Lectures and Oral Communications

L 15 — Friday Morning: Pasteur Auditorium — L 16

GENE REGULATION BY PEPTIDE NUCLEIC ACID (PNA)

Peter E. Nielsen
University of Copenhagen, IMBG, Laboratory B, The Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

The pseudopeptide DNA mimic PNA (peptide nucleic acid) has many of the properties desired of a gene targeting reagent. It binds strongly and with high sequence specificity to complementary RNA and to homopurine and AT-rich targets in double stranded DNA, and can thereby specifically and efficiently inhibit translation and transcription, respectively. Furthermore, PNA has high biostability and it is easy to chemically synthesize and modify. In the process of evaluating the prospects of developing PNA derived gene therapeutic drugs, recent results relating to targeting of double stranded DNA, antisense gene regulation in bacteria and eukaryotic cells as well as improved methods for cellular uptake of PNA will be presented.

References:

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Lohse, J., Dahl, O.& Nielsen, P.E.(1999) Double Duplex Invasion by Peptide Nucleic Acid: A general Principle for Sequnce Specific Targeting of Double Stranded DNA. *Proc. Natl. Acad. Sci. USA* **96**, 11804-11808.

Ljungstrom, T., Knudsen, H. & Nielsen, P.E. (1999) Cellular Uptake of Adamantyl Conjugated Peptide Nucleic Acids. *Bioconjug Chem.* **10**, 965-972.

OF MICE AND MAN: IDENTIFICATION OF TAA PEPTIDES OF HUMAN CANCER

Lea Eisenbach, Boaz Tirosh, Erez Bar-Haim, Lior Carmon, Adrian Paz, Irene Priel, Ezra Vadai, Michael Feldman, Mati Fridkin[1], Esther Tzehoval, François Lemonnier[2].
Departments of Immunology and Organic Chemistry[1], The Weizmann Institute of Science,Israel and Department of Retroviruses and AIDS Research, Pasteur Institute[2] France.

Cytotoxic T lymphocytes (CTL), directed against peptides presented by MHC class I molecules, constitute powerful effectors of the immune system against tumors or infectious agents. Antigens recognizable by CTL were isolated from human melanomas and from other human and murine tumors by a number of methods. Their efficacy in vaccination against tumors was demonstrated in animal models and lately in clinical trials. For identification of Tumor Associated Antigen (TAA) peptides, we have induced anti-tumor CTL in (H-2D$^{b-/-}$ x β_2 microglobulin$^{-/-}$) double knockout mice, transgenic for a single chain HLA-A2-β_2 microglobulin construct (HHD mice). Immunizations were performed with peptide fraction, isolated from tumors and loaded on antigen presenting cells (APCs), with human tumor cell lines transfected with the HHD construct or with synthetic peptides loaded on APCs. CTL induced against peptides from various tumors (Breast, colon, TCC, prostate) recognized tumor peptides more effectively than peptides extracted from normal tissues and also reacted with a serie of peptides derived from overexpressed candidate proteins. Comparison of CTL derived from HHD mice to CTL induced from patient's CTL showed overlapping recognition of candidate peptides. Using these HHD mouse derived CTL we identified novel peptide sequences from Prostate antigens like PSA, PSMA and PAP and Breast Carcinoma antigens MUC1 and BA46-1. Analysis of tumor differentially expressed genes by the SAGE method in colon, followed by screening for HLA-A2 binding peptides resulted in 500 candidate peptides for immunogenicity screening. We have identified 22 antigenic peptides of which 7 peptides were found to be immunogenic in HHD mice. Interestingly 3 of these peptides are derived from the same protein. Differential expression studies, using "DNA chips" were performed on prostate and bladder tumors versus normal tissues. Ten new candidate genes from TCC were analysed for expression and potential immunogenic peptides. Novel peptides from Uroplakins and from MAGE-8 were identified.

OC 65 — Pasteur Auditorium - Combinatorial Chemistry — OC 66

INHIBITION OF THE β-AMYLOID PEPTIDE NEUROTOXICITY AND AGGREGATION BY IMIDAZOL-PYRIDO-INDOLES

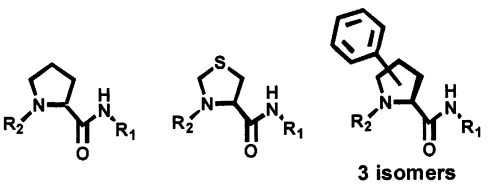
Sylvie E. Blondelle, Ema Crooks, Natàlia Reixach. Torrey Pines Institute for Molecular Studies, San Diego CA 92121 USA

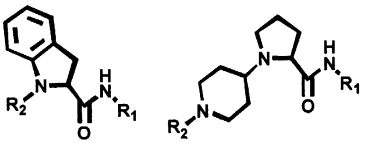
Alzheimer's disease is a progressive neurodegenerative disorder characterized by the deposit of amyloid fibrils in the brain that result from the self-aggregative polymerization of the β-amyloid peptide (A β). Evidence of a direct correlation between the ability of A β to form stable aggregates in aqueous solution and its neurotoxicity has been reported. The cytotoxic effects of A β have been attributed to the aggregation properties of a domain corresponding to the peptide fragment A β25-35. In an effort to generate novel inhibitors of A β neurotoxicity and/or aggregation, a mixture-based synthetic combinatorial library composed of 23,375 imidazol-pyrido-indoles was generated and screened for inhibition of A β 25-35 neurotoxicity toward the rat pheochromocytoma PC-12 cell line. The effect of the identified lead compounds on A β25-35 aggregation was then evaluated by means of circular dichroism (CD) and thioflavin-T fluorescence spectroscopy. Their activity against A β 1-42 neurotoxicity toward the PC-12 cell line was also determined. The most active imidazol-pyrido-indoles inhibited both A β 25-35 and A_1-42 neurotoxicity in the low to mid-micromolar range. Furthermore, inhibition of the random coil to β-sheet transition and self-aggregation of A β 25-35 was observed by CD and fluorescence spectroscopy, supporting the relationship between inhibition of the A β aggregation process and neurotoxicity. The deconvolution to individual inhibitors from the different SCLs as well as their effect on A β25-35 aggregation will be presented.

COMBINATORIAL MULTIPLE PARALLEL SYNTHESIS ON MODULAR GRAFTED SOLID SUPPORTS

Nicholas J. Ede and Andrew M. Bray
Mimotopes Pty. Ltd., 11 Duerdin St. Clayton Victoria. 3168 Australia

The synthesis of hundreds to thousands of individual compounds on a multi-milligram scale using newly designed modular supports will be presented. These next generation grafted supports called "lanterns" have loading and synthesis characteristics comparable to conventional PS resin. Synthesis performance and comparable analysis of the new solid support is presented. We have synthesised libraries based on a central proline template and used the versatile BAL linker to generate secondary amides as the first point of diversity. In addition to presenting analytical results, equipment developed for the simple manual handling of the lanterns from synthesis to cleavage will be presented.





Lectures and Oral Communications

OC 67 Pasteur Auditorium - Combinatorial Chemistry OC 68

COVALENT DNA-PROTEIN FUSION: A NOVEL TOOL FOR THE *IN VITRO* DISPLAY OF PEPTIDES AND PROTEINS

Markus Kurz and Peter A. Lohse,
Phylos, Inc., Lexington, MA 02421, USA

The extension of selection technology to the *in vitro* selection of peptides and proteins from large libraries presents an area of great interest. The difficulty in designing schemes for the *in vitro* selection of proteins is that there is no simple way to amplify protein molecules that have been selected for function. In order to select and identify proteins with a desired function, the genetic information must be kept topologically linked to the protein either in form of a coding sequence such as RNA or DNA, a set of chemical tags or a physical address.

