

## PA173 - A new class of peptide bioconjugates utilizes modified C-sugars to improve bioavailability

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Peptidomimetics and pseudopeptides both represent common strategies to improve peptide bioavailability. Often, the polyether, PEG, has been incorporated as a structure stabilizing bioconjugate, and this demonstrates another approach for enhanced drug delivery. Our recent work with pseudopeptides and pseudoglycopeptides has featured stabilized sugars (C-sugars) to augment peptide stability and targeting. Inadequate transport through various membranes and the blood brain barrier is an impediment to the use of peptides as drugs. Two of the main problems are believed to be the polarity of the peptide bond and the associated H-bonding, especially via solvation. The sugar moiety is also a storehouse of hydrogen bond donors and acceptors by virtue of its polyhydroxy structure.

In addition, the carbohydrates themselves tend to be as structurally labile as peptides, due in their case to the hydrolysis-vulnerable hemiacetal

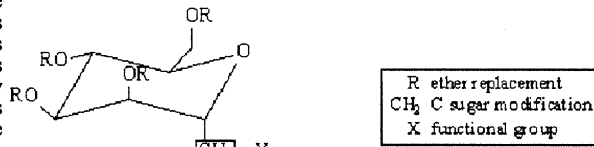


Figure 1. Alkylated derivative of  $\alpha$ -D-mannose

linkages. We have chosen to use C-sugars as a core, and O-alkylation as a hydroxyl replacement, in order to develop a new class of compounds as peptide-solubilizing bioconjugates. The C-sugar (Figure 1) incorporates a methylene replacement for the anomeric oxygen, multiple ether appendages, and a terminal functionality at C-1, typically an acid or amine.

Such derivatives are then easily linked to a wide variety of peptides. To assess the utility of this approach, we selected our recently described cyclic helicomimetic peptide analogs [1] which contain the LXXLL NR box motif [2]. We find that these variants can retain their bioactivities while offering promise for improved transport, especially against their nuclear receptor targets.

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## PA175 - On the synthesis of N- $\gamma$ -glycosylated $\alpha,\gamma$ -diamino acid containing peptides

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Deltorphins are opioid heptapeptides isolated from frog skin and are highly selective ligands at  $\delta$ -opioid receptors. When injected into the brain of mice and rats, deltorphin I and deltorphin II produce analgesia, locomotor stimulation, and motivational rewarding without development of physical dependence or respiratory depression. However the exploration of the therapeutic potential of these  $\delta$ -opioid agents has been hampered by the lack of derivatives active on peripheral injection.

In a previous paper [1] we described the synthesis of some deltorphin analogues  $\beta$ -O- and  $\alpha$ -C-glycosylated on the C-terminal amino acid residue and reported their  $\delta$ -opioid receptor affinity and selectivity as well as their analgesic potency after subcutaneous injection in mice. In order to further investigate the role of glycosylation in enhancing peptide stability in biological fluids and tissues, we prepared new deltorphin I [H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH<sub>2</sub>] analogues in which the aspartic acid residue in position 4 has been replaced by a sugar amino acid, ( $\beta$ -D-GlcNAc)Asn, N <sup>$\beta$</sup> -methoxy-N <sup>$\beta$</sup> -glucosyl- $\alpha,\beta$ -diamino (S) propanoic acid [2] or N <sup>$\gamma$</sup> -methoxy-N <sup>$\gamma$</sup> -glucosyl- $\alpha,\gamma$ -diamino (S) butanoic acid. The latter was prepared from Fmoc-Asp-OBzl which was first reduced to homoserine derivative and then oxidized to benzyl 2-fluorenylmethoxycarbonylamino-3-formyl propanoate by the Swern reaction. Reductive amination of the formyl group with NH<sub>2</sub>-OCH<sub>3</sub> / NaBH<sub>3</sub>CN yielded the benzyl N <sup>$\alpha$</sup> -Fmoc-N <sup>$\gamma$</sup> -methoxy- $\alpha,\gamma$ -diamino (S) butanoate. N-*tert*-Butyloxycarbonylation followed by catalytic hydrogenation yielded the N <sup>$\alpha$</sup> -Fmoc, N <sup>$\gamma$</sup> -Boc, N <sup>$\gamma$</sup> -methoxy- $\alpha,\gamma$ -diamino (S) butanoic acid which was directly used for peptide elongation by the solid phase procedure.

The final peptide was removed from the resin, purified and chemoselectively glycosylated by reaction with the unprotected monosaccharide [3].

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## PA174 - Enzymatically hydrolyzed chitosan as a carrier for presentation of bioactive peptides

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Glycoproteins consisting of carbohydrates covalently attached to proteins, are very commonly occurring protein types. Chitin, the second in abundance natural polymer, has found many potential applications in its deacetylated form, chitosan. Usual forms of chitosan have very long polymeric chains (average ~5000 units), however oligomeric chitosan molecules have distinct properties and applications. We treated chitosan (poly-D-glucosamine) with the enzyme chitosanase from *Streptomyces* sp. N174 to obtain a hydrolyzate with an average length of 10 D-glucosamine units per molecule. This water-soluble oligosaccharide was used as a carrier-matrix for a biologically active peptide: the bradykinin B1 receptor antagonist. Previous data suggested that the C-terminal part of this peptide is responsible for the interaction with its receptor. Therefore, we coupled the N-terminal part of the peptide to the oligosaccharide using a maleimidothiol reaction. 6-maleimidocaprylic acyl was coupled to the N-terminus of Lys[D- $\beta$ NaI<sup>7</sup>,Ile<sup>8</sup>]desArg<sup>9</sup> bradykinin on solid phase synthesis. Oligo-D-glucosamine was derivatized by mercaptopropionic amidation and then was conjugated with maleimided peptide. The conjugate kept its antagonistic property.

