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APPLICATION OF THE 4-METHOXYDIPHENYLMETHYL-BROMIDE RESIN IN THE SYNTHESIS OF PEPTIDES AND PEPTAIBOLS

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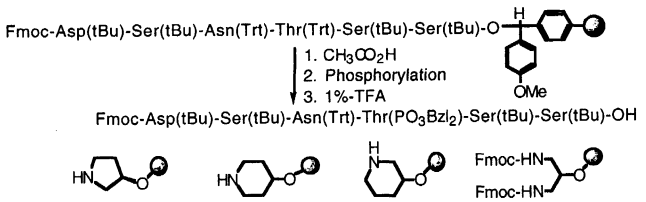
The 4-Methoxydiphenylmethyl resin (MDPR) was tested for various applications of peptide synthesis. The MDPR was proved extremely reactive and several acids, alcohols, thiols and amines were attached in high yield onto the resin. In contrary to the 2-chlorotrityl resin, the attachment of secondary alcohols proceed fast and in high yield, providing resins loaded with >1 mmol secondary alcohol/g. Examples of high loaded resins of some aminoalcohols which could not be attached on the 2-chlorotrityl resin are given below. The acid stability of the ester and ether bond of acids and alcohols to the resin, was determined. It was found that these bonds are cleaved quantitatively with 1-5% TFA within 30 min at room temperature. In contrary, treatment with acetic acid solutions let the bonds intact. This allowed the on the resin selective detritylation of amino acid side chains and their subsequent functionalization, e.g. their global phosphorylation. Subsequently the protected fragments obtained were cleaved from the resin by treatment with 1%-TFA in dichloromethane/triethylsilane (95/5). The cleavage proceeds in good selectivity in the presence of the acid sensitive peptide-resin bond and of the tBu-type amino acid side chain protecting groups. The obtained fragments were purified by HPLC and applied in the convergent synthesis of larger peptides. A synthetic example is given in the scheme.



SEQUENTIAL NUCLEOPHILIC SUBSTITUTION ON HALOGENATED AZAAROMATS: A NOVEL APPROACH TO CYCLIC PEPTIDES AND PEPTIDOMIMETICS

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Cyclic peptides and peptidomimetics that are involved in biological processes are rapidly emerging as important mechanistic probes and drug leads [1]. Based on their planarity, aromatic rings can impose severe conformational constraints on the structure of cyclic peptides. Thus incorporation of aromatic ring systems into peptide backbones displays an effective approach to mimetic drug design and may circumvent obstacles related to delivery and formulation of peptides and peptidomimetics [2].
In the present study we introduce a novel strategy on the generation of such cycles based on the sequential nucleophilic attack of two amino functions of a linear peptide or peptidomimetic onto halogenated azaaromats such as 1,3,5-trichloro-triazine. Graded reactivity of halogen atoms enabled selective and successive substitution by different nucleophiles of the same peptide.
The SPOT-synthesis technique [3] was applied to chemically screen the scope of the procedure to form a wide variety of ring sizes, possible directions of the cyclization reaction and compatibility of the method with side chains of commonly used amino acids. It was found that the method can generally and efficiently be applied for cyclization of peptides and peptidomimetics on solid support and in solution. The remaining halogen atoms at cyclic monochloro-triazinyl peptides not involved in the cyclization process enabled further modifications on the incorporated aromatic system subsequent to the ring closure reaction leading to highly diverse compound libraries.
Therefore, the described method is well suited to systematically vary molecular properties of peptidic and peptidomimetic compounds.

[1] Fairlie, D.P.; Abbenante, G.; March, D.R. *Current Medicinal Chemistry* **1995**, *2*, 654.
[2] Kieber-Emmons, T.; Murali, R.; Greene, M.I. *Current Opinion in Biotechnology* **1997**, *8*, 435.
[3] Frank, R. *Tetrahedron* **1992**, *48*, 9217.

P 51 P 52

Cyclic Tetrapeptides – Novel Scaffolds for Pharmacophore Design.

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Cyclic peptides are useful tools for epitope/pharmacophore studies by allowing a varied display of biologically relevant functionality around a core whose conformational mobility will depend on the size of the ring. Cyclic tetrapeptides are particularly interesting, in this context, providing a rigid 12-membered ring scaffold, with very few reported, naturally occurring or synthetic, examples. Several of these natural products are reported to possess *in vivo* activities, which is rare for small peptides and suggests a potential for therapeutic use.
We have prepared the tetrapeptide, apicidin, by biosynthesis and determined its structure. This structure is consistent with its distinctive chemical reactivity here reported. We also report the chemical synthesis of another naturally occurring cyclic tetrapeptide, and its biological activity.