Recently, two techniques have been developed that afford the functional selection of peptides or proteins entirely *in vitro*: mRNA-protein fusions and ribosome display [for a recent review see: R. W. Roberts, Curr Opin Chem Biol. 1999, 3: 268-273]. We have developed a novel protein display technology employing cDNA-protein fusions. The covalent linkage between the cDNA and the encoded protein allows to read and amplify a protein sequence after it has been selected based on its function.

We are presenting an efficient method to prepare cDNA-protein fusions from a novel type of a mRNA-puromycin construct. This construct was prepared by photoligation of a branched puromycin linker to the 3'-end of the mRNA. *In vitro* translation of the mRNA construct followed by reverse transcription yielded the DNA-protein fusion molecules.

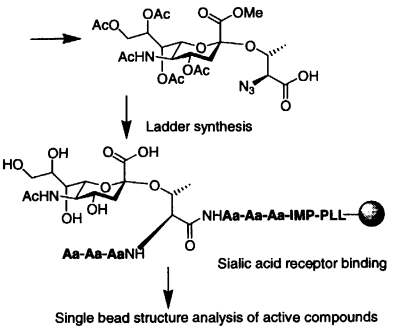
DNA-protein fusions show improved chemical and biological stability over RNA-protein fusions. The improved robustness and the facile preparation make DNA-protein fusions a useful new tool for the rapid discovery of peptide and protein ligands under a wide range of *in vitro* conditions. Applications of DNA-protein fusions to peptide and protein display will be discussed at the meeting.

COMBINATORIAL SPLIT SYNTHESIS LIBRARIES OF GLYCOPEPTIDES FOR THE IDENTIFICATION OF HIGH AFFINITY LIGANDS FOR CARBOHYDRATE RECEPTORS

Morten Meldal Koen Halkes and Phaedria St. Hilaire

Center for Solid Phase Organic Combinatorial Chemistry (SPOCC), Dept. of Chemistry, Carlsberg Laboratory, DK-2500 Valby, Denmark

The application of sialylated, mannosylated and glucosaminosylated building blocks in synthesis of split and combine libraries was accomplished using the principle of ladder synthesis on PEGA and POEPOP resins. The ladder synthesis was performed with labeling of the carbohydrate positions with long chain aliphatic carboxamides that allowed simple and immediate analysis of the glycopeptide structure. The libraries were screened with sialic acid and mannose binding receptors which had been obtained either by over expression or by isolation from natural sources and labeled with flourescent probes. A strong structure consensus of the active ligands was achieved and the active molecules were synthesized and their interaction with the receptor in solution studied in detail. For the sialic acid receptor the interaction was particularly specific and the presence of the carboxylate was important for binding . A similar library containing a lactam ring involving the sialic acid carboxylate was completely inactive.



OC 69

THE QUESTION OF MIXTURES IN COMBINATORIAL LIBRARIES: THE BALANCE BETWEEN COMPLETENESS AND EFFICIENCY - SINGLE COMPOUND ARRAYS, SMALL MIXTURES, OR LARGE MIXTURES?

Richard A. Houghten, Torrey Pines Institute for Molecular Studies & Mixture Sciences, Inc., 3550 General Atomics Court, San Diego, CA 92121 USA.

There is a clear division in the explosively growing field of combinatorial chemistry as to whether all compounds in a library should be examined individually, as small mixtures (5 to 50 compounds) or as large mixtures (100 to >10,000 compounds). We have been able to rapidly identify highly active, individual compounds using a wide range of assay systems and heterocyclic, peptidomimetic, or peptide combinatorial libraries made up of at least 100,000 compounds, in either the iterative or positional scanning format. While the separate examination of each individual compound in a library is clearly desired, the use of mixtures of varying numbers can greatly expedite the screening process. For example, a library of 1,000,000 compounds arrayed individually would require at least an equal number of screening points. The majority of laboratories carrying out screening assays are not capable of this level of high throughput. The same library, however, arranged in a positional scanning format with three diversity positions and 10,000 compounds per mixture would require only 100 separate mixtures per position, for a total of 300 screening points. The number of compounds screened and the activity level of identified compounds are key issues. The theoretical and practical considerations of the strengths and weaknesses of each approach will be discussed.

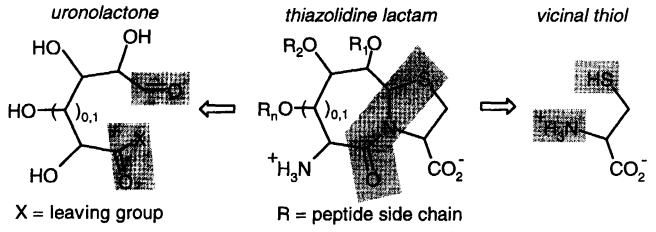
Lectures and Oral Communications

OC 70 — Einstein Auditorium - Peptidomimetics — OC 71

POLYOL PEPTIDOMIMETICS

A. Geyer, P. Tremmel
Faculty of Chemistry, University of Konstanz, Germany

Uronolactones and cysteine combine in a highly stereoselective manner to bicyclic thiazolidine lactams. The α-hydroxy group was exchanged for an amino function and rigid polyol dipeptides are accessible in only four high-yielding synthetic steps.



Tuning of ring sizes and stereochemistry by tailoring the carbohydrate moiety results in various novel amphiphilic β-turn mimetics. N- and C-terminal elongation leads to rigidified peptide analogues, coupling reactions proceed without the need to protect the secondary hydroxy groups. C-terminal elongation was alternatively performed by condensation of an uronolacatone with a peptide which bears a N-terminal cysteine. Thiazolidine lactams resist epimerisation or hydrolysis under the conditions of peptide synthesis.

Each bicyclic scaffold populates a single well-defined solution conformation. The NMR-based structural analyses are corroborated by crystal structures. Bicyclic polyol peptidomimetics have interesting ion-binding properties and form a new class of enzyme inhibitors.

One, two- or even three hydroxy groups mimic the side chains of hydrophilic amino acids. Alternatively, the hydroxy groups can be further functionalized – for example as ethers – to mimic peptide side chains.

Another topic is the oligomerisation of bicyclic thiazolidine lactams of differing ring geometries resulting in protein-like secondary structures.

A. Geyer, F. Moser, *Eur. J. Org. Chem.* **2000** *(in press).*

New Reactions and structures for peptidomimetic drug design

M. Goodman, T. J. Baker, Y. Rew and C. J. Creighton
[a]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, USA 92093-0343,

In this presentation, we cover new results with our guanidinylation reagents, novel bridged opioids and mechanistic studies of an unusual amide bond scission.

Diurethane-triflylguanidines are efficient new guanidinylation reagents.[1] The general applicability of these reagents are illustrated using *N,N'*-diBoc-*N''*-triflylguanidine and *N,N'*-diCbz-*N''*-triflylguanidine in reactions to form guanidine-containing peptides, aminoglycosides and heterocyclic drugs.[2]

Through the use of sulfur and nitrogen heteroatoms as bridges in cyclic opioids, we have obtained analogs with decreased conformational flexibility and enhanced bioactivity.[3] Specifically, Tyr-c[D-Val_L-Gly-Phe-D-Ala_L]-OH (where Val_L and Ala_L denote the termini of a lanthionine building block) is potent and highly δ-receptor selective and Tyr-c[(N^1CH_3)-D-A_2bu-Gly-Phe-$NHCH_2CH_2$-] (a methylamine-bridged enkephalin analog) is one of the most potent opioids prepared to date. These molecules are representative of our design of novel peptidomimetic opioids.