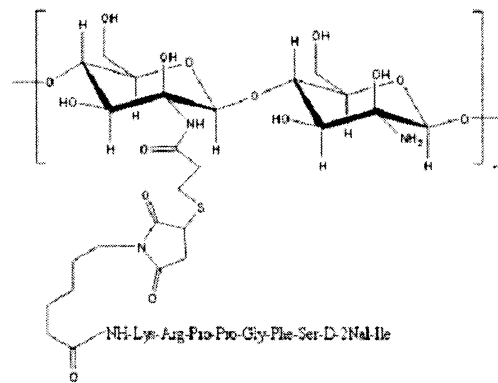


Fig. 1 - Structure of the glycoconjugate

## PA176 - Synthesis of Ig domain of emmprin carrying N-linked core pentasaccharide

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Tumor cell invasion and metastasis require the degradation of extracellular matrix components by matrix metalloproteinases (MMPs). These MMPs are mainly produced in tumor-associated fibroblasts, rather than in tumor cells themselves. Emmprin, a glycoprotein presents on the surface of tumor cells plays an essential role in this phenomenon, since it has been shown that emmprin stimulates nearby fibroblasts to produce MMPs. The functional site of this protein is the extracellular first Ig domain (34-94), which contains a consensus N-glycosylation site at Asn<sup>44</sup> [1]. Although the glycosylation is essential to exert MMP stimulation activity, the structure of sugar chain at Asn<sup>44</sup> has not yet been known. Thus, we intended to synthesize the Ig domain with various sugar chains at Asn<sup>44</sup> to identify the essential carbohydrate structure for its activity. We have already prepared the Ig domain carrying GlcNAc or chitobiose unit at Asn<sup>44</sup> [2]. In this report, the synthesis of Ig domain with a pentasaccharide core unit (Man<sub>3</sub>-GlcNAc<sub>2</sub>) will be reported. The sequence of the Ig domain was divided at Gly<sup>58</sup>-Gly<sup>59</sup> and N-terminal peptide thioester carrying the pentasaccharide and C-terminal peptide amide was prepared by the solid-phase method. In contrast to the previous synthesis [2], the use of Boc method for the N-terminal peptide synthesis was difficult, as the  $\beta$ -mannoside bond in the pentasaccharide unit was highly acid sensitive. Thus, following the procedure of Li et al. [3], the peptide thioester was synthesized using a cocktail composed of 1-methylpyrrolidine, hexamethyleneimine and HOBt as Fmoc deblocking reagent. The results of the synthesis of peptide thioester carrying pentasaccharide as well as the segment condensation, characterization of the final product will be reported.

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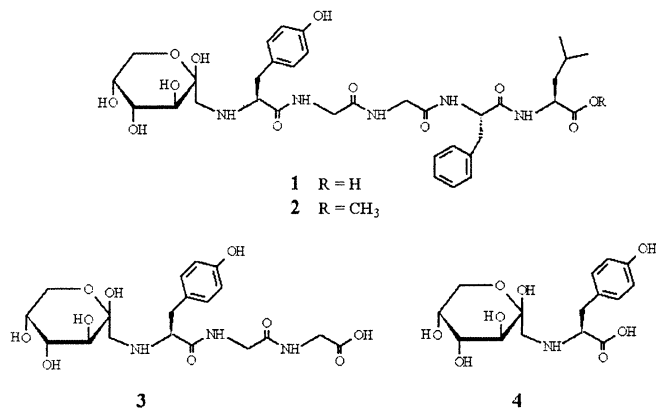
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## PA177 - Study of degradation pathways in sugar-peptide Maillard model systems

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The Maillard reaction is a complex multistep process initiated by the binding of reducing sugars to free amino groups of peptides or proteins resulting in adducts which undergo dehydrations and rearrangements to produce reactive compounds (advanced glycation end products = AGEs) with characteristic fluorescence and covalent crosslinking properties. The formation of AGEs *in vivo* is linked to various physiological and pathological changes, particularly in the elderly and diabetics, as well as in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. As defined models for studying the Maillard reaction *in vitro* we used Amadori compounds 1-4 in which a 1-deoxy-D-fructose moiety is linked to the endogenous opioid pentapeptide leucine-enkephalin (1), its methyl ester (2), Tyr-Gly-Gly (3) or to a single amino acid, Tyr (4).



The stability and degradation pathways of Amadori compounds 1-4 was studied in different solvents and at various temperatures by using reversed-phase HPLC, UV and fluorescence spectroscopies. In addition, the main decomposition products were isolated and characterized by NMR and mass spectrometries.

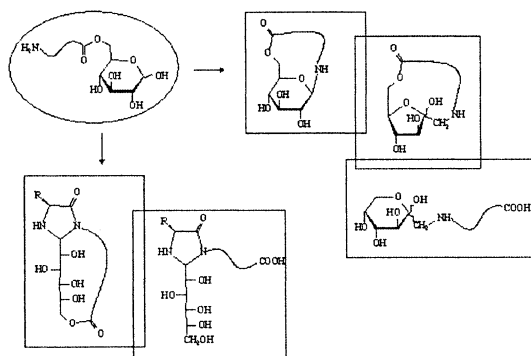
## PA179 - Glycopeptide mimetics as "puzzles" in understanding the biological phenomena

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In spite of the increasing knowledge of human biology and genetic information, it is becoming more evident that the complexity of biological systems lies predominantly at the level of proteins. Among protein conjugates involved in a variety of bioprocesses, glycoproteins are identified as key modulators of cell-cell communication events. Diversity and complexity of natural glycoproteins demand well-defined glycopeptide mimetics to probe the nature of biological processes.

Addressing these issues we have used structurally characterized monosaccharide esters of peptides as models for the study of non-enzymatic glycation reactions. *In vivo* glycation is associated with a number of late complications of aging, diabetes, vascular and Alzheimer's diseases. In our study glucose esters were used as tools to light up some of the open issues, namely the reactivity of such sugar-peptide adducts and the influence of the length and the composition of the peptide part on intra- and intermolecular reactions. We have revealed that, depending on the reaction conditions studied esters undergo cyclization reactions leading to the bicyclic Amadori compounds or bicyclic imidazolidiones. In addition to NMR spectroscopy, mass spectrometry was used as powerful analytical tool able to answer the challenge of biomedical science. MS studies on model compounds enabled correlation of distinct fragmentation pattern with specific sugar-peptide structure and established specific ions as markers for each type of compounds. Presented results can be considered as "puzzles" necessary for the identification and characterization of more complex "images", either naturally occurring or synthetically obtained.



