donor in the kinetically controlled peptide bond formation mediated by α-chymotrypsin [1]. Thus, major drawbacks associated with protease-catalyzed peptide synthesis - a narrow substrate specificity and the secondary hydrolysis of a growing peptide - can be overcome by employing this ester. We have also intended to utilize the catalytic activities of some microbial proteases for peptide synthesis [2]. As the continuation of this work, we have investigated the kinetically controlled peptide synthesis mediated by subtilisin Carlsberg (ex *Bacillus licheniformis*) using Cam esters as acyl donors. The superiority of the Cam ester was ascertained once again with this microbial protease in the couplings of a series of Z-L-amino acid esters with L-amino acid amides in acetonitrile without a buffer. A distinct "pH memory" [3] was observed in these couplings: the peptide yield increased significantly upon changing the pH of the buffer solution from which the immobilized (on Celite) protease was obtained from 8.0 to 10.7. Furthermore, the advantage of the Cam ester was confirmed also in several segment condensations mediated by the microbial protease: high peptide yields without racemization during the coupling.

References
[1] T. Miyazawa *et al.*, *J. Chem. Soc., Perkin Trans. 1*, 2001, 82; *ibid.*, 2001, 87; *ibid.*, 2002, 390; *ibid.*, 2002, 396.
[2] T. Miyazawa *et al.*, *Peptides 2000*, 335 (2001).
[3] *Cf.* K. Xu and A. M. Klibanov, *J. Am. Chem. Soc.*, 118, 9815 (1996).

P A50 - Chloroform-phenol mixed solvent for efficient segment condensation reaction of sparingly soluble protected peptides performed using the solution or solid-phase method

Y. Nishiuchi[(1)], H. Nishio[(1)], T. Inui[(1)], T. Kimura[(1)]

1. Peptide Institute Inc., Protein Research Foundation - Japan

We recently demonstrated the utility of a combined solid-phase and solution approach for protein synthesis by synthesizing green fluorescent protein (238 AAs) [1] and human leptin (146 AAs) [2]. This procedure is based on performing the segment condensation reaction in solution employing a maximum protection strategy with Boc chemistry. Each segment used in the subsequent segment condensation is prepared by solid-phase assembly on a base-labile *N*-[9-(hydroxymethyl)-2-fluorenyl] succinamic acid (HMFS) linker [3], which is cleaved by treatment with 20% morpholine in DMF to release fully protected segments with a free α-carboxyl group. To establish this procedure for general protein synthesis, however, we needed to solve several problems encountered during preparation of the protected segments as well as the segment coupling in solution and HF treatment [4]. Among them was the solubility problem of intermediates, particularly due to hampering of the chemical synthesis of large peptides or proteins. To overcome this obstacle, we employed a β-sheet disrupting solvent, a mixture of chloroform and phenol, possessing a much higher solubilizing potential than that of chloroform and 2,2,2-trifluoroethanol (TFE) [5]. The chloroform-phenol system proved essential for coupling sparingly soluble segments without danger of epimerization and of phenyl ester formation, if EDC was used as the coupling reagent in the presence of HOOBt [6]. Furthermore, the chloroform-TFE mixed solvent was found to be efficient enough to enable completion of amino acid-coupling reactions during chain-assembly on HMFS resin even in the case of peptides related to a difficult sequence. In the present study, we tried to synthesize human stresscopin (40 AAs) by coupling the segments on a solid support in the chloroform-phenol mixed solvent under the optimal conditions determined for the solution method (e.g., the concentration of phenol in chloroform as well as the amounts of a carboxyl component, EDC and HOOBt against an amino component). The present synthetic strategy used with the chloroform-phenol and chloroform-TFE mixed solvent permits facile synthesis of large peptides using not only the solution method but also the solid-phase one.

References
[1] Nishiuchi, Y., Inui, T., Nishio, H., Bódi, J., Kimura, T., Tsuji, F.I., Sakakibara, S. *Proc. Natl. Acad. Sci. USA* 95 (1998) 13549.
[2] Nishiuchi, Y., Inui, T., Nishio, H., Kimura, T. In Lebl, M. and Houghten, R.A. (Eds.) *Peptides: The Wave of the Future (Proceedings of the 17th American Peptide Symposium)*, in press.
[3] Rabanal, F., Giralt, E., Albericio, F. *Tetrahedron* 51 (1995) 1449.
[4] Nishiuchi, Y., Nishio, H., Inui, T., Bódi, J., Kimura, T. *J. Peptide Sci.* 6 (2000) 84.
[5] Kuroda, H., Chen, Y-N., Kimura, T., Sakakibara, S. *Int. J. Pept. Protein Res.* 40 (1992) 294.
[6] Inui, T., Bódi, J., Nishio, H., Nishiuchi, Y., Kimura, T. *Lett. in Peptide Sci.* (2002) in press.

P A51 - Aliphatic amino acids in helix VI of the AT1 receptor play a relevant role in agonist binding and signal transduction

A. C. Paiva[(1)], S. I. Shimuta[(1)], L. Oliveira[(1)], S. A. Correa[(1)], H. Zalcberg[(1)], S. W. Han[(1)], C. M. Costa-Neto[(2)]

1. Department of Biophysics / Universidade Federal de São Paulo, Escola Paulista de Medicina - Brazil
2. Department of Biological Sciences / UNESP, SP - Brazil