Unusual acidolysis reactions are reported for derivatives containing acylated *N*-methyl-α-aminoisobutyryl (*N*MeAib) residues.[3] The bond linking the *N*MeAib residue to the following amino acid is cleaved. Through X-ray diffraction studies of the *N*meAib-containing molecules, we have shown that the carbonyl of the preceding residue is in proximity to the carbonyl carbon of the *N*MeAib residue. Thus, it can act as an internal nucleophile leading to a cleavage reaction by way of an oxazolinium ion intermediate. Kinetic experiments for the cleavage reaction were carried out on a series of benzoyl dipeptide derivatives (*p*-X-$C_6H_4C(O)$-*N*MeAib-Phe-OMe) where X is varied from NO_2 to Cl. The value of $\rho = -1.335$ for the Hammett linear free energy relationship strongly supports the intermolecular oxazolinium intermediate proposed.

The topics described are indicative of our integrated program combining synthesis, biophysics and bioassays to obtain novel bioactive compounds.

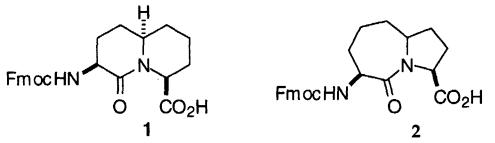
1. a) K. Feichtinger, C. Zapf, H. L. Sings and M. Goodman, *J. Org. Chem.* **1998**, 63, 3804-3805. b) K. Feichtinger, H. L. Sings, T. J. Baker, K. Matthews and M. Goodman, *J. Org. Chem.* **1998**, 63, 8432-8439.
2. T. J. Baker and M. Goodman, *Synthesis* **1999**, 1423-1426.
3. C. J. Creighton, T. T. Romoff, J. H. Bu and M. Goodman, *J. Am. Chem. Soc.* **1999**, 121, 6786-6791.

OC 72 — OC 73

"UNE PIERRE DEUX COUPS", SYNTHESIS OF QUINOLIZIDINONE AND PYRROLOAZEPINONE AMINO ACIDS FROM THE SAME LINEAR PRECURSOR.

Francis Gosselin and William D. Lubell*,
Département de Chimie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

The spatial requirements for protein chemistry and biology may be studied through the employment of azabicyclo[X.Y.0]alkane amino acids as rigid dipeptide surrogates that can restrain the back-bone geometry of the native peptide. Mimicry of a variety of peptide conformations may result from using azabicycloalkane amino acids of different ring sizes, because ring size can influence the ψ and ϕ dihedral angles constrained within the heterocycle. The construction of two azabicycloalkane ring systems **1** and **2** has now been achieved from the same linear α,ω-diaminodicarboxylate precursor by selective cyclization of the amino and carboxylate functions.



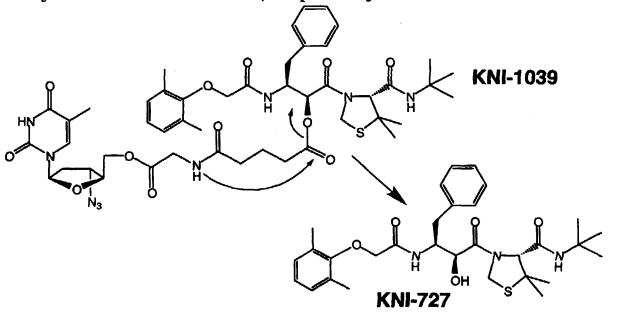
The linear precursor, (2*S*, 9*S*)-1-*tert*-butyl 10-benzyl 5-oxo-2-[*N*-(PhF)amino] 9-[*N*-(BOC)amino]dec-4-enedioate was first prepared by olefination of *N*-(PhF)aspartate β-aldehyde with a pyroglutamate-derived β-keto phosphonate (PhF = 9-phenylfluoren-9-yl). A combination of judicious employment of protecting groups and the (*E*)-double bond geometry was then used to control the first cyclization to provide either the 6-alkylpipecolate or 5-alkylproline intermediate that was subsequently transformed into the respective bicycle by a lactam cyclization. Protecting group shuffling provided finally the Fmoc-amino acid derivatives **1** and **2** suitable for solid-phase synthesis of peptide mimic libraries.

"DOUBLE-DRUGS": A NOVEL CLASS OF PRODRUG FORMS OF HIV PROTEASE INHIBITORS CONJUGATED WITH AZT BY SPONTANEOUSLY CLEAVABLE LINKERS

Yoshiaki Kiso, Hikaru Matsumoto, Tomonori Hamawaki, Hisashi Ota, Tooru Kimura, Yoshio Hayashi
Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, Kyoto Pharmaceutical University, Kyoto, 607-8412, JAPAN

We designed and synthesized a new series of prodrug type anti-HIV agents consisting of peptidomimetic HIV protease inhibitors conjugated with a nucleoside reverse transcriptase inhibitor, AZT in an effort to enhance the antiviral activity. For conjugation, a series of linkers that connect the two different classes of inhibitors have been investigated. Conjugates using a succinyl amino acid linker were shown to occur the faster release of the parent components via the spontaneous imide formation compared to conjugates using a glutaryl amino acid linker, as expected from the energetically favorable cyclization to the five-membered ring.

Herein we report a new "double-drug" (KNI-1039) with a glutarylglycine linker, which exhibited 920 and 62 times more potent anti-HIV activity than KNI-727 and AZT, respectively.



OC 74 Einstein Auditorium - Peptidomimetics

STRUCTURAL STABILIZATION AND MINIMIZATION OF THE POTASSIUM-CHANNEL BLOCKER, SHK TOXIN

M.W. Pennington[1], Y. Lefievre[1], R.S. Norton[2], M.D. Lanigan[2], H. Rauer[3], K.G. Chandy[3] [1] Bachem Bioscience Inc., King of Prussia, PA 19406, USA. [2] Biomolecular Research Institute, Parkville 3052, Australia. [3] Department of Physiology & Biophysics, University of California, Irvine, CA 92717 USA.

ShK toxin, a 35-residue polypeptide toxin containing three disulfide bonds, is a potent blocker of the voltage-gated potassium channel, Kv1.3. It is a compact molecule containing two short α-helices and a number of reverse turns. The essential residues for K-channel binding cluster on one face of the molecule to form a well-defined channel-binding surface. The compactness of this binding surface makes it feasible to design low MW mimetics of ShK.

The importance of the disulfide bonds in maintaining the structure of ShK was first probed using Abu substitutions at Cys3-Cys35, Cys12-Cys28 or Cys17-Cys28. Analogues lacking the internal 12-28 and 17-32 disulfides were largely unstructured and had poor activity, but the analogue lacking the 3-35 bond had a moderately well-defined solution structure, although different from ShK. This analogue still bound tightly to Kv1.3, suggesting that it may be flexible enough to adopt an ShK-like channel-bound conformation.

A truncated mimetic of ShK was synthesized that retains key K-channel binding residues and is stabilized by a non-native disulfide bond (Cys12-Cys24) as well as two lactam bridges, one in each of the α helices. Its structure was native-like, but differences in the relative orientation may account for its poor activity.

Lectures and Oral Communications

OC 75

Friday Afternoon: Pasteur Auditorium
New Synthetic Approaches

OC 76

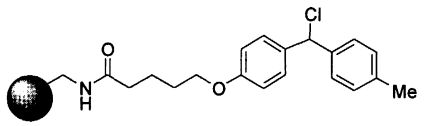
A VERSATILE 4-(4-TOLYL(CHLORO)METHYL)PHENOXY LINKER FOR THE SOLID-PHASE SYNTHESIS OF PSEUDOPEPTIDES

Gail E. Atkinson,[a] Peter M. Fischer [b] and Weng C. Chan [a]

[a] School of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, U.K. and [b] Cyclacel Ltd., James Lindsay Place, Dundee DD1 5JJ, U.K.

