POSTER SECTION B

P 112 SYNTHESIS AND CONFORMATION OF ANALOGUES OF THE ANTIVIRAL PEPTIDE HALOVIR A

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Recently, the isolation, amino acid sequence, chemical synthesis, anti-viral activity, and solution conformation of halovir A, a terminally-blocked pentapeptide extracted from a marine-derived fungus of the genus Scytalidium have been reported [1,2]. The primary structure of this amphiphilic helical lipopeptoid is as follows:

Myr-Aib5-Hyp-Leu-Val-Gln5-Lol

where Myr is the fatty acyl moiety myristoyl (C14) and Lol is the 1,2-amino alcohol leucinol. In addition to the naturally occurring peptide and its equally bioactive [Leu5-OMe] synthetic precursor, we have synthesized by solution-phase methods two analogues [([Me]Leu)4, Leu5-OMe] and [([Me]Val)4, Leu5-OMe] of halovir A with a potentially reinforced helicity and a substantially unmodified amphiphilicity. The preferred conformations of the three analogues in solution, as compared to that of halovir A, have been determined by a combination of FT-IR absorption, 2D-NMR, and CD techniques. Assays on the antiviral activity are currently under way.

[1] Rowley, D.C., Kelly, S., Kauffman, C.A., Jensen, P.R., Fenical, W., Bioorg. Med. Chem., 11, 4263 (2003).
[2] Rowley, D.C., Kelly, S., Jensen, P.R., Fenical, W., Bioorg. Med. Chem., 12, 4929 (2004).

P 113 TOTAL SYNTHESIS IN SOLUTION AND PRELIMINARY CONFORMATIONAL ANALYSIS OF TOAC-LABELED ALAMETHICIN F505 ANALOGUES

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Alamethicin, the most extensively investigated long peptoid, is still under debate and several, more or less convincing, models have been postulated. The primary structure of one of the most important members of alamethicin (F505) is shown below:

\[
\text{Myr-Aib}^{5-6}\text{Hyp-Leu-Val-Gln}^{5-6}\text{Lol}
\]

where Myr is the fatty acyl moiety myristoyl (C14) and Lol is the 1,2-amino alcohol leucinol. Its amino acid sequence is:

\[
\text{Myr-Aib}^{5-6}\text{Hyp-Leu-Val-Gln}^{5-6}\text{Lol}
\]

This amphiphilic helical lipopeptoid is as follows:

where TOAC is 2,2,6,6-tetramethylpiperidin-1-oxyl-4-amino-4-carboxylic acid and Fol is the 1,2-amino alcohol phenylalaninol.

While it is easy to covalently label the chromatographically purified alamethicin F505 at the C-terminus (on the primary alcoholic function), any N-terminal or C-terminal modification of the sequence requires the total synthesis of the peptoid. We have recently reported the total syntheses in solution of this terminally blocked, 19-mer peptoid and its [Glu(OMe)] analogue by an easy tunable segment condensation approach. Here we extend this research by describing the syntheses of four analogues, three mono-labeled with the stable free radical TOAC residue at either position 1, 8 or 16, and one bis-labeled at positions 1 and 16. A preliminary conformational analysis was performed by FT-IR absorption and CD. A membrane permeability study was also carried out.

P 114 A LIPID MONOLAYER MADE PERMEABLE TO TI(I) IONS BY THE LIPOPEPTAIBOL TRICHOGIN GA IV

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Trichogin GA IV is one of the shortest members of the family of peptaibols (antibiotic peptides containing Aib residues and a C-terminal 1,2-amino alcohol). Its amino acid sequence is: n-Oct-Aib5-Gly-Leu-Aib8-Gly-Leu-Aib2-Gly-Ile-Lol where n-Oct is n-octanoyl and Lol is leucinol. All peptaibols, the 3D-structure of which is highly folded (due to the heavy presence of the helicogenic Aib residues), exhibit membrane-modifying properties.

Since the length of a lipid monolayer (~20 Å) matches the main-chain length of trichogin, we synthesized and studied this peptoid in a dioleoylphosphatidylcholine (DOPC) monolayer self-assembled on a hanging Hg drop electrode. Incorporating trichogin in DOPC-coated Hg from its 4×10M solution in aqueous 0.1M KCl increases the capacity of the monolayer throughout the potential range of stability of the film, while leaving its resistance R practically unaltered. These results indicate that inorganic ions can move back and forth within the monolayer along pores created by this peptoid, but cannot be accommodated on the metal side of the monolayer. The lipid monolayer, which is not permeable to Ti(I) ions in the absence of trichogin, gives rise to a well-defined cyclic voltammogram due to the Ti(I)→Ti(II) couple in its presence, thus confirming the formation of pores.

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P 115 A UNIVERSAL INFLUENZA B PEPTIDE VACCINE

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The efficacy of conventional Influenza vaccines depends on the degree of antigenic “match” between the strains used for vaccine preparation and those circulating in the population. A universal influenza vaccine based on invariant regions of the virus would solve a major medical need. Since the temporal and geographical dominance of the influenza type and/or subtype cannot be predicted, a universal vaccine, like the vaccines currently in use, should include both type A and type B influenza components. However, while encouraging preclinical data are available for influenza A, no candidate universal vaccine is available for influenza B.

We show here that a peptide conjugate vaccine, based on the highly conserved maturational cleavage site of the HA0 precursor of the influenza B hemagglutinin, can elicit a protective immune response against lethal challenge with viruses belonging to either one of the representatives, non antigenically cross-reactive, influenza B lineages. We demonstrate that protection by the HA0 vaccine is mediated by antibodies, probably through effector mechanisms, and that a major part of the protective response targets the most conserved region of HA0: P1 residue of the scissile bond and the fusion peptide domain. In addition, we present preliminary evidence that the approach can be extended to influenza A, although the equivalent HA0 conjugate is not as efficacious as for influenza B.
**P 116** DEUTEREATED ANALOG OF THE ANTIMICROBIAL PEPTIDE TACHYPLESIN-1
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Tachypleus-I is a cyclic seventeen residue broad-spectrum β-sheet antimicrobial peptide, isolated from the hemocytes of *Tachypleus tridentatus*. It exerts anti-bacterial activity by permeabilizing the bacterial membrane. The four cysteine residues in Tachypleus-I are thought to play a structural role in imparting amphipathicity to the peptide, which has been shown to be essential for its activity. In order to understand the role of the cysteine residues in Tachypleus-I, a cysteine-deleted analog (CDT-I) was synthesized and its interaction with membranes was studied. Our studies show that CDT-I, like Tachypleus-I, exhibits antimicrobial activity and permeabilizes the outer bacterial membrane of *E. coli* at micromolar concentrations. These results suggest that the cysteine residues do not have any functional role, and simultaneously hydrophobicity and charge characteristics of the peptide may be the essential determinants of antimicrobial activity. This finding opens the door for the development of simpler, linear analogs.

**P 117** TOTAL SOLID-PHASE SYNTHESIS OF CYCLIC LIPODEPSIPETIDE ANTIBIOTIC FUSARICIDIN A
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Fusaricidin A is naturally occurring cyclic lipodepsipeptid antibiotic isolated from *Bacillus polymyxa* strain KT-8. Its simple peptide sequence ([L-Thr1-D-Val2-L-Val3-D-Ala4]-D-Thr5-D-Asn6-D-Ala7), strong activity against various kinds of fungi and Gram-positive bacteria including *S. aureus*, and indications that it may be active against MRSA, make this natural product particularly interesting as a lead compound for development of new antimicrobial agents. Total solid-phase synthesis of fusaricidin A represent the first step toward complete exploitation of its antibacterial potentials. Our synthesis commenced with side-chain anchoring of Fmoc-D-Asp-OAllyl to Temcel S RAM amide resin. This strategy affords unpumped peptide chain assembly by standard solid-phase Fmoc chemistry, yet also leaves the α-COOH group free for on-resin head-to-tail cyclization with corresponding α-NH2 group. Standard Fmoc-chemistry was used throughout. The last amino acid in the linear peptide sequence, Fmoc-D-Ala7, was coupled via ester bond to the hydroxyl group of Thr-L-Thr residue using DIC/DMAP coupling methodology. After selective removal of Fmoc and Allyl protective groups, the linear peptide was cyclized between D-Ala7 and D-Asn6 residues with an excess of PyBop/HOBt/DIEA mixture. Tetrt(Trt) protecting group was then selectively removed from Nα-L-Thr residue with 0.2% TFA in CH2Cl2, and 15-guanidino-3-hydroxypentadecanoic acid was attached. Final deprotection and cleavage of fusaricidin A from the resin was carried out with TFA/H2O/Thioanisole mixture.

**P 118** SYNTHESIS OF DIMERIC QUORUM SENSING PEPTIDES TO PROBE VIRULENCE IN *S. AUREUS*
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The induction of virulence in *Staphylococcus aureus* is controlled by a two-component system encoded by the agr operon. A receptor histidine kinase (AgrC), is activated upon binding to its cognate autoinducing peptide (AIP), which facilitates expression of downstream virulence factors [1]. Dimerization has been shown to be necessary for activation of histidine kinases such as EnvZ in *E. coli* [2]. Due to sequence homology and previous pharmacological studies, we hypothesize that AgrC is a dimer. In order to study AgrC’s oligomeric state and create more potent quorum sensing pheromones, we designed dimeric AIP’s using a double ligation strategy. The macrocycle essential for activity is formed first via an intramolecular transthioesterification, and two AIP’s are then ligated using the copper (I) catalyzed [3+2] azide-alkyne cycloaddition [3]. In addition to being agonists, AIP’s are inhibitors of non-cognate agr groups, and our ligation approach can be applied to making inhibitor AIP’s. The biological characterization of the dimeric AIP’s will be described here.

1Dunny, G.M., Leonard, B.A.B. *Annu. Rev. Microbiol.*, 51, 527 (1997).
2Tomomori, C., et al. *Nat. Struct. Biol.*, 8, 729 (1999).
3Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. *Angew. Chem. Int. Ed.*, 41, 2596 (2002).

**P 119** TETRACATIONIC AND HEXACATIONIC GRAMICIDIN S ANALOGS WITH REDUCED HEMOLYTIC ACTIVITY
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Although gramicidin S (GS), cyclot-Val-Orn-Leu-D-Phe-Pro-, is strongly active against gram-positive bacteria, it is highly toxic to human blood cells, which has limited its therapeutic applications. Polycationic analogs of GS possessing additional amino groups are good candidates of antibacterial agents which exhibit high activity against both gram-positive and gram-negative bacteria and low hemolytic activity. Tetracationic analogs possessing NH2 groups at 4α-β-positions of the two Pro residues effectively permeabilized the outer membrane of gram-negative bacteria but their antibacterial activity was much lower than parent GS [1]. Hexacationic analogs possessing L-Lys-NH2 groups at the Pro residues were also only weakly active. However, their hemolytic activity was very low. The analogs possessing D/L-Phe-NH2 instead of L-Lys-NH2 groups exhibited considerably reduced hemolytic activity and also high antibacterial activity against both gram-positive and gram-negative bacteria [2].

1Kawai, M., Tanaka, R., Yamamura, H., Yasuda, K., Narita, S., Unemoto, H., Ando, S., Katsu, T. *Chem. Commun.*, 2003, 1264 (2003).
2Kawai, M., Yamamura, H., Tanaka, R., Unemoto, H., Ohmizo, C., Higuchi, S., Katsu, T. *J. Peptide Res.,* 65, 98 (2005).

**P 120** BIOLOGICAL AND STRUCTURAL CHARACTERIZATION OF A NEW LINEAR GOMESIN ANALOGUE
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Gomesin (pGlu-CRRLCYKQRCVTYCRGRNH2 - Gm) is a potent antimicrobial peptide isolated from the hemocytes of the spider *Acanthoscurria gomesiana*. The two intramolecular disulfide bridges Cys6,11 help the molecule to fold in a β-hairpin structure [1] and confer on it a high stability in human plasma (85% intact after 24 h incubation). The two intramolecular disulfide bridges Cys2,15 and Cys4,9 assist in the formation of a hairpin structure [1] and confer on it a high stability in human plasma (85% intact after 24 h incubation). Here we describe the properties of a new linear Gm analogue. [D-Thr6,11,15, Pro3]-D-Gm is only 4-fold less active than Gm against *S. aureus* and *E. coli*, but remains as potent as Gm against *C. albicans*. The antimicrobial activity of this analogue is less salt-resistant than that of Gm. On the other hand, the analogue is 4-fold less hemolytic and as stable as Gm in human plasma. Its CD spectra collected in different solvent mixtures are typical of a β-sheet structure. 1H-NMR experiments in the presence of SDS micelles showed that [D-Thr6,11,15, Pro3]-D-Gm presents a conformation very similar to that of Gm. Both molecules presented comparable hydrophobicity, despite the analogue is slightly more electropositive. [Supported by CNPq and FAPESP]

1Mandard, N., Bulet, P., Caille, A., Daflu, S., Vovelle, F. *Eur. J. Biochem.*, 269, 1190 (2002).
P 121 MONOMIC ANALOGUES OF HALOCIDIN
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Bacterial resistance to traditional antibiotics has become a dramatic problem. The occurrence of multidrug-resistant pneumococci in community, and methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci in hospital environment has increased greatly, and has culminated in the first documented case of infection caused by vancomycin-resistant S. aureus recently reported. The undisclosed use of antibiotics in medicine and agriculture has led to this situation. Much research has been devoted to new antibiotics, amongst which antimicrobial peptides.

Halocidin is a heterodimeric antimicrobial peptide isolated from a tunicate, Halocynthia aurantium. We used the most active of the two monomers, an 18 residue amidated peptide as lead structure and determined the role of each amino acid with alanine scanning. The results obtained led to the synthesis of a first generation of analogues with antimicrobial activity. The selectivity towards bacterial versus mammalian cells has been explored, as well as the specificity for gram positive (S. aureus ATCC 25923) versus gram negative bacteria (E. coli ATCC 25922). The hydrophobic moment was used to analyze the results.

P 122 FRAGMENT OF HUMAN LYSOZYME CONJUGATED ON THE N-TERMINUS AND DISPLAYING ANTIBACTERIAL PROPERTIES
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There is a great need for new antibiotics as resistance in bacteria is becoming a dramatic problem both in community and in hospital environment. This urgent quest is directed toward antibiotics with a new mode of action. Antimicrobial peptides and proteins belong to such a class and have a promising potential.

Lysozyme is a small sized enzyme (14.4 KDa, 129 amino acids) widely distributed in living organisms. This basic protein is implicated in many biological processes amongst which antimicrobial activity. This activity is due to two different mechanisms: a) an enzymatic antimicrobial activity targeting gram positive bacteria, and b) an antimicrobial activity against gram positive and negative bacteria due to a domain located in the loop structure at the upper lip of the enzymatic site.

Using this loop sequence and its reverse as a lead structure, we have conjugated a number of acids on their N-terminus. Their antimicrobial activity on gram positive (S. aureus ATCC 25923) and gram negative bacteria (E. coli ATCC 25923) has been studied.

P 123 SYNTHESIS AND RAMAN SPECTROSCOPIC STUDIES OF THE ANTIMICROBIAL PEPTIDE CECROPIN B2
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The wide-spread use of antibiotics for treatment and prevention of bacterial infections has resulted in an alarming increase in multi-resistant pathogenic bacteria. In recent years, much research in this area has focused on naturally occurring antimicrobial peptides, which rapidly kill a wide range of pathogenic microorganisms. Cecropin B2, an antimicrobial peptide originally isolated from the silkworm Bombyx mori, was synthesized manually using Fmoc chemistry. Raman spectroscopy was used to monitor the secondary structure of the resin bound peptide at every other step of the synthesis. Furthermore, the secondary structure of cecropin B2 was investigated in solid state, aqueous solution, and 50% aqueous TFE. The antibacterial activity of cecropin B2 towards American Type Culture Collection (ATCC) S. aureus ATCC 25923 and E. coli ATCC 25922 was investigated in solid state, aqueous solution, and used to monitor the secondary structure of the resin bound peptide at synthesis. Raman spectroscopy was focused on naturally occurring antimicrobial peptides, which rapidly kill a wide range of pathogenic microorganisms. Cecropin B2, an antimi-

Finally, Raman spectroscopy was used to study the differences between the aforementioned bacterial strains and for probing the interaction of cecropin B2 with E. coli at different concentrations.

P 124 ALAMETHICIN INTERACTION WITH LIPID MEMBRANES: A SPECTROSCOPIC STUDY ON SYNTHETIC ANALOGS
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Alamethicin is one of the most extensively studied membrane-active antibiotic peptides. However, several aspects of its mechanism of action are still debated. We have employed synthetic analogs of alamethicin F50/5 (Ac-Alb-Pro-Alb-Alb-Alb-Gln-Alb-Val-Alb-Gly-Leu-Alb-Pro-Val-Alb-Alb-Gln-Gln-Phol), to investigate the effect of varying peptide hydrophobicity on its membrane-perturbing activity. Small variations in the sequence cause drastic changes in the aggregation and partition behavior of the analogs, which, in turn, modulate their activity and selectivity toward different membranes. In particular, substitution of Gln16 and Gln17 by Gln(OMe) causes a 6-fold increase in activity, while the selectivity of the natural peptide for cholesterol-free membranes is maintained.

Other analogs, labeled with fluorophores at the N- or C-terminus, were employed to investigate both the position and orientation of this peptide in the membrane. Energy-transfer, depth-dependent quenching and polarization experiments indicate that, in the absence of a membrane potential, alamethicin inserts its N-terminus into the membrane, while the C-terminus is exposed to the aqueous phase. No peptide translocation to the inner leaflet of the lipid bilayer occurs, and the peptide populates different orientations with respect to the membrane normal.

P 125 RATIONAL DESIGN OF GRANULYSIN DERIVATIVES
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Granulysin is a 74 amino acid polypeptide secreted by human natural killer cells (NK) and cytolytic T lymphocytes (CTL) 8-10 days after activation through the T cell receptor (TCR). Granulysin is broadly lytic against mammalian cells, bacteria, fungi, and parasites. Structurally, granulysin consists of 5 a-helices separated by short loop regions. The crystal structure of granulysin reveals two intra-molecular disulfide bonds. Using a panel of synthetic peptides corresponding to different regions of granulysin, we previously found that the lytic activity is confined to helices 2-4.

A series of granulysin peptide mutants was prepared. Some of these peptides show a 2-4 log increase in anti-bacterial properties and no lytic activity against mammalian cells. In general, lytic activity against mammalian cells correlated with high hydrophobicity and helicity or the ability of peptides to form disulfide bonds. Substitution of some or all residues with D-amino acids greatly improved antimicrobial activity. In addition, formation of cyclic peptides or linear multimers resulted in enhancement of antibacterial activity. Mechanisms of lysis by selected derivative peptides will be discussed.

P 126 EFFECT OF THE HOST-DEFENSE PEPTIDE BAC7(1-35) ON MEMBRANE PERMEABILITY AND VIABILITY OF GRAM-NEGATIVE BACTERIA
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A large number of gene-encoded, host-defense peptides (HDPs), playing a defense role throughout the living organisms, has been described over the past decade. Although many of these peptides interact with bacterial membranes and permeabilize them, it is not clear whether this is the cause of their lethal effect. To address this issue, we studied the interaction of Bac7(1-35), a fragment of the Pro-rich peptide Bac7, and...
of some analogues and truncated forms, with Escherichia coli and Salmonella enterica by using killing kinetics assays, flow cytometry and immunogold electron microscopy. In the flow cytometry assays, bacterial cells were stained with different fluorochromes sensitive to changes in either membrane integrity (propidium iodide) or membrane potential (DiBAC4(3)), and the data obtained compared to the bacterial killing kinetics and the capacity of the different peptides to enter bacterial cells. Results indicate that Bac7(1-35) is able to penetrate target cells and rapidly inactivate them without any initial appreciable membrane permeabilization, which is evident only later. A rapid membrane depolarization was observed by treating the Gram-negative bacteria with Bac7(1-35) as well as with its inactive all-D enantiomer, indicating a poor correlation between cell viability and changes in membrane potential. Differences in the degree of permeabilization and depolarization were also found between the two bacterial species tested. Results are discussed in terms of a different mechanism of action of Bac7(1-35) with respect to other classes of HDPs.

P 127 DESIGN OF BACTERICIDAL SELF-ASSEMBLING CYCLIC D,L-α-GLYCOPEPTIDES

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The rapid emergence of new strains of bacteria that are resistant to existing drugs underscores the urgent need for novel antibacterial agents. We have shown previously that appropriately designed cyclic D,L-α-peptides can self-assemble in bacteria cell membranes and exert potent in vitro and in vivo activity against multidrug-resistant bacteria [1]. Here we show how glycosylation of specific residues in cyclic D,L-α-peptide sequences can improve their physical properties and ameliorate their biological activity. We demonstrate that cyclic glycopeptides form β-sheet-like hydrogen bonded tubular assemblies that are oriented roughly parallel to the plane of the lipid membrane. We also show that glycosylation and the resulting increase in hydrophilicity of the cyclic D,L-α-peptide does not influence cell membrane uptake or antibacterial activity. Moreover, depending on glycosylation position and the type of glycosyl moiety employed, the toxicity of the peptide toward human red blood cells could be significantly attenuated. Among the cyclic D,L-α-glycopeptides tested, those bearing β-Gal side modification were found to be the most active antibacterial peptides against methicillin-resistant S. aureus (MRSA) and vancomycin-resistant E. faecalis (VRE) and had the lowest in vitro toxicity against human red blood cells.