Pharmacological inhibition of angiotensin II (AngII) production and action are major targets for drug development because it plays a fundamental role in controlling the functional and structural integrity of the arterial wall and is an important factor in hypertension and other cardiovascular diseases.
Most of the AngII physiological actions are mediated by the G-protein coupled receptor AT1, and mutagenic studies provided information about the importance of different amino acid residues of this receptor for its interaction with AngII and subsequent activation. Concerning the latter, it has been shown that the ligand's interaction with residues V254 and His256, in the receptor's helix VI is important for the initiation of signal transduction [1,2]. In a preliminary screening of AT1 receptor sequences, two peculiar motifs were found in the distal end of helix VI and the adjoining segment of the EC-3 loop: (1) a conserved series of Leu residues placed at intervals of 3 positions (262, 265 and 268), thus likely to point to the same side of the helical structure; (2) an insertion of 8 residues in the loop containing Leu268 and a conserved Cys274 which is likely to form a second extracellular disulphide bond with the receptor's N-terminal domain. To investigate whether the aliphatic side-chains in this region of the receptor might play a role in receptor-agonist interactions, we prepared AT1 mutants in which Leu was replaced either by negatively (Asp) or positively (Arg) charged residues. The triple mutant [L262D-L265D-L268D]AT1 (LD3) and the point mutants L262D, L265D, L268D and L268R, as well as the wild-type AT1 receptor, were stably transfected into CHO cells for ligand binding and signaling assays. LD3 showed a marked reduction of the binding affinity for AngII and for non-peptide (DuP753) and peptide ([Sar1,Leu8]AngII) antagonists, and a reduced inositol phosphate (IP) response to AngII. Replacement of Leu262 (in the EC-3 loop) or Leu268 (in the transmembrane domain) by Asp or Arg did not cause significant changes in the receptor's affinity for the ligands and in IP production. In contrast, the point mutation L265D (at helix VI) markedly decreased affinity and ability to stimulate IP production. According to molecular modeling of the AT1 receptor [3] based on a recent crystal structure of rhodopsin [4], the side-chain of Leu265, at helix VI, is turned towards helix V side-chains, allowing an interaction that would stabilize the receptor structure near Lys199, in the agonist binding site, which is necessary for full activity.

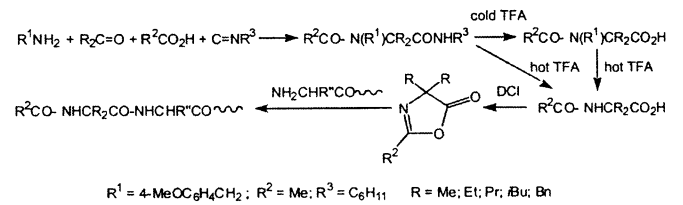
References
[1] Noda K, Saad Y and Karnik SS (1995) J Biol Chem 270:28511.
[2] Han HMCB, Shimuta SI, Kanashiro CA, Oliveira L, Han SW and Paiva ACM (1998) Mol Endocrinol 12:810.
[3] Oliveira, L. (2001) *GPCRDB*.
[4] Palczewski K, Kumazaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M (2000) Science 289:739.

P A52 - A simple and efficient approach to the synthesis of N-acyl-α,α-dialkylglycines and their esters and amides by Ugi's four-component reaction

S. A. Pereira-Lima[(1)], S. P. Costa[(1)], H. S. Maia[(1)]

1. Department of Chemistry, University of Minho, Gualtar, P-4710-350 Braga - Portugal

Owing to steric hindrance, syntheses of and reactions with α,α-dialkylglycines are very slow; thus, most methods of peptide synthesis are of little use to handle these compounds when the α-alkyl groups are larger than methyl. Ugi's four-component reaction is appropriate to synthesise these amino acids [1], but for peptide synthesis it presents two drawbacks, i.e. (i) peptide isonitriles racemise above -20 °C and (ii) an unavoidable alkyl group in the reaction product needs to be cleaved [2]. Reactions with α,α-dialkylglycines are too slow to allow performing them at -20 °C. Isonitriles have been devised to allow mild cleavage of the amide bond at the C-terminus of the Ugi adduct, but in α,α-dialkylglycine derivatives the amide bond generated by the isonitrile is sufficiently labile [3] to allow mild cleavage. Thus, as shown in the scheme below, we devised a simple and fairly efficient approach to routine synthesis of peptides containing α,α-dialkylglycine residues, using commercially available reagents.



R^1 = 4-MeOC$_6$H$_4$CH$_2$; R^2 = Me; R^3 = C_6H_{11} R = Me; Et; Pr; *i*Bu; Bn

References
[1] H.L. Maia, B. Ridge and H.N. Rydon, *J. Chem. Soc.* 1973, 98
[2] G. Gokel, P. Hoffmann, H. Kleimann, H. Klusacek, G. Ludke, D. Marquaerding and I. Ugi, in I. Ugi (Ed.) *"Isonitrile Chemistry"*, Academic Press, New York and London, 1971, p. 201
[3] S.P. Costa, H.L.S. Maia and S.M.A. Pereira-Lima, communication no. P022 to the 16th American Peptide Symposium, Minneapolis, 1999; C.J. Creighton, T.T. Romoff, J.H. Bu and M. Goodman, *J. Am. Chem. Soc.* 1999., *121*, 6786

P A53 - Chemoselective oligosaccharide chain elongation in synthetic neoglycopeptides

F. Peri[1], F. Nicotra[1]

1. Department of Biotechnology and Biosciences, University of Milano-Bicocca - Italy

Chemoselective synthetic strategies allow the preparation of complex macromolecules such as proteins and neoglycoproteins in a convergent and efficient way. In particular, the possibility to obtain neoglycoproteins and neoglycopeptides in a chemically pure form and in hundred milligram quantities is attractive in order to investigate the influence of the sugar part on the conformation of the bound peptide [1]. We developed a procedure for the chemoselective and stereoselective assembly of neoglycopeptides in solution based on the unique reactivity of a methylamino-oxy group on an unprotected peptide and the aldehyde group of the free reducing end of a mono- or oligosaccharide [2]. D-Glucose, *N*-Acetyl-D-glucosamine and di- and trisaccharides were covalently linked to a tripeptide as β-glycosides (Figure 1). We report here an extension of the same reactivity to the synthesis of oligosaccharides with a non-natural methoxy-amino interglycosidic bond. We investigated the chemoselective elongation of the oligosaccharide chain of a glycopeptide by reacting the C-6 methoxy-amino sugar bound to peptide with an unprotected sugar thus forming neoglycopeptides bearing disaccharide units (Figure 2). The disaccharide analogues were formed in good yields with β-stereoselectivity in the case of Glucose, Galactose and *N*-Acetyl-glucosamine. The *N-OMe* intergylcosidic linkage is an isostere of the natural ether bond and, interestingly, it is stable to glycosidases and acids.