## PA178 - Multiple antigen glycopeptides (MAGs) with Tn tumour antigens and incorporated adjuvant: synthesis and immunobiological activities

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MAGs with Tn antigens [H-Ser/Thr(α-D-GalNAc-1→O)-OH] and norMurNAc-Abu-D-iGln as adjuvant were prepared using SPPS on Rink Amide AM resin. First, [Fmoc-Lys(Dde)]<sub>2</sub>-Lys-Gly-NH-resin was prepared. The Fmoc groups were deprotected and the two α chains with perbenzylated norMurNAc-Abu-D-iGln were built up. The normuramic acid was condensed as benzyl 2-acetamido-4,6-di-O-benzyl-3-O-carboxymethyl-2-deoxy-α-D-glucopyranoside. Then the Dde groups were split off by hydrazine in methanol and the ε chains with Ac or stearyl-Tn-Tn-γ-Abu were prepared. The O-acetyl groups from Tn antigens were split off by hydrazine in methanol and the MAGs were detached from the resin by 95% TFA in CH<sub>2</sub>Cl<sub>2</sub>. The benzylated MAGs were purified, hydrogenolyzed on Pd/C in acetic acid and again HPLC purified. The obtained [norMurNAc-Abu-D-iGln-Lys(X-Y-Y-γ-Abu)]<sub>2</sub>-Lys-Gly-NH<sub>2</sub> (I, X = Ac, Y = serine Tn; II, X = Ac, Y = threonine Tn; III, X = stearyl, Y = serine Tn; IV, X = stearyl, Y = threonine Tn) were studied and characterized by MALDI-TOF MS, NMR and AAA. Compounds with lipophilic stearyl residues were used for incorporation into liposomes. Immunobiological data of all compounds will be presented.

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## PA180 - Synthesis of a mannosyl peptide as an acceptor substrate for a new N-acetylglucosaminyltransferase

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Glycoproteins play an important role in biological processes, such as cell recognition, cell adhesion, immunogenic recognition and so on. In order to study these roles precisely, the syntheses of glycopeptides and their mimics are important. It is known that O-mannosylated glycoproteins, which are a kind of O-glycoprotein, are abundant in the yeast cell wall. Endo and co-workers found a new O-mannosyl glycan in the bovine peripheral nerve glycoprotein called as α-dystroglycan [1]. α-Dystroglycan is an extracellular peripheral membrane glycoprotein and anchored to the cell membrane by binding to a transmembrane glycoprotein, β-dystroglycan [2]. This novel mannosyl glycan contains the Ser/Thr(GlcNAcβ1→2Man) linkage, which is a rare type of protein modification in mammal. We have prepared several N- and O-glycopeptides by solid-phase synthesis using an Mpt-MA method without protection of the carbohydrate hydroxyl groups [3-5]. In the glycopeptide synthesis utilizing the Mpt-MA method, a deprotection step for the carbohydrate moiety that has a risk of side-reaction (e.g., β-elimination, racemization, and so on) can be avoided. In order to investigate a new GlcNAc transferase which transfers UDP-GlcNAc to the Man residue and forms the GlcNAcβ1→2Man linkage, we synthesized a mannosyl peptide [Ac-Ala-Ala-Pro-Thr(Man)-Pro-Val-Ala-Ala-Pro-NH<sub>2</sub>] using Fmoc-Thr(Man)-OH 2 as a building block by the Mpt-MA method. When the UDP-[<sup>3</sup>H]GlcNAc and the neonatal rat brain extract were incubated with the mannosyl peptide 1, the GlcNAc transferase activity was detected. The obtained radioactive mannosyl peptide was characterized and the GlcNAc-Man linkage was elucidated to be GlcNAcβ1→2Man. Furthermore, it is found that this GlcNAc transferase is different from the GlcNAc transferase I (GnT-I) and GnT-II, which are essential to the biosynthesis of complex N-glycans.

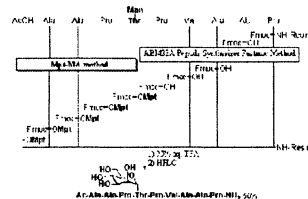


Fig. 1 - Synthesis of mannosyl peptide

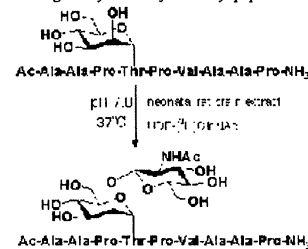


Fig. 2 - The activity of new glycosyl-transferase

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## P A181 - Kinin B1 receptor antagonists with metabolic stability

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The kinin B<sub>1</sub> receptor has been implicated in a variety of pathological states including diabetes, inflammation and painful syndromes. Potent, selective, specific and possibly long lasting antagonists to this receptor are in high demand. In the last three years we have developed several potent B<sub>1</sub> receptor antagonists containing various substitutions intended to protect peptide from enzymatic degradation and increase half-life *in vivo*. This was obtained by protecting from amino peptidases with acetylating or/and replacement of the first amino acid (R-715, R-892, R-955, R-954, R-725, R-724) combined with changes in positions 2, 5, 7 and 8 on bradykinin.

	pA <sub>2</sub>	PA/A	ACE+h plasma
R-732 Lys[Leu <sup>8</sup> ]desArg <sup>9</sup> BK	8.27	PA	+++
R-715 AcLys[D-βNal <sup>7</sup> ,Ile <sup>8</sup> ]desArg <sup>9</sup> BK	8.4	A	+
R-725 D-Lys[D-βNal <sup>7</sup> ,Ile <sup>8</sup> ]desArg <sup>9</sup> BK	7.7	PA	+
R-724 Sar[D-βNal <sup>7</sup> ,Ile <sup>8</sup> ]desArg <sup>9</sup> BK	7.4	PA	+
R-892 AcLys[(αMe)Phe <sup>2</sup> ,D-βNal <sup>7</sup> ,Ile <sup>8</sup> ]desArg <sup>9</sup> BK	8.6	A	0
R-955 AcLys[Oic <sup>2</sup> ,(αMe)Phe <sup>2</sup> ,D-βNal <sup>7</sup> ,Ile <sup>8</sup> ]desArg <sup>9</sup> BK	8.5	A	0
R-954 AcOrn[Oic <sup>2</sup> ,(αMe)Phe <sup>2</sup> ,D-βNal <sup>7</sup> ,Ile <sup>8</sup> ]desArg <sup>9</sup> BK	8.64	A	0

pA<sub>2</sub>: apparent affinity in rabbit aorta; PA: partial agonist; A: antagonist; +++ metabolized; + partially protected; 0 - non metabolized

Compounds were found to be highly potent (pA<sub>2</sub> > 8.0, for R-732, R-715, R-892, R-955 and R-954), pure antagonists (R-715, R-892, R-955 and R-954), protected from degradation by purified ACE and human plasma (R-892, R-955 and R-954). Moreover, *in vivo*, R-954 showed a prolonged duration of action. This compound was found to be active on inflammatory and painful experimental diseases. It is expected to be active in human.