[1] Fernandez-Lopez S. et al. Nature, 412, 452 (2001).

P 128 HB-50: A PRE-CLINICAL STUDY OF A FABRICATING PEPTIDE FOR WOUND INFECTION

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Topical prophylaxis against wound infection by an agent that is active against multi-resistant bacteria does not generate resistance and is rapidly cidal would be of great clinical benefit. Peptides of the innate immune system have long been known to protect a wide range of organisms from attack by bacterial and fungal pathogens. Helix BioMedix Inc. has developed a short bioactive peptide antimicrobial modelled after these peptides. HB-50 is an amphipathic cationic alpha-helical peptide that has broad spectrum activity and is rapidly microbicidal. These attributes make HB-50 an ideal candidate for wound infection prophylaxis. In vitro studies have demonstrated HB-50 to be active against both gram-positive and gram-negative bacteria killing 5-7 log orders of bacteria within minutes. In addition, this peptide has potent activity against Vancomycin and Mupirocin resistant S. aureus. Preliminary testing of the peptide in a rat abraded skin infection model has shown the peptide’s effectiveness in preventing wound infection while not inhibiting wound healing. Additionally, the HB-50 sequence has been specifically developed to be cost effective to manufacture and therefore is well suited for use as a topical antimicrobial agent.

P 129 DESIGNER MULTIFUNCTIONAL ANTIBACTERIAL PEPTIDES KILL FLUOROQUINOLONE-RESISTANT CLINICAL ISOLATES

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Approximately 10-25% of E. coli and K. pneumoniae strains, bacteria responsible for most urinary tract infections (UTI), are resistant to fluoroquinolones, the currently preferred UTI treatment. Native antibacterial peptides destroying the membrane structure are potentially toxic and peptides with intracellular targets are usually not potent enough when the in vitro assays are run in microbiology approved media. To overcome these limitations, we designed proline-rich antibacterial peptides that maintain their DnaK-binding ability to inhibit protein folding in bacteria and low toxicity in eukaryotes, but enter bacterial cells much more avidly than any earlier derivative. The basis of the design process was a multiple alignment of all known proline-rich sequences and sequence optimization as a second step. The resulting chimeric and combinatorial analogs exhibit 8-16 microgram/ml MIC efficacies against a series of UTI pathogens, collected from urological infections all over the world. Significantly, the best peptide, A3-AP0, resists serum degradation and retains full activity in the presence of mouse serum. Across a set of 8 fluoroquinolone-resistant clinical isolates, the A3-AP0 peptide is 4 times more potent than Ciprofloxacin and 20-fold more active than the best single peptide-based analog.

P 130 CHEMICAL SYNTHESIS, RECOMBINANT EXPRESSION, AND ANTIMICROBIAL PROPERTIES OF HUMAN β-DEFENSINS

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Three antibiotic human beta-defensins (hBD1, hBD2 and hBD3) have been isolated and described in the literature, while an hBD4 sequence (37 residues) was predicted by screening of genomic sequences [1]. To facilitate biological and physico-chemical studies of hBDs (hBD 1-4 and a hybrid peptide hBD2/3), we devised an Fmoc-based SPPS protocol for their chemical synthesis followed by oxidative folding. In some cases we carried out the disulfide formation using pairwise orthogonal Cys-protection [2]. We also developed an efficient recombinant expression procedure for preparation of hBD1 (36, 42 and 47 residues), hBD2, hBD3, and hBD4 (44 residues). Synthetic and/or recombinant peptides were used to produce antibodies that have been useful for localizing hBD expression in various human tissues. Structural studies reveal that hBD 1-3 share similar β-sheet-dominated folds, but with differently oriented positive charges. We evaluated the antimicrobial properties of these peptides against E. coli and S. aureus, demonstrating that hBD3 had the strongest bactericidal activity even in the presence of high salt concentrations. Studies of an hBD2/3 hybrid indicated that the carbonyl half of hBD3 confers a portion of this salt insensitivity.

[1] Conejo Garcia, J.-R., et al., FASEB J., 15,1819 (2001).
[2] Osapay, G., Osapay, K., Selsted, ME., “Synthesis and disulfide array determination of human β-defensin-1.” The Protein Society Symposium, San Diego (1998).

P 131 SOLUTION STRUCTURE OF STOMOXIN AND SPINIGERIN TWO ANTIMICROBIAL PEPTIDES FROM INSECTS

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Stomoxyn and spinigerin belong to the class of linear cysteine-free insect antimicrobial peptides that kill a range of microorganisms, parasites and some viruses but without any lytic activity against mammalian erythrocytes. The structure of stomoxyn and spinigerin in aqueous
solution and in TFE/water mixtures was analyzed by CD and NMR spectroscopy and molecular modeling. Stomoxyn and spinigerin adopt a flexible random coil structure in water while both assume a stable helical structure in the presence of TFE. In 50% TFE, the structure of stomoxyn is typical of cecropins, including an amphipathic helix at the N-terminus and a hydrophobic C-terminus with helical features. In contrast to stomoxyn, spinigerin acquires very rapidly a helical conformation. In 10% TFE the helix is highly bent and the structure is poorly defined. In 50% TFE, the helical structure is well defined all along its sequence, and the slightly bent α-helix displays an amphipathic character, as observed for magainin 2. The structural similarities between stomoxyn and cecropin A from *Hyalophora cecropia* and between spinigerin and magainin 2 suggest a similar mode of action on the bacterial membranes of both pairs of peptides. Our results also confirm that TFE induces helix formation and propagation for amino acids showing helical propensity in water but also enhances the helix propagation propensity of non polar β-branched residues.

In an attempt to assess the presence of a regular conformation in protic solvent as well as to correlate far-UV chiroptical properties and conformational preferences of oligoureas, we have undertaken a detailed conformational investigation of oligoureas of varying length utilizing both CD and NMR spectroscopy in MeOH [3]. The body of structural information now available provides a rationale for the design of oligoureas with useful biological (antimicrobial) properties.

[1] Semetey, V. et al. Angew. Chem. Int. Ed. 2002, 41, 1895-1898.
[2] Guichard, G. in *Pseudopeptides in Drug Development*; Nielsen, P. E., Ed.; Wiley-VCH, 2004, 33-120.
[3] Violette, A. et al. *J. Am. Chem. Soc.* 2005, 127, 2156-2164.

**P 132 SYNTHESIS OF PLUSBACIN A₃ AND KATANOSIN B: DEPSIPEPTIDE ANTIBIOTICS THAT INHIBIT BACTERIAL CELL WALL BIOSYNTHESIS**

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Bacterial resistance to commonly used antibiotics has created an urgent need for identification of novel antibacterial agents capable of treating infections due to resistant pathogens. The synthesis of plusbacin A₃ and katanosin B, depsipeptide antibiotics that disrupt bacterial cell wall biosynthesis [1] will be reported.

[1] Maki, H., Miura, K., Yamano, Y. *Antimicrob. Agents Chemother.*, 45, 1823 (2002).

**P 133 OLIGOUREA FOLDAMERS AS ANTIMICROBIAL PEPTIDOMIMETICS**

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Interested by secondary structure elucidation and bioactivity investigation of urea-based peptidomimetics, we have shown that enantiopure N,N’-linked oligoureas 2 adopt a well defined 2.5 helical structure [1], reminiscent of the 2.614 helix of γ-peptides 1 [2].

In an attempt to assess the presence of a regular conformation in protic solvent as well as to correlate far-UV chiroptical properties and conformational preferences of oligoureas, we have undertaken a detailed conformational investigation of oligoureas of varying length utilizing both CD and NMR spectroscopy in MeOH [3]. The body of structural information now available provides a rationale for the design of oligoureas with useful biological (antimicrobial) properties.

[1] Semetey, V. et al. Angew. Chem. Int. Ed. 2002, 41, 1895-1898.
[2] Guichard, G. in *Pseudopeptides in Drug Development*; Nielsen, P. E., Ed.; Wiley-VCH, 2004, 33-120.
[3] Violette, A. et al. *J. Am. Chem. Soc.* 2005, 127, 2156-2164.

**P 134 THE DE NOVO DESIGN OF ANTIMICROBIAL PEPTIDES WITH BROAD SPECTRUM ACTIVITY AND SPECIFICITY BETWEEN BACTERIAL AND HUMAN CELL MEMBRANES**

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The emergence of pathogenic bacteria with clinically significant resistance to conventional antibiotics is a major public health concern. The development of a new class of antibiotics has become critical. The designed cationic antimicrobial peptides whose sole target is the cytoplasmic membrane could represent such a class since the development of resistance is not expected because this would require substantial changes in the lipid composition of the cell membranes of microorganisms. Utilizing a structure-based rational approach to antimicrobial peptide design in two structural classes of peptides (cyclic β-sheet and α-helical) we were able to develop antimicrobial peptides with improved activity, specificity and clinical potential as broad spectrum antibiotics. The controlled disruption of β-sheet and α-helical structure by placing a positively charged residue in the center of the non-polar face of these amphipathic molecules (disruption of structure and dimerization under benign conditions but inducible structure in hydrophobic conditions) is related to the strong antimicrobial activity against a variety of Gram-negative and Gram-positive bacterial strains and specificity (no detectable toxicity to normal cells (hemolytic activity)). In addition, we have further investigated these lead compounds, first, by modulating the hydrophobicity, the number of hydrophobic interactions and hydrophobic clusters on the non-polar face and second, by changing the amphipathy without changing the amino acid composition of the polar and non-polar faces on the biological/biophysical properties.

**P 135 RAPID IN VIVO CONTROL OF ENZYME FUNCTION THROUGH CONDITIONAL PROTEIN SPlicing**

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Conditional Protein Splicing (CPS) allows two polypeptides to be joined together post-translationally with a native peptide bond. Rapamycin induced dimerization of the FKBP and FRB domains is exploited to complement inactive intein fragments and trigger the splicing reaction.[1] The reaction is rapid, occurring on the time scale of one or two hours and can be tuned by varying the amount of rapamycin used.[2] Here we show that the CPS reaction can be exploited to rapidly activate enzymatic function by splicing inactive fragments of the enzyme luciferase in vivo. This provides a method for studying the effects of enzymes in their native context on a time scale unattainable with genetic means such as inducible promoters or RNAi.

[1] Mootz, H.D. and Muir, T.W. *J. Am. Chem. Soc.*, 124, 9044 (2002).
[2] Mootz, H.D., Blunn, E.S., Tyszkiewicz, A.B., Muir, T.W. *J. Am. Chem. Soc.*, 125, 10561 (2003).
P 136 SYNTHESIS OF 5-HYDROXYLYSINE DERIVATIVES FOR USE IN SOLID-PHASE PEPTIDE SYNTHESIS

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The synthesis of 5-hydroxylysine (Hyl) derivatives for incorporation by solid-phase methodologies presents numerous challenges. The goals of this work were two-fold: first, develop a convenient method for the synthesis of O-protected Fmoc-Hyl; second, evaluate the efficiency of methods for the synthesis of O-glycosylated Fmoc-Hyl [1]. The 5-O-tert-butylmethylisilyl (TBDMs) fluoro-9-ylmethoxy carbonyl-Hyl (Fmoc-Hyl) derivative synthesis was significantly improved by incorporating the TBDMs group directly to copper complexed Hyl[2-tert-butylxycarbonyl(Boc)] using pyridine as solvent. This “one pot” procedure provided Fmoc-Hyl[e-Boc,OBz]-OBz[2] and peracetylated galactosyl bromide were added to silver trifluoromethane, and Koenigs-Knorr methods. The most efficient approach was found to be Koenigs-Knorr under inverse conditions, Fmoc-Hyl[e-Boc,OBz] was compared for the thioglycoside, trichloroacetimido glycosides, respectively. Research on the enzymology of protein splicing reaction, the VMA intein has been artificially split in two, and the intein fragments have been fused to either Phytochrome B (PhyB) or PIF3, a phototransductable and photoreactive pathway activation (input) and biological pathway activation (output). These studies are expected to lead to a deeper understanding of how the dose, timing, and localization of active R-Smads influence cellular and organismal behavior.

P 137 CHROMISM-BASED ASSAY (CHROBA) TECHNIQUE FOR IN SITU DETECTION OF PROTEIN KINASE ACTIVITY

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The sequencing of the human genome has successfully completed, offering the chance to obtain a large amount of valuable information in a single experiment for simply and rapidly understanding the complex cellular events. In order to address these proteomic studies, there is interestingly increasing the importance of protein-detecting microarray technology [1]. Especially, studies on post-translational modifications of proteins are essential for development of the microarray technology. A unique chromism-based assay technique (CHROBA) using photogenic spiroopiran-containing peptides has been firmly established for detection of protein kinase A-catalyzed phosphorylation [2]. The alternative method has advantages that avoid isolation and/or immobilization of kinase substrates to remove excess reagents including non-reactive isotope-labeled ATP or fluorescently-labeled anti-phospho-antibodies from the reaction mixture. The novel technique based on thermocoloration of the spiroopiran moiety in the peptide can offer not only an efficient screening method of potent kinase substrates but also a versatile analytical tool for monitoring other post-translational modification activities.

P 138 LIGHT-ACTIVATED PROTEIN SPlicing

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Protein splicing is a naturally occurring process in which a protein domain, known as an intein, autocatalytically cuts itself out of the flanking protein domains, called exteins. The exteins are then ligated with a native peptide bond. To gain temporal and spatial control over protein splicing reaction, the VMA intein has been artifically split in two, and the intein fragments have been fused to either Phychrome B (PhyB) or PIF3, a phototransductable and photoreactive pathway activation (input) and developmental fate (output). These studies are expected to lead to a deeper understanding of how the dose, timing, and localization of active R-Smads influence cellular and organismal behavior.

P 139 SYNTHESIS AND BIOLOGICAL ASSESSMENT OF SULFONIC ACID-BASED GLUCAGON ANALOGS

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The structure-activity relationship of glucagon has been studied with a particular emphasis on the identification and refinement for selective receptor antagonism. While the Cterminal α-helical region is believed to be important for receptor recognition there are a set of Nterminal amino acids that collectively serve the key role in signal transduction. Replacement of Asp9 with Glu
[1], in addition to the deletion of His[2] yields a potent antagonist [desHis[3], Glu[4],glucagon amide, which is purported to retain weak partial agonist activity.

Sulfonic acid-based amino acids are structurally and electronically homologous to the more native carboxylic acid containing amino acids. The dramatic biological significance exhibited by the subtle replacement of Asp9 with Glu[2] attracted our attention to explore the suitability of a set of sulfonic acid homologs for Asp and Glu. These structural substitutions when inserted in known glucagon agonists and antagonists yield fully efficacious and highly potent peptide hormones. The structural basis for the observed biological activities appears to be a function of proper interaction of the side-chain acidity with the peptide backbone and the glucagon receptors. Additional amino acid modifications to the native glucagons sequence were explored to further enhance the biological, physical and synthetic aspects of the more optimal glucagon analogs.

P 140 PHOTACTIVATION OF SIGNAL TRANSDUCTION IN LIVE CELLS AND ANIMALS

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Using Expressed Protein Ligation, we have prepared caged versions of Smad2, a principle signaling component of the cellular response to cytokines and morphogens of the TGFβ superfamily. Caging groups of the 3-nitrobenzyl class were conjugated site-specifically to activating phospho-serine residues or the C-terminal carboxylate of Smad2, rendering it photoactivatable. A conditional fluorescence system was developed allowing the protein to be visualized only in the active state, a key feature for microscopic evaluation. Biochemical and cell biological assays validated the approach. Spatially and temporally defined activation of these proteins with UV light in live cells and vertebrate embryos will permit quantitative analysis of protein function in native contexts. Issues that are being addressed with these proteins include the location of activation of R-Smads, the kinetics of nuclear translocation, and the relationship between levels of TGFβ pathway activation (input) and developmental fate (output). These studies are expected to lead to a deeper understanding of how the dose, timing, and localization of active R-Smads influence cellular and organismal behavior.
vealed its acylation to different degrees [1]. It has now been discovered that the influenza HA acylopeptides isolated from virions digested by bromelain in the absence of 2-mercaptoethanol, predominantly contain three residues of palmitate/stearate. The fatty acids detach completely from the influenza HA acylopeptide by 10mM dithiothreitol solution for 120 minutes at 50°C. Experiments on model cysteine containing peptides, S-modified by either acetic or succinic anhydride, or palmitic acid, has shown that the thioester bond is extremely sensitive to thiol treatment and becomes broken by 10mM dithiothreitol after 10-20 minutes of incubation at room temperature. So, in model acylopeptides the thioester bond is even more labile than it was found for the HA C-terminal peptide. [1] Kordyukova, L.V., Serebryakova, M.V., Ovchinnikova, T.V., Ivanova, V.T., Baratova, L.A., in Peptides 2004, Proc. 3rd IPS and 28th EPS, Prague, Czech Rep., M.Flegel, M.Fridkin, C.Gilon, I.Slaninova (Eds.) (2005) in press.

This work is supported by the ISTC/BTEP grant #2816p.

P 143 MOLECULAR DYNAMICS SIMULATIONS OF MUC1 VARIANT GLYCOPÆPTIDES
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MUC1 is a glycoprotein that is differently glycosylated in normal and cancer cells. The extracellular domain of muc1 consists of tandem repeats of a 20 amino acid motif AHGVTASPDPTRPGSTAPP that includes serine and threonine residues, which are potential sites of O-glycosylation. MUC1 demonstrates the presence of concerted replacements (DT > ES) in the tandem repeat peptide sequence from normal and cancer cells [1]. To evaluate structural effects of these polymorphisms and O-glycosylation on the polypeptide backbone conformations, the conformational propensities of the 21-residue nonglycosylated peptide AHGVTASPDPTRPGSTAPP and its differentially O-glycosylated analogs were studied by molecular dynamics (MD) simulations. Structural and conformational parameters derived from the 4.5 ns MD simulations for the nonglycosylated and glycosylated peptides with the GalNAc residue at positions T5, S10, and T17 were compared with the results of the MD simulations for the AHGVT- SAPDPTRPGSTAPP peptide and its O-glycosylated analogs. Comparison of structural properties of the glycopeptide fragments VTSA, PESR/PDT and GSTA with their nonglycosylated counterparts demonstrated differential conformational propensities near glycosylation sites that may explain antigenic properties of these peptides.

[1] Muller, S.; Alving, K.; Peter-Katalinic, J.; Zachara, N.; Gooley, A.A.; Hanisch, F.G. J. Biol. Chem., 272, 24780 (1999).

P 144 CHEMICAL SYNTHESIS OF MUC2 TANDEM REPEAT MODEL CARRYING MULTIPLE O-GalNAc MOIETIES
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Mucins cover epithelial surfaces of various organs. Within their tandem repeat region, they are rich in O-linked carbohydrates, which play essential roles in many biological processes, such as fetal development, epithelial differentiation and carcinogenesis. However, the structure of the O-glycan is highly heterogeneous, which prevents the analysis of these functions in detail.

To overcome the problem, we designed a simple method to obtain a homogeneous mucin model based on the repetitive segment coupling. First, a peptide thioester carrying 2 O-GalNAcs corresponding to the sequence of MUC2 tandem repeat unit composed of 23 amino acid residues 2 by the Fmoc method. Then the segment condensation was carried out 6 times by the thioester method to obtain tandem repeat model composed of 141 amino acid residues carrying 42 O-GalNAc moieties 1.

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Chemoselective glycosylation, acylation, and alkylation of completely unprotected peptides can be accomplished by incorporating N-alkylaminooxy amino acids into the peptide sequence. The N-alkylaminooxy side chains react selectively with reducing sugars, activated alkyl halides, and various acylating agents in mildly-acidic aqueous buffers (pH 4) to furnish neoglyco- and neolipopeptides. A key feature of the approach is that a single parent peptide can be quickly reacted with a variety of agents to provide a large number of "post-translationally"-modified peptides. The ability to easily synthesize arrays of modified peptides allows comprehensive studies of the effects that glycosylation and lipidation have on peptide structure and function. Here we present an overview of the methodology and initial results on its application to studying problems of biological interest.

P 146 PHOSPHORYLATION-DEPENDENT PROTEIN DESIGN AND PROTEIN STRUCTURE
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Protein phosphorylation is a ubiquitous signaling mechanism whose target is frequently a nonglobular protein region. We have used protein design to develop novel functional protein architectures, termed protein kinase-inducible domains (pKIDs), whose structures are dependent on phosphorylation by specific protein kinases. We have designed kinase-

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P 142 CHARACTERIZATION OF D-AMINO-ACID-CONTAINING CONOTOXINS: PREDICTING POSTTRANSLATIONAL EPIMERIZATION
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Posttranslational isomerization of L- to D-amino acids has been documented in only few gene products, mostly small peptides under 10 AA. This is particularly stealthy modification undetectable by standard proteomic methods, such as the Edman sequencing or mass spectrometry. Accurately predicting posttranslational epimerization requires more examples of naturally modified peptides. Diverse disulfide-rich toxins from Cone snails (conotoxins) are greatly enriched in various posttranslational modifications. The I-conotoxin superfamily comprises larger peptides (35-45 AA) with four disulfides that appear to be excitoconotoxins; several target K+ channels. We have demonstrated that three I-conopeptides have a single D-amino acid at the third position from the C-terminus; this modification is important for biological activity.