Fig. 1 -

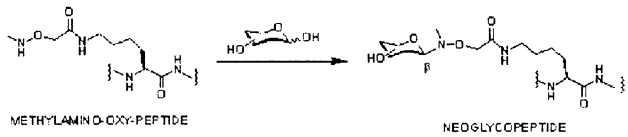
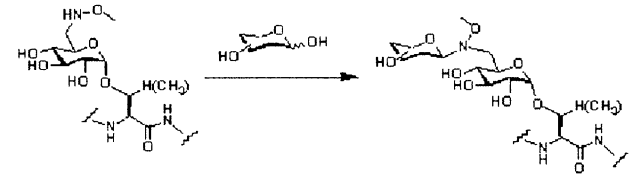


Fig. 2 -



References

[1] H. C. Hang, C. R. Bertozzi *Acc. Che. Res.* 2001, 34, 727-736.
[2] F. Peri, P. Dumy, M. Mutter *Tetrahedron* 1998, 54, 12269-12278.

P A54 - The binding of endotoxin-neutralizing peptides to LPS studied by NMR and molecular modeling

P. Pristovšek[1], L. Szilágyi[2], J. Kidrič[1]

1. National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana - Slovenia
2. University of Debrecen, Dpt. of Organic Chemistry, H-4010 Debrecen, Pf. 20. - Hungary

Lipopolysaccharide (LPS, endotoxin) induced Gram-negative sepsis and septic shock remain lethal in up to 60 % of cases, and LPS antagonists that neutralize its endotoxic action are the subject of intensive research. In the last decade peptidic antagonists have become increasingly important in providing leads for treatment of LPS-mediated diseases [1]. In an effort to determine the molecular motifs of LPS/ lipid A specific binding by peptides that neutralise LPS pathogenicity we are investigating their complexes by NMR and molecular modeling. We have determined the LPS-bound structure of the natural antibiotic peptides polymyxin B (PmB) and E (PmE) with the aid of the transferred NOE effect and proposed a model for the complex [2]. We have employed a similar methodology with antiseptic peptides that are fragments of proteins that bind and neutralize LPS in vitro. The preliminary models for the bound structures of the peptides are compared with that of PmB and used to refine the picture of the binding motif employing molecular modeling (Fig. 1). The proposed models are expected to be useful for the design of peptides with improved antiseptic activity.

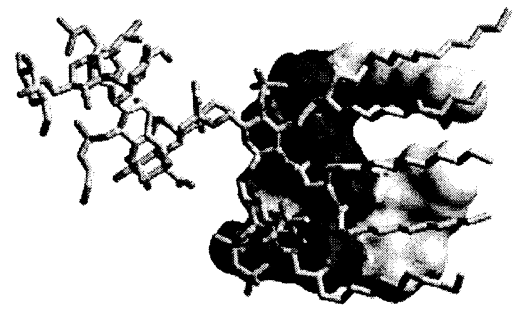


Fig. 1 - Refined model of the LPS (sticks) - PmB (surface) complex.

References

[1] Pristovšek P., Kidrič, J., *Mini Rev. Med, Chem.*, 1, (2001) 409-416.
[2] Pristovšek P., Kidrič J., *J. Med. Chem.* 42 (1999) 4604.

P A55 - Synthesis of "difficult sequences" of highly aggregating peptides

M. Ruvo[1], F. Rossi[1], P. Palladino[1], E. Benedetti[1], C. Pedone[1]

1. Istituto di Biostrutture e Bioimmagini and Dipartimento di Chimica Biologica, via Mezzocannone, 6, I-80134, Napoli - Italy

A major problem experienced in Solid Phase Peptide Synthesis (SPPS) is the occurrence of aggregation that, affecting both chain assembly and purification, often leads to extremely poor yields of desired products [1]. Aggregation is sequence-dependent and is mainly due to H-bonds-promoted intra- and inter-chain associations which, during solid phase chain assembly, strongly reduce solvation of the peptide-resin complex lowering reaction rates and coupling yields [1, 2, 3]. Aggregating peptides also exhibit very low solubility in water-acetonitrile mixtures and in some cases cannot be analyzed using classical reverse phase columns [3]. Problems associated with these so-called "difficult sequences" have been partly overcome using Hmb-aminoacid derivatives but they still remain very expensive and not amenable to automatization [1, 3].
We have devised a combination of resin, coupling reagents, solvents and chromatographic conditions to obtain such sequences straightforwardly and with high yields. The strategy imply the use of PEG-based resins, uronium salts-based condensing agents (HBTU, TBTU), 20% piperidine, classical Fmoc protected aminoacids and does not require any manual synthetic step. For the purification and chromatographic characterizations, polymer-based HPLC columns (POLIMERX) have been utilized using classical water-acetonitrile-TFA gradients. The strategy has been successfully applied to the preparation of some well-known "difficult sequences" like β[1-42] and fragments of the Prion Protein, with high yield and purity levels of final products.

References

[1] Hyde, C., et al., (1994) *Int. J. Peptide Protein Res.*, 43, 431-440.
[2] Bedford, J., et al., (1992) *Int. J. Peptide Protein Res.*, 40, 300-307.
[3] Quibell, M., et al., (1994) *J. Org. Chem.*, 59, 1745-1750.