Combinatorial methods for the generation of compound libraries are revolutionising the drug discovery process. The most widely used synthetic techniques in combinatorial chemistry rely on solid-phase organic synthesis, in which substrates are covalently attached to the solid support *via* a linker molecule. As such, the selection of an appropriate linker is paramount to the success of any solid-phase synthetic strategy. Indeed, the variety of chemistry that can be employed is pre-determined by the linker stability and cleavage conditions.

In this context, we report a facile approach for the synthesis of 5-[4-(4-tolyl(chloro)methylphenoxy]pentanoyl aminomethylated polystyrene **1** and its application as an anchor for the synthesis of structurally diverse modified amino acids and pseudopeptides. A diverse array of challenging solid-phase chemical transformations will be described. For example, we have established the robustness of the linker-resin **1** to conditions employed in Mitsunobu and Suzuki reactions. The range of conditions required for the mild acidolytic release of assembled compounds have been investigated and these will be described in detail.



1

TOTAL SYNTHESIS BY COMBINED SOLID PHASE AND SOLUTION APPROACH OF THE P41 FRAGMENT

Cristina Chiva[a], Fernando Albericio[a], Ernest Giralt[a], Franck Molina[b], Claude Granier[b], Tatsuya Inui[c], Hideki Nishio[c], Yuji Nishiuchi[c], Terutoshi Kimura[c], Shumpei Sakakibara[c].

[a]Departament de Química Orgànica, Facultat de Químiques, Universitat de Barcelona, Spain. [b] CNRS UMR 9921, Faculté de Pharmacie, Montpellier, France. [c]Peptide Institute Inc., Osaka, Japan.

Major histocompatibility complex (MHC) class II molecules are associated, during intracellular transport, with a protein termed invariant chain (Ii) [1]. Ii exist in two alternative spliced forms p31 and p41 [2], both forms prevent premature peptide binding, facilitate class II assembly, folding, exit from the endoplasmatic reticulum and enhance class II localisation to endosomal antigen-processing compartments. Splicing of a 64 amino acid fragment produces the p41 variant of Ii. The Ii p41 fragment specifically inhibits cathepsin L [3] and its sequence is homologous to the thyroglobulin type 1(Tg type 1) domain originally found in thyroglobulin [4].

In order to study the function and the structure-activity relationship of the p41 fragment and in general of the Tg type 1 domain we have synthesised the p41 fragment using a combined solid-phase and solution approach [5]. The entire molecule was assembled from fully protected peptides in the size-range of ten residues. After the deprotection, the free peptide was subjected to folding reaction to obtain the native conformation. Circular dichroism studies and biological activity of the synthetic p41 fragment will be presented.

H-LTKCQEEVSHIPAVHPGSFRPKCDENGNYLPLQCYGSIGYCWCVFPNGTEVPNTRSRGHHNCSES-OH

Sequence of the p41 fragment

1 Cresswell P., *Anu. Rev. Immunol.*, **12**, 259-293, (1994).
2 Strubin M et al., *EMBO J.*, **5**, 3483-3488, (1986).
3 Ogrinc T et al., *FEBS Lett.*, **336**, 555-559, (1993).
4 Molina F. et al., *Eur. J. Biochem.*, **240**, 125-133, (1996).
5 Nishiuchi Y. et al., *Proc. Natl. Acad. Sci. USA*, **95**, 13549-13554, (1998).

OC 77

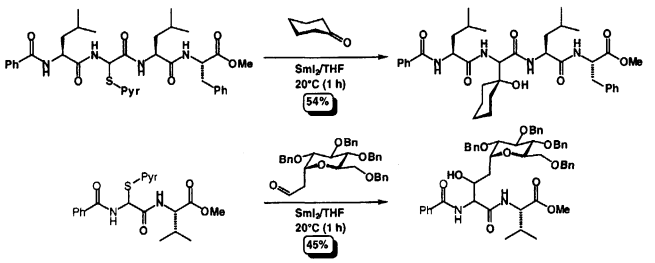
Reductive Samariation as a Means for the Selective *C*-Alkylation of Glycine Residues: Application to the Synthesis of Unnatural Peptides.

Marina Ricci, Peter Blakskjær and Troels Skrydstrup*

Department of Chemistry, University of Aarhus, Langelandsgade 140, 8000 Aarhus C, Denmark

The selective introduction of side chains to a specific glycine residue in a peptide strand represents a challenging task for the preparation of unnatural peptides. Instead of employing a stepwise approach where commercially available or synthetic amino acids are incorporated via a traditional peptide synthesis, application of a direct peptide modification step is justified by the numerous analogs which may be quickly synthesized from a single and intact peptide.

With our interest in the application of transition metal based single electron reducing agents,[1] we have recently disclosed our preliminary results, demonstrating that reductive samariation of glycine pyridyl sulfides in the presence of carbonyl substrates (Barbier Conditions) is a viable approach for the selective incorporation of carbinol side chains onto glycine residues in peptides.[2] In this paper, we present a more elablorate investigation illustrating the scopes and limitations of this approach for the *C*-alkylation of peptides. As illustrated below, such couplings are reasonably efficient considering that alkylation occurs at room temperature and in the presence of the amide protons. Key to the success of these couplings is the high stability of the α-glycine radical intermediates.



1. (a) T. Hansen, Sussie L. Krintel, K. Daasbjerg, T. Skrydstrup, *Tetrahedron Lett.* **1999**, *40*, 6087; (b) S. L. Krintel, J. Jiménez-Barbero, T. Skrydstrup, *Tetrahedron Lett.* **1999**, *40*, 7565; (c) T.B. Christensen, D. Riber, K. Daasbjerg, T. Skrydstrup, *Chem. Commun.* **1999**, 2051.
2. M. Ricci, L. Madariaga, T. Skrydstrup, *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 242.

OC 78

O-SUCCINIMIDYL CARBAMATE DERIVATIVES FROM AMINO ACIDS AND PEPTIDES : A GENERAL ENTRY TO UREA-BASED PEPTIDOMIMETICS

Vincent Semetey[a], Arnaud-Pierre Schaffner[a], Michel Marraud[b], Claude Didierjean[c], André Aubry[c], Marc Rodriguez[d], Jean-Paul Briand[a], and Gilles Guichard[a].

[a]Laboratoire de Chimie Immunologique, UPR 9021 CNRS, IBMC, Strasbourg, France, [b]LCPM, UMR-CNRS 7568, ENSIC-INPL, Nancy, France; [c]LCM3B, ESA-CNRS 7036, Université Henri Poincaré, Vandœuvre lès Nancy, France, [d]Neosystem, Strasbourg, France.

The creation of novel linear and cyclic urea-based scaffolds with specific biological functions and/or defined secondary structures is becoming an exiting new focus of peptidomimetic chemistry. For example, *N,N'*-substituted ureas are key elements of several drug candidates including potent HIV inhibitors [1]. Recently, cystine-based macrocyclic bisureas have been found to form nanotubes in the solid state [2]. In the field of non-natural oligomers, oligoureas have been introduced as scaffolds for the creation of artificial β-sheets [3] and as novel peptide backbone mimetics [4]. The expected increased resistance to enzymatic degradation as compared to peptides as well as hydrogen bonding properties of the urea backbone make this class of compounds particularly suitable for drug discovery.