Previously we reported a peptide having D-Phe, namely r11a. We have characterized two other I-conotoxins with D-Phe or D-Leu at the same position. Based on cDNA cloning, we identified over 20 conotoxins highly homologous in sequence to those confirmed to contain the D-amino acid. This provides an opportunity to define parameters that determine when this posttranslational modification occurs. Neither the chemical nature of the side chain nor the precise vicinal sequence around the modified residue seem to be critical, but there may be favored loci for isomerization to a D-amino acid.

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P 145 N-ALKYLAMINOOXY AMINO ACIDS AS VERSATILE DERIVATIVES FOR "POST-TRANSLATIONAL" MODIFICATIONS OF SYNTHETIC PEPTIDES
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Chemosselective glycosylation, acylation, and alkylation of completely unprotected peptides can be accomplished by incorporating N-alkylaminooxy amino acids into the peptide sequence. The N-alkylaminooxy side chains react selectively with reducing sugars, activated alkyl halides, and various acylating agents in mildly-acidic aqueous buffers (pH 4) to furnish neoglyco- and neolipopeptides. A key feature of the approach is that a single parent peptide can be quickly reacted with a variety of agents to provide a large number of "post-translationally"-modified peptides. The ability to easily synthesize arrays of modified peptides allows comprehensive studies of the effects that glycosylation and lipidation have on peptide structure and function. Here we present an overview of the methodology and initial results on its application to studying problems of biological interest.
P 147 DRUGS FOR THE BRAIN FROM THE BRAIN: ANALGESIA & BEHAVIORAL EFFECTS OF GLYCOCEP TIDES BASED ON ENKEPHALINS & ENDOPHINS

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A series of glycopeptides based on the Leu-enkephalin analogue Yt-GFS*-CONH2 led to greatly enhanced stability in vivo and effective penetration of the BBB. Transport through the BBB hinges on the bioavailability of the glycopeptides—the glycopeptides have two conflicting conformational manifolds, a H2O soluble state, and an amphipathic state at H2O-membrane phase boundaries. Multiple lines of evidence suggest that the BBB transport mechanism is absorptive endocytosis. Mixed µ-opioids showed antinociceptive potencies greater than morphine, and lacked many of the side effects generally associated with classical µ-selective opiate analgesics. The bioavailability was extended to larger glycopeptides (16 residues) related to β-endorphin, which also penetrated the BBB and produced antinociception in mice. Plasma membrane waveguide resonance (PWR) studies showed that the amphipathic helices bound to membrane bilayers with µM to low nM KD's. The presence of diverse endogenous neuropeptide transmitters and neuromodulators in the human brain is potentially applicable to the treatment of a wide range of behavioral disorders.

P 151 A PHOTO-CONTROLLED β-HAIRPIN

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We have been able to design, synthesize and characterize a hairpin based on the tryptophan zipper motif[1] that incorporates an azobenzene-based photo-switch allowing for time-resolved folding studies of β-structures with unprecedented temporal resolution. At room temperature the trans-azo isomer exhibits a mostly disordered structure, but light-induced isomerization to the cis-azo form leads to formation of a β-hairpin, where the two peptide parts are linked by the novel photo-switch [3-(3-aminomethyl-phenylazo)-phenyl]-acetic acid (AMP). While in the original sequence the dipeptide Asn-Gly nucleates the type 1 β-turn that connects the two strands of the hairpin, this role is taken in our photoresponsive β-hairpin by the AMP chromophore that apparently can act as a β 1-turn mimetic. The β-hairpin structure was confirmed and determined by NMR spectroscopy, but folding can be monitored by pronounced changes in the CD, IR and fluorescence spectra.

[1] Cochrane, A.G., Skelton, N.J., Starovaskin, M.A., Proc. Natl. Acad. Sci U.S.A 98, 5578-5583 (2001).
**P 154 MEMBRANE ASSOCIATED STRUCTURE OF A GHRÈL- LIN INVERSE AGONIST AS DETERMINED BY NMR**

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We have undertaken the structural characterization of a ghrelin inverse agonist, sequentially related to substance P, using high-resolution NMR methods. To mimic the membrane associated structure, the study was carried out in the presence of zwitterionic micelles made of dodecyolphosphocholine. The undecapeptide readily associates with the micelles and a number of negative NOEs are detected. At least two β-turn-like conformations have been localized in position DPhε-Gln9, and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined and a number of negative NOEs are detected. At least two fragments encompassing residues Pro1-Gln6 and DTrp9-Leu10, respectively. Altogether the backbone is well defined.

**P 155 SCAFFOLD, DENTRIC AND METAL-ASSISTED ASSEMBLY OF COLLAGEN-LIKE BIOMATERIALS**

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A series of collagen-like peptides were synthesized through scaffold (TRIS, Boc-CysOH; 4)AcLys(Biotin-CysOH; 5)Ac-D-Lys(Biotin-CysOH). In these cysteine and bioactive molecule was changed (modulated).

**P 156 [3,3]-SIGMATROPIC REARRANGEMENTS, AND CHIRAL AZIRIDINES FOR THE ASYMMETRIC SYNTHESIS OF NOVEL AMINO ACIDS**

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α- Unsaturated amino acids are important synthetic building blocks in organic synthesis and peptidomimetics. The introduction of a β-substituent to such chiral amino acids can retain the functionalities of the original amino acid that are usually important for bioactivities, and at the same time provide the unsaturated site necessary for further syntheses. Recent studies show that the anti-β-substituted γ,δ-unsaturated amino acids can be synthesized by [3,3]-sigmatropic Eschenmoser or thio-Claisen rearrangements. The enantiomeric synthesis can be started in different ways. The synthesis showed excellent anti/syn selectivities and very good diastereoselectivities, and similar α-unsaturated amino acids can be synthesized using these methods. Furthermore, a novel methodology is being developed for the design and synthesis of α,β-chiral aziridines via β-hydroxy α-amino acids, which can be obtained from γ,δ-unsaturated amino acids. The potential synthetic value for these aziridines has been investigated for their conversion to α- or β-thio-amino acids using mercapto nucleophiles under different conditions. The syntheses of these novel amino acids and their applications to syntheses of bicyclic β-turn dipetide mimetics and other macrocyclic peptides are being investigated. [Supported by grants from the USPHS and NIDA.]

**P 157 A CONFORMATIONALLY PEPTIDE UPRIFIED α-HE- LICAL PEPTIDE LIBRARY**

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A conformationally purified peptide library was constructed based on a unique idea combining the combinatorial library concept with de novo peptide design. This peptide library was designed to allow peptides to acquire metal-binding ability when they fold into helix-loop-helix structure. Therefore, a mixture of randomized peptides was purified by immobilized metal affinity chromatography (IMAC) to provide a library of peptides with the homogenous conformation.

**P 158 A LIBRARY OF CYSTEINE-BIOTINE DERIVATIVES USEFUL FOR PRETARGETING AVIDIN-BIOTIN RA- DIOIMMUNOSCINTIGRAPHY**

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Incorporation of a bioactive molecule into a nitrido99mTc-complex has been successfully achieved by using [Tc(N)(PNP)]2 metal fragment approach (PNP=tetraphosphine) [1]. With this method the strong electrophilic Tc(N)(PNP)]2 moiety efficiently reacts with bifunctional ligand carrying π-donor atoms, such as N-functionalized cysteine [O.S.]. We present here a potential application of this new labeling procedure in antibody pretargeting technology using the avidin/biotin system (KD=10−15 M).

A series of Te-nitrido complex conjugate with cysteine functionalized biotine was prepared. To minimize the steric and the electronic influence of the Pe-carrying moiety on the biotin-avidin interaction, the following different N-functionalized cysteine biotin derivates were synthesized: 1)Biotin-CysOH; 2)Biotin-Abu-CysOH; 3)Biotin-Abz-CysOH; 4)AcLys(Biotin)-CysOH; 5)Ac-D-Lys(Biotin)-CysOH. In these bifunctional ligands the length and the flexibility of the spacer between cysteine and bioactive molecule was changed (modulated). Thus, different dissymmetrical nitrido-[Tc(V)]2[Tc(N)(BIOT-Cys-OH)] complexes were synthesized and their biological profile evaluated by in vitro avidin binding assays and by in vivo biodistribution in mice.

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P 159 A METHIONINE SCAN OF REGION [168-176] OF THE PARATHYROID HORMONE RECEPTOR 1 – THE “MAGNET EFFECT”

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Parathyroid hormone (PTH) 1–34 contains two α-helical domains associated with specific functions: the N-terminal helix is required for activation of its G protein-coupled receptor, and the C-terminal helix is responsible for receptor binding. We recently focused on the role of the mid-domain of PTH. From photoaffinity-crosslinking studies, we found that a β-benzoxylphenylalanine (Bpa) residue in both positions 11 and 21 crosslinked to the same receptor region [165-189]. We now present a methionine scan of receptor region [168-176]. The two ligands, [Bpa]-PTH, and [Bpa]-PTH, were crosslinked to the mutant receptors (S168M). [E169M]; [V171M]; [K172M]; [F173M]; [L174M], and [N176M] PTHR1, transiently expressed on COS-7 cells. The ligand-receptor conjugates were then isolated, digested with cyanogen bromide (CNBr), and analyzed by SDS-PAGE. Interestingly, a band similar in size to free ligand was obtained for the whole range of mutants when crosslinked to [Bpa]-PTH, and up to mutant [L174M]PTHR1 when crosslinked to Bpa-PTH. Such a ligand fragment is generated by crosslinking to the e-methyl group of methionine [1]. We conclude that Bpa has increased reactivity towards methionine compared to other amino acids. We call this the “Magnet Effect” of methionine. Outside of the above-mentioned range, i.e. with receptor mutant [V183M], we do not observe the characteristic band with neither Bpa-PTH nor Bpa2-PTH.

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P 160 A MS-BASED ENCODING METHOD FOR OBOC COMBINATORIAL BRANCHED PEPTIDE LIBRARIES

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We recently reported a novel PAD (“Partial Alloc-deprotection”) approach [1] to encode “one bead one compound” (OBOC) combinatorial peptide libraries [2]. This approach allows successive deprotection of Alloc group on beads (starting from head surface towards bead interior), controlled by the exposure time of water-swollen beads to palladium reagent. To further exploit the utility of this approach, we have developed a novel MS-based encoding method for a three-arm branched peptide library. In this method, the full-length branched peptide library compounds are constructed on the outer bead layer, and coding tags consisting of a series of pseudo-ladders are generated in the bead interior using the PAD approach. The released coding tags are then analyzed by MALDITOF MS. To simplify the interpretation of the mass spectra, a bromine-containing amino acid is incorporated into the coding tags of one arm, thus generating doublet peaks, which can easily be differentiated from the coding tags derived from the other two arms with singlet peaks. Fifty beads were randomly isolated from a model branched peptide library and decoded with the above approach. A high sequencing success rate of over 90% validated the utility of this encoding method to peptide libraries with complicated configurations.

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P 161 A NEW APPROACH TO THE SYNTHESIS OF POLICYCLIC DIPETIDE DERIVATIVES AS POTENTIAL ANTUMORAL AGENTS

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Several biologically active compounds, of both natural and synthetic origin, present a tetrahydroisoquinoline or β-carboline moiety. The broad spectrum of biological activity and the rigid heterocyclic skeleton of these unique pharmacophores present an excellent opportunity for combinatorial application targeted at drug discovery. Here we report the development of a simple, one-pot method for the synthesis of these derivatives in good to excellent yield using single-mode microwave irradiation. From the stand point of synthesis, the Picet-Spenger cyclization reaction is one of the most obvious way for construction of these systems. Incorporation of such derivatives into peptide or non-peptidic structures can usually bring new insights into SAR analysis, and lead to the development of compounds with improved pharmacological profiles.

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P 162 A NOVEL CLASS OF ANTI-THROMBOTIC AGENTS

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Irocipptide (ITF169), a CRP-derived tetrapeptide now in phase II trials as anti-atherogenic agent, is a weak (IC50 3 mM) antagonist of ADP-induced platelet aggregation and thromboxane release. To improve on this activity a series of analogues were made and tested as anti-aggregating agents. Furthermore binding studies using SDS mi-celles or liposomal preparations enriched in negatively charged phospholipids, were carried out and a correlation between the anti-platelet activity of the analogues and their ability to bind the negatively charged surfaces was established. From these studies, ITF1952 emerged as the most potent derivative of the series with an IC50 of about 10 μM in the platelet aggregation assay and an association constant of about 107 M-1 in the binding assays. In vivo, ITF1952 was a potent inhibitor of thrombosis (ED50, 8 μg/kg) with very little bleeding activity observed at the active doses. Its mechanism of action was further studied and found to involve the metabolism of phospholipids. In particular, ITF1952 specifically inhibited phospholipase D-mediated platelet aggregation and the release of thromboxane from activated platelets. Thus a new class of anti-thrombotic agents was established which appears to be superior in vivo to existing anti-thrombotic therapies. A comprehensive description of the structural and biological properties of these new antagonists will be presented.

P 163 A PEPTIDE INHIBITOR OF REGULATORS OF G PROTEIN SIGNALING

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Objective: To test the hypothesis that a peptide inhibitor of RGS (Regulator of G protein Signaling) proteins binds to RGS4 at the Gα interaction site to prevent GAP (GTPase Accelerating Protein) activity. Methods: YJ34, a cyclic octapeptide inhibitor (Ac-Val-Lys-Cys-Lys-Thr-Gly-Ile-Cys-NH2,) was designed[1] based on the RGS4-Gα crystal structure.[2] We tested YJ34 in Gαo single turnover GAPase assays with purified RGS4, RGS7 and RGS8. Results: YJ34 inhibits RGS4-enhanced Gαo GTPase activity, but has no effect on the GTPase activity of Geo alone. Y34 inhibits RGS4, RGS7 and RGS8 with IC50 of 9 μM, 43 μM, and 11 μM respectively Also, BR2, a YJ34 analogue with a Gly5 to Ser6 change was made to mimic an RGS insensitive Gαo mutant[3]. BR2 did not inhibit RGS4-stimulated GTPase. Conclusions: The activity and specificity of YJ34 against purified RGS proteins supports the hypothesis that the peptide is binding directly to the RGS protein. Loss of activity of the Gly5 to Ser peptide suggests that YJ34 is binding RGS in the same manner that Gα do. These results show the feasibility of designing RGS inhibitors with at least some degree of specificity. Structure-activity relationship studies are underway in order to further characterize this interaction.

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P 164 A PURIFICATION STRATEGY FOR SYNTHETIC PEPTIDES THAT UTILIZES PH TO OPTIMIZE SELECTIVITY

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For economic analysis and purification of synthetic peptides an exceptionally robust HPLC column is required. Due to the diversity in type/nature of the synthetic peptides currently being synthesized, not all separations can be achieved using the conventional 0.1% TFA eluents. An HPLC packing is needed that can be operated under acidic, neutral and basic conditions. In addition to the chemical stability, a range of particle sizes is also needed; high performance small particles for screening and early stage method development, and larger particle size preparative and process packing materials for scale up.

Data will be presented to show how a single polymeric reversed phase column can be used for analytical method optimization using acidic, neutral and basic eluents. Separations will be used to demonstrate the scalability of the purification schemes, by showing that selectivity is independent of particle size.

From the data presented a reversed phase purification strategy will be proposed for synthetic peptides.

P 165 AGONIST ACTIVATION OF THE ANGIOTENSIN II TYPE 1 RECEPTOR ALTERS THE SPATIAL PROXIMITY OF TRANSMEMBRANE 7 TO THE BINDING POCKET

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Agonist activation of seven-transmembrane domains (TMDs) G protein-coupled receptors (GPCRs) involves important movements of TMDs following binding of an agonist. The underlying molecular mechanism by which GPCRs activation takes places is largely unknown and may be inferred by the photoaffinity labeling method. We have combined photoaffinity labeling with a X-methionine (Met) directed mutagenesis strategy followed by Met-specific CNBr cleavage to study the implication of position eight of Angiotensin II (AngII). This position is the so called aromatic agonist switch, responsible for the activation of the AngII type 1 receptor (AT1). For this purpose, we compared a partial agonist photolabel, [125I]-[sar1,Tdp8]AngII with an antagonist photolabel, [125I]-[sar1,Bpa8]AngII. Both labeling probe AngII analogues showed equilibrium binding properties similar to native AngII on Wild-type and constitutively active hAT1-WT as well as on the constitutively active hAT1-N111G mutant receptors.125I-[Sar1,Tdp8]AngII was shown to incorporate in TMD7 only, whereas 125I-[Sar1,Bpa8]AngII was shown to incorporate into TMD3 and TMD6 only, whereas 125I-[sar1,Bpa8]AngII was shown to incorporate in TMD7 only. These data, combined with others (1), suggest that upon activation, TMD7 of AT1 receptor is moving away from the binding pocket. This study adds to the accumulating evidences that an outward movement of TMD7 is part of the class A GPCRs activation mechanism.

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P 166 AN ASYMMETRIC SYNTHESIS OF (R)-AND (S)-o-CYANO-PHENYLALANINE, LEADING TO CHIRAL, CONSTRAINED PHENYLALANINE DIPETIDE MIMETICS

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The use of conformational constraints in peptides is a valuable tool to improve metabolic stability, alter receptor selectivity, enhance binding affinity and to examine binding models.

o-Cyano-phenylalanine is a precursor for constrained phenylalanine dipeptide mimetics. (S)-and (R)-o-CN-Phe (3, 4) were obtained in good yields and enantiomeric purity by an optimised asymmetric phase transfer catalysed alkylation of benzophenone imine protected glycine t-butyll ester 2 with o-cyano-benzyl bromide 1, using 3rd generation Cnochona derived catalysts.[1] Neo-phthaloyl protection of o-CN-Phe, followed by nitrile to aldehyde reduction, reductive amination with amino acids and ring closure provides aminobenzazepinones Aba-XX 5, which are constrained Phe-Xxx dipeptide mimetics.

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P 167 ANALOGUES OF MULTIFUNCTIONAL LIGANDS FOR OPIOID AND CCK RECEPTORS

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Recently, our group introduced a new paradigm in drug design for the treatment of pain in the disease state [1]. CCK is known to have an anti-opioid effect and thus can be used to alleviate pain. We therefore, are developing chimeric peptide ligands which have enhanced opioid efficacy by also acting as antagonists to CCK receptors and agonists at opioid receptors. The design of the ligands was based on the hypothesis of targeting multiple receptors with overlapping pharmacophores. As a lead compound, RSA402 which was highly potent at opioid receptors was modified. One of the analogues, HW2044 showed high δ opioid binding affinities, less than 1 nM (0.50 ± 0.04). For GTP binding activities, δ opioid EC50: 3.68 ± 0.32 nM and Emax: 88 ± 1.0% and μ opioid EC50: 4.22 ± 0.12 nM and Emax: 87.1 ± 10.4%. Recent research has shown that an antagonist of the MC4, can produce an anti-allodynic effect. The opioid pharmacophore is bridged through a linker to the N-terminus of the MC4-CCK pharmacoophores which share a Trp residue. This project is supported by grants from the USPHS and NIDA.

RSA402 Tyr-c[D-Glu-Gly-Trp-Glu]-Asp-Phe-NH₂, HW2044 Tyr-c[D-Glu-Gly-Phe-c[Trp-Lys]]-Asp-Phe-NH₂

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P 168 ANTIGENE EFFECTS OF PEPTIDE NUCLEIC ACIDS ON HIF-1 ALPHA

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The hypoxia inducible factor 1 alpha (HIF-1α) is frequently overexpressed in many common human tumors.[1] Peptide nucleic acids (PNAs) are DNA mimics with the potential to downregulate gene expression. [2] We evaluated inhibition of HIF-1α expression with mono- and bis-PNAs targeting the 5'-untranslated region. The PNAs followed by nitrile to aldehyde reduction, reductive amination with amino acids and ring closure provides aminobenzazepinones Aba-XX 5, which are constrained Phe-Xxx dipeptide mimetics.

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P 169 ANTI-PROLIFERATIVE EFFECTS OF NOVEL GLYCO-LIPOAMINOACID-MODIFIED ARSENICALS (III) ON MCF-7 HUMAN BREAST CANCER CELLS

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Reduction of p-arsanilic acid I, As-protection and conjugation to a lipooaminoacid and glucuronic acid afforded a novel, non-toxic, highly soluble arsenical (III) II with significant anti-cancer activity. Incubation of compound II with MCF-7 human breast cancer cells induced cytoxicity and cell-death by apoptosis at 10 μM.

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P 170 ANTISENSE PNA AND PNA-PEPTIDE CONJUGATES FOR THE MODULATION OF THE β-GLOBIN GENE SPlicing

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β-thalassemia is a very common genetic disease due to mutations causing defective β globin gene expression and deficiency of β globin and haemoglobin A. More than 100 thalassemic mutations have been identified so far, the most common being those causing aberrant splicing1. Antisense oligonucleotides targeting aberrant splice sites in β-globin pre mRNA are able to restore correct splicing and production of haemoglobin A2. In order to repair splicing defects, antisense molecules are required not to promote target RNA cleavage by RNase H and to accumulate in the cellular nuclei. Antisense PNA are not able to activate RNase H, thus resulting good candidates for the modulation of gene splicing. Aim of this work is to synthesis PNA and PNA-peptide conjugates in order to test their ability to correct aberrant splicing in β globin genes. PNA complementary to the a mutated region of the β globin pre mRNA and PNA conjugated to a NLS peptide have been synthesized on solid phase. The affinity of these molecules toward the globin pre mRNA and PNA conjugated to a NLS peptide have been evaluated in appropriate bioassay systems. The insect kinin analogs were evaluated in an insect diuretic assay; and the pyrokinin analogs in papuariation and pheromonotropic bioassays.