SOLUTION AND SOLID-PHASE SYNTHESIS OF UREIDOPEPTIDES AND OLIGOUREA/PEPTIDE HYBRIDS

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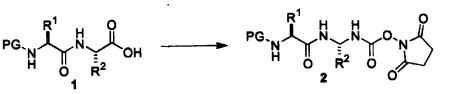
Modification of peptides by introducing a ureido unit ψ[NH-CO-NH] in the backbone is known for more than 20 years. A number of angiotensin analogues containing a urea fragment were prepared between 1975-1980 by the Chipen's group *via* direct condensation of a peptide isocyanate with the free amino group of a second peptide fragment. However, synthesis on solid-support from more appropriate monomeric precursor would be a prerequisite for rapid screening of potentially bioactive ureidopeptides.
We previously described an efficient stepwise synthesis of oligoureas on solid support using *O*-succinimidyl carbamate derivatives as activated monomers [2a]. These building blocks were readily prepared starting from *N*-protected β^3-amino acids via Curtius rearrangement of the corresponding acyl azides and treatement of the resulting isocyanate with *N*-hydroxysuccinimide [2b]. Herein we apply the same strategy to *N*-protected dipeptides **1** which were transformed according to the above procedure to afford the desired *O*-succinimidyl carbamates **2**.



O-succinimidyl carbamates are crystalline, stable and react readily with various amino derivatives to form ureas. These activated intermediates thus represent novel building blocks for the efficient solid-phase synthesis of ureidopeptides and oligourea/peptide hybrids.

[1] Antsan, Y. E.; Chipens, G. I. *Bioorg. Chem.(URSS)* **1975**, 1,998. Mishnev, A. F., Bleidelis, J.; Ancans, Y. E.; Chipens, G. I. *Chem. Abstr.*, **1981**, 95: 195520m.
[2a] Guichard, G.; Semetey, V.; Didierjean, C.; Aubry, A.; Briand, J.-P.; Rodriguez, M. *J. Org. Chem.* **1999**, 64, 8702. [2b] Guichard, G., Semetey, V.; Rodriguez, M.; Briand, J.-P. *Tetrahedron Lett.* **2000**, (in press).

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APPLICATION OF BORNANE-10,2-SULTAM IN ASYMMETRIC AMINO ACIDS SYNTHESIS

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α-Amino acids are among the most important compounds in living organisms. Amino acids are not only constituents of peptides and proteins but they play an important role in many reactions in living cells. There are many excellent methods of the asymmetric synthesis of α–amino acids, but only some of them are versatile. In this work we present an application of one of the most versatile method, the Oppolzer's method [1], for a preparation of noncoded amino acids. The method introduced by Oppolzer et al. [1] is based on an alkylation of a metalated chiral Schiff's base. In their syntheses of amino acids they applied a derivative of glycine attached to bornane-10,2-sultam (the derivative of camphor). The yield of preparation of amino acids by Oppolzer's method (alkylation of the Schiff's base) is very high (>83%) and optical purity of the final product is excellent (enantiomeric excess, (ee) > 95%).
There was a need for analogues of proline, amino acids bearing chromophores (for preparation of analogues of active peptides and conformational studies of peptides by means of fluorescence) and aminophosphonic acids in our laboratory. In our synthesis of proline analogues and aminophosphonic acids first we alkylated the Schiff's base with bromojodo- or bromochloroalkyl of type: X-$(CH_2)_n$-Y, where n = 1Ø4 and (X,Y) = Cl, I or Br, and then we performed basic hydrolysis, and Arbuzov reaction, respectively. For the preparation of potentially useful chromophoric amino acids we used bromo- or iodoalkyls like: p-nitrobenzyl bromide, 2-iodomethyl-naphtalene and 8-iodomethyl-quinoline. We obtained the desired compounds with high yield (> 70%) and optical purity (ee>95%). Full synthetic details and characteristics of the compounds (IR, NMR, optical rotations, photophysical parameters) will be presented.