We have developed a practical and versatile preparation of *O*-succinimidyl carbamates starting from *N*-protected α- and β-amino acids as well as from *N*-protected dipeptides. These derivatives are stable compounds that react readily with amines to form substituted ureas. The mild conditions required for their preparation are compatible with most functionalized side chains of amino acids and with Fmoc/tBu chemistry. The use of *O*-succinimidyl carbamates as activated monomers for the solution and solid phase synthesis of oligoureas [5], ureidopeptides and oligourea/peptide hybrids as well as for the synthesis of novel urea-based scaffolds will be presented. Conformational properties of short chain ureido-peptides in solution and in the solid state have also been investigated and will be discussed.

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Lectures and Oral Communications

OC 79

Friday Afternoon: Pasteur Auditorium
New Synthetic Approaches

OC 80

EVALUATION OF RING CLOSING METATHESIS (RCM) REACTIONS FOR THE PREPARATION OF PEPTIDE CYCLIC ESTERS IN THE SOLID PHASE

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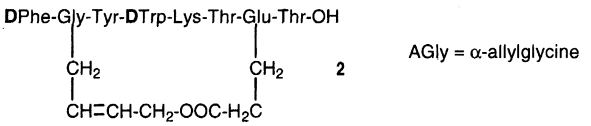
In recent years, ring closing metathesis (RCM) reactions have been utilized in the formation of C-C bonds in chemistry[1,2]. With the introduction of efficient metal carbene complexes, significant advances have been made in the synthesis of natural products, as well as cyclic peptide derivatives. These results prompted us to explore the use of RCM reaction in the construction of cyclic esters of various peptides. When resin-bound, protected peptide **1** was treated with Grubbs' catalyst (bis(triscyclohexylphosphine)benzylidine ruthenium (IV) dichloride), the cyclic ester **2** was obtained in high yield.

Boc-**D**Phe-AGly-Tyr-**D**Trp-Lys-Thr-Glu(γ-OAll)-Thr-O-RESIN, **1**



2

AGly = α-allylglycine

Formation of the cyclic ester in the above case is dependent upon the initial loading of the resin. While **2** was obtained in quantitative yield with a lower loaded resin (0.18 – 0.2 mmol/g), the course of the reaction was altered significantly using a resin with a higher initial loading. An intermolecular metathesis reaction was observed resulting in the formation of a dimeric peptide. Along with details of RCM applications, methods to reduce the unsaturated products to their saturated counterparts will also be presented.

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COMBINATORIAL LIBRARIES FOR SENSOR ARRAYS

Günther Jung[a], Rolf Tünnemann[a] Dietmar Leipert[a], Jürgen Mack[a], and Karl-Heinz Wiesmüller[b], [a]Institute of Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, FRG, [b]EMC microcollections GmbH, Sindelfinger Str. 3, 72070 Tübingen.

Synthetic receptor collections [1] on transducers have been developed based on the virtual library of 10^{16} cyclopeptides. From subcollections attached to gold (quartz microbalance [2]) and glass (reflectometric interference spectroscopy [3]) transducers we deduced selective cyclohexapeptides. As for natural receptor proteins the specifity of single synthetic receptor molecules comprises a multitude of structurally related ligands. Therefore arrays of chemosensors, pattern recognition and multiparameter analysis are used in chemosensorics. Successful applications of cyclopeptide monolayers and of functional matrix supported bilayers are illustrated.

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Lectures and Oral Communications

OC 81

Friday Afternoon: Einstein Auditorium
Structure-Activity Studies

OC 82

RXP 407, A SELECTIVE INHIBITOR OF THE N-DOMAIN OF ANGIOTENSIN I- CONVERTING ENZYME, BLOCKS THE *IN VIVO* DEGRADATION OF THE HEMOREGULATORY PEPTIDE ACETYL-SER-ASP-LYS-PRO WITH NO EFFECT ON ANGIOTENSIN I HYDROLYSIS.

Christophe Junot[a], Joël Cotton[b], Marie-Françoise Gonzales[c], Annie Michaud[d], Gilles Vazeux[b], Stamatia Vassiliou[e], Athanasios Yiotakis[e], Michel Azizi[f], Eric Ezan[a], Pierre Corvol[d] and Vincent Dive[b]

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The phosphinic peptide RXP 407 has been recently identified as the first potent selective inhibitor of the N-active site (domain) of angiotensin converting enzyme (ACE) *in vitro*. The aim of this study was to demonstrate that RXP 407 inhibits selectively the ACE N-domain *in vivo* and to compare its inhibitory effects to those of lisinopril, a non-selective ACE inhibitor of both the N- and C-domains. In mice infused with increasing doses of RXP 407 (0.1 to 30 mg/kg/30 min), plasma concentrations of AcSDKP, a physiological substrate of the N-domain, increased significantly and dose-dependently towards a plateau 4 to 6 times the basal levels. RXP 407 significantly and dose-dependently inhibited *ex vivo* plasma ACE N-domain activity, whereas it had no inhibitory activity toward the ACE C-domain. RXP 407 (10 mg/kg) did not inhibit the pressor response to an i.v. angiotensin I bolus injection in mice, thus confirming its N-selectivity *in vivo*. In contrast, lisinopril infusion (5 and 10 mg/kg/30 min) affected the metabolism of both AcSDKP and angiotensin I. The differential effects of RXP 407 on the metabolism of the AcSDKP hemoregulatory peptide and on that of angiotensin I, suggest that this drug could be used to protect hematopoietic stem cells during antitumor chemotherapy without affecting blood pressure regulation. In conclusion, this work demonstrates for the first time that the activities mediated by the two ACE active sites can be dissociated.

GLUCAGON RECEPTOR INTERACTIONS WITH TWO $G\alpha_s$ SPLICE VARIANTS.

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New York, NY 10021 USA

Glucagon is known to exhibit both high and low binding affinities for the glucagon receptor (GR), which is thought to be due to the existence of a heterogeneous population of receptors in cells, one having a high affinity and the other a low affinity for ligand. Glucagon binding to GR, a member of a distinct class within the superfamily of G protein-coupled receptors (GPCRs), activates the G protein G_s which leads to an increase in cAMP and intracellular Ca^{+2}. Two major molecular weight forms of the α subunit of G_s, termed short and long ($G\alpha_s$-S and $G\alpha_s$-L), are present in most cells but their proportions vary. To analyze functional differences in the interactions of GR with the two predominant forms of $G\alpha_s$ the rat glucagon receptor (GR) was covalently linked to the short and the long variants of $G\alpha_s$ to produce the fusion proteins GR-$G\alpha_s$-S and GR-$G\alpha_s$-L. The 3'-terminal codon of the open reading frame of a synthetic GR gene was joined directly to codon 2 of the cDNAs encoding the two $G\alpha_s$ variants. Following expression in transiently transfected COS-1 cells, the pharmacological characteristics of the fusion proteins in response to glucagon and a glucagon antagonist were examined. Our results confirm that $G\alpha_s$-S and $G\alpha_s$-L have different effects on GR-mediated signaling and indicate that receptors precoupled to $G\alpha_s$-L account for GTP-sensitive high affinity glucagon binding. In addition, both GR-$G\alpha_s$-S and GR-$G\alpha_s$-L displayed elevated levels of cAMP even in the absence of glucagon. Our observations are compatible with the expanded ternary complex model of receptor activation which postulates that G protein-coupled receptors exist in an equilibrium between two interconverting states which are stabilized by association with G proteins, even prior to ligand binding. GR in the fusion proteins is precoupled to either $G\alpha_s$-S or $G\alpha_s$-L and exists in a partially activated state. Our results show that the efficacy and potency of GPCR agonists and antagonists are not only dependent on their intrinsic structural properties but also on the type and relative amounts of G proteins within the cell.