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[3] Cheng, R.P., Gellman, S.H., DeGrado, W.F. Chem. Rev., 101, 7219 (2001).

P 171 BACTERICIDAL MECHANISM AND CYTOCIDAL SELECTIVITY OF RHEUS THETA DEFENSINS

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Chemokines and their receptors are implicated in a wide range of human diseases such as Acquired Immune Deficiency Syndrome (AIDS). In a collaborative study, we developed a chemical approach to generate a new family of unnatural chemokines termed SMM (synthetically and modularly modified)-chemokines chemically engineered with high receptor selectivity and affinity, and reduced toxicity. A proof of the concept is shown by applying this strategy to transform the viral macrophage inflammatory protein (vMIP)-II, a very nonspecific che- makine, into new analogs with enhanced selectivity and potency for CXCR4 or CCR5, two principal coreceptors for human immunodefi- ciency virus type 1 (HIV-1) entry. Such novel molecules were shown to be valuable probes that provide insights into receptor binding and signaling mechanisms and HIV-1 inhibitors with higher potency and structural features with protegrins, members of porcine neutrophil cathelicidins. To determine whether the shared structural features correlate with similar peptide functions, we compared the biological properties of RTD 1-3 and protegrin 1 (PG-1) in assays for antimicrobial activities, bacterial membrane permeabilization, and toxicity to human cells. RTD 1-3 and PG-1 have similar microbicidal potencies against Escherichia coli, Staphylococcus aureus, and Candida albicans in the absence of salt, calcium chloride, magnesium chloride, or serum. In the presence of these additives, RTD 1-3 exhibit differential bactericidal activities. ONPG-hydrolysis experiments demonstrated that killing of E. coli by RTD 1-3, similar to PG-1, is linked with the permeabilization of the bacterial cell envelope that is followed by peptide internalization and inhibition of the cytoplasmic β-galactosidase. While PG-1 was toxic to human fibroblasts and caused marked hemolysis, theta-de- fensins were non-cytotoxic and nonhemolytic. Thus, all three theta- defensins are more selective for microbial targets than protegrin even though the four peptides have nearly identical microbicidal potencies.

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[2] Nachman, R.J., Roberts, V.A., Holman, G.M., Beier, R.C. Regul. Peptides, 57, 359 (1995).
[3] Cheng, R.P., Gellman, S.H., DeGrado, W.F. Chem. Rev., 101, 7219 (2001).
Proprotein Convertases (PCs) are Ca^2+. J. Gillies,2 and V. J. Hruby1

The specific targeting of tumor cells has been the main challenge in the research on cancer therapy and diagnosis. Receptors for different endogenous regulatory peptides, like the neuropeptide neurotensin, are re-expressed or over-expressed in different human cancers, therefore they may be used as binding sites for selective peptide ligands. Peptides carrying cytotoxic moieties or radiotracers and able to bind membrane receptors on tumor cells, can act as drugs or diagnostics. The main drawback in the use of peptides in vivo is their extremely short half life. We recently demonstrated that neurotensin, like several other endogenous peptides, when synthesized in a dendrimeric form retains its biological activity and becomes resistant to blood proteases 2. We synthesized neurotensin (NT) and its short analogue NT(8-13) in a tetrabranched form and conjugated it to fluorescent dyes or to chelators for radionuclides. We tested their ability to bind cell membrane receptors and studied subcellular localization on HT29 human adenocarcinoma cell line. Tetrabranched NT(8-13) molecules are more efficient than monomeric NT in binding membrane receptors, they are internalized within 2 hours, and localize in the lysosomes. Membrane receptors are re-exposed on the plasma membrane and thus newly accessible to neurotensin peptide.

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The affinity of ligands for the cell-surface receptors can be increased by having an oligomeric ligand bind to several receptors simultaneously. These multivalent ligands have shown higher binding affinities and apparent positive cooperativity compared to the monomers. As a model, we have used CCK/MSH receptors, which are involved in a wide variety of physiological processes including cancer. Several small ligands for the CCK receptor were designed that can be modified at the N-terminus and serve as an anchor for multimeric ligands, and for chelating lanthanides for fluorescence emission based detection of ligands. These Eu-labeled ligands provide an attractive alternative to traditional radiolabeled methods. From two series of compounds synthesized, malonic acid linked to the N-terminus was found to be best. Besides, as part of our approach to obtain hetero-multimeric ligands for diagnostic and therapeutic applications in cancer, we have designed MSH and CCK heterodimers using various spacers. The synthesis and bio-assay results of these multimeric ligands based on this design and Eu-coordinated CCK-8 will be discussed. Supported by grants from the USPHS, NCI.

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P 177 CHEMICAL BIOLOGY AND BIOMEDICAL APPLICATION OF SYNTHETIC MOLECULES TARGETED TO APOPTOSIS REGULATED BY THE BCL-2 FAMILY

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Members of the Bcl-2 family play a critical role in regulating the process of apoptosis and are implicated in the resistance of cancer cells to many of the currently available drugs. As such, small molecule agents blocking or mimicking the biological function of Bcl-2 family members could be used as novel regulators of apoptosis and potential anticancer agents. We designed and synthesized various molecules targeted to the surface functional sites of Bcl-2 family proteins. In a series of biological and biochemical studies, we demonstrated that these analogs antagonized the biological function of Bcl-2 and induced apoptosis in human cancer cells. These small molecules that bind Bcl-2 or related family member proteins are promising leads for the development of a new class of therapeutics for the treatment of human diseases such as cancer and neurodegenerative disorder and useful probes of the mechanism of Bcl-2 regulated signaling pathways. The use of these synthetic molecules to study the mechanism of protein-protein interactions and functional role of the Bcl-2 family in apoptosis and human diseases is presented.
P 179 CHEMICALLY ENGINEERED SMM-CHEMOKINES AS PROBES AND INHIBITORS OF HIV-ASSOCIATED NEURONAL APOPTOSIS

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As the main coreceptors for HIV-1 entry, CCR4 and CCR5 play important roles in HIV-1 pathogenesis, including HIV-associated dementia (HAD). HIV-1 gp120 contributes to HAD by directly inducing an apoptotic pathway in neurons and/or indirectly stimulating glial cells to release neurotoxic factors. In contrast to the natural ligands of CCR5 that are known to prevent gp120 neurotoxicity, SDF-1α, the only natural ligand of CCR4, cannot protect neurons. To further study the role of CCR4 and CCR5 in HAD and develop new therapeutics for neuroprotection, we have recently generated a new family of unnatural chemokines, termed synthetically and modularly modified (SMM)-chemokines, which display enhanced receptor selectivity, binding affinity and anti-HIV activities than natural chemokines. Here, using rodent cerebrocortical cultures as a model system, we have demonstrated that SDF-1α and vMIP-II, both of which are neurotoxic, can be modified to protect neurons from gp120-induced neuronal apoptosis. Interestingly, the neuroprotective effects of such CCR4 inhibitors were accomplished without activating the protein kinase Akt, Erk or JNK. We also found that the inhibition of CCR5 by CCR5-selective SMM-chemokines led to neurotoxicity by activating p38 MAP kinase. These results demonstrate a new chemical strategy for understanding and treating neuronal death caused by HIV-1 infection.

P 180 CHEMISTRY, BIOLOGY AND MEDICINE OF CHEMOKINES: NEW INSIGHTS INTO LIGAND-RECEPTOR INTERACTIONS AND PROGRESS TOWARDS ANTI-HIV DRUG DEVELOPMENT

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Chemokines and their receptors play important roles in normal physiological functions and pathogenesis of a wide range of human diseases including the entry of HIV-1. Here, we report a novel D-amino acid containing chemokine designed based on vMIP-II. The incorporation of non-natural D-amino acids enhances the affinity of this molecule for CCR4 receptor but significantly diminishes that for CCR2 and CCR5 receptors, thus yielding much more selective recognition for CCR4 than the wild type molecule vMIP-II. Furthermore, this D-amino acid-containing chemokine showed correspondingly higher and more specific inhibitory activity of HIV-1 entry via CCR4 than natural chemokines, demonstrating its value for developing highly potent and selective HIV-1 entry inhibitors. High resolution crystal structure was determined for this D-amino acid containing chemokine, which provides the structural basis for its biological activity and suggests a mechanism for the selective interaction between the ligand and chemokine receptors.

P 181 COMPARATIVE IMMUNOGENICITY OF COMMON AND RARE HIV MUTANTS

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Immunogenicity studies of HIV have revealed that CTL responses are directed primarily to the conserved regions of various HIV proteins. Interestingly, common mutations, i.e., those found in 30-40% of viral isolates, represent a few different sequences, while numerous different rare mutations are found only in single or very few individuals. One interpretation of these facts may be that the common mutants represent weak immunogenic sequence variations that have most successfully evaded the selective pressure of CTL recognition, and that more immunogenic rare mutants have not. Furthermore, the principal basis for enhanced immunogenicity of the rarer CTL epitope mutants may be their increased content of non-conservative amino acid substitutions. In support to these premises, ELISpot analysis with blood from 19 different A201 positive HIV patients showed that rare mutants of the Gag epitope SL9 sequence were broadly recognized by all donors having reactivity to SL9. Similarly, using HLA-A2+ transgenic mice, we showed that two rare mutant peptides were more potent in inducing a CTL response than the cognate, original SL9 sequence.

P 182 CONFORMATIONAL STUDIES OF AGOUTI-RELATED PROTEIN (AGRP)-MELANOCORTIN CHIMERIC PEPTIDES

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The melanocortin system consists of five G-protein coupled receptors (MC1R-MC5R), endogenous agonists (α-, β-, γ-melanocyte stimulating hormones) and endogenous antagonists – agouti protein and agouti-related protein (AGRP). Our previous studies identified a melanocortin-AGRP chimera possessing subnanomolar agonist activity at MC1 and MC3-5 receptors [1]. We generated a peptide library utilizing this new template by substitution of the His, DPhc and Trp residues with different natural and unnatural amino acids. These compounds resulted in novel melanocortin receptor pharmacology. Based on 2D 1H NMR and computer assisted molecular modeling studies we propose specific bioactive conformations as responsible for agonist versus antagonist activity of these chimeric ligands at the mouse melanocortin receptors.

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P 184 CYCLIC PHENYLSTATINE-BASED TETRAPEPTIDES AS INHIBITORS OF β-SECRETASE

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Extracellular amyloid β-peptide (Aβ) deposition into plaques is one of the characteristic histopathological lesions found in brains of Alzheimer’s disease patients. The 40- to 42-membered peptide Aβ is generated by the successive proteolysis of the integral membrane protein APP, the amyloid precursor protein, by γ- and β-secretase (BACE1)[1]. The 1.9 Å crystal structure of the catalytic domain of BACE complexed with the inhibitor OM99-2 revealed the characteristic architecture of the active-site cleft of aspartic proteases of the pepsin family to which substrates and related transition state analogues bind in a β-extended conformation[2]. A series of macrocyclic peptides of the general structure shown in Fig. 1 was designed, where the side chain to side chain clamp R² served to constrain the tetrapeptide to the active conformation.

Synthesis, conformational properties and bioactivities of the cyclic molecules will be presented.

\[ \text{Fig 1} \]

R¹-Asp-(Phes)Sta-Val-Asp-NH₂

R²

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P 185 DESIGN AND STRUCTURE DETERMINATION OF A SODIUM LABELLED PEPTIDE ANTIMICROBIAL

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Molecular interactions between pathogens and host-cells are necessary for infection. The capsular F1 antigen from Yersinia pestis plays the role of targeting the site of infection on a human cell.[1] Recently, the X-ray structure of the capsular F1 antigen has been elucidated.[2] To design a compound that can selectively bind to the F1 antigen and then kill the bacteria is a feasible way to protect human cells from infection. In our laboratory we used phage expression libraries to successfully screen for a peptide that can specifically bind to the F1 antigen of Y. pestis. Seleno-cyanate coupled to this peptide was able to kill over three logs of E. coli which expressed the F1 antigen in 15 minutes at micromolar concentrations, by the selenium catalyzed production of superoxide radicals on the bacterial cell surface. Yet, the peptide does not kill E. coli that does not express the F1 antigen. 2-D proton NMR was then used to elucidate the peptide’s structure. Based on the NMR data, the solution structure of this peptide was shown to have a restricted conformation rather than a random coil. A predominant conformation of the solution structure of this peptide was shown to have a restricted conformation.

In this report, we will describe the strategy to identify highly potent and specific ligands of alpha4 beta1 from design, synthesis and screening of an initial OBOC peptidomimetic library based on a known LDV motif [2], to a much more focused library with just several thousand permutations. In these topological-segregated libraries, the testing compound displays on the surface of the beads, and the coding tag resides in the interior of the beads which eliminates interference with the screening ligands [3]. The decoding is easily achieved by Edman sequencing. The application of the ligands will also be discussed.

[1] Lam, K.S. Lebl, M., Krchnak, V. Chem. Rev. 97, 411-448 (1997).
[2] Lin, K.C., Ateeq, H.S., Hsiung, S.H., et al. J. Med. Chem. 42, 920 (1999).
[3] Liu, R., Marik, J., Lam, K.S. J. Am. Chem. Soc. 124, 7678 (2002).

P 187 DESIGN AND SYNTHESIS OF HISTONE DEACETYLASE INHIBITORS BY SIDE CHAIN MODIFICATION OF 2-AMINO-(n-1)-ALKENOIC ACIDS

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Ethyl Boc-DL-2-amino-(n-1)-alkenoes were conveniently synthesized from diethyl Boc-aminomalonate and corresponding bromoalkane. The resolution was successfully carried out by the action of subtilisin. The fully protected amino alkenoic acids were converted to diols, and then hydroxymethyl ketones. The olefin was also conveniently changed to the epoxide, which was reacted with sodium methoxide to get the ether. The further oxidation with Dess-Martin reagent gave methoxymethyl ketone. As a demonstration of the usefulness of this method, the side chain of chloramydcin was equipped with hydroxy- or methoxymethyl ketone. The analogs showed significant inhibitory activities toward human histone deacetylases.

P 186 DESIGN AND SYNTHESIS OF ENCODED ONE-BEAD ONE-COMPOND PEPTIDOMIMETIC LIBRARIES FOR IDENTIFICATION OF ALPHA4 BETA1 INTEGRIN LIGANDS

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Integrins are cell surface receptors that mediate cell-cell and cell-matrix interaction and regulate cell motility, migration, survival and proliferation. Alpha4 beta1 integrin is an important target for drug development. The “one-bead one-compound” (OBOC) combinatorial library method [1] provides a powerful tool for rapid identification of ligands for alpha4 beta1 integrin through on-bead cell binding as says. In this report, we will describe the strategy to identify highly potent and specific ligands of alpha4 beta1 from design, synthesis and screening of an initial OBOC peptidomimetic library based on a known LDV motif [2], to a much more focused library with just several thousand permutations. In these topological-segregated libraries, the testing compound displays on the surface of the beads, and the coding tag resides in the interior of the beads which eliminates interference with the screening ligands [3]. The decoding is easily achieved by Edman sequencing. The application of the ligands will also be discussed.

[1] Lam, K. S. Lebl, M., Krchnak, V. Chem. Rev. 97, 411-448 (1997).
[2] Lin, K.C., Ateeq, H.S., Hsiung, S.H., et al. J. Med. Chem. 42, 920 (1999).
[3] Liu, R., Marik, J., Lam, K.S. J. Am. Chem. Soc. 124, 7678 (2002).
**P 189** DESIGN AND SYNTHESIS OF NOVEL GLP1 ANALOGUES WITH SIGNIFICANTLY PROLONGED TIME ACTION

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Glucagon-like peptide-1 (GLP-1) is a 30-amino acid, C-terminally amidated peptide hormone secreted from gut endocrine cells in response to nutrient ingestion. It binds to and activates a type II G protein-coupled receptor (GLP-1r), stimulating glucosedependent insulin secretion, and inhibiting food intake, gastric emptying, and glucagon secretion. These actions additively promote reduction of fasting and postprandial glycemia in subjects with type 2 diabetes. In vivo, native GLP-1 undergoes rapid inactivation by dipeptidyl peptidase IV and is also cleared from the circulation via renal filtration. As a result, levels of circulating GLP-1 fall rapidly after subcutaneous injection, posing a considerable challenge to its utility as therapy for type 2 diabetes.

Longer-acting GLP-1 analogues would therefore suit the therapeutic demands in type 2 diabetes better than native GLP-1. We will describe the discovery of C-terminally extended, proteolytically-stable GLP-1 receptor agonists which demonstrate reduced clearance and increased insulinotropic activity in pre-clinical animal models. * Contributed equally to the work.

**P 190** DESIGN OF A LIBRARY OF HISTONE DEACETYLASE INHIBITORS BASED ON CHLAMYDOCIN

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Chlamydocin is a cyclic tetrapeptide, which contains unique (2S,9S)-2-amino-8-oxo-9,10-epoxydecane acid (Aoe). Based on its cyclic tetrapeptide framework, cycl(o-L-Aoe-Aib-L-Phe-D-Pro-), we designed and synthesized the focused library to find out specific inhibitors toward histone deacetylases (HDAC), which may relate to various diseases. The library includes the varieties in the zinc ligands, Aib related amino acids, aromatic amino acids and imino acids. The library was profiled by the inhibition of HDAC1, HDAC4, HDAC6, and HDAC8. The p21 promoter assay was also employed to evaluate in vivo effects. Some selected synthetic peptides were subjected to the conformation analyses.

P 191 DESIGN OF AN ANTIBODY FOR EARLY DETECTION OF OVARIAN CANCER

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Phosphoserine phosphatase (PSP), a catalytic enzyme that phosphorylates certain proteins within a cell, is shown to be over-expressed in ovarian cancer tissues. Phosphorylation of cellular proteins allows regulation of enzymatic activities by mediating allosteric conformational changes or by blocking access to enzyme catalytic sites. The aim of this project is to design antibodies that would allow for early detection of ovarian cancer. Algorithms for sequence prediction were used to find antigenic sites within the PSP peptide that are surface exposed. The sequence we identified is for amino acids 135-157 of the PSP protein. The peptide has been synthesized as a chimeric construct with the promiscuous T-cell epitope MVF, and pairs of rabbits were immunized using this construct. High titers of antibodies produced by the rabbits have been purified and are being tested against a variety of ovarian cancer tissue and cell lines. The antigenicity of the epitope is being characterized by FACS analysis using the Ishikawa cell line. The results of these experiments will be presented along with data from investigations into the use of these antibodies as a possible vaccine to block phosphorylation within cancerous tissues.

This work was supported by a GYN Cancer Center Grant.

**P 192** DESIGN OF SUBTYPE SELECTIVE OPIOID RECEPTOR LIGANDS

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Previously developed potent mu- and delta-selective cyclic tetrapeptides with low affinity toward kappa-opioid receptors were modified to explore structural features of residues in the third position essential for recognition of these peptides by kappa-receptors. The tetrapeptides synthesized with Phe3 replacements by small, basic, and aromatic side chains, were cyclized through disulfide or disulfide bridge, contained free amidated C-terminus and some variations in the forth residue (D-Phe, L-D-Cys). The binding affinities of cyclic tetrapeptides were determined for the stably expressed mu-delta- and kappa-receptors. In general the resulting analogues failed to exhibit appreciable affinity for the kappa receptor, with the exception of the tetrapeptide Tyr-(L-D-Cys-Phe-D-Cys)-NH2, cyclized via a disulfide bond, which demonstrated high binding affinity toward all opioid receptors (Ki(mu)=1.26 nM, Ki(delta)=16.1 nM, Ki(kappa)=38.7 nM) [1].

Modeling of the kappa-receptor/ligand complex in the active state reveals that the receptor binding pocket for residues 3 and 4 of the tetrapeptide ligands is smaller than that in the mu receptor and requires, for optimal fit, that the tripeptide cycle of the ligand assume a higher energy conformation. The magnitude of this energy penalty depends on the nature of the fourth residue of the peptide (D-Pen or D-Cys) and correlates well with the observed kappa receptor binding affinity.