## P A182 - Main-chain length effect on conformation-activity relationship of membrane active lipopeptaibol antibiotics

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*Trichoderma* fungi biosynthesize antimicrobial peptides (peptaibols) that are known as efficient membrane modifiers. Peptaibols are linear peptides characterized by the C<sup>α</sup>-dimethylated α-amino acid Aib and a C-terminal 1,2-aminoalcohol. A sub-class of short-sequence peptaibols (lipopeptaibols) has a fatty acyl chain at the N-terminus replacing the usual acetyl group present in peptaibols. Lipopeptaibols show interesting antimycoplasmic activity.

Here we describe the chemical synthesis *via* fragment condensation in solution, conformational analysis by IR absorption, CD and bidimensional NMR in a number of solvent systems, and membrane permeability measurements and antibiotic properties of the C-terminal L-Leu-OME analogue of trichogin GB IX, the longest sequence among lipopeptaibols. To test the main-chain length effect on conformation-activity relationship, the results were compared with those of the C-terminal L-Leu-OME analogues of shorter sequences (trichodeceniin I, and trichogin GA IV) and the longer sequence -U-(GLUG)<sub>4</sub>-I-L. An additional comparison was made between the trichogin GA IV analogue and the "aneurism" peptide, characterized by a comparable overall main-chain length, but with three UGGL quartets instead of two GLUG quartets.

<i>trichodeceniin I</i> analogue	Dc-G - GLUG - I-L-OME	(7-peptide)
<i>trichogin GA IV</i> analogue	Oc-U-(GLUG) <sub>2</sub> -I-L-OME	(11-peptide)
<i>trichogin GB IX</i> analogue	Oc-U-(GLUG) <sub>3</sub> -I-L-OME	(15-peptide)
<i>longer analogue</i>	Oc-U-(GLUG) <sub>4</sub> -I-L-OME	(19-peptide)
<i>"aneurism" peptide</i>	Oc - (UGGL) <sub>3</sub> - OMe	(12-peptide)

where Dc is *n*-decanoyl, Oc is *n*-octanoyl, U is Aib, and OMe is methoxy. All of the peptides examined, except the shortest homologue (GLUG 7-peptide), are partially helical in solvents like CHCl<sub>3</sub>, CH<sub>3</sub>CN and MeOH. Helical percentage tends to increase with main-chain lengthening. The GLUG 11- and GLUG 15- peptides exhibit the highest membrane-modifying and antibiotic properties, whereas the least active in these two assays are the GLUG 19- and UGGL 12 ("aneurism") peptides. To summarize, although peptide helicity undoubtedly plays a role on bioactivity, helix amphiphilicity seems also to be a significant factor.

## P A183 - Synthesis of glycopeptides from PSGL-1 containing sialyl Lewis X

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Glycoproteins play an important role in cell-adhesion processes. In particular, the ligand for P-selectin, PSGL-1, was shown to carry an oligosaccharide side-chain (sialyl Lewis X/A) within the key N-terminal peptide fragment. [1,2] Our goal was to provide a strategy for the linear synthesis of the sialyl Lewis X carrying N-terminal fragment of PSGL-1. To this end, fully protected sialyl Lewis X azide was converted to the anomeric amine and coupled to Fmoc-protected aspartic acid allyl ester. After removal of all protecting groups except for the neuraminic acid methyl ester the Fmoc group was re-introduced and the resulting building block was used in SPPS on chlorotrityl resin. The procedure for the elongation, cleavage, isolation and purification of the product will be described.

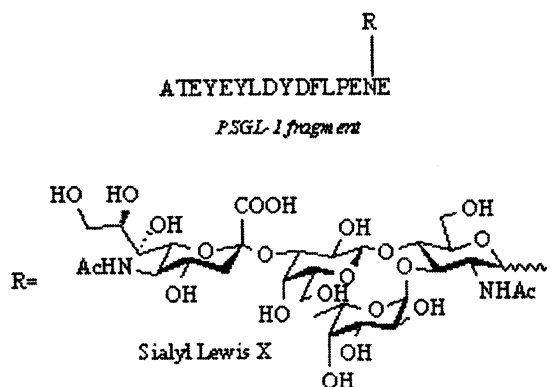


Fig. 1 - PSGL-1 and sialyl Lewis X

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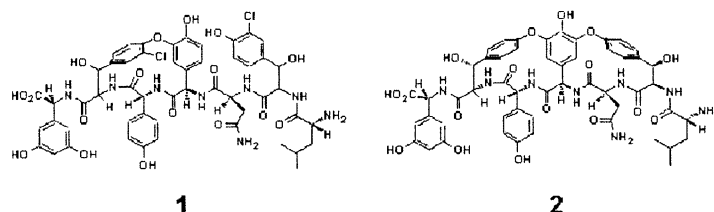
## P A184 - The aglycon formation of tricyclic glycopeptide antibiotics of the vancomycin type from linear peptide precursors

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The glycopeptide antibiotic vancomycin is the most prominent representative of a class of microbial metabolites comprising several hundred compounds. Vancomycin has gained considerable interest due to its function as an antibiotic of last resort for infections of methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Moreover, glycopeptides are versatile phases for chiral separations.

The structural motif, which is characteristic for most glycopeptide antibiotics, is the side-chain bridging of a linear heptapeptide to form two biarylether and one biaryl bond. The ring assembly is performed by the consecutive action of three oxygenases (OxyA/B/C) converting the linear heptapeptide into the tricyclic aglycon, which is then further modified e.g. by glycosylation. Our investigations focused on the isolation and structure elucidation of putative intermediates with different cyclization degrees. In our approach we generated mutants bearing mutations in the respective oxygenase genes (*oxyA/B/C*) of the balhimycin producing bacterium *Amycolatopsis mediterranei*. From these mutants so far unknown mono- (1) and bi-cyclic (2) peptides were isolated, which are key-intermediates in order to understand the stepwise ring assembly of glycopeptide antibiotics [1,2]. We consider these results as representative for all vancomycin-type glycopeptide antibiotics. We assume that similar peptides can be isolated from respective mutants of type III (ristocetin) and type IV (teicoplanin) glycopeptide antibiotics.