P A56 - A comparative study of coupling reagents for automatic multiple peptide synthesizers

G. Sabatino[1], B. Mulinacci[1], M. C. Alcaro[1], M. Ginanneschi[1], M. Chelli[2], P. Rovero[3], A. M. Papini[1]

1. Dipartimento di Chimica Organica "Ugo Schiff", Università di Firenze, I-50019 Sesto Fiorentino (FI) - Italy
2. CNR-ICCOM, Dipartimento di Chimica Organica "Ugo Schiff", Università di Firenze, I-50019 Sesto Fiorentino (FI) - Italy
3. Dipartimento di Scienze Farmaceutiche, Università di Salerno, I-84080 Fisciano (SA) - Italy

The efficiency of the coupling reactions is a key issue in the successful use of an automated multiple peptide synthesizer. Accordingly, protocols based on the use of potent coupling reagents and/or large excess of the acylating mixture are routinely used. On the other hand, it is well known that over-activation may lead to undesired side reactions. Moreover, the high cost of some protected amino acids and coupling reagents suggests maintaining their consumption to a reasonably low level, compatibly with the success of the synthesis.
Prompted by these considerations, we have compared the performances of a series of coupling reagents using a batch multiple peptide synthesizer (Advanced ChemTech Apex 396). We choose as a test peptide the fragment 65-74 of the Acyl Carrier Protein (ACP: H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2), which is a good example of sequence-dependent problems encountered in peptide synthesis, due to the development of internal secondary structures, and therefore it has been previously used in similar studies.
We selected the following coupling reagents: HOBt/TBTU and PyBOP (uronium and phosphonium type coupling reagents), TCTU and HCTU (two novel HOBt-based uronium type coupling reagents, kindly provided by Luxembourg Industries Ltd, Tel Aviv, Israel), and DMTMM, a triazine-based reagent [1].
Syntheses were performed using a Fmoc/tBu protection scheme. In order to emphasize the differences, in terms of reactivity, between coupling reagents, we reduced the excesses of the acylating mixture and used a single coupling procedure.

Reference

[1] Kaminski et al., *J. Org. Chem.*, 1998, *63*, 4248-4255

P A57 - Synthesis of methionine containing phosphopeptides with mono-benzyl protected phospho- amino acids

W. R. Sampson[1], N. Ede[1], M. Fitzgerald[1], K. Turner[1]

1. Mimotopes - Australia

The solid phase synthesis of peptides containing phosphotyrosine, phosphoserine and phosphothreonine is now readily accomplished using preformed mono benzyl protected derivatives of each of the amino acids using optimised coupling protocols [1-4]. However we have recently observed a benzylation side reaction when these derivatives are incorporated adjacent to or in the vicinity of a methionine residue. We have observed up to 30% benzylation of the methionine side chain, resulting from alkylation by the benzyl carbocation. The carbocation is released from the adjacent benzyl protected side chain of the phospho-amino acid. Using the model sequence YRSP(pAA)MPENL (1-3) derived from Human M-Phase Inducer Phosphatase 3, we describe this side reaction and several strategies for eliminating it during solid phase synthesis.
To confirm this side reaction was occurring on methionine, peptides replacing methionine with alanine, YRSP(pAA)APENL (4-6) were prepared. Preparation of the target phosphorylated peptides (1-3) devoid of the benzylated side reaction was then carried out by incorporating methionine oxide in place of methionine to give peptides YRSP(pAA)-Met(O)-PENL (7-9). Following standard cleavage and workup, reduction of the methionine oxide residue was achieved using cleavage with TMSBr [5] to give (1-3).

References
[1] Wakamiya, T., Surata, K., Yasuoka, J., Kusumoto, S., *Chem. Lett.* 1099 (1994)
[2] Perich, J., Ede, N., Eagle, S., Bray, A., *Lett. Pept. Sci.* 6, 91 (1999)
[3] White, P., Beythien, J., *"Innovations and Perspectives in Solid Phase Synthesis and Combinatorial Libraries"*, 4th International Symposium, Mayflower Scientific Ltd, Birmingham, pp557 (1996)
[4] White, P., Poster 159 presented at the 17th American Peptide Symposium, San Diego, California, June 2001.
[5] Albericio, F., Annis, I., Royo, M., Barany, G., In: *Fmoc Solid Phase Peptide Synthesis, A Practical Approach* (eds. Chan, W.C. and White, P.D.) Oxford University Press, 2000, p77.

P A58 - An expeditious peptide synthesis in solution with the use of Nsc-amino acids

V. V. Samukov[1], P. I. Pozdnyakov[1], N. N. Karpyshev[1], D. V. Lebedeva[1], H. J. Kim[2]

1. State Research Center of Virology and Biotechnology Vector, Koltsovo, Novosibirsk Reg. - Russian Federation
2. A&Pep Inc., Bucheon, Kyunggi-Do - South Korea

The major obstacle to successful employment of the Fmoc N(α)-protection in solution phase peptide synthesis seems to be a relatively slow and reversible reaction of the Fmoc cleavage product, dibenzofulvene, with an excess of the cleaving reagent, secondary aliphatic amine. It makes difficult efficient trapping of dibenzofulvene with the amine and its subsequent removal from a reaction mixture. In contrast to the Fmoc, the cleavage of the Nsc group with secondary amines leads to the formation of 4-nitrophenyl vinyl sulfone which then undergoes almost instant and virtually irreversible Michael addition to the excess of an amine giving corresponding tertiary amine as an adduct. Relying on this fact, we developed a fast and flexible procedure for solution phase peptide synthesis employing Nsc-amino acids. Leucine-enkephalin chosen as an initial model peptide was prepared by the step-wise method starting from H-Leu-OBu^t. Couplings were performed in DMF using small (8-10%) molar excesses of Nsc-amino acids and BOP as a condensing reagent. Intermediate peptides were used further without isolation, after usual extraction workup. Cleavage of N(α)-Nsc-protection was achieved by the treatment with the 20-25% solution of a volatile secondary amine (piperidine or diethylamine) in DMF followed by evaporation. Thus formed equimolar mixture of N(α)-deblocked peptide and the vinyl sulfone-amine adduct was further introduced directly into the next synthetic step. The adduct which served as a base in the following coupling reaction could then be easily removed by acidic wash during the extraction workup. The protected final pentapeptide was isolated in 65% overall yield and then deblocked with TFA giving the crude enkephalin of 94% purity. The whole synthesis including deprotection took less than two days. An another model peptide, THF-γ2 (LEDGPKFL), was synthesized and deprotected similarly, however, the final crude octapeptide contained about 12% of an impurity which was identified as an aspartimide derivative. The repeated assembly of the peptide using Boc-Leu-Glu(OBu^t)-Asp(OBu^t)-OH as an N-terminal building block improved the situation and led eventually to the target octapeptide in 52% yield and 95% purity prior to purification.