Supported by Polish Scientific Research Committee (KBN)
[1] W. Oppolzer, R. Moretti, Ch. Zhou, Helv. Chim. Acta (1994)77, 2363

REDUCTION OF METHIONINE SULFOXIDE WITH TETRABUTYLAMMONIUM BROMIDE AND TETRABUTYLAMMONIUM IODIDE: A COMPARATIVE STUDY

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The main problems associated with methionine in solid phase peptide synthesis are alkylation and oxidation to methionine sulfoxide. Carbocation formation in the deprotection of N^{α}-groups with TFA before the coupling reactions and cleavage of the peptide-resin bond using strongly acidic conditions can lead to alkylation at sulphur. On the other hand, it has been observed that oxidation of methionine can be provoked by the presence of oxygen during or after peptide synthesis. Generally speaking, these drawbacks can be overcome by using scavengers or performing the synthesis under an inert atmosphere. In spite of these limitations, methionine-containing peptides can be recovered by heating the alkylated derivatives or reduction of the sulfoxide. However, by-products other than those with alkylated methionine or methionine sulfoxide are formed irreversibly in some cases even after taking the precautions mentioned above.
A solution to these problems is to use methionine sulfoxide, since the deprotection of this amino acid can be carried out at the end of the peptide synthesis. Recently, we have reported on the reduction of methionine sulfoxide containing peptides using NH_4I / Me_2S in TFA and on the scope of application of the method. We describe in this communication our results on the deprotection of the methionine residues with the quaternary ammonium salts tetrabutylammonium bromide and tetrabutyl-ammonium iodide. Unlike NH_4I, full solubility of the salts in TFA was achieved. On the other hand, the cleavage of the peptide from the resin and the methionine sulfoxide reduction were performed in a one-pot process. The suitability of these reagents as an alternative to the use of NH_4I / Me_2S as well as their compatibility with peptides containing sensitive residues is discussed.

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AMINOXY ACID-CONTAINING ANALOGUES OF A HUMAN LEUKOCYTE ELASTASE SUBSTRATE. SYNTHESIS, BIO-ACTIVITY AND STRUCTURE

Laurent Thévenet[a], Regis Vanderesse[a], Michel Marraud[a], Nicole Boggetto[b] and Michèle Reboud[b]
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Chronic inflammatory diseases, such as pulmonary emphysema, are associated with a functional or genetic deficiency of plasmatic inhibitor (α_1-PI) which regulates a serine protease, the human leukocyte elastase (HLE). The Z-Ala^1-Ala^2-Pro^3-Val^4-Ala^5-Ala^6-NH^iPr hexapeptide, a substrate of HLE, is cleaved between the Val^4 and Ala^5 residues. Hydrazinopeptide analogues containing one ψ(CO-NH-NH) amide surrogate have revealed interesting properties (Collet *et al.*, 1998). For example, the [Pro^3ψ(CO-NH-NH)Val^4]-hydrazinopeptide binds HLE without being cleaved.
The α-aminoxy acids are easily obtained from the corresponding α-amino acids and do not require particular N-protection before coupling. Moreover, the ψ(CO-NH-NH) hydrazide and ψ(CO-NH-O) amidoxy links are isosteric amide surrogates which induce quite similar local folded structures of the γ-like turn type. Besides the ψ(CO-NH-O) amidoxy link, an α-aminoxy group also reacts with aldehydes to give the ψ(CH=N-O) oxime link. The latter in turn may be reduced into the ψ(CH_2-NH-O) reduced amidoxy link which is not protonated at the physiological pH.
The amidoxy and oxime links have been successively introduced in the Pro^3-Val^4 and Val^4-Ala^5 positions of the Z-Ala-Ala-Pro-Val-Ala-Ala-NH^iPr hexapeptide using a fragment condensation procedure in the liquid phase. The [Pro^3ψ(CO-NH-O)Val^4]-amidoxy analogue has also been obtained by a recurrent step-by-step synthesis using an oxime resin and isopropylamine as cleaving reagent. The two reduced amidoxy analogues have been synthesized by reduction with $NaBH_3CN$ of the corresponding oxime bond.
Biological tests on the six pseudopeptide analogues and proton NMR experiments are in progress. Preliminary spectroscopic data suggest that the amidoxy link induce a local folded structure. We are presently investigating whether the *trans* or *cis* conformation of the oxime link may be isolated and present any influence on the biological and structural properties.

$EXPO_{3000}$ – A NEW EXPANDABLE RESIN FOR ORGANIC SYNTHESIS AND ENZYMATIC ASSAYS.

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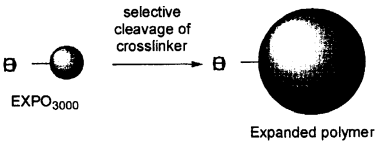
A new resin for solid-phase chemistry has been developed which combines the best properties of the low-swelling polystyrene resins and the water compatible poly(ethyleneglycol) (PEG)-based resins.
Cationic copolymerization of [bis(3-methyl-oxetanylmethyl)]-PEG_{3000} and a highly functionalized core with oxetanylgroups (crosslinker) gave the $EXPO_{3000}$ resin (EXpandable POlymer) in high yield which displayed low swelling in all solvents.