Structures of mono- and bi-cyclic glycopeptides isolated from *A. mediterranei* mutants.

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**PA185 - Structure activity relationship of synthetic lipopeptides inducing cell activation through toll-like receptor-2**

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Cell activation by lipoproteins and shorter lipopeptides is induced through their interaction with Toll-like receptors (TLR) representing a molecular link between microbial products and host defense mechanisms. TLRs are type I transmembrane receptors triggering signals which finally lead to the transcription of cytokine genes [1]. Ligands for most of the ten TLRs have been identified, representing predominantly distinct bacterial or viral components. Among others, ligands for TLR2 are bacterial lipoproteins and lipopeptides, which are well known to induce proliferation of B-lymphocytes and to induce cytokine production in monocytes/macrophages. Synthetic analogues of the N-terminal part of these lipoproteins constitute potent immunoadjuvants in vivo and in vitro. Immunisation in the presence of these adjuvants leads to a long-lasting protection against virus challenge and to efficient priming of virus-specific cytotoxic T cells [2]. Potent hapten-specific immune responses in different species were obtained by immunization with a conjugate consisting out of a lipopeptide, a hapten and a haplotype-specific T helper-cell epitope [3]

In this study, a systematic approach was followed to investigate the influence of the different structural elements of lipopeptides with respect to their ability to induce cytokine release via activation of TLR2. To dissect the contribution of the peptide moiety to receptor stimulation, hundreds of fully synthetic combinatorial N-Palmitoyl-S-[2,3-bis(palmitoyloxy)-propyl]-cysteine-peptide collections containing defined amino acids and fully degenerated amino acid positions were synthesized and investigated in cellular assays. Deconvolution led to defined lipopeptides with improved activity. Additionally numerous lipopeptide analogues differing in number, length and structure of amide and ester-bound fatty acids attached to the dihydroxypropylcysteine scaffold were synthesized and investigated for biological activity. The dependence of TLR-mediated cellular responses on the chirality of carbon atom C-2 of the dihydroxypropyl moiety was investigated as well as was the impact of modifications of the dihydroxypropylcysteine core structure. Fluorochrome-labeled lipopeptides have been synthesized and their interaction with the cell surface will be presented.

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**PA187 - HRMAS-NMR Monitoring of the assembly of resin-bound aggregating sequences: effect of the peptide loading, amine group ionisation and the solvent system.**

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The solid phase peptide synthesis method has been intensively investigated as a convenient model for improving the knowledge of physicochemical details that govern chemical processes occurring throughout a polymeric matrix. In this context we have studied the solvation of resins and peptide-resins either through swelling measurements of beads and its relationship with the polarity of the medium [J.Org.Chem. (1996), 61, 8992], or applying the EPR method. In the latter case, resin-bound fragments of the model aggregating (65-74) acyl carrier protein (ACP) sequence have been labeled with a spin probe for investigating mobility details of peptide chains in the resin network [J. Org. Chem. (1999) 64, 9118]. In this case the paramagnetic amino acid TOAC (2,2,6,6-tetramethylpiperidine-N-oxyl-4-amine-4-carboxylic acid), properly derived for labeling peptide sequences was used as probe [J. Am. Chem. Soc. (1993) 115, 11042]. Aiming at extending this study of aggregating sequences in a variety of conditions and with an alternative approach, we herein designed a (HRMAS)-NMR study for monitoring the chain growth of the well known aggregating 10-residue ACP fragment (VQAAIDYNG) bound to a solid support. This effort indeed follows our initial work [J. Magn. Res (1997), 125, 185] where some types of peptide-polymers were comparatively examined towards their physicochemical features using this spectroscopy. To examine the peptide loading effect, BHAR batches with substitution degrees up to 3 mmol/g were used for ACP sequence assembly.

<sup>1</sup>H-NMR spectra of the C-terminal 3-residue fragment (ING) in CDCl<sub>3</sub> and in DMSO-d<sub>6</sub> showed that in low peptide loading condition, improved mobility of chains assigned by peaks at 7-8 ppm range occurred in the former solvent, thus stressing the dominant influence of the apolar polystyrene backbone of the resin. As expected from the increase in the polarity of resin induced by the presence of larger amount of polar peptide bonds, the higher the peptide content of resins, the better the chain mobility in the polar aprotic DMSO. Obviously an opposite effect is observed in the apolar CDCl<sub>3</sub> with the appearance of very immobilized populations of chains. However this solvation rule in DMSO seems to be peptide-content dependent: higher chain mobility is observed in resins with near 30% than with 50% where strong interchain associations occur throughout polymeric matrix. When the solvation study is extended specifically in DMSO to several minor fragments of the larger VQAAIDYNG peptide, the <sup>1</sup>H-NMR monitoring of this 10-mer sequence revealed that (Figure 1) the highest chain mobility occurs at 7th position (Ala) but becomes much more associated after the incorporation of the last residue (Val). The decreasing order of aggregation in DMSO displayed by <sup>1</sup>H-NMR spectra seems to be 3 > 10 > 7. These findings are indicative of a significant sequence-dependent aggregation effect that is aggravated by the very high peptide loading condition, deliberately employed in the present study. Lastly, the influence of the ionization state of the amine group of the ING and VQAAIDYNG sequences were examined towards solvation behavior in DMSO. Relevant for instance in the potential application for the "in situ" coupling procedure where amine function in protonated form is used, the <sup>1</sup>H-NMR data depicted a sequence-dependent influence of the amine group ionization state upon the dynamics of peptide chains. Contrariwise to the higher peptide motion observed with the shorter ING segment, the protonation of the amine group induced significant aggregation in the longer VQAAIDYNG sequence. These results suggest precautions when aiming at improving coupling reactions based upon protonation strategy of the peptide segment to be acylated as is the case of the "in situ" coupling strategy. Taken together these findings stress the highly sensitivity of the (HRMAS)-NMR spectroscopic procedure for revealing chemical physical features regarding clusters of microenvironments spread throughout polymer backbone. Supported by FAPESP and CNPq.