P A59 - An appraisal of polymer-bound coupling reagents and amine-scavenger for solution-phase and peptide chemistry

M. Sanna[1], P. D. White[2], B. W. Bycroft[1], W. C. Chan[1]

1. School of Pharmaceutical Sciences, University of Nottingham - United Kingdom
2. CN Biosciences UK Ltd. - United Kingdom

Following the successful introduction and extensive development of solid-phase peptide and organic syntheses, attention has been focused in recent years to the development of robust polymer-supported reagents for use in both traditional solution-phase chemistry and peptide synthesis. We herein describe a new polymer-supported scavenger resin and a novel series of resin-bound coupling reagents for solution-phase synthesis.
The synthesis of a new amine-scavenger resin and its use for the purification of solution-phase reactions is reported. Specifically, a highly poly-functionalised polymer-supported anhydride was found to be an effective scavenger for the removal of primary and secondary amines from solution, generally requiring a 2-fold excess of resin over a 2-4 hour reaction period. In addition, scavenging of substituted anilines was effected with longer times (18 h) at 60° C. The macroporous matrix of the resin results in low swelling (2.4 mmol/g) and compatibility with most commonly used solvents. Such physical properties, together with its high substitution level (5 mmol/g), make this resin an effective and convenient electrophilic scavenger for solution-phase synthesis; *e.g.* the solution-phase synthesis of small peptides, and subsequent scavenging of excess reagents was demonstrated.
The rational design and synthesis of a series of polymer-supported coupling reagents for peptide synthesis are also reported. The new compounds, which are structurally analogous to the well-established phosphonium reagents (BOP, PyBrOP, PyClOP) were tested for the coupling of amino acids and small molecules in solution.

P A60 - Enzymatic peptide synthesis using inverse substrates: comparison of catalytic efficiency of trypsins from cold-adapted species (chum salmon and atlantic cod) at low temperatures

H. Sekizaki[1], K. Itoh[1], E. Toyota[1], K. Tanizawa[1]

1. Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293 - Japan

Many studies on the characterization of trypsins from cold-adapted species have been reported.These trypsins display substantially higher catalytic efficiency than their mammalian counterparts. Thus, trypsins from cold-adapted species could be expected effective catalyst for enzymatic peptide synthesis.
It is known that enzymatic peptide synthesis is more advantageous than chemical synthesis in many respects; it is highly stereoselective and racemization-free, and requires minimal side-chain protection. There are, however, several problems to be solved with this method. Secondary hydrolysis of the resulting peptide may arise from the inherent nature of the protease. Moreover, the method is limited to the use of amino acid derivatives which meet the enzymatic specificity as the coupling component. These difficulties of enzymatic peptide synthesis can be largely overcome by using inverse substrate.
In our previous work, it was shown that *p*-amidino- and *p*-guanidinophenyl esters behave as specific substrates for trypsin and trypsin-like enzymes and allow the specific introduction of an acyl group carrying a non-specific residue into the enzyme active site. Such substrate is termed an "inverse substrate" by us and "substrate mimetic" by Bordusa *et al.* The characteristic features of inverse substrates suggested that they are useful for enzymatic peptide synthesis. Recently, we demonstrated successful application of inverse substrates for chum salmon trypsin-catalyzed coupling. Herein, we describe comparison of catalytic efficiency between the chum salmon and Atlantic cod trypsins.
Boc-amino acid *p*-guanidinophenyl esters (Boc-AA-OGp) were prepared by condensation of the appropriate Boc-amino acid and *p*-[*N*',*N*"-bis(Z)-guanidino]phenol, and sequential deprotection by catalytic hydrogenation following the previously reported method. The coupling reaction was carried out by incubating an acyl donor (inverse substrate, 1 mM) with an acyl acceptor (amino acid *p*-nitroanilide, 20 mM) and enzyme (10 μM) in a mixture of GTA buffer (50 mM, pH 8.5, containing 20 mM $CaCl_2$) and DMSO (1:1) at 0°C. The progress of the coupling reaction was monitored by RP-HPLC. Both trypsins were effective for the condensation reaction. Atlantic cod trypsin was more slightly efficient than the chum salmon trypsin. Detail of this method with typical example will be discussed.

P A61 - Bioassay of CSF production from rat lung in the presence of lead nitrate

A. Shabani[1], A. Rabbani[2]

1. Department of Biology, Faculty of Science, Azzahra University, Vanak Place, Tehran - Iran
2. Institute of Biochemistry and Biophysics, University of Tehran, Tehran - Iran

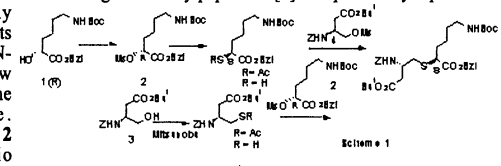
The colony stimulating factors (CSFs) are a group of glycoproteins which in vitro act specifically to control the proliferation, differentiation, and end-cell activation of hemopoietic cell populations.in vivo studies using purified bacterially synthesized CSFs have confirmed that these proteins have actions on normal hemopoietic cells similar to those observed in vitro. Lead is one of the toxic metals that are widely distributed in the atmosphere, soil, and groundwater. Thus, human exposure to low, relatively nontoxic concentrations of this metal is unavoidable. Lead poisoning is well-recognized clinical entities, but the possible health risks from exposure to low concentrations of this metal remain controversial. In this study the effect of different concentrations of lead nitrate on granulocytes-macrophage colony stimulating factors (CSFs) production by lung rat was investigated. Using semisolid colonogenic assay as well as long-term bone marrow culture. Results obtained indicate that CSF production decreased as the lead concentration was increased. Up to 6hours,the amount of CSF was not enough to produce colonies from bone marrow cells, and produce clusters. However after 12 hours of incubation, the CSF production was increased and it was enough to produce colonies. Another case in this experiment is to analyze the production of CSF by addition of different concentrations of lead nitrate after 6,12,24 hours. In these cases the number of colonies increased as the time of exposure was decreased. The mechanism by which lead nitrate exerts its action on CSF production is not understood. However, our preliminary work on the effect of low concentrations of methylxanthines such as Caffeine (2mM) on bone marrow cells and CSF production from lung rat condition medium pretreated with 60μM lead nitrate, shows that Caffeine modulates the number of colonies remarkably. Caffeine is known to inhibit phosphodiesterases reaction and elevates cAMP levels in the cell. It is therefore suggested that lead nitrate presumably exerts its action via cAMP processes. Of course the measurement of cAMP levels in the treated cells would support the above phenomena.by which mechanism lead nitrate exerts its function on CSF production remains to be elucidate.