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**P 193** DESIGN, SYNTHESIS AND APPLICATIONS OF CELL-PENETRANT PEPTIDES AS SIGNAL TRANSDUCTION MODULATORS

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The regulatory and catalytic domains of intracellular proteins are common targets for experimental manipulations. Protein-protein interactions are also a fundamental feature of signal transduction pathways. Agents that specifically mimic or inhibit protein interactions are, therefore, valuable research tools with proven clinical utility. Moreover, the development of agents capable of manipulating signalling events may be also promote the discovery of additional protein partners that represent novel drug discovery targets. Our recent studies have utilised the cell-penetrating vectors transportan-10 and Tat for the effective intracellular delivery of a range of mimetic peptides that represent the partial sequences of the regulatory domains of intracellular and transmembrane proteins. Specific targets for these studies include heterotrimeric G proteins, protein kinase C, phospholipase D and proliferating cell nuclear antigen. Data indicate that this approach can selectively modulate a range of cellular activities, including secretion, MAP kinase phosphorylation and cell fate. Moreover, the sequence-dependent effects of cell penetrating peptides may be stimulatory or inhibitory. Our aim now is to identify the molecular targets of biologically active peptides that include sequences derived from the CB1 cannabinoid receptor and the C-terminal domain of the a subunit of the Gs protein.
**P 194** DESIGN, SYNTHESIS OF NOVEL MTII/AGRP HYBRID ANALOGUES AND THEIR BIOLOGICAL ACTIVITIES

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AGRP, a natural endogenous antagonist for MCRs, has a cysteine-rich COOH-terminal domain. Recently, nuclear magnetic resonance studies (PDB: 1HYK) demonstrated that the cysteine residues in AGRP adopts a structural motif known as an inhibitor cystine knot. This knot has been shown in vitro to be an inverse agonist with a potential in vivo to regulate the various MCRs, even in the absence of melanocortins. MTII is a super agonist and it has been implicated in the treatment of sexual dysfunction and obesity. To further study the 3D topographical structure of agonist and antagonist, a group of MTII/AGRP hybrid analogues has been designed and synthesized, and their biological activities determined. The superimposed NMR structure of the AGRP knot (Cys115-Arg-Phe-Phe-Asn-Ala-Phe-Cys118) and NMR based molecular modeling derived structure of the hybrid analogues were compared. Though the primary sequence of AGRP (111-118) is totally different from that of MTII, the 3D topological structures of both pharmacophores are similar and their structure fits quite well when observed either from the α-carbon or from their backbone structure.

Supported by Grants from the USPHS-H9251.

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**P 195** DESIGN, SYNTHESIS, AND EVALUATION OF GLUTEN PEPTIDE ANALOGS AS SELECTIVE INHIBITORS OF HUMAN TG2

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Recent studies have implicated a crucial role for tissue transglutaminase (TG2) in the pathogenesis of Celiac Sprue, a dietary disorder due to the gluten. Starting from a short peptide of sequence PQPQLPY, known to be a good substrate of human TG2, we designed new analogs modified at Pro6 by replacing it with constrained amino acids to develop specific inhibitors of TG2. We report a preliminary structural study and the biological evaluation of these new compounds.

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**P 196** DEVELOPING BLUE-AND RED-SHIFTED INTRAMOLECULARLY QUENCHED FLUOREOGENIC PEPTIDE SUBSTRATES FOR RAPID PROTEOLYTIC “FINGERPRINTING” OF SARS-CORONAVIRUS 3CL PROTEASE AND HIGH-THROUGHPUT DRUG SCREENING

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In late 2002, severe acute respiratory syndrome-coronavirus (SARS-CoV) became the first new highly pathogenic and easily transmissible human virus to emerge in the 21st century. By analogy with other CoVs, SARS-CoV encodes a chymotrypsin-like protease (3CLpr) that plays a pivotal role in virus replication, making 3CLpr a prime target for the development of therapeutic agents. We report here our success in developing blue-[1] and red-shifted [2] decapeptidyl internally quenched fluorogenic substrates (IQFSs) based on resonance energy transfer between the donor/acceptor couples (Abz, Y(NO2);[1]) or (CaiRed, BH2Q2; [2]). All IQFSs prepared in this study are derived from the 11 SARS-CoV 3CLpr-dependent proprotein cleavage sites. Our kinetic analyses performed with recombinant SARS-CoV 3CLpr revealed that the sequence encoded by the cleavage site between nps4/nps5 in the SARS-CoV proprotein pp1ab is the most efficiently cleaved. Drawing on this information, we developed a high-throughput (HT) dual-substrate continuous 3CLpr assay including our best blue-and red-shifted IQFSs and are applying this unique HT protease assay to the identification of 3CLpr small-molecule inhibitors. Supported by NCE/PENCE (F. Jean).

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**P 197** DEVELOPMENT OF A TRAIL-R2 SYNTHETIC AGONIST FOR CANCER THERAPY

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The TRAIL-R2 ligand triggers tumor cell apoptosis independently of the p53 tumor-suppressor gene[1-5]; thus, peptide agonists may offer a complementary approach to conventional cancer therapy. A novel linear peptide sequence identified through peptides-on-plasmids primary screening was found to bind the TRAIL-R2 receptor with 120 μM binding affinity. The sequence of this peptide is unrelated to that of native or recombinant TRAIL, yet the sequence was found to compete with TRAIL for binding the TRAIL-R2 receptor. Specific architectural modifications of the peptide structure enhanced the binding affinity from 50 to 1000-fold depending on the architecture. Modification of the peptide sequence or architecture significantly affected the functional activity, resulting in peptides with potencies ranging from no functional activity to low micromolar apoptotic activity.

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**P 198** DEVELOPMENT OF PEPTIDE VACCINES AGAINST HPV-16 ASSOCIATED CERVICAL CANCER AND GROUP A STREPTOCOCCI

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To develop novel vaccines for the treatment of HPV-16 associated cervical cancer, and preventative vaccines against Group A Streptococci (GAS) using the Lipid-Core Peptide (LCP) system. Methods: LCP vaccines were synthesised by chemical ligation or by stepwise solid-phase synthesis. Mannose residues were coupled to the HPV-16 vaccine candidate to examine dendritic cell mannose receptor targeting. Peptide
lipidation enables peptide internalisation, with MHC class I presentation and induction of CD8+ cytotoxic lymphocytes (CTLs). This type of response is required to reduce tumour burden in HPV-16 associated cervical cancer.

P 199  DISULFIDE AS A CONSTRAINT TO BUILD POTENT AND SELECTIVE MELANOCORTIN-4 RECEPTOR (MC4R) AGONISTS

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Using disulfide as a constraint and the core sequence motif His-d-Phe-Arg-Trp, we designed, synthesized, and in vitro evaluated a series of MC4R agonists. A number of potent and selective MC4R agonists have been developed (e.g. 11). Compared with a well-known macro lactam melanocortin agonist, the peptide with Lilly’s 21-membered disulfide ring system was ten fold more potent. We have also shown that selectivity and potency of these disulfide cyclic peptides could be manipulated by fine tuning the ring size, the ring hydrophobicity, and the substitute on the d-Phe side chain. With these studies, we have demonstrated that disulfide bond ring system is an excellent alternative scaffold of lactam to develop both potent and selective MC4R agonists.

P 201  EFFECT OF COUNTERANIONS AND MEMBRANE POTENTIAL ON THE CELLULAR UPTAKE OF ARGinine-RICH PEPTIDES

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Arginine-rich peptides, including those derived from human immunodeficiency virus (HIV)-1 Tat protein, have been reported to translocate through cell membranes and to be utilized as carriers for the intracellular delivery of various oligopeptides and proteins in vitro and in vivo. In the recent research focused on the internalization mechanism of these peptides, significant contribution of endocytosis to the cellular uptake has been suggested. However, the translocation mechanisms through the endosomal membrane are still unclear. In addition, participation of a non-endocytic, energy-independent process can not be excluded because the cellular uptake of these peptides is not completely inhibited at 4°C [1]. Recently we have reported that organic anions such as pyrenebutyrate can accelerate the cellular uptake of octaarginine (R8) by the HeLa cells [2]. Here we show that this anion-mediated internalization is also observed for the cells incubated at 4°C and that the membrane potential plays a critical role in this process.

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P 202  EFFECT OF DIMERIZATION AND TETRAMERIZATION ON THE POTENCY OF HIV-1 INTEGRASE INHIBITORY PEPTIDES

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The HIV-1 integrase (IN) is essential for HIV replication cycle and ability of HIV virus to infect nondividing cells. Therefore it is a good target for HIV antiviral chemotherapy, also because it has no counterpart in mammalian cells. During the last few years we have carried out detailed structure-activity studies of several peptide sequences with IN inhibitory activity[1]. We will present results of these studies, especially results for different covalent dimers and tetramers of these sequences. The peptides were dimerized using different thioether type linkers, or using amino acid with two -NH2 groups (e.g. lysine) as a C-end linker 1. Tetramers were synthesized using three residues of amino acid with two -NH2 groups each at the C-end of a peptide 2.

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P 203  EFFECTS OF STABLE BRADYKININ RECEPTOR 2 AGONIST B9972 ON PULMONARY VASCULATURE IN VITRO AND IN VIVO

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The nine-amino-acid vasoactive peptide bradykinin (BK) has a two-fold action profile by exerting pathophysiological as well as pronounced beneficial physiological effects. Since bradykinin itself has a very short lifetime, we synthesized a novel stable B2-receptor agonist B9972 to study the effects of this compound on human pulmonary microvascular endothelial (HPMVEC) and pulmonary artery smooth muscle cell (HPASMC) cells in vitro and in our animal model of severe pulmonary hypertension (SPH) in vivo. In HPMVEC B9972 caused induction of caspase-3 activity as well as eNOS and MAP kinase (MAPK) activation. Treatment of HPMVEC with vasculard growth factor inhibitor SU5416 resulted in inhibition of eNOS and MAPK activity while B9972 attenuated this inhibition, suggesting a protective effect of the bradykinin agonist on endothelial cells. In PASM, B9972 induced eNOS activation, but had no effect on MAPK or caspase-3 activation. In vivo in our SPH model, B9972 caused reversal of pulmonary vascular remodeling as well as reduction in the number of plexiform lesions in lung arterioles. Our data suggest that stable bradykinin B2 receptor agonist B9972 can repair vascular remodeling and reopen obliterated vessels in SPH.
**P 205** FLUORESCENCE RESONANCE ENERGY TRANSFER SUBSTRATES FOR DETERMINING CATHESPIN B pH SPECIFICITY

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Cathespin B can catalyze the cleavage of peptide bonds by two mechanisms: endopeptidase activity with a pH optimum around 7.4, and both peptide- and peptidyl-peptidase activity at an acidic pH of 4.5-5.5. This specific activity is related to the presence of an additional occluding loop Ile105-Pro126 which makes access to the S4.5-5.5. This specific activity is related to the presence of an additional occluding loop Ile105-Pro126 which makes access to the S4.5-5.5. This specific activity is related to the presence of an additional occluding loop Ile105-Pro126 which makes access to the S4.5-5.5. This specific activity is related to the presence of an additional occluding loop Ile105-Pro126 which makes access to the S4.5-5.5. This specific activity is related to the presence of an additional occluding loop Ile105-Pro126 which makes access to the S4.5-5.5. This specific activity is related to the presence of an additional occluding loop Ile105-Pro126 which makes access to the S4.5-5.5. 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P 208 GHERLIN ATTENUATES BURN-INDUCED CALEXIA
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Major burn injury results in increased production of catabolic hormones and inflammatory cytokines, and lower levels of anabolic hormones, causing prolonged hypermetabolism, loss of lean body mass and muscle wasting. Since ghrelin, a 28-residue peptide isolated from the stomach, has been shown stimulate the synthesis and release of orexigenic peptides NPY & AGRP and the anabolic hormones, GH & IGF-1, and because plasma ghrelin and its mRNA levels in the stomach are down regulated in burn-injured rats, we hypothesized that ghrelin treatment may attenuate burn-induced anomalies. In our initial studies, rats with 30% surface area burn injury were treated overnight using implanted osmotic pumps with either saline, or ghrelin (2.4 mg/kg or 24 mg/kg); EDL muscles were isolated to measure protein breakdown rates in vitro.
Compared to saline, ghrelin, at either dose, significantly inhibited protein breakdown to a comparable degree. In the second experiment, we measured food intake, food weight-and composition in mice subjected either to a 20% surface area burn or to a sham procedure, and treated either with ghrelin (25 μmol/kg/day, s.c.) or saline-vehicle daily for 7 days. Ghrelin significantly increased body weight and food intake over 7 days in both sham and burn groups compared to controls. Ghrelin-treated mice also had greater fat stores than saline-treated controls. Since ghrelin retains its ability to favorably alter both the peripheral anabolic signals and the central energy homeostasis system after burn, ghrelin based compounds may be developed to treat weight loss and muscle wasting in cachectic diseases including burn, sepsis, cancer, and AIDS.

P 209 HIGH AFFINITY HIGH SPECIFICITY ALPHA4 BETA1 INTEGRIN TARGETING PEPTIDES FOR LYMPHOID CANCERS
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Alpha 4 beta 1 integrin plays an important role in inflammation, cancer development, and metastasis. Blocking alpha 4 beta 1 integrin interactions with vascular cell adhesion molecule-1 and fibronectin has been used as a therapeutic strategy for inflammation and autoimmune diseases. A growing body of literature suggests that alpha 4 beta 1 integrin may be an excellent target for imaging and treatment of lymphoid malignancies. The one-bead one-compound (OBOC) combinatorial library method offers a powerful technique to identify and optimize cancer-specific peptides. Here we report on the design and synthesis of a focused OBOC peptide mimetic combinatorial library, in conjunction with a high stringency-based screening method, to rapidly optimize the LDV binding motif for alpha 4 beta 1 integrin. Using this approach, high-affinity high-specificity targeting peptidomimetics against activated alpha 4 beta 1 integrin on both T- and B-lymphoma cells have been identified. The molecular interactions between the targeting agents and a number of alpha 4 beta 1 mutant cell lines were analyzed by flow cytometry, and we were able to show that Trp188 and Gly190 are crucial for binding. Furthermore, using a murine xenograft model we demonstrated that one of these ligands, when conjugated to a near infrared dye, was able to image lymphoma with high sensitivity and specificity.

P 210 HIGHLY N-METHYLATED SOMATOSTATIN ANTAGONISTS: SYNTHESIS, BIOLOGICAL ACTIVITY AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES
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Somatostatin, a tetradecapeptide, is a major endocrine hormone with multiple physiological actions which are modulated by one or more of the five known G-protein-coupled receptor subtypes: sst1-sst5 [1]. The biological role as well as the cellular distribution of each receptor subtype is far from being completely understood. For this reason, the search for synthetic analogues of somatostatin which exhibit selective affinities for the five receptor subtypes is of considerable basic and therapeutic interest. We were interested in the potential effects of one or many peptide bond N-methylation on the selectivity and pharmacological properties of some somatostatin analogues. Indeed, N-Methyl amino acids are well-known to improve pharmacokinetically useful parameters such as membrane permeability, proteolytic stability, and conformational rigidity [2].
A full N-methyl scan of the Veber peptide [c(Phe-Pro-Phe-D-Trp-Lys-Thr)] [3] was aided by the introduction of N-Me groups during regular solid phase peptide synthesis. Although a FRET depsipeptid, Ac-DED(Edans)-EE-α-Abu(D-COO)ASK/Daberyl-NH2 (substrate I) is widely used for detecting HCV NS3/4A serine protease activity [1], its low sensitivity and short detection wavelength limit the use of this Dabcyl/Edans FRET substrate for high throughput screening. We have recently developed a sensitive FRET HCV protease substrate for the high throughput screening of HCV protease inhibitors. This new FRET substrate, Ac-DE-Dap(QXL185)-EE-α-Abu(D-COO)ASK/Daberyl-NH2 (substrate II) incorporates 5-FAM (donor) and QXL[185]260 (quencher). QXL185 is proven to be the most an excellent quencher for fluorescens such as FAM and FITC. This new FRET peptide offers the following advantages: a) Stronger absorption and emission intensity at longer wavelength (490 nm/520 nm) of 5-FAM compared to Edans. Assays using this substrate exhibit lower background due to less autofluorescent interference from cell components and test compounds. b) QXL[185]260 is more water-soluble than Dabcyl. c) Substrate II provides better assay sensitivity, Ks, is 21-fold lower than I. Substrate II is 8-fold more sensitive than substrate I, and can detect femtomol of HCV NS3/4A protease. The Synthesis was accomplished using a combination synthesis of the Fmoc solid phase and solution phase.
[1] Talian, M., et al. Anal. Biochem. 240, 60 (1997).

P 211 HIGHLY SENSITIVE FRET SUBSTRATE FOR DETECTION OF HCV PROTEASE
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Inhibiting the activity of HCV protease serves as an important method to prevent HCV infection caused by the multiplication of HCV virus. However, the development of effective HCV protease inhibitors is limited by the lack of sensitive HCV protease detection reagents. Although a FRET depsipeptid, Ac-DED(Edans)-EE-α-Abu-D-COO)ASK/Daberyl-NH2 (substrate I) is widely used for detecting HCV NS3/4A serine protease activity [1], its low sensitivity and short detection wavelength limit the use of this Dabcyl/Edans FRET substrate for high throughput screening. We have recently developed a sensitive FRET HCV protease substrate for the high throughput screening of HCV protease inhibitors. This new FRET substrate, Ac-DE-Dap(QXL185)-EE-α-Abu-D-COO)ASK/Daberyl-NH2 (substrate II) incorporates 5-FAM (donor) and QXL185260 (quencher). QXL185 is proven to be the most an excellent quencher for fluorescens such as FAM and FITC. This new FRET peptide offers the following advantages: a) Stronger absorption and emission intensity at longer wavelength (490 nm/520 nm) of 5-FAM compared to Edans. Assays using this substrate exhibit lower background due to less autofluorescent interference from cell components and test compounds. b) QXL185260 is more water-soluble than Dabcyl. c) Substrate II provides better assay sensitivity, Ks, is 21-fold lower than I. Substrate II is 8-fold more sensitive than substrate I, and can detect femtomol of HCV NS3/4A protease. The Synthesis was accomplished using a combination synthesis of the Fmoc solid phase and solution phase.
[1] Talian, M., et al. Anal. Biochem. 240, 60 (1997).

P 212 IDENTIFICATION AND CHARACTERIZATION OF SYNTHEITIC PEPTIDE SUBSTRATES AND SMALL MOLECULAR INHIBITORS OF NON-RECEPTOR TYROSINE KINASES ETK
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Epithelial and endothelial protein tyrosine kinase, Etk/Bmx, is a member of the Btk family of non-receptor tyrosine kinase. Like other members of Btk family of kinases, it is comprised of a pleckstrin homology (PH) domain, a Src homology 3 (SH3) domain, a Src homology 2 (SH2) domain, and a kinase domain. As implicated by its modular structure Etk plays an important role in signal transduction involving cell migration, proliferation, differentiation, and apoptosis. Etk is commonly expressed in epithelial and endothelial cell including prostate cells. Interestingly it is highly expressed in metastatic prostate and breast cancer cells. Identification of Etk substrates and inhibitors presents opportunity to study effects of regulated activity of Etk in prostate cancer cells as well as development of potential therapeutic agent for metastatic prostate cancer. Through screening OBOC combinatorial peptide libraries, several Etk substrate peptides and small molecule inhibitors have been identified. We have also screened peptidomimetic libraries for Etk ligands and identified several compounds with strong consensus.
motif. While a few of these shows weak enzyme inhibitory activity, computer stimulated automated docking placed the small molecule compounds at the ATP/substrate binding pocket.

**P 213 IDENTIFICATION OF HIGH AFFINITY ANTI-MCP-1 ANTIBODIES USING SYNTHETIC PROTEINS**

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We used synthetic human (h) MCP-1 and several synthetic analogs to identify and characterize highly potent anti-MCP-1 antibodies using antibody phage display technology in collaboration with MorphoSys, AG. Synthetic MCP-1 was equipotent with recombinant hMCP-1. For identification of antibodies from the HuCAL™ phage library, the MCP-1(Ile11) analog was biotinylated using a biotin-succinimide reagent enabling its use as the panning antigen. The heterogenous, chemically biotinylated MCP-1(Ile11) was only partially active in calcium mobilization assay, presumably due to the biotinylation of lysine residues involved in receptor interactions. In order to generate homogeneous, fully biologically active biotinylated MCP-1(Ile11), we designed and synthesized site-specific biotinylated hMCP-1 analogs. Two MCP-1(Ile11) analogs with biotin-PEG4, attached to lysine residue at positions 69 or 75 were chemically synthesized by stepwise solid phase synthesis. Site-specific biotinylated MCP-1(Ile11) was shown to be as active as recombinant hMCP-1. This compound was used in the identification of anti-MCP-1 antibodies with picomolar affinity using the MorphoSys antibody phage technology.

**P 214 IN SITU eIF4E-TEMPLATED CLICK REACTION - eIF4GDERIVED eIF4E BINDING PEPTIDES AS ANCHORING FRAGMENTS IN SEARCH OF PUTATIVE EXOSITES**

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Eukaryotic initiation factor (eIF) 4E binds to the scaffolding protein eIF4G an interaction that is critical for the formation of the heteromeric eIF4F translation initiation (TI) complex (eIF4E/eIF4G/eIF4A). An abnormally high level of eIF4E is found in several human cancers, suggesting the importance of the eIF4E/eIF4F interaction in the excessive translation of oncogenic proteins. eIF4G binds to eIF4E through a motif: While a few of these shows weak enzyme inhibitory activity, computer stimulated automated docking placed the small molecule compounds at the ATP/substrate binding pocket.