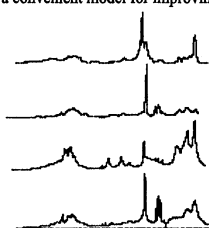


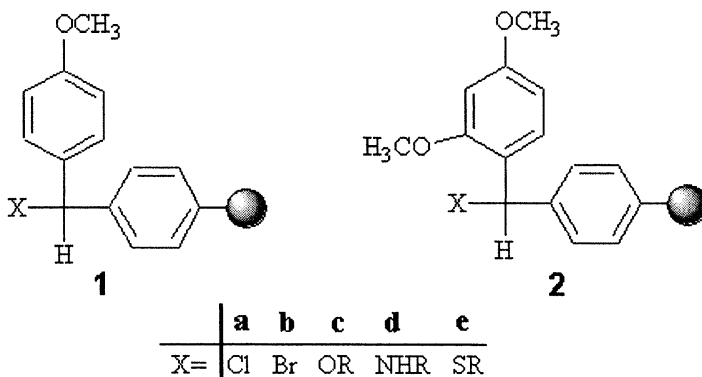
Figure 1. HRMAS RMN 1D spectra at 400 MHz of segments of the acyl carrier protein (65-74) bound to a 3 mmol/g BHAR in DMSO. Spectra were recorded in a 4 mm rotor with ~ 5 mg of peptide-resin.

**PA186 - Application of mono- and dimethoxybenzhydryl halides and amines in solid phase synthesis**

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1. University of Patras/Department of Chemistry - Greece

Amines, alcohols and thiols were attached in high yield onto the 4-methoxy- and 2,4-dimethoxybenzhydryl halide resins. The obtained resin-bound derivatives were tested in solid phase synthesis. In particular, the benzhydrylamin resin **2d** was successfully applied in the synthesis of calcitonin and the anti-HIV peptide T-1249.



**PA188 - Peptide cyclisation using polymer supported reagents.**

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We are currently involved in a project concerning  $\beta$ -sheet forming cyclic peptides and wished to develop an easy cyclisation procedure. To this effect, we used gramicidin S as a model. This antibacterial cyclodecapeptide, cyclo-(Val-Orn-Leu-D-Phe-Pro)<sub>2</sub>, is well known and has been shown to produce an antiparallel  $\beta$ -sheet capped at both ends with a type II'  $\beta$ -turn defined by Val-Pro-D-Phe-Leu residues. The structure is stabilised by four transannular hydrogen bonds between the carbonyl and NH groups of valine and leucine, two of these hydrogen bonds being an integral part of the  $\beta$ -turn.

For the manual solid phase peptide synthesis, we used a preloaded Boc-Orn(Z)-PAM resin, 3 equiv Boc-AA-OH, 3 equiv TBTU, and 6 equiv DIPEA in NMP. Cleavage of the peptide from the resin with concomitant deprotection was effected using the low-high TFMSA procedure. The crude linear peptide was cyclised in solution using various polymer supported diimide derivatives. PS-Carbodiimide was found to be the most efficient. Thus gramicidin S and four new analogues where D-Phe is replaced with D-1-Nal, D-2-Nal, D-4-Pya, and D-Hof were conveniently synthesised.

## PA189 - Combinatorial approach to the design of new grafted hydrophilic polymeric surfaces optimised for peptide synthesis

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Over the past 10 years, the solid phase synthesis of small molecule compounds has emerged from being a curiosity to being a ubiquitous tool for preparing large sets of compounds for lead finding and optimisation in the pharmaceutical industry. Since the mid 1980's, our group has been performing multiple solid phase synthesis of both peptide and small molecule compounds on grafted surfaces (SynPhase™ Crowns and Lanterns). The fundamental advantage of this technology, which is an alternative to beaded cross-linked resins, is the ease of handling large numbers in multiple parallel synthesis. There have been many improvements in the underlying system to allow for increased target diversity, purity and loading. These improvements include the introduction of new graft polymers that are optimised for solid phase organic synthesis. There have been several generations, each with improved surface (hence loading) / volume ratios. Graft polymers have been optimised on the basis of several factors including reaction kinetics, loadings and compatibility with broad chemical and physical reaction conditions. Taking advantage of the multiple parallel handling efficiencies, we have developed a new hydrophilic lantern by systematically varying parameters such as type, concentration and ratio of monomers, radiation dose rate and linker loading. This combinatorial approach to polymer design has yielded a Lantern specifically optimised for peptide synthesis. Examples of peptide/peptidomimetic syntheses including libraries of FRET peptides will be presented.

## PA190 - Synthesis of a new biocompatible resins containing poly(ethylene glycol)-dicarboxylic acid

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The spreading of combinatorial chemistry and of high throughput biological screening methods have greatly extended the claim to develop new biocompatible solid supports. The large number of samples synthesized by split-mix solid phase combinatorial chemistry needs to be tested by high throughput, ideally on-bead biological screening methods for the efficient drug discovery. The known poly(ethylene glycol) containing resins (e.g. TentaGel, ArgoGel, SPOCC) have excellent chemical and swelling properties in biological systems and can be used for on-bead testing. The most of it was made by copolymerisation of styrene and ethylene oxide. The disadvantages of PEG-based resins are the low capacity and the high price due to the advanced technology employed in their production. It was our aim to synthesize a new type of biocompatible resins, which have high capacity and sufficient acid stability for Boc chemistry. We have prepared these solid supports from simple building blocks, including the commercially available PEG-dicarboxylic acid. The starting aminomethyl-PS resin (200-400 mesh; 1.4 mmol/g; 1% DVB) was derivatized with poly(ethylene glycol) dicarboxylic acid (M=600Da). Ethylene diamine and lysine were introduced to extend the PEG dicarboxylic acid chains. The presence of lysine compensated the decrease of resin capacity by increasing PEG chain length. Branched and linear chain resins were prepared with various length of poly(ethylene glycol) blocks attached to the support. Coupling conditions were optimised to obtain the highest resin capacity. The acid stability of the PEG-derivatized resins was determined by cleavage in liquid hydrogen fluoride. Essentially no chains loss was observed. The resins were tested in Boc and Fmoc peptide synthesis. The bioavailability of the resins were assayed by the determination of the efficacy of enzymatic cleavage of a peptide substrate and compared to TentaGel.

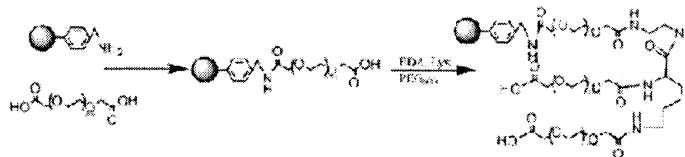


Fig. 1 - Synthesis scheme and structure of the resin

This work was supported by grants from the Hungarian Research Foundation (OTKA No: T 030841) and the Foundation for Hungarian Peptide and Protein Research, Budapest.