OC 83

EXPRESSION, AMYLOIDOGENICITY AND CYTOTOXICITY OF HUMAN PRO-ISLET AMYLOID POLYPEPTIDE (PROIAPP)

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The major constituent of pancreatic amyloid, found in more than 95% of type II diabetes patients, is the 37-residue peptide islet amyloid polypeptide (IAPP). The mechanism that underlies *in vivo* IAPP insolubilization into cytotoxic amyloid still remains to be understood. IAPP is expressed as a 89-residue precursor protein (PreProIAPP) in the β-cells of the pancreas. Cleavage of the signal peptide yields ProIAPP that consists of a 67-residue sequence that contains IAPP, an 11-residue N- and a 19-residue C-terminal propeptide sequence. ProIAPP is processed into IAPP and stored together with insulin in secretory granules. To date, little is known about the role of ProIAPP and its processing in the formation of pancreatic amyloid. This is due in part to the experimental difficulties that are commonly encountered during both the chemical synthesis and the recombinant expression and purification of amyloidogenic polypeptides.

Here, we present a method for efficient recombinant expression and purification of ProIAPP and a ProIAPP mutant, mutProIAPP, that was designed to be devoid of the Lys-Arg proteinase cleavage sites and that is not processed *in vivo* into IAPP. The procedure is based on the expression of these proteins as N-terminally tagged thioredoxin-(Trx)/$(His)_6$ fusion proteins. We also present our studies on the conformation and amyloidogenicity of ProIAPP, mutProIAPP, and the Trx-tagged fusion proteins as assessed by circular dichroism spectroscopy (CD), electron microscopy (EM), and Congo red staining (CR). Our results suggest that ProIAPP and mutProIAPP contain significant amounts of β-sheet structure and that both proteins are capable of self-assembling into amyloid. However, the conformational transitions of ProIAPP or mutProIAPP that occur during aging are distinct from the random coil-to-β-sheet transition that IAPP undergoes in the process of aging and amyloidogenesis [Kayed et al., *J. Mol. Biol.* 1999]. Also, both proteins are less amyloidogenic than IAPP. Of note, the Trx-fusion proteins are completely devoid of amyloidogenicity. *In vitro* cytotoxicity studies on pancreatic cells reveal that ProIAPP is markedly less cytotoxic than IAPP, whereas mutProIAPP lacks any cytotoxic potential. Together, our studies suggest that the pro region significantly affects the amyloidogenic and cytotoxic properties of IAPP.

OC 84

INHIBITORS OF METALLOENDOPEPTIDASE EC 3.4.24.15 STABILISED AGAINST PROTEOLYSIS BY THE INCORPORATION OF β-AMINO ACIDS

D. L. Steer[1], R A Lew[2], S Gerreyn[2], M. Harte[3], S.Bond[3], P. Perlmutter[3], A. I. Smith[2] and M. I. Aguilar[1]
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Endopeptidase EC 3.4.24.15 (EP 24.15) is a thermolysin-like metalloendopeptidase which is expressed widely throughout cells and tissues, with the highest concentrations in the brain, pituitary and testis. The exact role of EP 24.15 remains unknown, but is thought to participate in the regulated metabolism of a number of specific neuropeptides. Of the limited number of inhibitors of EP 24.15 that have been developed, N-[1-(*R,S*)-carboxy-3-phenylpropyl]-Ala-Ala-Tyr-*p*-amino benzoate (CFP) is the most widely studied. CFP is a potent and specific inhibitor, but is unstable *in vivo* due to its cleavage between the alanine and tyrosine residues by the enzyme neprilysin (EP 24.11). The cpp-Ala-Ala N-terminal product of this cleavage is a potent inhibitor of angiotensin converting enzyme, which limits the use of CFP *in vivo*. In order to generate specific inhibitors of EP 24.15 that are resistant to *in vivo* proteolysis by EP 24.11, β-amino acids have been incorporated into the structure of CFP. We have prepared racemic mixtures of β-amino acids containing proteogenic side chains which are Fmoc protected and several analogues of CFP have been synthesised using solid phase peptide synthesis. The results of the stability and inhibitory actions of these new analogues show that the incorporation of β-amino acids adjacent to the scissile bond can indeed stabilise the peptides against cleavage by EP 24.11 and still inhibit EP 24.15. The results obtained in these studies demonstrate the potential of these amino acids in the synthesis of peptidomimetics and in the design of new stable and specific therapeutics.

Lectures and Oral Communications

OC 85

Friday Afternoon: Einstein Auditorium
Structure-Activity Studies

OC 86

STRUCTURE-FUNCTION STUDIES ON HUMAN GHRELIN, A NEW GROWTH-HORMONE RELEASING PEPTIDE

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Recently, a 28 amino acid peptide isolated from human stomach extract, has been recognized as an endogenous ligand specific for growth-hormone secretagogue receptor (hGHSR1a). In primary-culture pituitary cells, this new peptide, ghrelin, induces growth hormone release without affecting the release of other pituitary hormones.

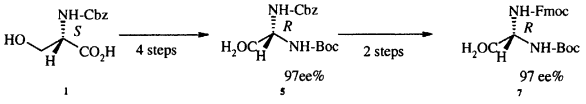
$CO\text{-}(CH_2)_6CH_3$
|
GSSFLSPEHQRVQQRKESKKPPAKLQPR

In order to elucidate the structural features of human ghrelin which participate in strong ligand-hGHSR1a interaction, number of analogs of the peptide hormone were synthesized and tested for activation of hGHSR1a:
- truncated analogs of human ghrelin,
- analogs in which the hydroxyl group of Ser^3 is esterified with various aliphatic or aromatic acids,
- analogs in which the hydroxyl group of Ser^2 or Ser^6 or Ser^{18} is esterified with n-octanoic acid,
- analogs in which the ester group in the side chain of Ser^3 is replaced by an acylamino group.

The N-terminal region of human ghrelin, encompassing the n-octanoylated Ser^3 residue, appears to be critical for activation of hGHSR1a. Also, bulky hydrophobic moieties at the side chain of Ser^3 seem to be necessary for efficient interaction of ghrelin peptides with hGHSR1a. For example, in the GHSR1a activation assay, analogs of human ghrelin with undecanoyl or 2-propylpentanoyl groups in place of the octanoyl group, but not with acetyl or benzyl groups, were as potent as the parent compound. Similarly, in the same assay, several grelin peptides in which the octanoyl-ester group in the side chain of Ser^3 was replaced by an octanoyl-amide group were equipotent to ghrelin.

SYNTHESIS OF (*R*)-BOC-(FMOC)-AMINOGLYCINE AND ITS USE IN THE DESIGN OF RECEPTOR-3 SELECTIVE SOMATOSTATIN (SRIF) ANTAGONISTS.

Jean Rivier[a], Michal Sypniewski[a], Judit Erchegyi[a], Carl Hoeger[a], Botond Penke[b], Lajos Simon[b], Dean Kirby[a], Ryan DeBoard[a], Beatrice Waser[c], Jean-Claude Schaer[c] and Jean Claude Reubi[c].
[a]The Clayton Foundation Laboratories for Peptide Science, The Salk Institute, La Jolla, CA, USA; [b]Albert Szent-Györgyi Medical University, Hungary; [c]Institut de Pathologie, Université de Berne, Switzerland.