P A62 - Mesylates and aziridines derived from α-aminoacids. Application to pseudopeptides synthesis

J. Thierry[1], V. Servajean[1]

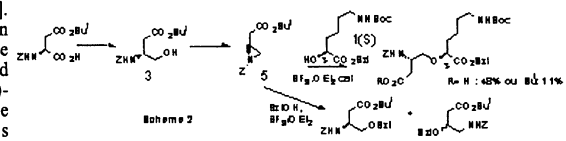
1. Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette - France

We are currently investigating the reactivity of sulfonates (triflates and mesylates) of α-hydroxyacids derived from α-aminoacids. Our purpose is to introduce various substituents on C-2. Our final goal is to get fragments to prepare pseudodipeptides. We focused on the preparation of the pseudodipeptide -AspΨ[CH2X]Lys-, where X could be diverse heteroatoms such as S or O. This fragment is the central unit of the tetrapeptide NAcSDKP, a biological molecule we have been studying for several years, making analogues stable towards degradation by peptidases[1]. We previously reported the activation of the hydroxy compound **1** as a triflate and its various substitutions with N-nucleophiles [2]. We are now studying the reactivity of the mesylate derivative. Substitution of the mesylate **2** was successful with thio nucleophiles (thioacetate or thiol derived from Z-Asp(OBu^t)ol) (Scheme 1) but failed with N-nucleophiles.Use of aziridines as activated species has recently received increasing attention. Aziridines can be easily prepared through intramolecular Mitsunobu reaction of β-amino alcools and be opened under mild conditions with a range of nucleophiles such as alcools, amines, thiols or C-nucleophiles[3].



Scheme 1

A Mitsunobu reaction performed on the alcohol **3** obtained from Z-Asp(OBu^t)-OH gave easily the aziridine **5**. This aziridine was opened in presence of BF_3/ OEt_2 as a catalyst with benzyl alcohol to give two regioisomers in a 1/1 ratio and regioselectively with the secondary alcohol **1**(S) yielding the desired pseudodipeptide Z-Asp(OBu^t)Ψ[CH_2O]Lys(Boc)-OBzl (Scheme 2). The aziridine **5** was also opened with benzylamine and $LiClO_4$ in acetonitrile at 80°C or in toluene without any catalyst at 80°C. Similar conditions have been applied to the aziridine obtained from Z-Ser(Bu^t)-ol. It was opened up in presence of BF_3/OEt_2 as a catalyst with benzyl alcohol to give two regioisomers in a 1/1 ratio and regioselectively with (±)-1-phenylethanol. It was also opened with benzylamine using Yb(OTf)$_3$ as a catalyst . The use of mesylates or aziridines derived from amino acids provides a new and easy access to pseudodipeptides.



Scheme 2

References

[1] S. Gaudron, M.-T. Adeline, P. Potier and J. Thierry *J. Med. Chem* 1997, 40, 3963-68.
[2] I.Weber, P. Potier and J.Thierry, *Tetrahedron Lett.* 1999, 40 ; 7083-7086.
[3] W. McCoull, F.A. Davis, Synthesis 2000, 10, 1347-65, B. Zwanenburg, P. ten Holte, In: *Topics in Current Chemistry* Vol.216, Springer-Verlag, Berlin, Heidelberg, 2001, 94-124, D. Tanner, *Angew. Chem. Int. Ed. Engl.* 1994, 33, 599.

P A63 - Synthesis and characterization of fullerene-C60 derivatives

V. Tsikaris[1], P. Sofou[1], Y. Elemes[1], A. Stavrakoudis[1], C. Sakarellos[1], M. Sakarellos-Daitsiotis[1], C. Toniolo[2], F. Formaggio[2]

1. University of Ioannina, Department of Chemistry - Greece
2. University of Padova, Department of Organic Chemistry - Italy

In an attempt to study the biological activity of peptide fullerene-C60 derivatives, we synthesized the fullerene-C60 derivative (4) by thermal 1,3-dipolar cycloaddition of the azomethine ylide generated in situ from the decarboxylation of the imine of N-sudstituted glycine with H2CO. After the isolation of product (4) we performed esterification with Boc-Pro-OH. Deprotection of the amine group and coupling of product (5) with the protected peptide Boc-PPGMR(Pmc)P-OH, followed by removal of the protecting groups, led to the isolation of the final product (8), which gave the correct ESI-MS, and characterized with NMR spectroscopy.
The final target is to test the biological activity of product (8). The proline rich peptide sequence PPGMRPP, found in several copies in Sm and U1RNP autoantigens, is the main target of the anti-Sm and anti-U1RNP autoantibodies in systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD) patient's sera. It is expected that the presence of the C60 moiety will differentiate the anti-Sm and anti-U1RNP activity of the above peptidic sequence.