## PA191 - Peptide array synthesis on a glass chip using micromirror array (MMA) and protein binding assay

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With recent developments in microfabrication processes and surface modification, on-site analysis, which is a powerful method for diagnosis of diseases, can be achieved by biochips of protein or peptide array. Here, we synthesized HPQ containing pentapeptides on a glass chip and used streptavidin as a model protein to study the binding assay since HPQ containing pentapeptides are well known to bind streptavidin [1]. We have previously reported an efficient method to quantify different binding activities of HPQ containing pentapeptides on a resin bead and found that a spacer between the ligand peptides and the resin matrix is important to reveal maximum binding activities [2]. We performed similar experiments on a chip and proved that peptide array synthesis on a chip is much simpler than SPPS and can save reagents, time and labor to perform the binding assay.

We utilized nitroveratryloxycarbonyl (NVOC) group as a photolabile protecting group for peptide synthesis. The glass surface, which was protected by NVOC group was selectively irradiated by a UV illuminator (360 nm) using micromirror array (MMA.). After removing the NVOC group, we coupled NVOC amino acids successively and synthesized a series of HPQ containing pentapeptides on a chip. After incubating the peptide array chip with FITC-streptavidin, we obtained different intensities of fluorescence images (50x50 μm<sup>2</sup>), which correspond to the binding activities of the peptides to the protein on a chip.

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## PA192 - Chemoselectivity in reductive alkylation on the solid phase

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Secondary amines can commonly be synthesised by reductive alkylation of a primary amine with an aldehyde or ketone. This two-step reaction proceeds via the formation of an imine intermediate, obtained in acidic medium (1%AcOH/DMF) or by using a dehydrating agent (trimethylorthoformate, TMOF), followed by the reduction with either borohydride reagents or a borane-pyridine complex. On solid support, a large excess of aldehyde is used, which may lead to overalkylation.

We are currently investigating the reaction of either amino acids (eg. H-Arg(Pbf)-resin) or dipeptides (eg. H-γ-Abu-Arg(Pbf)-resin) on solid support (PAL-PEG-PS resins) with a range of substituted benzaldehydes. Preliminary results, using either the traditional method 1%AcOH/DMF:NaCNBH<sub>3</sub> or the combination TMOF:DCM:Na(OAc)<sub>3</sub>BH gave a mixture of the unchanged dipeptide, the imine and the amine, which indicate that both the imine formation and the reduction step do not proceed to completion. Using molecular sieves gave the imine quantitatively, however the reduction with Na(OAc)<sub>3</sub>BH was unsuccessful. Increasing the quantity of reducing agent (from 4 to 20 eq.) or the solvent (DMA, DCE, DCM vs DMF) still lead to a mixture of the imine and the amine. Acid catalysis of iminium ion formation to promote reduction will be discussed.

Some sequences were found to give dialkylation with the above one-pot procedures. Therefore we have compared the two-step reaction, where the resins are washed after imine formation before the reduction, with the one-step method and observed that in the former case, no amine was synthesised. Study of the reduction of the peptidic imines, utilising different reducing agents, acid catalysts and conditions, is in progress.





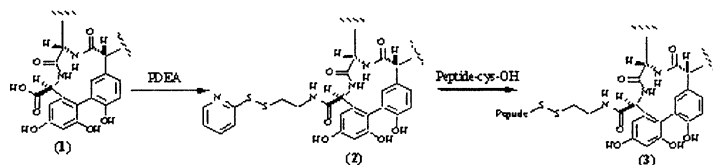
## PA197 - Synthesis and activity of membrane-targeted glycopeptide antibiotics

A. P. Beevers<sup>(1)</sup>, M. Cooper<sup>(2)</sup>, Y. Gong<sup>(2)</sup>, D. F. Brown<sup>(3)</sup>, C. P. Chapman<sup>(1)</sup>, G. Smith<sup>(1)</sup>, R. A. Smith<sup>(1)</sup>, J. R. Betley<sup>(1)</sup>

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2. Department of Chemistry, Cambridge University - United Kingdom
3. PHLS, Addenbrooke's Hospital - United Kingdom

Vancomycin (**1**), a naturally occurring cyclic glycopeptide, is the drug of last resort used to treat many MRSA infections. However it has a significant levels of toxicity and the isolation of a variety of strains of Vancomycin resistant enterococci (VRE) is now common. At Adprotech we have designed a variety of peptides consisting of a hydrophobic N-terminal group (e.g. myristoyl or biphenyl), a positively charged lysine-rich region, and a C-terminal cysteine. A combination of membrane-insertive and electrostatic interactions can direct such peptides to bacterial cell membranes, whilst the free sulfhydryl present in the cysteine sidechain allows for easy derivatisation via pyridyl disulfide-activated exchange. These peptides are conjugated to vancomycin in a simple two-step process starting from commercially available starting materials.

Vancomycin is derivatised specifically and in good yield at the carboxyl terminal with 2-pyridyldithioethylamine (PDEA). The product (**2**) then reacts with membrane-directed peptides in quantitative yield to afford the bioactive conjugates (**3**). Striking decreases in MIC values are observed *in vitro* against a range of VRE strains of varying phenotypes, as well as against a variety of other bacteria. These data are presented in addition to toxicology data and *in vivo* efficacy data against a relevant clinical isolate.



## PA199 - Semi-combinatorial approach for the preparation of linear comb-like glycopeptide dendrimers with Tn antigen using maleimide ligation

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Modular maleimide approach can be effectively used in the preparation of high molecular peptides and artificial proteins. Combination of solid phase synthesis and chemoselective ligation permitting rapid assembly of target compounds in high purity and yield, followed by easy work-up are the major advantages over the classical solid phase approach. Tn antigen [H-Ser/Thr( $\alpha$ -D-GalNAc-1 $\rightarrow$ O)-OH], a marker of human adenocarcinoma, is a potential target for vaccine design and development. A series of glycopeptide dendrimers bearing 1) dimer of Tn antigen (a B-cell epitope) and triglycine spacer and 2) dimer of Tn antigen, triglycine spacer and an antigenic sequence 307-319 derived from *Influenza virus hemagglutinin* (T-cell epitope) of growing molecular weight was prepared in order to study immunological response to this marker in mouse model.