**P 215 LIPO-P59: THE ROLE OF THE MEMBRANE ON THE BEHAVIOR OF FIV-T CELL FUSION**

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Many evidences suggest that detergent-insoluble membrane microdomains, referred to as lipid rafts, play an important role in virus budding. [1]. In a previous work, we reported that a synthetic 20-mer peptide, P59, derived from a conserved region of feline immunodeficiency virus (FIV) gp36 induced FIV infection of CrFK and lymphoid cells. Circular dichroism and NMR analysis [2] showed that the peptide adopted a structure containing an amphipathic-helical segment of approximately 7 residues, corresponding to 2 helical turns, in presence of a hydrophobic environment. Here, we study the conformational behaviour of lipo-P59, containing an aliphatic tail in C-terminal, in the presence of membrane mimetic environments. The data deriving from fluorescence, EPR and NMR spectroscopy show a relevant capacity of the lipophilic chain to drive the interaction respect to the membrane.

[1] Nieva J.L, et al, J. of Biol. Chem, 277, 21776 (2002).
[2] D’Ursi, A.M., et al, Peptides, 722 (2002).

**P 216 MAPPING CELL BINDING USING COLLAGEN III “TOOLKIT”**

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The triple-helical domains of the collagens interact with both cell-surface receptors and extracellular matrix proteins. The triple-helical structure of collagen can be exploited in short collagen-like peptides by inserting a stretch of Gly-Pro-Pro triplets before and after a specific collagen binding sequence; this is referred to as the “host-guest” strategy. Collagen-related peptides (CRPs) and GFOGER peptides [1] have been shown to induce platelet activation through GpVI [2] and to act as antagonists of platelet recognition of collagen through α2β1[3], respectively. We have prepared a synthetic “toolkit” using a Fmoc strategy to allow us to map and then define the collagen III motifs responsible for interactions. We have cut the 163-1196 collagen III sequence into 27-AA sequences with a 9-AA overlap which gives us 57 peptides. To ensure a triple helical structure, we use the “host-guest” strategy and we obtain in this way, GPC-(GPP)5-(GXX')9-(GPP)5-GPC-NH2 peptides of 63 AA, purified by HPLC and characterized by MALDI-TOF. We have found that GROGER is the best motif in Col III for recognition of α2β1 integrins. The studies have shown that the motif GPGPO is not sufficient to bind GpVI and the best combination is GPOXXXXXXXGG-GPGPO. We have also seen that sequences without any GPO can strongly bind GpVI that might contain a new binding site.

[1] Knight CG et al. J. Biol. Chem., 275, 35, (2000).
[2] Emsley J et al. Cell, 100, 47 (2000).
[3] Knight CG et al. Cardiovasc. Res., 41, 450 (1999).

**P 217 MECHANISM OF INTERACTION BETWEEN THE (17-31) BINDING DOMAIN OF PTH AND THE PTH RECEPTOR**

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The parathyroid hormone receptor (PTHR) is a class 2 G protein-coupled receptor that plays a critical role in bone and calcium metabolism. PTH is thought to interact with the PTHR via a multi-step mechanism: The C-terminal a presumed amphipathic α-helix, docks to the amino-terminal extracellular (N) domain of the receptor, and this docking enables the N-terminal portion of the ligand to engage the extracellular loop/heptahelical core, or juxtamembrane (J) domain of the PTHR and induce activation. The specifics of the interaction are still unclear, however. For example, it is not known whether the C-terminal region of the ligand contributes to the J domain interaction. To further explore the PTH/PTHR interaction mechanism, we performed an alanine scan analysis of the (17-31) segment of
P219 MODELLING OF THE NEUROPEPTIDE Y Y1 RECEPTOR COMPLEXES WITH NON-PEPTIDE ANTAGONISTS

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Postsynaptic Y1 receptors mediate potent vasoconstrictor effects of Neuropeptide Y (NPY) in coronary and cerebral vessels, and are involved in the simulation of food intake by NPY. Potent non-peptide antagonists of the Y1 receptor have been designed by mimicking the C-terminal tetrapeptide of NPY. A model of the transmembrane domain of the human Y1 receptor was built using the crystal structure of bovine rhodopsin. Docking simulations have been used to estimate the binding affinities of complexes alpha1-adrenergic antagonists/alpha1-adrenoceptor. The results were supported by mutagenesis of the residues involved in the binding and by the binding constants determined by the radioligand binding assay. The results of the simulations were compared with the experimental data obtained for the binding of Y1 receptor antagonists to the Y1 receptor. The simulations showed that the binding of Y1 receptor antagonists can be modeled with a high accuracy.

P220 MODULATION OF THE BIOLOGICALLY ACTIVE PEPTIDES BY DIRECTED PEPTIDE LIBRARIES

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Phage display method is a powerful tool to produce lead structures for the design of new therapeutic molecules. The prerequisites of the binding can be studied by using directed peptide libraries. In this method critical amino acids are replaced with a mixture of amino acids with selected properties [1]. In one batch thousands of peptides are synthesized. Synthesized peptides are selected with affinity chromatography. Previously we have identified three cyclic peptides, which promote the proteolytic activity of prostate specific antigen (PSA) [2]. Conformation and binding properties of these peptides were studied by using MSMS mass spectrometry. We were able to isolate the original peptide from the hydrophilic set. In addition one peptide with two prolines in the beta-turn region was identified.

P221 NEUTRALIZATION OF HEPARIN ANTICOAGULANT ACTIVITY BY SYNTHETIC HFP PEPTIDES

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Heparin is the only agent used clinically to inhibit blood coagulation in open heart surgery. However, heparin has side effects of post-operative bleeding. Generally, protamine is used to neutralize the anticoagulant activity. In this work, a series of HFP (Heparin interacting protein) [3] analog peptides were synthesized by using solid phase FMOC method-ology and studied as potential therapeutic neutralization agents. The interaction between the synthetic peptides and heparin was characterized in detail in terms of a) specific amino acid residues involved in the binding; b) the effect of the binding on the pKa values of lysine side chain of ammonium groups; c) the binding constant and thermodynamic parameters for the binding of each analog peptide by heparin; d) the relative binding strengths of the synthetic peptides with heparin by affinity chromatography; e) the effect of binding on the structure and conformation of the peptides and heparin; and f) the effect of NaCl concentration on the binding affinity.

In addition, the binding of D-amino acid analog peptides with the same sequences was studied in order to address the feasibility of peptides-resistant peptide-based drugs. Coagulation assays were performed for both the L peptides and D peptides to investigate the efficacy of the peptides for neutralization of the anticoagulant activity of heparin.

[1] Liu, S., Zhou, F., Hôo, M., and Carson, D. D. Proc. Natl. Acad. Sci. U.S.A. 94, 1739 (1997).
[2] Rabenstein, D. L., Robert, J. M., Hari, S. FEBS Letters 376, 216 (1995).
[3] Rabenstein, D. L., Bratt, P., Schierling, T. D., Robert, J. M., and Guo, W. (1992) J. Am. Chem. Soc. 114, 3278-3285.
P 222 NEW UROTENSIN-II ANALOGS MODIFIED AT THE 4 POSITION
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Recently, we have reported a superagonist at UT receptor (PSU) and the first peptide antagonist (Urantide), which represent important tools to elucidate the role played by this peptide-hormon in human body [1,2]. In order to elucidate the importance of 4 position in biological activity and for receptor interaction we synthesized new analogues where Asp4 was replaced with residues of natural and unnatural amino acids. We report the preliminary pharmacological experiments and SAR study of this new set of compounds.

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[2] Patacchini, R.; Santicioli, P.; Grieco, P.; Rovero, P.; Novellino, E.; Maggi, C.A. British J. of Pharmacology, 140, 1155 (2003).

(4-11)-U-II  H-Asp-[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH
PSU H-Asp-[Pen-Phe-Trp-Lys-Tyr-Cys]-Val-OH
Urantide H-Asp-[Pen-Phe-DTrp-Om-Tyr-Cys]-Val-OH
New H-Xaa-[Pen-Phe-Trp-Lys-Tyr-Cys]-Val-OH

P 223 NEW UROTENSIN-II ANALOGS WITH A CON- 
STRAINED TRP-7 SIDE CHAIN
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Recently, we have reported a superagonist (PSU) and a full antagonist (Urantide) at UT receptor [1,2] and we have elucidated the structural bases of the agonist/antagonist functional switching of these ligands [3]. In particular, we found that the side chain orientation of the Trp-7 residue is crucial for that switching. We now report the synthesis and the preliminary pharmacological and conformational analysis of a new set of compounds with a constrained Trp-7 side chain structure.

[1] Grieco, P.; Carotenuto, A.; Campiglia, P.; Zampelli, E.; Patachini, R.; Maggi, C.A.; Novellino, E.; Rovero, P. Journal of Medicinal Chemistry, 45, 4391 (2002).
[2] Patachini, R.; Santicioli, P.; Grieco, P.; Rovero, P.; Novellino, E.; Maggi, C.A. British J. of Pharmacology, 140, 1155 (2003).
[3] Carotenuto, A.; Grieco, P.; Campiglia, P.; Marinelli L.; Novellino, E.; Rovero, P., European Peptide Symposium (2004), P387.

PSU H-Asp-[Pen-Phe-Trp-Lys-Tyr-Cys]-Val-OH
Urantide H-Asp-[Pen-Phe-DTrp-Om-Tyr-Cys]-Val-OH
New H-Xaa-[Pen-Phe-Lys(Om)-Tyr-Cys]-Val-OH

P 224 NOVEL GnRH ANTAGONISTS DERIVED FROM DE- 
garelix: EXPLORATION OF THE GnRH ANTAG-
ONIST PHARMACOPHORE
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Degarelix (FE2004846, 1) is a potent and very long acting antagonist of gonadotropin-releasing hormone (GnRH) after subcutaneous (sc) administration and is currently in phase II clinical development for the treatment of prostate cancer. Degarelix is three times longer-acting than the two structurally closely related GnRH antagonists acyline (2) and azaline B (3). Since such a subtle structural modification in degarelix resulted in an unexpected increase in duration of action without signif-
icant influence on the antagonistic potency, we hypothesized that other substitutions may exist that would be even more favorable. Herein, we describe an iterative approach to the discovery of novel GnRH antagonists substituted at positions 3, 5, 6, 7, 8 and the N-terminus in the structure of degarelix. The effect of these substitutions on antagonist potency in vitro, and duration of action in inhibiting the release of luteinizing hormone in vivo in castrated male rats will be presented.

1. degarelix, X = L-hydroxycyl, Y = carboxamidyl
2. aclyline, X = Y = acetyl
3. azaline B, X = Y = 5’-(3-amino-1H-1,2,4-triazolyl)
oped QXL™ dyes to eliminate these limitations. QXL™ dyes are excellent dark quenchers that are individually optimized to pair with all of the popular fluorescent dyes such as fluoresceins and rhodamines. Our QXL™ series of nonfluorescent dyes cover the full visible spectrum with unusually high efficiency. QXL™ 520 has absorption maximum perfectly matching the emission of FAM while QXL™ 570 is proven to be the best quencher for TAMRA.

AnaSpec has used QXL dyes to develop a number of FRET peptide substrates for high throughput analysis of protease activities and screening of protease inhibitors. For example, HiLyte Fluor™ 488 (or FAM)/QXL™ 520-based HIV protease substrates have demonstrated significantly enhanced performance. Excellent performance has also been observed for our new HCV protease substrates that incorporate QXL™ 520 as acceptor. We have also used QXL/HiLyte Fluor pairs to develop novel protease substrates for analysis of secretases, caspases and various MMP activities.

**P 227**

**ONE-BEAD ONE-PEPTIDE LIBRARY: DIFFERENT TYPES OF SCREENING FOR ANTI-BACTERIAL ADHESION**

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The adhesion of bacteria to human tissue surfaces and implanted biomaterial surfaces is an important step in the pathogenesis of infection [1]. The alarming increase in drug-resistant bacteria makes a search for a novel means of fighting bacterial adhesions imperative. In this study, we employed one-bead one-peptide (OBOC) approach [2] to identify anti-bacterial adhesion compounds. Gram negative E. coli and gram positive Enterococcus were labeled with fluorescent, biotin or culturing on beads and screened with OBOC library. The anti-adhesion compounds were decoded by Edman micro sequencer. Compounds toxicity on beads and screened with OBOC library. The anti-adhesion compounds for two days without toxicity to bacteria and human blood cells. These results demonstrate that novel anti-adhesion compounds can be easily identified from OBOC combinatorial libraries. Long-lasting anti-adhesion compounds might be constructed using this approach and serve as a new mean to fight infectious diseases.

[1] Cook, A.D., Sagers, R.D. J Biomol Application, 2, 89 (1993).

[2] Lam, K. S., Salmon, S. E., Hersh, E. M. Nature, 7, 82 (1991).

**P 228**

**PEPTIDE-MEDIATED DELIVERY OF SRNA VIA COVALENT CONJUGATES AND NONCOVALENT COMPLEXES**

Renata T. Wittkowska, Mohammad Ahmadian, James W. Dattilo, Lafe J. Purvis, Lishan Chen, Sasha J. Mayer, Kunyuuan Cui, Ken W. Farber, Michael E. Houston, Paul H. Johnson and Steven C. Quay

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Thrombosis is a primary triggering mechanism in many cardiovascular diseases. There are at present no agents in clinical use that target the earliest upstream event in thrombus formation, i.e. the platelet adhesion. Disrupting platelet adhesion is achieved by inhibiting platelet-receptor glycoprotein GPIIb-IIIa (vWF) interactions [1]. The crystal structure of the GPIb-IIIa-vWF complex has been reported [2] and showed that the interaction surface is extended, making this interface a difficult target for small molecules. We have prepared β-hairpin peptidomimetics modelled on a surface portion of GPIIb-IIIa using peptides reproducing the ApoA-I sequence 141-164. They also demonstrated that these peptidomimetics are robust lead compounds for the development of novel antiplatelet agents targeting the initial phase of thrombus formation.

[1] Jackson, S.P., Schoenwaelder, S.M. Nature Rev. Drug Discov, 2, 1 (2003).

[2] Huijinga, E.G., Tsuji, S., Romijn, R.A.P., Schiphorst, M.E., de Groot, P. G., Sistus, J. S., Gros P. Science, 297, 1176 (2002).

**P 229**

**PEPTIDOMIMETIC INHIBITORS OF PLATELET ADHESION AS POTENTIAL NOVEL ANTIPLATELET AGENTS**

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Thrombosis is a primary triggering mechanism in many cardiovascular diseases. There are at present no agents in clinical use that target the earliest upstream event in thrombus formation, i.e. the platelet adhesion. Disrupting platelet adhesion is achieved by inhibiting platelet-receptor glycoprotein GPIIb-IIIa (vWF) interactions [1]. The crystal structure of the GPIb-IIIa-vWF complex has been reported [2] and showed that the interaction surface is extended, making this interface a difficult target for small molecules. We have prepared β-hairpin peptidomimetics modelled on a surface portion of GPIIb, by transplanting 12 to 19 residues from the protein onto a β-turn stabilising motif. The conformational stability was further increased by the introduction of an internal bridge. The results obtained have clearly shown the strict requirement for the inhibitory activity of a stabilised secondary structure. They also demonstrated that these peptidomimetics are robust lead compounds for the development of novel antiplatelet agents targeting the initial phase of thrombus formation.

[1] Jackson, S.P., Schoenwaelder, S.M. Nature Rev. Drug Discov, 2, 1 (2003).

[2] Huijinga, E.G., Tsuji, S., Romijn, R.A.P., Schiphorst, M.E., de Groot, P. G., Sistus, J. S., Gros P. Science, 297, 1176 (2002).
loblastoma and a novel binding site on U373MG astrocytoma for the C-terminal heptapeptide of gastrin (G7; H-AYGWMDF-NH2), were exploited as molecular targets for either G7 or the SSTR2-specific analogue, Phe-Cys-Tyr-gly-Pro-Cys-Val-Cys-Thr-NH2. Data indicate that our chimeric constructs can specifically deliver peptide cytotoxins (the receptor-mimetic, pore-forming tetradecapeptide mastoparan, MP, H-INLKLAAALAKKL-NH2 and the pro-apoptotic peptide, 6[KLAK-LAK]K), to human astrocytoma. Moreover, as an N-terminal extension of MP and 6[KLAK-LAK]K, the G7 address motif enhanced the cytotoxicity of each peptide cytotoxin tested. We have also identified more cytotoxic analogues of MP to enhance the efficacy of our chimeric analogues. Finally, we have demonstrated that in human astrocytoma, MP specifically targets intracellular signaling proteins, besides that of heterotrimeric G proteins and that MP does not promote cell death of human astrocytoma by random pore formation, but initiates apoptosis as detected by in situ TUNEL staining. Thus, the authors anticipate further development of brain tumour-specific cytotoxic peptide chimeras and continue to resolve intracellular signaling mechanisms leading to tumour cell death.

It also weakly binds to the active site of thrombin. However, the inhibitory concentrations of RPPGF are fairly high making it not practical as a therapeutic agent. Substitutions to the sequence have been prepared, aiming to make it a better thrombin receptor activation inhibitor for the management of acute coronary syndrome. To date, compounds have been produced with improved inhibition of thrombin activation of platelets and thrombin itself.

**P 234 PROTEIN KINASE C (PKC) ISOFORM PEPTIDE ACTIVATOR/INHIBITORS EXERT CARDIOPROTECTIVE EFFECTS IN POLYMORPHONUCLEAR LEUKOCYTE (PMN)-INDUCED ISCHEMIA/REPERFUSION (I/R) INJURY**

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Myocardial ischemia followed by reperfusion results in cardiac contractile dysfunction, and is characterized by a decrease in nitric oxide (NO), and an increase in superoxide (SO) release from endothelial cells and PMNs. PKC is a key enzyme that regulates NO and SO release. We tested the hypothesis that PKC beta II and zeta peptide inhibitors would attenuate PMN-induced cardiac contractile dysfunction in isolated perfused rat hearts following 20 min (I) and 45 min (R) separately and in combination. We also evaluated PKC delta peptide activator and inhibitor as separate studies. Comparison of left ventricular developed pressure (LVDP) time courses among the five studies revealed differences between the rate of LVDP recovery. The combination of PKC beta II/zeta inhibitors restored LVDP faster than either peptide inhibitor tested separately relative to control I/R hearts. The PKC delta activator significantly restored LVDP faster at five minutes reperfusion than the delta inhibitor compared to I/R controls (p<0.05). All three PKC isoform inhibitors significantly augmented NO release from rat aorta compared to basal values (p<0.01). PMN SO release was significantly inhibited by the PKC delta activator and beta II/zeta inhibitors from controls (p<0.01). These results suggest that the PKC delta activator restores postreperfusion LVDP by inhibition of PMN SO release and the delta activator by augmenting endothelial NO release, whereas the beta II/zeta inhibitors restore LVDP by both mechanisms.

**P 233 RATIONAL DESIGN OF SMALL MOLECULES FOR A NOVEL CLASS OF ANTI-CANCER DRUGS USING A PHENYLALANINE LIBRARY**

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During this decade we have developed highly potent, orally active peptide bradykinin B2 (B10238), B1 (B10324) and a peptide dimer B1/B2 (B9870) receptor antagonists and their small molecule mimetics (M-570 and M-638) with excellent growth inhibition of small cell lung cancer (SCLC) in vitro and in vivo (68-91%) [1]. Interestingly, most of these compounds act via multiple mechanisms: they stimulate programmed cell death (apoptosis), they inhibit angiogenesis and tumor migration. Certain analogs also act as cyclooxygenase (COX) inhibitors. The mimetic lead-compound, M-570, is a simple acyl-tirosine amide derivative. Because tyrosine could be considered as a phenylalanine analog we set up a phenylalanine library with the hope of obtaining more potent, less toxic and more specific anti-cancer analogs of M-570 with improved solubility. We report the synthesis of the new small molecules and their biological activities, focusing on a better understanding of the structure-activity relationships. We compare the anti-cancer action of these compounds on lung and prostate cancers.

[1] Gera, L., Chan, D.C., Heffrich, B., Bunn, P.A. Jr., York, E.J. and Stewart J.M. In “Peptides 2000”. Edited by J. Martinez and J.-A. Fehrentz. Edk, Paris, 2001, pp. 637-638.
P 236 REPAIR OF PHOTODAMAGING EFFECTS ON HUMAN MELANOCYTES BY 4-PHENYL BUTYRYL-HIS-D-PHE-ARG-TRP-NH2, A SUPERANTIGEN ANALOG OF α-MELANOCORTIN.

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Cultured human melanocytes (hMC) respond to the α-melanocortin (α-MSH) with increased proliferation and melanogenesis, and survival after challenge with apoptosis inducers (e.g. ultraviolet radiation [UV] or hydrogen peroxide). In our laboratory we have found that Ac-His-D-Phe-Arg-Trp-NH2 was almost equally potent to α-MSH in stimulating tyrosinase activity on hMC while 4-Phenylbutyryl-His-D-Phe-Arg-Trp-NH2 (LK-184) had an EC50 value about 10 fold less. The effects of LK-184 on hMC proliferation and tyrosinase activity were found to be mediated by binding to the MC1R, since concomitant treatment with a fragment analog of the physiological MC1R antagonist, human agouti signaling protein, totally abolished these effects. LK-184 had a more prolonged effect and a more remarkable anti-apoptotic effect than α-MSH on UV-irradiated hMC, as determined by Annexin V staining. LK-184 also mimicked the effects of α-MSH on repair of UV-induced DNA photoproducts and reduction of release of hydrogen peroxide from hMC. We propose that LK-184 may be a useful agent in the prevention of skin cancer, since it reduces the photodamaging effects of sun exposure and contributes to photoprotection by increasing cutaneous pigmentation.