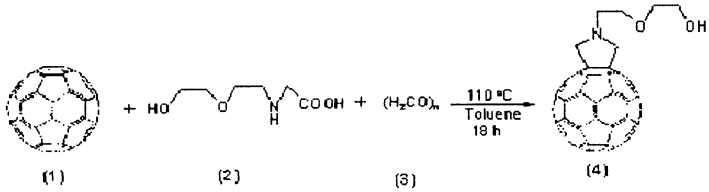


Fig. 1 - Synthesis of fullerene compound (4)

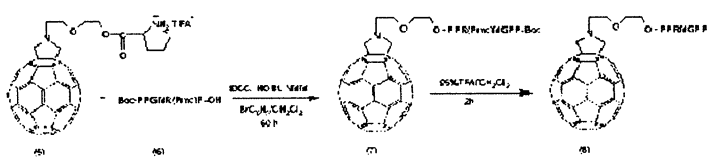


Fig. 2 - Synthesis of fullerene-peptide derivative (8)

P A64 - Non-proteinogenic components in subtilisin catalized peptide bond formation

T. L. Voyusina[1]

1. V.M. Stepanov Laboratory of Protein Chemistry - Russian Federation

Among the enzymes applied as a catalyst in the peptide synthesis proteinases of subtilisin family are used the most frequently. These enzymes are capable to exhibit catalytic activity in concentrated water-organic solution and in practically anhydrous organic solvents. Stability of the enzymes in organic media could be increased by their chemical modification or by introducing corresponding alteration in the protein structure by site-directed mutagenesis. Sorption of proteinase on macroporous support also prevent the enzyme from inactivation. In our opinion the later method is the most convenient for preparative synthesis, because the catalyst easily separated from the reaction mixture and could be used many times again. A broad specificity of subtilisin especially of their S_1'-subsite allow to use derivatives of practically all proteinogenic amino acids and their analogues (such as amino alcohols or semicarbazone of amino aldehydes) as the amino components in peptide synthesis. We investigated the possibility of enzymatic acylation of different amines with sorbed subtilisin in a model reaction

Z-Ala-Ala-Leu-OCH_3 + NH_2-R ⟶ Z-Ala-Ala-Leu-NH-R + CH_3OH

where R = $(CH_2)_2$-OH, DNP-NH-$(CH_2)_2$, DNP-NH-$(CH_2)_6$, C_6H_5-NO_2, imidasole, piperidine etc.

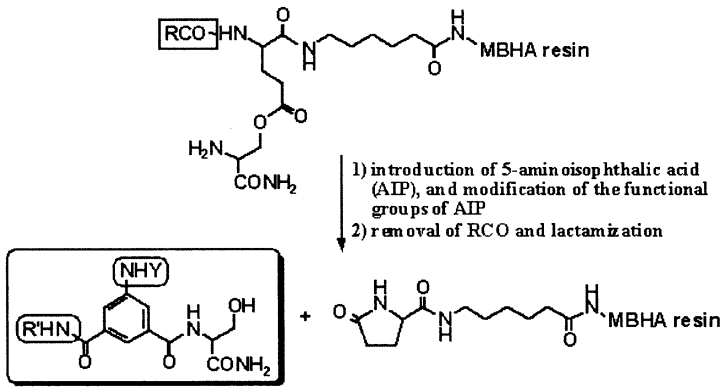
Acyltripeptide ester is the most effective acylating agent in subtilisin catalyzed reaction, therefore acyl enzyme is formed very quickly and its deacylation is rate limiting stage of the process as for other serine proteinases. The yield of the reaction product significantly depends on nucleophility of amino component. Nevertheless sometimes such weak nucleophile as p-nitroaniline could be enzymatically acylated in a good extent that would be very useful when a "difficult" p-nitroanilide e.g. Z-Ala-Ala-Met-pNA should be obtained.
Recently we found that in spite of an extended active site of subtilisin amino acid ester hydrochlorides could serve the carboxyl components in the reaction of peptide bond formation catalyzed by this endopeptidase. We examined acylation of Leu-pNA with non proteinogenic amino acid esters and organic acid esters catalyzed by subtilisin. In these cases formation of acyl enzyme is rate limiting step therefore the composition of the reaction products and their yield strongly depend on the carboxyl component. To insure a good yield structure of ester must correspond to the spatial requirement of S_1-subsite of the enzyme. Nevertheless this reaction is interesting from practical point of view because it offer the enzymatic method of introduction of N-protective groups, if benzoic, anthranilic or pyroglutamic acid esters are used as acylating agents.

P A65 - A novel methodology for solid-phase synthesis utilizing the characteristics of glutamic acid

T. Wakamiya[(1)], D. Yoshida[(1)], Y. Mizuki[(1)], K. Hanatani[(1)], Y. Yamaguchi[(1)], T. Shimamoto[(2)]

1. Faculty of Science and Technology, Kinki University, Higashi-osaka, Osaka 577-8502 - Japan
2. Suntory Biomedical Research Limited, Shimamoto-cho, Mishima-gun, Osaka 618-8503 - Japan

Glutamic acid forms easily γ-lactam structure under suitable conditions to produce pyroglutamic acid. In the present study, we focused on the development of a novel cleavage reaction of synthetic compounds, which are comprised of hydroxyamino acids or saccharides as the fundamental structure, from solid support by taking advantage of the characteristics of glutamic acid. At first, the synthesis of Ser-containing compounds was elucidated as shown in the following scheme.



Next we planed to apply this methodology to develop the preparation of library related to glucosamine that is a typical example of hydroxy compounds with multi functional groups.

P A66 - On-resin formation of the GlyΨ[CH$_2$NH]Aaa type reduced peptide bond by Mitsunobu alkylation.