Acknowledgements: This work was supported by the Grant Agency of the Czech Republic No. 303/01/0690 to T.T.

## PA198 - Ligand protection strategies for contact point determinations by Edman degradation of photolabeled peptide receptors.

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The spatial organisation of peptide ligands in their cognate receptors has been the object of many past and ongoing efforts. Classical structure-activity relationship studies, together with more receptor-oriented site-directed mutagenesis strategies, in combination with various *in silico* approaches, are the rule. All those approaches do study implicitly the access pathways of a given ligand and not only its binding locus. One particular technology, photoaffinity labeling, is however a typical binding locus tool and we have applied this tool to peptidergic receptors. The challenge in this technology is however the determination of the exact contact point. Successful ligand contact determination is a powerful tool in receptor proteomics. (Bioch. 39, 9662). Contact point information is however difficult to obtain and, in many cases, it was pure luck and residue-selectivity. Systematic approaches need the isolation of an appropriate receptor protein fragment with the label positioned in the N-terminal part and step-wise Edman degradation for the identification of the labeled residue. The main problem is concomitant digestion of the labeling peptide that may produce release of the radiotracer before the respective label location in the receptor is attained. We have solved this problem by several approaches and present these strategies in the present contribution on the two angiotensin II (AngII) receptors AT<sub>1</sub> and AT<sub>2</sub> with photoactivable and radioiodinated AngII analogues.

In a first approach on hAT<sub>1</sub> the CNBr generated N-terminal receptor fragment 284-334 was to be sequenced and precocious radioactivity release could be prevented by per-acetylation of the labeled receptor prior to fragmentation, identifying pos. 293/294 as contact points. In a second approach on hAT<sub>2</sub>, degradation resistance was obtained through N-terminal quaternisation (betain-structure) or acetylation of the ligand and the fragments to be sequenced were generated through CNBr or Endo-Lys digestion on the labeled receptor.

The information gained allowed the verification and confirmation of *in silico* generated models of both receptors. The AngII molecule is organised in an extended transmembrane orientation in both AT<sub>1</sub> and AT<sub>2</sub> receptors.

Acknowledgements: Research supported through grants from the Canadian Institutes of Health Research.

## PA200 - Orthogonal chemical ligation for biomolecule engineering

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Chemoselective ligation has recently established itself as a method for the synthesis of functional biological macromolecules and also of engineered proteins and peptides. In particular in the native chemical ligation [1], a C-terminal peptide thioester reacts with an N-terminal cysteine peptide to form an amide bond. We [2] have recently described a novel method for the solid phase synthesis of thioesters which is amenable to both Fmoc and Boc chemistries. The method is based on the use of the 3-carboxypropanesulfonamide safety catch linker.

Here we present some applications of this method. First we have focused on the synthesis of tandem repeat antigen molecules useful for the development of peptide vaccines. Our method is based on the use of the Fmoc/t-Bu chemistry, the safety catch linker and the use of a temporary protecting group for the N-terminal cysteine that enabled the repetitive application of the chemical ligation reactions to the synthesis of our target molecule. The modular assembly of synthetic peptides expands enormously the opportunities of chemical synthesis in the field of biomolecules. Second we have applied our method to the production of molecules useful for non-viral gene delivery. The molecules consist of two moieties: a DNA-binding portion and a ligand for receptor targeting, nuclear localization, etc. The precursors were assembled by Boc or Fmoc chemistry and then condensed through a flexible linker by various chemoselective reactions which include thioether bond formation and thioester-mediated amide bond formation (native ligation).

Chemoselectivity was also exploited in the context of DNA based vaccines. The goal was to increase DNA immunogenicity through its selective delivery to the Dendritic Cell compartment, mediated by binding to the mannose receptor. As ligand to this receptor we employed a cluster mannose with a long flexible linker which was ligated to the suitably derivatized DNA binding molecules such as bis-PNA and pyrrole-imidazole polyamides

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## A8 - Ligation chemistry/protein modification

### PA201 - Chemical modification of peptides and proteins for the fabrication of microarrays on semicarbazides glass slides: application to the serodiagnostic of pathogen infections

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Microarrays formed by the covalent binding of unprotected peptides onto a functionalized support have some advantages over the in situ synthesis methods in which no control of the resulting probes can be realized. However, this approach represents a demanding challenge in comparison with the oligonucleotides or DNA immobilization chemistries given the diversity of functionalities displayed by all the amino acid side chains. In this poster we present the site specific ligation of glyoxylyl peptide on semicarbazide glass slide (figure 1). This strategy has been applied to the immobilization of synthetic peptide epitopes for the simultaneous detection of many different pathogen infections in a miniaturized assay. Our preliminary results demonstrate that sensitivity and selectivity is higher than ELISA methods.

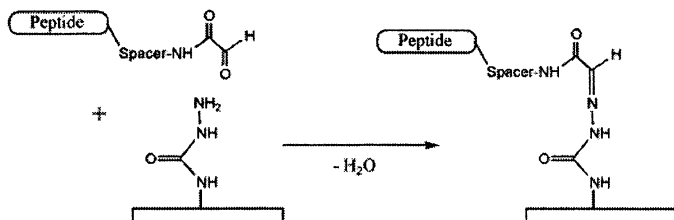


Fig. 1 - Chemical ligation between a glyoxylyl peptide and a semicarbazide glass slide

### PA202 - Chemical construction of the O-linked Sialyl Lewis a - Threonine conjugate liposomes as ligands for the investigation of cell adhesion processes

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The carbohydrate moieties of glycoproteins play key roles in biological selection processes. For investigations of these biological recognition phenomena, glycopeptides of exactly specified structure are required (ligands of cell adhesion components, virus envelope structures or potential tumor-associated antigens). Novel Sialyl Lewis<sup>x</sup> conjugates have been synthesised and evaluated as inhibitors of E- and P-selectin mediated cell adhesion in cell culture assays. In this context, it appears interesting to also include the modified carbohydrate ligand Sialyl Lewis<sup>a</sup> in the investigation of glycopeptide ligands. In contrast to Sialyl Lewis<sup>x</sup> glycopeptides, synthetic glycopeptides with Sialyl Lewis<sup>a</sup> have not been described so far.