P 237 RGD-LABELLED QUANTUM DOT FOR IMAGING TUMOR CELLS OVEREXPRESSION αVß3 INTEGRIN

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αV-integrins are heterodimeric transmembrane cell adhesion molecules involved in cell migration, cell growth, tumor invasion/metastasis and angiogenesis. Particularly, integrin αvß3 expression is upregulated in newly formed blood vessels and it recognizes the tripeptide sequence RGD at low-nanomolar affinity. Quantum dots are a brand new class of fluorescent bio labeling reagents with size- and composition-tunable fluorescence emission from visible to near-infrared wavelengths and very high levels of brightness and photostability suitable for optical multiplexing. A series of potent RGD peptides were conjugated with various near-infrared fluorescent quantum dots and the resulting conjugates were tested for in vitro staining of several cell lines with different levels of integrin αvß3 expression. The optimal quantum dot-RGD probes were subjected to preclinical xenograft models to test the in vivo stability, tissue/organ distribution, and tumor targeting efficacy. [1] Hood John, D.; Cheresch David, A. Nat. Rev. Cancer 2002, 2, 91-100. [2] Rusolalhi, E. Annu. Rev. Cell Dev. Biol. 1996, 12, 697-715. [3] Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; S. L.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. Science 2005, 307, 538-44.

P 238 RIGID CYCLIC TETRAPEPTIDES AS PROBES AND MIMICS OF REVERSE TURNS

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Cyclic tetrapeptides, much like β-turns, form a ~180° turn between the first and second amino acid and therefore inherently possess bonds similarly situated to the Cα-Cβ bonds of a β-turn. We have found certain sequences of cyclic tetrapeptides (e.g. Figure 1) that have one unique conformation as well as bonds that overlap the Cα-Cβ bonds found in the β-turns of the PDB[1][2]. The use of proline in these compounds adds rigidity, prevents internal hydrogen bonding, reinforces cis conformations, and extends rigidity beyond the Cα-Cβ bond (i.e. Cβ-Cy, Cy-Cy). These compounds can be used to mimic known β-turn pharmacophores and probe unknown β-turn conformations.

P 239 SITE-DIRECTED BLOCKING AND LABELLING OF THROMBIN EXOSITE-I

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Enzymes in the blood coagulation pathway are typically composed of a sequence specific peptide cleaving active site. However, much of the specificity and regulation of these enzymes is dictatated by several protein binding surface (exosites) that mediate specific protein/protein interactions. For example, thrombin is specific for cleavage at –PFR- sequences but gains specificity for cleavage of fibrinogen through interactions in exosite-I. Fibrinogen cleavage can be blocked by interaction with thrombomodulin that competes for binding to exosite 1. Similarly, the anticoagulant leech peptide hirudin binds to the catalytic site and exosite-I of thrombin. In order to better understand the relationship between active site and exosite binding between these serine proteases, we are developing methods to introduce a site specific, covalent label at exosite I in a manner that blocks binding and introduces a fluorophore. A series of hirugen analogs (hirudin 54-65) were synthesized that contain benzoylphenylalanine and fluorecin at the N-terminus. The peptides were tested for inhibition of thrombin mediated fibrinogen clotting. The strongest inhibitor was crosslinked to thrombin and the complex purified on an antifluorescin column. This exosite-I blocked thrombin demonstrated normal activity towards substrates requiring active site and/or exosite-II interactions, but was deficient in assays requiring exosite-I function.

P 240 SOLID-PHASE SYNTHESIS AND ANTIBACTERIAL EVALUATIONS OF N-DEMETHYLVAOCYCMICIN DERIVATIVES

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Vancomycin, a potent glycopeptide antibiotic, is the last resort against methicillin-resistant Staphylococcus aureus. Unfortunately, vancomycin-resistant bacteria have emerged and pose a serious threat to public health. N-Demethylvancomycin, an analogue of vancomycin that has been used clinically in China since 1967, differs from vancomycin at the N-terminus, where the N-methyl-leucine is replaced by leucine[1][2]. This free primary amine at the N-terminus allows one to add another point of diversity for building vancomycin libraries. Both solution-phase and solid-phase methods have been used in the past to modify vancomycin. We herein report a new solid-phase synthesis strategy for N-demethylvancomycin derivatives, which involves chemoselective protection of amino groups by Fmoc at the N-terminus and subsequent deprotection. The present method allows us to efficiently and regioselectively reduce allylkyte N-demethylvancomycin at both the vancomycin and the N-terminal sites. Twenty-five N-demethylvancomycin derivatives were successfully synthesized on solid-support. Furthermore, their antibacterial activities were evaluated to determine the contribution of hydrophobic substituents to antibiotic activity.

[1] Ling, D. K., Yu Zh., Su Ch. Acta Pharmacuetica Sinica, 21, 208 (1986).
P 241 STEREO-CONTROLLED SYNTHESIS OF [L-ARG, L/D-3-(2-NAPHTHYL)ALANINE]-TYPE (E)-ALKENE DISUBSTITUTED AMINO ACID CONTAINING PEPTIDES

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The β-amylloid peptide (Aβ) is thought to aggregate via a nucleation process where micellar aggregates propagate to form oligomers (protofibrils) which then polymerizes into insoluble fibrils. This fibrillogenic process has been linked to the pathogenesis associated with Alzheimer’s disease (AD). We have previously shown that novel peptide based inhibitors, Aβ-disubstituted amino acids incorporated at alternating positions in the hydrophobic core of Aβ (KLVFFA) were capable of binding to the Aβ sequence. The current study elucidates the Aβ aggregation mechanism in the presence of the peptide based inhibitors. Using these inhibitors, we have discovered an alternate Aβ aggregation pathway. Our experimental results showed that in the presence of the peptide based inhibitors, Aβ aggregation was facilitated more rapidly as compared to the control. CD, SFM, and TEM results showed that of the rhodopsin-retinal interaction. The same experiments on the trans/cis cinnamoyl peptides. Our molecular modeling studies support the hypothesis that tetrapeptides differing in one single amino acid at P1 position adopt different conformations into active site of thrombin which might be related to significant experimentally determined differences in their inhibitory activity.

P 244 STRUCTURE OF THE ANGIOTENSIN II TYPE-1 RECEPTOR BY THE METHIONINE PROXIMITY ASSAY

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The C-terminal residue of angiotensin II (AngII) binds within the transmembrane domains of the hAT1 receptor. The complete molecular environment of this binding locus in the receptor remains however to be elucidated. The preferential reaction of photoactivated benzophenone photolabels to methionine residues in the target structure has enabled us to design an experimental approach for this purpose the methionine proximity assay (MPA), based on systematic receptor mutagenesis and photolabeling. We first optimized Met selectivity by synthesizing 8 peptides with modified benzophenone groups in position 8 of AngII. Then a series of 44 transmembrane X→M mutants were constructed in TMD’s III, VI, and VII. Photolabeling of these receptor mutants with the best Met selectivity ([1]-(Sar1,p'-benzoyl-L-Phe')AngII and [2]-(Sar1,p'-methoxy-p'benzoyl-L-Phe')AngII, followed by CNBr digestion and SDS-PAGE produced for several mutants a new receptor contacts two in TMIII, four in TMVI and five in TMVII. Homology modeling and incorporation of those contacts allowed for an evidence-based molecular model of the liganded hAT1, which is very similar to that of the rhodopsin-retinal interaction. The same experiments on the constitutively active hAT1 receptor (N111G) yielded essentially the same contact points suggesting a high similarity structure.

P 245 STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF A NOVEL CXC4 Chemokine ANTAGONIST: REVEAL UNIQUE ACTIVITY PROFILE

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Antagonists at the CXC4 chemokine receptor could have broad reaching implications as therapeutic agents in a myriad of disease states including breast cancer metastasis. Few CXC4 antagonists have been reported which provides a significant impediment to the development of this potential class of therapeutic agents. In order to understand how a peptide antagonist identified in our laboratory interacts with its G-protein coupled receptor, we performed structure-activity relationship (SAR) studies to determine critical residues that could be part of a CXC4 antagonist pharmacophore. The receptor affinity of the peptides for CXC4 indicates that basic residues at two key positions are critical, and several additional residues significantly contribute to receptor affinity. Ala substitution in other positions enhanced receptor affinity, suggesting that further sequence optimization is possible. Supported by the NIH COBRE Award 1P20RR15563, and matching support from the State of Kansas and the University of Kansas (SV-C) and Howard Hughes Medical Institute (Biological Sciences Education Grant) and Kansas State University (Terry C. Johnson Center for Basic Cancer Research) (J-PP).
P 246 STUDY OF TPP-1 SUBSTRATE SPECIFICITY BY COMBINATORIAL PEPTIDE LIBRARY AND MASS SPECTROMETRY

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Classical late-infantile neuronal ceroid lipofuscinoses (LINCL) is a fatal hereditary neurodegenerative disease caused by mutations in the CLN2 gene, which encodes a lysosomal protease with tripeptidyl-peptidase I (TPP-1) activity [1]. To investigate the pathophysiological process of LINCL and the biological role of TPP-1, we characterized the substrate specificity of the protease. Standard combinatorial peptide libraries and...
fluorescent peptide libraries incorporating 7-aminomethylcoumarin (ACC) [2] were constructed. Then the kinetics of TFP-I digestion were analyzed using fluorescence and LC/MSMS-based TFP-I activity assays. By stepwise optimization of each position, the specificities at P3, P2, P1, P1' and P2' were obtained. An improved fluorescent substrate, ArgNleLeu-ACC, was then designed that exhibits a 6-fold higher specificity for TFP-I and considerably lower hydrolysis by other proteinases compared to the widely-used substrate AlaAlaPhe-AMC. Based on these results, improved clinical and biochemical assays are in the development process.

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P 251 SUNFLOWER DERIVED TRYPsin INHIBITORS AS ANTIMETASTATICS

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Matriptase is a multi-domain trans-membrane receptor with multiple cellular functions. A serine protease domain on its extracellular domain has been shown to function in cancer metastasis by virtue of its proteolytic action on the extracellular matrix. Early on a potent inhibitor of that protease domain was discovered, by identification of a 14 amino acid long bicyclic peptide termed sunflower derived trypsin inhibitor, SFTI-1 [1]. In structure optimization studies we designed a number of structural analogs in which the bisecting cystine linkage is replaced by redox-stable analogs, such as for example, SFTI-9. These new agents may function as anti-metastatic agents in epithelial cells.

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P 252 SURFACE PLASMON RESONANCE- AND QUARTZ CRYSTAL MICROBALANCE-BASED METHODS FOR DETECTING GRB2 SH2 PEPTIDE INTERACTION

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Biosensor technology [1, 2] has been applied for analyzing of biomolecular interactions in recent years [3, 4] for its advantages of high sensitivity, rapid analysis and experimental simplicity. In this study, quartz crystal microbalance (QCM)- and surface plasmon resonance technology have been applied for analyzing of biomolecular interactions between Grb2 SH2 and synthetic peptides. GST-Grb2 SH2 has been trapped by the ligand, anti-GST antibodies, which was immobilized on the surface of sensor chip using standard amine coupling method. Followed by the injection of various concentrations (1.78 * 10^-8, 1.44 * 10^-7, 0.22 * 10^-6, 0.11*10^-6) of synthetic peptides (Fmoc-Glu-Tyr-Aib-Asn and Glu-Tyr-Aib-Asn), the peptide-Grb2 SH2 interactions were detected by progressive cleavage of the glycine moieties showing measurable activity. The in-vitro and in-vivo activity of the peptides has been tested. In binding assays, terlipressin itself has very low potency compared to LVP. In a functional in-vitro assay and in-vivo pressor assays in rodents, the putative metabolites produced by progressive cleavage of glycine N-terminal residues resulting in active intermediates including LVP that bind to vasopressin V1α receptors to induce vasoconstriction. In order to further investigate the mechanism of terlipressin action, the peptide and its putative metabolites were synthesized by solid phase synthesis. The in-vitro and in vivo activity of the peptides was tested. In binding assays, terlipressin itself has very low potency compared to LVP. In a functional in-vitro assay and in-vivo pressor assays in rodents, the putative metabolites produced by progressive cleavage of the glycine monomers showed measurable activity. The in-vitro and in vivo data in these experiments are consistent with a pro-drug hypothesis and suggest that the diglucose and monoglucose intermediates and LVP may contribute to the pressor responses induced by terlipressin clinically.

[1] Liu Y.; Yu X.; Zhao R.; Shangguan D.-H.; Bo, Z.; Liu G. Biosensors and Bioelectronics 18, 1419-1427 (2003).

[2] Cui X.; Yang F.; Sha Y.; Yang X. Tantalya 60, 53-61 (2003).

P 253 SWITCH ON AMYLOID β PEPTIDE SELF-ASSEMBLY BY ENZYME-TRIGGERED ACYL MIGRATION

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Conformational transitions as origin of peptide aggregation is considered as a fundamental molecular event in early processes of degenerative diseases. We have recently developed a new generation of switch-peptides for the controlled induction of conformational transitions at physiologic pH using O → N acyl migrations in situ [1]. Here, we explore the sequential triggering of O → N acyl migrations in amyloid β derived switch-peptides as a general tool to study the onset and inhibition of polypeptide folding, self-assembly and aggregation (Figure). As specific cleavage sites (Y) a series of orthogonal systems including chemical, photolytic and enzymatic triggers (T) are developed. As shown by conformational and structural analyses, the sequential “switching on” of S-elements in Aβ 1-42 allows for evaluating the impact of individual peptide segments upon folding and self-assembly as well as its specific inhibition.

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P 254 SYNTHESIS AND BIOLOGICAL ACTIVITY OF TERLIPRESSIN AND ITS PUTATIVE METABOLITES

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Terlipressin (H-Gly3-LVP) is a peptide drug approved in some European and Asian countries for the treatment of bleeding esophageal varices and in France for hepatorenal syndrome. Previous studies suggest that terlipressin acts as a pro-drug with cleavage of glycine N-terminal residues resulting in active intermediates including LVP that bind to vasopressin V1α receptors to induce vasconstriction. In order to further investigate the mechanism of terlipressin action, the peptide and its putative metabolites were synthesized by solid phase synthesis. The in-vitro and in vivo activity of the peptides was tested. In binding assays, terlipressin itself has very low potency compared to LVP. In a functional in-vitro assay and in-vivo pressor assays in rodents, the putative metabolites produced by progressive cleavage of the glycine monomers showed measurable activity. The in-vitro and in vivo data in these experiments are consistent with a pro-drug hypothesis and suggest that the diglucose and monoglucose intermediates and LVP may contribute to the pressor responses induced by terlipressin clinically.
P 255 SYNTHESIS AND EVALUATION OF NEUROPROTECTIVE \(\alpha,\beta\)-UNSATURATED ALDEHYDE SCAVENGER HISTIDYL-CONTAINING ANALOGS OF CARNOSINE

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Carnosine and related histidine-containing dipeptides are known to react with high efficiency with the products of lipid peroxidation, namely 4-hydroxy-trans-2,3-nonenal (HNE) and other \(\alpha,\beta\)-unsaturated aldehydes, preventing their reaction with nucleophilic residues in proteins and nucleic acids. Several compounds synthesized in our laboratories demonstrated higher aldehyde-sequestering efficiency than carnosine, and were also efficient in protecting SH-SYSY neuroblastoma cells and rat hippocampal neurons from HNE-mediated death. The results confirm the postulated reaction mechanism of carnosine-like scavengers, where the histidine \(\varepsilon\) nitrogen and an aldehyde-reactive nucleophile are both involved in a 2-steps mechanism leading to \(\alpha,\beta\)-unsaturated aldehyde inactivation. The cytoprotective efficacy of these compounds suggests their potential as therapeutic agents for disorders that involve excessive membrane lipid peroxidation.

P 256 SYNTHESIS AND EVALUATION OF NOVEL Dipeptide Benzoyloxyethylketones as Caspase Inhibitors

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The development of small molecule inhibitors of caspases as therapeutics has been a target of intense research. Herein we report the preparation and biological evaluation of a group of aryl- and acyl-oxymethyl ketones of \(N\)-protected dipeptides. They were synthesized by modified procedure described by Dole [1]. Brieﬂy \(Z\)- or FMOC-dipeptides were converted to the corresponding bromomethyl ketones via the intermediate diazomethyl ketones. Displacement of bromide by carboxylate followed by deprotection of aspartyl-\(\text{OBu}\) group gave the desired aryl/acyl-oxymethyl ketones of \(N\)-protected dipeptides in good yield. The enzymatic activity of the synthesized compounds in the inhibition of caspase-3 was tested using a standard fluorometric assay with Ac-DEVD-AFC used as substrate. The results showed that the inhibitory activity of 1 was comparable with the activity of the commercially available caspase-3 inhibitors. So it provides the opportunity be used as a clinically attractive target to inhibit the apoptosis in neurodegenerative diseases.

Dole, R., Hoyer D. J. Med. Chem, 37, 563 (1994).

P 257 SYNTHESIS, CHARACTERISATION, STRUCTURE AND REACTIVITY OF HUMANIN, AN ALZHEIMER’S DISEASE ASSOCIATED PEPTIDE

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Humanin (HN) has recently been found and shown to be able to abolish neurotoxicity by all types of familial Alzheimer’s disease (AD) mutants and by different \(\beta\)-amyloid (A\(\beta\)) species, therefore, HN represents a promising neuroprotective factor with therapeutic potential in AD [1]. However, mechanisms behind the protective effects of HN are still unclear. In this work, complete HN was synthesized by SPPS using special conditions in prolonged deprotection steps and long coupling times, amido acid sequence and homogeneity of humanin were confirmed by mass spectrometry (MS). HN with >90% homogeneity after RP-HPLC purification was characterized by NMR and H/D exchange MS. Preliminary results have indicated a strong binding between HN and A\(\beta\), which lead to understanding how HN ceases neuron death in AD. The HN-A\(\beta\) complex was studied by NMR and MS, and binding sites between HN and A\(\beta\) were determined using affinity chromatography in combination with high resolution Fouriertransform ion cyclotron resonance mass spectrometry (FTICR MS).

[1] Hashimoto, Y., Niikura, T., Tajima, H., Yasukawa, T., Sudo, H., Ito, Y., Kita, Y., Kawasumi, M., Koyama, K., Doyu, M., Sobue, G., Koide, T., Tsuji, S., Lang, J., Kurokawa, K. and Nishimoto, I. Proc. Natl. Acad. Sci., 98, 6336 (2001).

P 258 SYNTHESIS OF 4-AMINO-1,2,4,5-TETRAHYDRO-2-BENZAZEPINE-3-ONES AND STUDY OF THEIR \(\beta\)-TURN INDUCING PROPERTIES

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Freidinger reported the use of 5-membered lactams to induce a \(\beta\)-turn conformation. [1] An unsaturated 7-membered lactam has been proposed as a \(\beta\)-turn mimic.[2] We have prepared the 1,2,4,5-tetrahydro-2-benzazepine-3-one scaffold as a conformational constrained Phe residue, starting from L-\(\alpha\)-formyl-Phe and an amino acid benzyl ester. The benzazepinone, as well as its N\(^4\)-methyl derivative were incorporated in a tetrapeptide mimic and we have investigated their turn inducing properties by NMR and molecular modeling.

Figure 1: \(\beta\)-turn tetrapeptide mimic

Although NMR did not indicate a preferential turn structure, molecular modeling indicated several low energy conformers corresponding to a \(\beta\)-turn. Evidence is presented that the conformation of the 7-membered ring influences the turn inducing properties of the scaffold.

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[2] Gmeiner P. et al., JOC, 68 (1), 62-69. (2003).
P 259 SYNTHESIS OF A RGD PEPTIDE-PEG HYBRID FOR CARRYING ADENOVIRUS VECTORS INTO CELLS

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The adenovirus vector is a promising carrier for the efficient transfer of genes into cells. To make the adenovirus vector practicable for clinical use, it is necessary to design an auxiliary transporter. The present study describes the use of RGD-related peptide, a peptide that bind to integrines, as a tool to modify the adenovirus such that the risk of side effects incurred during clinical application was reduced. The present study describes the design, preparation and use of (Ac-YGGRGDTP₆)-PEG₁₋₄₆⁴ as an efficient peptide-PEG transporter tool for carrying adenovirus vectors into cells. (Ac-YGGRGDTP₆)-PEG₁₋₄₆⁴ was coupled with 6-maleimidohexanoyl acid N-hydroxysuccinimide ester and the resulting 6-[Ac-YGGRGDTP₆]-PEG₁₋₄₆⁴ was shown to exhibit high gene expression even in a coxsackie adenovirus receptor-negative cell.

P 260 SYNTHESIS OF CYCLIC IMINO ACIDS FROM α-AMINO-ω-BROMOALKANOIC ACIDS

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Cyclic imino acids are key constituents of many synthetic and natural bioactive molecules and useful building blocks for the preparation of peptides and peptidomimetics. It is important to find convenient and efficient synthetic routes to cyclic imino acids. We have developed a method for the synthesis of enantiomers of cyclic imino acids. The key step in the synthesis is the spontaneous intramolecular cyclization of α-amino-ω-bromoalkanoic acids. The protected derivatives of cyclic imino acids containing the neighboring hydroxy group were also successfully employed for hydroxyproline and hydroxyprolineic acid derivatives.