Cbz-*S*-serine **1** was reacted with diphenylphosphoryl azide in the presence of Et_3N to yield a cyclic carbamate. In step 2, the ring nitrogen of the carbamate was protected with a Boc group. In step 3, the cyclic carbamate was hydrolyzed with benzyl-trimethylammonium hydroxide to yield the (R)-enantiomer of the alcohol. In step 4, the oxidation of the alcohol with pyridinium dichromate yielded the enantiomerically enriched (97% ee) (*R*)-Boc-(Cbz)-aminoglycine **5**, which after hydrogenolysis was converted to (*R*)-Boc-(Fmoc)-aminoglycine **7** with retention of optical purity. Independently, the methyl ester and amide of racemic **7** were resolved using papain. The stereochemistry of the isolated acid was determined to be (*R*) by coelution on HPLC of its adduct with Marfey's reagent and that of **7**. Introduction of this scaffold with N-methylation in SRIF analogs yielded a potent antagonist (affinity equal to that of SRIF-28) that is highly selective for SRIF receptor-3 (>500-fold).

L 17

UNNATURAL AMINO ACID AND PEPTIDE SYNTHESIS

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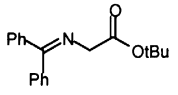
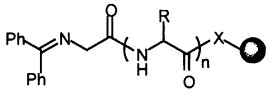
Schiff base esters of glycine have been used for the preparation of a variety of α-amino acid derivatives. Our studies using the benzophenone imines of glycine alkyl esters as either anionic or cationic glycine equivalents will be discussed. Catalytic enantioselective variants have been developed for both systems: alkylation of the benzophenone imine of glycine t-butyl ester (**1**) using phase-transfer catalysis, and palladium-catalyzed reaction of the α-acetoxy derivative of **1** with soft nucleophiles (e.g. malonates). Recently this chemistry has been extended to the resin-bound synthesis of unnatural amino acids and peptides in a combinatorial fashion. The methodology involves introduction of an unnatural amino acid side chain, starting from substrates such as **2**, during a normal Solid-Phase Peptide Synthesis.



1



2 (n = 0-2; X = O, NH, NOMe)

Posters: topic A1

P 01 New Synthetic approaches and Strategies P 02

SOLID - LIQUID PHASE SYNTHESIS OF SOME ARGINYL-GLYCYL-α-ASPARTYL PEPTIDES

Mohamed Abo-Ghalia, Somia Abdel-Rahman*
Azaa El-Kafraway* and Atef Kalmouch
National Research Centre, Cairo and Fac. of Science
Zagazig University, Zagazig*, EGYPT.

The confirmed versatile biological significance of the title peptide sequence (RGD Peptides), located in several inter-cellular matrix (adhesive) proteins, e.g. Fibronectin, Vitronectin, Fibrinoge and Collagen (1) and the antagonistic biological activity of the abstracted RGD synthetic short peptides vis'a vis the parent molecule is still a considerable research stimulus (2).

-------{- Arg-Gly-α-Asp-}--------
(RGD)

For a potentiated synthesis of some "RGD" peptides, while attempting to maximize the advantages and to minimize the disadvantages of both the currently utilized polystyrene/divinylbenzene polymer (PS, Merrifield Poylmer Solid Phase Peptide Synthesis, SPPS) and polyethylene glycol polymers (POE, Liquid Phase Peptide Synthesis, LPPS) herein, a PS-POE graft co-polymer (*Tantagels, Rapp Polymers™ Germany)*, was successfully manipulated . The following ten peptides were synthesized, purified and characterized:

1. **Arg-Gly-α-Asp-Ser**
2. **Gly-Arg-Gly-α-Asp-Ser**
3. **α-Asp-Gly-α-Asp-Ser**
4. **Gly-α -Asp-Gly-α-Asp-Ser**
5. **D-Ala-Gly-α-Asp-Ser**
6. **Gly-D-Ala-Gly -α-Asp-Ser**
7. **Ala-Gly-α-Asp-Ser**
8. **Gly-Gly-α-Asp-Ser**
9. **Pro-Gly-α-Asp-Ser**
10. **Gly-Pro-Gly-α-Asp-Ser**

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SYNTHESIS OF A GLYCOSYLATED BICYCLIC PEPTIDE, MEN11420, BY DOUBLE CYCLIZATION USING CHLOROIMIDAZOLIDIUM COUPLING REAGENT CIP

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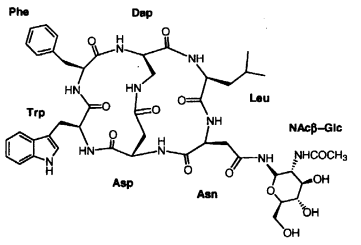
Glycosylated bicyclic peptide, MEN11420 [cyclo{[Asn(β-D-GlcNAc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)}], is a potent and selective tachykinin NK2 receptor antagonist[1]. We have synthesized MEN11420 by applying a chloroimidazolidium coupling reagent CIP[2] for intramolecular double cyclization.

A linear precursor containing a glycosylated Asn residue was constructed by Fmoc-based solid-phase procedure. The following double cyclization was conducted in solution using CIP as a coupling reagent. $CuCl_2$ was used as an additive to reduce the extent of racemization during the cyclization. The optical purity of the product was confirmed by GC-analysis using a chiral capillary column.

Thus, CIP reagent developed by us was successfully applied for the formation of sterically hindered intramolecular amide bond.

MEN11420



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2. K. Akaji, et.al., *J. Org. Chem.*, **1999**, 64, 405; and references cited therein.

P 03 P 04

PEPTIDES BASED ON A CROWN-CARRIER $C^{\alpha,\alpha}$-DISUBSTITUTED GLYCINE

Vittoria Azzini[a], Michel Wakselman[a], Jean-Paul Mazaleyrat[a], Marco Crisma[b], Fernando Formaggio[b], Claudio Toniolo[b]

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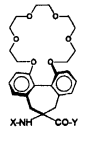
The exploitation of $C^{\alpha,\alpha}$-disubstituted Gly residues with well-defined stereochemical properties have greatly enhanced the scope of peptide design by imposing local restrictions on backbone conformation, thus conferring structural stability on potential peptide-based drugs. In this connection, *inter alia* we have previously shown that novel $C^{\alpha,\alpha}$-disubstituted Gly residues possessing only an axial chirality (Bip and Bin) are helix inducers and can give rise to efficient rigid fluorophores which have been explited in the design of the first peptide-based system of rigid donor - rigid interchromophore spacer - rigid acceptor. Here, we present our results on the synthesis and a preliminary conformational study of peptides based on [20-C-6]-Bip, in which the Bip architecture may be used as a rigid and chiral frame to carry a crown-ether effector.



Peptide	X	Y	Isomers
1	Boc	OMe	racemic R,S
2	Boc-Gly	OMe	racemic R,S
3	Boc	Gly-OMe	racemic R,S
4	Boc-Gly	Gly-OMe	racemic R,S
5	Boc-[20-C-6]-Bip-Gly-Gly	Gly-OMe	mixture of two racemic isomers
6	Boc	$(Aib)_6$-OfBu	(S) major enantiomer

The N-protected amino ester Boc-[20-C-6]-Bip-OMe (**1**) was obtained from both racemic and chirally resolved 2,2'-*bis*-(bromomethyl)-6,6'-dimethoxy-1,1'-diphenyl. Peptides **2-6** were prepared from **1** in good yields by solution synthesis, in which $(Boc\text{-}Gly)_2O$ was used for coupling at the N-terminus of the [20-C-6]-Bip residue, while couplings of both H-Gly-OMe and H-$(Aib)_6$-O*t*Bu at the C-terminus were performed by the EDC/HOBt method. Pentapeptide **5** (mixture of two racemic isomers) was obtained by fragment condensation of C-deprotected **3** with N-deprotected **4** by the EDC/HOBt method. The helical conformation of peptide **6** in chloroform solution was characterized by FT-IR absorption and ^1H NMR techniques.