K. Wisniewski[(1)]

1. Ferring Research Institute - U.S.A

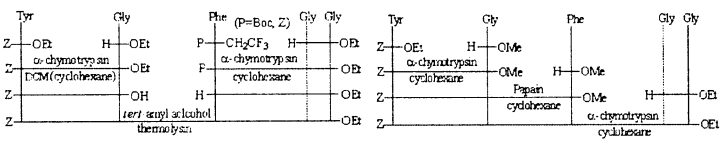
The Mitsunobu reaction is a very useful tool for the synthesis and modification of biomolecules, such as carbohydrates, PNA's, amino acids and peptides due to its mildness and compatibility with other functional groups. The Mitsunobu alkylation of sulfonamides as an alternative method for the synthesis of the Gly-Aaa type reduced peptide bond in solution, using N-Tos- and N-Pmc-protected amino acid esters as substrates, has been recently described. In the present communication on-resin formation of GlyΨ[CH$_2$NH]Aaa type of the peptide bond surrogate, using a similar approach is reported. Fukuyama's o-NBS (orthonitrobenzene-sulfonyl) group that is compatible with acid-labile resins, was selected to convert a resin-bound peptide into an acidic component of Mitsunobu reaction.
The alkylation reaction was optimized using resin bound o-NBS-amino acids (i.e. Trp, His, Lys), Fmoc-Gly-ol, and various combinations of azo- compounds and phosphines. Several solvents typically used for solid phase Mitsunobu reaction were tested. The best results were obtained when the reaction was carried out in 1,2 dimethoxyethane with the use of the TPP/DEAD redox system. To minimize the amount of the N-ethyl by-product, the TPP/DEAD complex was preformed at 0C or DEAD was replaced with less reactive DIAD. The replacement of Fmoc-Gly-ol with -substituted Fmoc-β-amino alcohols turned out to be unsuccessful, presumably due to enhanced steric hindrance.
Since the secondary amine of the reduced peptide bond remains protected throughout the synthesis, branching of the peptide chain during its elongation is prevented. Detailed description of the syntheses and analytical data for selected compounds will be provided. Some important aspects such as stability of the Fmoc-protecting group in the alkylation step as well as stability of peptide trityl resin ester linkage during the removal of the o-NBS group will also be discussed.

P A67 - Synthesis of some precursors of bioactive peptides by different proteases in non-aqueous media

Y. H. Ye[(1)], P. Liu[(1)], G. W. Xing[(1)], G. L. Tian[(1)], K. S. Lee[(2)], M. S. Wong[(2)], W. H. Lo[(2)]

1. The Key Laboratory of Bioorganic Chemistry and Molecular Engineering, Ministry of Education, Department of Chemistry, Peking University, Beijing 100871 - China
2. Open Laboratory of Chirotechnology and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong - China

Bioactive pentapeptide OGP(10-14) (TyrGlyPheGlyGly), is a C-terminal fragment of osteogenic growth peptide which shares the OGP-like *in vitro* mitogenic effect and *in vivo* stimulation of osteogenesis and hematopoiesis as well as retains the endogenous tetradecapeptide's full proliferative activity. Protected OGP(10-14) (Z-TyrGlyPheGlyGly-OEt) (I), Leu-enkephalin (Z-TyrGlyGlyPheLeuOH) (II) and precursors of two sweeteners: (Z-AspPhe-OMe) (III), (Z-AspAla-OcHex) (IV) were synthesized successfully by different proteases in different organic solvents with a little water. (I) was accomplished using papain, -chymotrypsin and thermolysin via 2+3 or 3+2 synthetic routes (see Scheme) in DCM, cyclohexane, *tert*-amyl alcohol for the first time. (II) was synthesized by -chymotrypsin and thermolysin in DCM, *tert*-amyl alcohol. The expected optically pure (III) and (IV) were obtained when racemic amino acid esters were used as amino components in starting materials. The side chain of Tyr and Asp were not protected during all enzymatic reactions. The optimum reaction conditions including different enzymes, solvents, substrates, amount of essential water and observed pH were studied systematically. The results indicated that the amount of essential water needed depended on different enzymes and different solvents. For synthesis of Z-PheGlyGly-OEt catalyzed by -chymotrypsin in cyclohexane, 0.5% water (V/V) was enough for the coupling reaction. For synthesis of Z-TyrGlyGly-OEt by -chymotrypsin in DCM, the optimum water was 0.15-0.25%(V/V). The optimized water content was 6-8%(V/V) for synthesis of Z-AspPhe-OMe and Z-AspAla-OcHex catalyzed by thermolysin in *tert*-amyl alcohol. The expected product was not obtained when there was no any water in anhydride organic solvents. Immobilized proteases on molecular sieve MCM-22 or different Y zeolites by adsorption for synthesis of protected OGP(10-14), Z-TyrGlyGly-OEt and the precursor of sweetener, Z-AspPhe-OMe were also studied. The results showed that immobilized enzymes were more stable and could be reused for more times than free enzymes. Compared with free enzyme, the reaction rate catalyzed by immobilized enzyme was remarkably enhanced and its synthetic yield was also increased in most cases. Our study broadens the range of the application of proteases in the synthesis of peptides and provides some useful information for enzymatic synthesis of peptides.



Scheme 2+3 and 3+2 synthetic routes of the precursor of bioactive pentapeptide OGP(10-14)

P A68 - P1 side chain diversification of phosphinic peptide analogues

A. Yiotakis[(1)], Á. Mores[(1)], M. Matziari[(1)], A. Makaritis[(1)]

1. Department of Chemistry, Laboratory of Organic Chemistry, University of Athens, Panepistimiopolis Zografou, 15771, Athens - Greece

Phosphinic peptides are transition state analogues, which have been proven to be potent inhibitors of zinc metalloproteases, a class of proteolytic enzymes that are involved in a variety of both physiological and pathological processes. It has been shown that phosphinic peptides engage in several tight interactions with enzyme active sites. P_1 and P_1' side chains of phosphinic inhibitors are considered to strongly affect inhibitor potency and selectivity. In order to avoid the use of cumbersome parallel synthetic routes which constitute the only access to such molecules reported to date, we present herein a strategy that would allow easy diversification of the P_1 position of a suitably functionalised pseudopeptidic precursor. To this end, the aminophosphinic serine analogue has been synthesized and the key pseudodipeptidic unit of general type SerΨ[P(O)(OH)CH$_2$]Xaa was obtained, by means of a Michael-type addition. This dipeptidic unit can easily undergo diversification to a great extent, applying transformation of the hydroxyl group into a good leaving group and further nucleophilic substitution. Using methanesulfonyl and methyl groups for activation of the hydroxyl group and protection of the hydroxyphosphinyl moieties respectively, several nucleophiles such as thiols can be introduced to the basic pseudodipeptidic unit in high yields and purities.