[1] Watanabe, L.A., Jose, B., Kato, T., Nishino, N., Yoshida, M., Tetrahedron Lett., 45, 491 (2004).
[2] Watanabe, L.A., Haranaka, S., Jose, B., Yoshida, M., Kato, T., Moriguchi, M., Soda, K., Nishino, N., Tetrahedron: Asymmetry, 16, 903 (2005).

P 261 SYNTHESIS OF QUANTUM DOT LABELED SHORT PEPTIDES AND THEIR APPLICATION IN CHARACTERIZATION OF THE INTERACTION BETWEEN IMMUNE PEPTIDES AND T CELL SURFACE RECEPTORS

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Semiconductor quantum dots (QDs) that conjugate with bio-recognition molecules have recently attracted widespread interest in biology and medicine. We set out to explore the feasibility of signal peptides targeting in cells using QDs. Nuclear targeting signal peptide (NTS) and endoplasm targeting signal peptide (ETS) were synthesized using Fmoc-Lys(tfa)-OH. The terminal amino groups of the peptides were linked with the carbosyl groups of 3-Mercaptotributyl acid-stabilized CdTe nanoparticles in organic phase. After deprotected Tfa group by pyridine in water, QDs coated with NTS and ETS can accumulate selectively in organelles of different cell lines by endocytosis. Furthermore, the cells remained stably labeled for over a week as they were maintained in the culture. These results indicate the construction of more complex bioconjugates with capabilities not only for targeting in living cells, but also for diagnostics and drug delivery.

P 262 SYNTHESIS OF QUANTUM DOT-SIGNAL PEPTIDES BIOCONJUGATES AND TARGETING IN LIVING CELLS

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Semiconductor quantum dots (QDs) that conjugate with bio-recognition molecules have recently attracted widespread interest in biology and medicine. We set out to explore the feasibility of signal peptides targeting in cells using QDs. Nuclear targeting signal peptide (NTS) and endoplasm targeting signal peptide (ETS) were synthesized using Fmoc-Lys(tfa)-OH. The terminal amino groups of the peptides were linked with the carbosyl groups of 3-Mercaptotributyl acid-stabilized CdTe nanoparticles in organic phase. After deprotected Tfa group by pyridine in water, QDs coated with NTS and ETS can accumulate selectively in organelles of different cell lines by endocytosis. Furthermore, the cells remained stably labeled for over a week as they were maintained in the culture. These results indicate the construction of more complex bioconjugates with capabilities not only for targeting in living cells, but also for diagnostics and drug delivery.

P 263 SYNTHESIS OF SYMMETRICAL DICARBOXYLIC ACID LINKED PEPTIDES ON SOLID SUPPORT

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We developed a new method for the synthesis of dimeric peptides of biological relevance on solid support using dicarboxylic acid coupling as the key step. The use of Rink resin for the synthesis allows for the facile construction and sidechain deprotection of the resultant peptide dimers. Using MBHA resin to synthesize the dimeric tetrapeptides (-A-Lys-Pro-Phe-), the suberic acid linked dimers produced the highest yield of covalent dimers, without producing carboxylated monomeric peptides. The selectivity and yield of the covalently linked tetrapeptides is highly dependent on the length of the dicarboxylate linker.

[1, 2, 3]

P 264 SYNTHESIS RADIOLABELING AND EVALUATION OF A NOVEL CARDIOACTIVE PEPTIDE ANALOG

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Cardioactive peptides present in the nervous system of Aplysia have been found to be potent cardioactive neuropeptides in several moluscan species. To find out whether these peptides can also be effective in mammals, we have prepared and evaluated one small cardioactive peptide (SCP) in an attempt to develop a peptide-based imaging agent for cardiac function studies. The 13-amino acid peptide, MAG₃-Abu-Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met-CONH₂ (MAG₃-SCP),
was prepared by solid-phase peptide synthesis following standard Fmoc/HBTU methodology. γ-Abu residue was inserted as a spacer between the cardioactive peptide sequence and MAG3 chelating moiety. Labeling with $^{99m}$Tc was achieved by stannous-tartrate exchange method. In vitro stability was evaluated in human plasma and in vivo biodistribution was performed in Balb/C mice. Radio-HPLC analysis revealed that MAG3-peptide labeled efficiently with $^{99m}$Tc and resulted in the formation of one stable radioactive species (≥ 90%). The radiopeptide displayed a good in vitro stability when incubated with human plasma at 37°C for 6 h. In vivo biodistribution in mice showed that the radiopeptide cleared rapidly from the blood. However, elimination of the radiopeptide proceeded mainly via the hepatobiliary system. Uptake in the heart was found to be somewhat low. No other organ appeared to be significantly involved in uptake or clearance of the radiopeptide. The preliminary results suggest that the poor pharmacokinetics of the radiopeptide is probably due to its high lipophilicity. Appropriate modifications in the sequence together with the use of a more suitable spacer group may improve the pharmacokinetics of the peptide and could make it a useful cardiac imaging agent.

P 265 SYSTEMATIC STUDY ON THE STRUCTURE-ACTIVITY RELATIONSHIP OF PYRAZINONE RING-CONTAINING BIOACTIVE OPIOID LIGANDS

K. Shiotani, 1 A. Miyazaki, 2 T. Li, 1 Y. Tsuda, 1-3 A. Ambo, 4 Y. Sasaki, 4

In vitro stability of the radiopeptide is probably due to its high lipophilicity. Ap-

propriate modifications in the sequence together with the use of a more suitable spacer group may improve the pharmacokinetics of the peptide and could make it a useful cardiac imaging agent.

P 265 SYSTEMATIC STUDY ON THE STRUCTURE-ACTIVITY RELATIONSHIP OF PYRAZINONE RING-CONTAINING BIOACTIVE OPIOID LIGANDS

K. Shiotani, 1 A. Miyazaki, 2 T. Li, 1 Y. Tsuda, 1-3 A. Ambo, 4 Y. Sasaki, 4

Previously, we reported that the pyrazinone ring-containing opioid mimetics, 3, 6-bis[Dmt-NH-(CH$_2$)$_n$]-5-methyl-2(H)pyrazinones ($n = 1-4$) exhibited considerably potent antinociceptive activity after icv, sc and po administration in mice [1]. These compounds bound to mu-opioid receptors with a high affinity and a high selectivity over delta opioid receptor. In this presentation, synthesis and bioactivity of the full set of 3-[Dmt-NH-(CH$_2$)$_n$]-5-methyl-2(H)pyrazinones ($n = 1-4$) and their struc-
ture-activity relationship will be discussed.

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P 266 T CELL SPECIFICITY EXAMINED IN A SYSTEMAT-IC AND COMPREHENSIVE MANNER USING PEP-TIDE POSITIONAL SCANNING LIBRARIES

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Historically, most studies of T cell specificity and degeneracy have relied on the determination of the effects on T cell recognition of amino acid changes at individual positions or MHC binding residues, and thus they have been limited to a small set of possible ligands. Synthetic combinatorial libraries (SCLs), and in particular positional scanning synthetic combinatorial libraries (PS-SCLs) represent collections of millions to trillions of peptides which allow the unbiased elucidation of T cell ligands that stimulate clones of both known and unknown specificity. PS-SCLs have been used successfully to study T cell recognition and to identify and optimize T cell clone (TCC) epitopes in infectious diseases, autoimmune disorders and tumor antigens. PS-SCL-based biometrical analysis then allows the screening data to be combined with information derived from protein sequence databases to identify natural peptide ligands. PS-SCL-based biometrical analysis provides a method for the determination of new microbial antigen and autoantigen sequences based solely on functional screening data rather than sequence homology or motifs. Therefore, this strategy is ideally suited for the prediction and identification of both native and cross-reactive epitopes by virtue of its ability to integrate the examination of trillions of peptides in a systematic manner with all of the protein sequences in a given database.

P 267 TARGETING EFFECTOR MEMORY T CELLS WITH A SELECTIVE PEPTIDE INHIBITOR OF Kv1.3 CHANNELS: IMPLICATION FOR THERAPY OF AUTOIMMUNE DISEASES

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Autoreactive effector memory (T$_{EM}$) T lymphocytes play a major role in the pathogenesis of autoimmune diseases. Therapies that selectively target T$_{EM}$ cells would have significant value. Blockers of the voltage-gated Kv1.3 $\pm$ channel preferentially inhibit T$_{EM}$ cells without impairing the activity of other T cells. In proof-of-concept studies, Kv1.3 inhibitors have been shown to ameliorate disease in a rat model of multiple sclerosis, prevent bone resorption associated with periodontal disease and suppress the delayed type hypersensitivity response. Here we describe the design and characterization of ShK(L5), the most selective Kv1.3 blocker. ShK(L5) exhibits picomolar affinity for the channel and is $>100$-fold selective for Kv1.3 over the closely related Kv1.1 channel and $>700$-fold selective over other related channels. It suppresses the proliferation of human and rat T$_{EM}$ cells and inhibits IL-2 production at picomolar concentrations. Naïve and central memory T cells that constitute the bulk of T cells in the blood, are initially 100-fold less sensitive to ShK(L5) and then become resistant to the peptide during activation by up-regulating the calcium-activated $\mathrm{IKCa1}$ $\pm$ channel. Administration of ShK(L5) by subcutaneous injection (10µg/kg/daily) results in blood levels of $>300$ pM over a five day period that are sufficient to suppress T$_{EM}$ cells without affecting other T cell populations. This treatment regimen prevents experimental autoimmune encephalomyelitis in rats, an animal model for multiple sclerosis caused by the transfer of myelin-specific T$_{EM}$ cells. It also suppresses delayed type hypersensitivity in rats mediated by T$_{EM}$ cells. ShK(L5) might have use for the therapy of autoimmune disorders.

P 268 TETRA AND PENTAPEPTIDE DERIVATIVES OF HEMIASTERLIN, SYNTHESIS AND ACTIVITY STUDIES.

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Hemiamsterlin I is a natural tripeptide toxin isolated from the marine sponge Hemiamsterella minor [1]. Hemiamsterlin acts as an extremely potent inhibitor of tubulin polymerization, and is active against leuke-
mia, ovarian carcinoma, and breast cancer. We investigated a very active hemiamsterlin analogue 2 [2] as a toxin in receptor-mediated, enzyme dependent drug delivery approach. Since the lysosomal processing of the receptor-targeted prodrg add iso 2 amino acid residues to the C-terminus of the toxin, it was necessary to select residues that would lead to the most active final products. We have generated a small library of hemiamsterlin tetra- and pentapeptide deriv-
atives using both natural and unnatural aminoacids in the fourth and fifth positions of modified hemiamsterlin. MTI cytotoxicity assay re-
vealed that most of the C-terminus extensions abolished cell toxicity of 2. However, tetrapeptides containing cyclohexylalanine A or either 1-
or 2-naphthylalanine at the C-terminus, kept a significant level of activity. We found that there was no simple correlation between tetra-
and pentapeptides cell toxicity and activity in tubulin polymerization
inhibition, suggesting that other factors, like membrane permeability
may also be essential.
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P 269 THE (L/D) CONFIGURATION OF AMINO ACIDS AT P1' POSITION IN PEPTIDES [D-PHE-PRO-D-ARG-P1'-CONH2] DETERMINES THE INHIBITORY ACTIVITY FOR THROMBIN

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A structure-activity relationship (SAR) of tetrapeptides [D-Phe-Pro-
D-Arg-P1'-CONH2] as reversible inhibitors toward thrombin is reported.
The P1' position was varied with D and L amino acids. The significant
differences between the inhibitory constants (Kis) of tetrapeptides from
the series DPhe-Pro-DArg-P1'-CONH2 suggest that the interaction
between the amino acid at P1' position and the S1' subpocket in
thrombin is very specific. There is a 2 to 500 fold experimentally
determined difference between the Kis of peptide inhibitors having one
single variation at P1' and the in vitro inhibition of thrombin showed
that the P1' position requires small hydrophobic and polar amino-acids.
In addition, there is a significant change in the affinity for interaction
with thrombin as the configuration for the same amino-acid in P1'
position is changing from L to D. Specifically, a switch from L-Thr into
D-Thr in P1' was correlated with a 15 fold increase in the peptide
inhibitory activity. Similarly, a switch from L-Ala to D-Ala in P1'
increased the affinity of peptide binding to thrombin by 8 fold. These
differences in the binding affinities upon switching from L into D of
amino-acids in P1' were confirmed both kinetically and through iso-
thermal titration calorimetry (ITC). The structural basis for this favor-
able switch in the affinity was further investigated through molecular
modeling of docked peptides into the thrombin template 1ABJ.pdb
using the software SCULPT from MDL.
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P 270 THE CHEMENOZYMIC SYNTHESIS OF OLIGOSACCHARIDE-LINKED PEPTIDES AIMED AT IMPROVED DRUG DELIVERY

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Central nervous system peptide drugs were glycosylated to utilize active
transport systems and target the compounds. A series of glycosyltrans-
ferase enzymes, derived from the bacterial pathogen Neisseria menin-
gitidis, were employed in the chemo-enzymatic synthesis of oligosac-
charide-linked endorphin peptide drug candidates [1]. The in vitro
caco-2 cell monolayer permeabilities and enzymatic stabilities of each
compound were measured to compare the suitability of the glycopeptide
derivatives for oral administration.
[1] Johnston, K.D., Dieckmann, M., Jennings, M.P., Toth, I.,
Blanchfield, J.T., Current Drug Delivery, in press (2005).

P 271 THE COMPARISON OF ZINC(II) AND COPPER(II) COMPLEXES OF A NOVEL POLYHISTIDINE TYPE LIGAND

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Histidines play an essential role in binding of biological metal ions,
either in small or macromolecular chelating molecules, e.g. in met-
alloenzymes. Therefore the low molecular weight polystyridine type
ligands are of potential importance for use as model substances.
Continuing our investigations on a novel branched oligopeptide type
ligand prepared by solid phase peptide synthesis – we investigated
the metal ion binding with zinc(II) and copper(II). The eight primary
metal-binding sites are the four imidazole and four ammine groups on
the ligand. pH-potentiometric titrations revealed, that up to pH 8 all
these donor atoms loose their protons on increasing pH. The competi-
tion between the protons and the metal ions results in the decrease of pK
values to about 1-3 in the case of copper(II) and to about 4-6 in the case
of zinc(II) ions. This reflects the higher stabilities of the complexes
formed with copper(II) in spite of the weak axial coordination that
seems to occur in zinc(II) complexes. Combined potentiometric, spec-
trophotometric, CD and NMR spectroscopic methods were utilized to
investigate the speciation and the structure of the complexes formed in
aqueous solution.
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P 272 THYMODEPRESSIN - A NEW IMMUNOSUPPRESSIVE DRUG FROM THE EW-FAMILY

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As a result of intensive studies of the EW-peptide family, a new
effective immunosuppressive analogue – γ-D-Glu-D-Trp – has been
developed [1]. This iso-dipeptide has exhibited significant immunosup-
pressive activity in numerous in vitro, in vivo and clinical studies [2].
We studied the effects of two structurally related peptides (Thymo-
gen-L-Glu-L-Trp and Thymodepressin γ-D-Glu-D-Trp) in a number of
scenarios: in vitro on blood neutrophils, monocytes, and lymphocytes,
as well as thymocytes, thymic epithelial cells and endothelial cells of
human vessels. We evaluated the effects of the peptides on the phago-
cytic activity of cells, active oxygen generation, expression of function-
ally important membrane molecules, including co-stimulating ones and
markers of cell activation, mitogen-stimulated proliferation and apop-
tosis, cytokine production. In vivo studies were performed in mice to
prevent chronic GVHD after allogenic bone marrow or spleen trans-
plantation.
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psoriasis and other autoimmune diseases will be presented and dis-
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P 273 TISSUE SPECIFIC PEPTIDES AS CELL PROTEIN KINASES AFFECTORS

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As reported earlier [1], tissue specific peptide pools are comprised of fragments of functional proteins. Most of them were shown to affect cell proliferation in vitro by unknown mechanisms. Neokorytophin, a proliferative peptide cleaved off alpha-hemoglobin chain was shown earlier to increase Ca2+ influx through L-type channels [2]. In this work we used various modulators of protein kinases activity to investigate the role of the kinases in neokorytophin effect realization. In the case of PKC down-regulation with phorbol ester or specific inhibition with bis-maleymide XI the proliferative effect of neokorytophin in tumor L929 cells is not reduced. In contrast, the specific inhibition of PKA with H89 resulted in full suppression of the peptide’s effect. On the other hand, the proliferative effect of neokorytophin was mimicked by PKA activator 8-Br-CAMP. The results obtained point at the essential role of PKA in the realization of neokorytophin effect. The same methodic approach was used to reveal the role of protein kinases in the action of antiproliferative tissue specific peptides.

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P 274 TOWARDS INHIBITION OF AMYLOID \( \beta \)-PROTEIN OLIGOMERIZATION

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Mounting evidence suggests a central role for amyloid \( \beta \)-protein (A\( \beta \)) oligomers as primary neurotoxic agents in Alzheimer’s Disease (AD). In particular, oligomers derived from the 42-amino acid form, A\( \beta \)42, are neurotoxic at nanomolar concentrations and are more potent neurotoxins compared to fibrillar A\( \beta \)42 or to aggregates of the more prevalent 40-amino acid alloform, A\( \beta \)40. We showed that A\( \beta \)42 oligomers differ from A\( \beta \)40 oligomers in their oligomer size distribution and morphology (1). Spherical A\( \beta \)42 units containing 5/6 monomers were shown to self-assemble into larger oligomers and were therefore termed “paranuclei.” In contrast, A\( \beta \)40 oligomers had amorphous morphology and existed as an equilibrium among monomer, dimer, trimer, and tetramer. Recent computer simulations have suggested that assembly differences between the two A\( \beta \) alloforms result primarily from conformational differences in the C-terminal region (2). Based on these findings we have designed and prepared A\( \beta \)42 C-terminal fragments and studied their conformation and their capability to inhibit paranucleus formation. Our data indicate that peptide conformation and inhibitory activity are related, and are dependent on peptide length. These data provide necessary baseline database for rational design of high-potency paranucleus inhibitors, which have potential to become new drugs for AD.

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P 275 TUMOR HOMING PEPTIDES: TOOLS FOR TARGETING, IMAGING AND DESTRUCTION

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We have isolated a panel of tumor homing peptides by using a phage display technology. These peptides target different substructures in different types of tumors. Our tumor homing peptides are e.g. able to differentiate between the blood and lymphatic endothelial cells in the same tumor, while they also bind to the tumor cells [1-2]. We have also isolated peptides that can distinguish the vasculature of a premalignant stage from the vasculature of a fully malignant tumor or from the vasculature of a corresponding normal organ in a mouse model of multistage tumorigenesis [3]. Some of our peptides that become internalized by cells are similar to the Tat and other cell penetrating peptides with an important distinction: our peptides are cell-type specific and deliver a payload to a specific target cells of the peptides are internalized by the cells they bind to [1-2].

LyP-1 is a peptide that specifically binds to tumor and endothelial cells of tumor lymphatics in certain tumors. Fluorescein-conjugated LyP-1 and a related peptide, LyP-1b, strongly accumulated in primary MDA-MB-435 breast cancer xenografts and their metastases from i.v. peptide injections, allowing visualization of orthotopic tumors in intact mice. LyP-1 induced cell death in cultured human breast carcinoma cells that bind and internalize the peptide. Systemic LyP-1 peptide treatment of mice with xenografted tumors induced with the breast cancer cells inhibited tumor growth. The treated tumors contained foci of apoptotic cells and were essentially devoid of lymphatics. These results reveal an unexpected antitumor effect by the LyP-1 peptide that seems to be dependent on a proapoptotic cytotoxic activity of the peptide [4]. As LyP-1 affects the poorly vascularized tumor compartment, it may complement treatments directed at tumor blood vessels. Our tumor homing peptides can be used in identification of vascular biomarkers, in tumor and premalignant lesion imaging as well as targeted delivery of tumor therapies.

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P 276 USE OF BETIDAMINO ACIDS IN DRUG DESIGN

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The G-protein coupled five receptors of somatostatin (H-Ala-Gly-Cys-Lys-Asp-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH, SRIF were described in the mid-nineties. The actual function, the cellular distribution and specificity of the different SRIF receptor subtypes are still not completely understood. SRIF receptors are overexpressed on certain tumor cells therefore synthetic SRIF analogs that selectively bind to these cells are potentially interesting for tumor diagnosis and treatment. Hundreds of SRIF analogs have been designed and tested for their binding affinity and selectivity at the five receptors but only a few of them are potent, labelable and selective to a SRIF receptor. Peptides generated from the use of unnatural scaffolds have been found to have physico-chemical, structural, biological, metabolic, and absorptive properties that differ from those of the parent peptides. We will describe betidamino acid containing SRIF analogs. The versatility of betidamino acids (acylated and/or alkylated aminoglycine scaffold) was demonstrated in the design of GnRH antagonists.[1] The introduction of betidamino acids in shortened chain analogs of SRIF resulted in highly receptor selective analogs (>100-fold) with high binding affinities (IC50<<10 nm). Importantly, the NMR structure of some of these selective analogs was determined and others were amenable to specific labeling.

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