NEW DMPU-BASED PEPTIDE COUPLING REAGENTS

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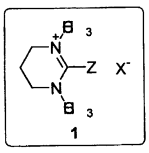
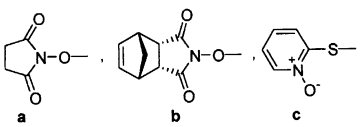
The development of new peptide coupling reagents is an important topic nowadays due the growing interest in the use of peptides as pharmaceuticals.[1] Amongst these reagents, aminium/uronium salts are probably the most effective and broadly used. Many of these compounds derive from 1,1,3,3-tetramethylurea (TBTU, HBTU, HATU, etc.), a compound which has shown toxicity as a mutagen and presenting reproductive effects.[2] As part of our ongoing project on the preparation of new peptide coupling reagents,[3] we present in this communication the development of new aminium/uronium salts **1** derived from the common organic solvent 1,3-dimethylpropyleneurea (DMPU). Thus, new hexafluorophosphate and tetrafluoroborate salts derived from DMPU and *N*-hydroxysuccinimide (**1a**), *N*-hydroxy-5-norbornene-2,3-dicarboxylic acid imide (**1b**) or 2-mercaptopyridine-1-oxide (**1c**) have been prepared and employed as efficient coupling reagents.



1

Z = 

a b c

X = PF_6, BF_4

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1. Albericio, F.; Carpino, L. A., en *Methods in Enzymology,* Academic Press, Orlando, **1997**, *289*, 104-126.
2. RTECS no. YU2625000.
3. Bailén, M. A.; Chinchilla, R.; Dodsworth, D. J.; Nájera, C. *J. Org. Chem.* **1999**, *64*, 8936-8939.

P-ONSu as a recoverable additive for peptide synthesis

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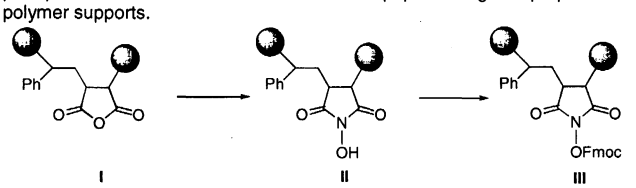
Polymer supported reagents are receiving increasing attention both for the ease of recycling and ease of work-up.[1] In a recent publication [2] we reported the preparation and applications of P-TBTU in peptide coupling reactions and the encouraging results obtained with this polymer bound reagent have prompted us to extend our studies to other peptide reagents prepared on polymer supports.



I II III

Thus, reaction of a styrene/maleic anhydride co-polymer (**I**) with hydroxylamine gave the corresponding polymer bound *N*-hydroxy-succinimide (P-ONSu,**II**) which was then used as an additive in DCC promoted peptide coupling reactions. Thus, the use of equimolar amounts of protected amino acid, aminoester, DCC, **II** and excess of pyridine gave good yields of the corresponding dipeptides with only limited amounts of racemization as shown by Anteunis' test. As a further extension of the use of this polymer-supported reagent, the reaction with Fmoc-Chloride under mildly basic conditions gives the corresponding P-Fmoc-ONSu (**III**). The preparation and initial applications of these novel polymer bound reagents will be described.

References:

1. See for example, James I.W., *Tetrahedron*, **1999**, *55*, 4855-4946
2. Chinchilla R., Dodsworth D.J., Nájera C., Soriano J.M., accepted for publication in *Tetrahedron Lett.*

3. We are grateful to Luxembourg Industries (Pamol) Ltd., Tel-Aviv, Israel for a generous gift of starting materials and moral support.

Late Deprotection Difficulties and Peptide Structure during Long-Mer Synthesis

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We wish to draw attention to the onset of Fmoc deprotection difficulties that arise late in the course of SPPS, and to propose that these late regions of deprotection trouble correspond to intrachain interactions of the growing peptide. What has been commonly observed for many years of SPPS, both by ninhydrin monitoring in tBoc chemistry and by UV monitoring in Fmoc chemistry, is that coupling and deprotection difficulties arise early in the course of synthesis, i.e. in the range of 7 - 15 residues from the C-terminus. The interpretation of these difficulties has been that sufficient chain length has accumulated to develop secondary structure, e.g. beta-sheet or alpha-helix, that inhibits access to the growing chain-end.

What we have observed during the course of long-mer synthesis, via the PE Biosystems 433A with UV monitoring of the Fmoc deprotections, is the onset of difficulties late in the synthesis, e.g. after 30, or after 50, or after 80 residues have been coupled. The late deprotection troubles are sequence dependent, and are not observed for all sequences.

We wish to focus attention on the beta-chemokines Rantes and MCP-1, which have similar structures in free solution but which show distinctly different UV deprotection profiles in the course of SPPS. Rantes displays no deprotection difficulties throughout its 68-mer synthesis, while MCP-1 runs into a region of serious deprotection troubles starting 34 residues from the C-terminus. Changes in the amino acid sequence in one area can lead to changes in the UV deprotection profile later on. Other chemokines and other long-mer sequences display unique profiles of deprotection difficulty during SPPS.

The NMR spectra of the chemokine peptide-resins before and after the onset of deprotection difficulties show marked differences and are consistent with structural changes in the peptide-resin environment. We wish to propose that the late structural changes represent intrachain peptide interactions, and that they may represent the development of tertiary structure in the growing peptide chain.

P 07 P 08

SYNTHESIS OF TRIPEPTIDE VINYL SULFONES USING POLYSTYRENE-BOC-RESIN

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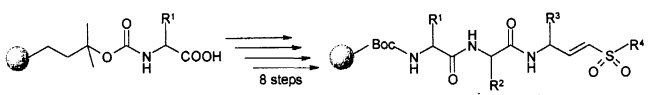
Starting from amino acids esters attached to PS Boc resin [1] we synthesised in a 8 step reaction sequence polymer bound tripeptide vinylsulfones. The first steps were an inverse tripeptide synthesis. This species was modified to a Weinreb amide which was then reduced using $LiAlH_4$ [2]. These aldehydes were used in a solid phase Horner Emmons [3] reaction with different sulfone phosphonates to form the corresponding resin bound vinyl sulfones. After cleavage from the solid support these tripeptides can be N-terminally modified with carboxylic acids using resin bound carbodiimide. In summary a solid phase synthesis protocol was developed for potential cysteine protease inhibitors [4].



Scheme : Solid phase synthesis of tripeptide vinyl sulfones

[1] T. Redemann, H. Bandel, G. Jung, *Mol. Diversity*, **1998**, 4, 191-197.
[2] S. Nahm, S. M. Weinreb, *Tetrahedron Lett.*, **1981**, 22, 3815-3818.
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[4] D. Brömme, J. Klaus, K. Okamoto, D. Rasnick und J. Palmer, *Biochem. J.*, **1996**, 315, 85-89.

SOLUBLE PRODUCTION OF A DEFECTIVE FOLDING MUTANT OF THE MALTOSE-BINDING PROTEIN, MALE31 WITH THE RAPID TRANSLATION SYSTEM (RTS)

Jean-Michel Betton

The Rapid Translation System (RTS) from Roche Molecular Biochemicals allows an efficient in vitro protein production by a coupled transcription/translation reaction. The key improvement of the system is the continuous supply of substrates and energy components, and the dilution of inhibitory reaction by-products through a semipermeable membrane. In order to evaluate the RTS, we produced the maltose-binding protein (MalE), an exported protein of Escherichia coli which serves as periplasmic receptor for the high affinity transport of maltodextrins, and a defective folding mutant (MalE31) that forms inclusion bodies in the bacterial periplasm. The yield of MalE purification was 0.35 mg/ml. Remarkably, when expressed in the RTS, MalE31 remains soluble and active even at 37°C. The RTS could provide a useful source for soluble proteins which otherwise form insoluble aggregates in a bacterial expression system.