$EXPO_{3000}$ can be used for organic solid-phase synthesis because of the high concentration of reactive sites due to the low swelling. The crosslinker is stable to most organic reaction conditions and after synthesis a selective expansion of the resin allow screening in enzymatic assays. It has previously been shown that intermediate sized enzymes are compatible with the PEG-backbone of the resin and they easily penetrate the expanded polymer because of the large swelling (>15 mL/g).

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SYNTHESIS OF TYROSINE SULFATE-CONTAINING PEPTIDES USING THE MASKED PHENACYL ESTER RESIN

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Solid phase synthesis of peptides containing multiple tyrosine sulfate residues is still one of the difficult problems. Since no practical protecting group for the strongly acidic sulfate group is available, incorporation of 9-fluorenylmethyloxycarbonyl (Fmoc)-tyrosine sulfate residue has been done using its barium and sodium salts. However, preparation of these building blocks is of low reproducibility and difficult to scale up. Recently, we found that isolation of Fmoc-tyrosine sulfate in the form of tetrabutylammonium (TBA) salt dissolved all these problems. Fmoc-Tyr(SO_3TBA)-OH with good solubility property in organic solvents can be activated in the usual manner in a reaction vessel. Usefulness of this new building block has already been shown through solid phase syntheses of phytosulfokine α [H-Tyr(SO_3H)-Ile-Tyt(SO_3H)-Thr-Gln-OH] containing two tyrosine sulfate residues (the 36th Japanese Peptide Symposium, Kyoto, 1999). These syntheses were successful using a super acid-labile trityl linker resin. However, use of the trityl-type resins restricts freedom of selection of side chain protecting groups especially for synthesis of protected peptide segments containing tyrosine sulfates. To this problem and to further improve the stability of the sulfate moiety during the peptide release from the resin support, a new anchoring bond with complete orthogonality with the trityl-type side-chain protecting groups is necessary. Recently, we also found that the base labile phenacyl ester group was stable enough to be applicable to the Fmoc synthesis when its carbonyl group was protected as acetal or dithioacetal. This idea was extended to development of a masked phenacyl linker, 4-(2-(1-hydroxyethyl)-1,3-dithiacyclohex-2-yl)-phenoxyacetic acid (HETPA) (First International Peptide Symposium, Kyoto, 1997). The dithioacetal masking can be easily removed by treatment with iodine/2,6-lutidine in aqueous tetrahydrofuran to generate a photolabile α-methylphenacyl linkage. Since work-up after photolysis (350 nm) of the resin suspension in *N,N*-dimethylformamide was simple, synthesis of the phytosulfokine α using the HETPA linker resin gave a better result than that obtained using the trityl linker resin.

A SYNTHETIC STRATEGY TOWARD CONSTRAINED HEAD-TO-TAIL CYCLOPEPTIDES LIBRARIES BY AMINO ACID SIDE CHAIN ANCHORING TO TRITYL RESINS

Jacques Uziel,[1,2] Maria C. Alcaro,[1] Giuseppina Sabatino,[1] Mauro Ginanneschi,[1] Mario Chelli,[1] Paolo Rovero,[3] and Anna M. Papini,[1] from [1]Dipartimento di Chimica Organica "Ugo Schiff" and CNR-CSCEA, Università degli Studi di Firenze, I-50121 Firenze, Italy; [2]Unité mixte Université de Cergy-Pontoise-ESCOM FRE CNRS, 2126 Synthèse Organique et Chimie Organométallique, F-95031 Cergy-Pontoise, France; [3]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, I-84080 Fisciano (SA), Italy.

Cyclic peptides are important tools in medicinal chemistry because they exhibit improved metabolic stability and increased potency, receptor selectivity and bioavailability. Their constrained geometry allows conformational investigations.
We recently set up the synthesis of Fmoc-Xaa(Trt-resin)-OAl (Xaa = His, Asp), where tri-functional amino acids are anchored to the solid support *via* side-chain. We previously used these building-blocks to obtain cyclodi-, tetra-, and hexapeptides [Papini *et al.*, *Tetrahedron Lett.* 40 (1999) 809-812]. We succeeded the synthesis of cyclo(-His-Gly-)$_2$ (usually prone to cyclodimerization) performing the cyclization step with a low level of resin substitution, according to the *pseudo-dilution* principle.
We have now optimized the solid-phase synthesis and head-to-tail on resin cyclization of several constrained cyclic peptides from 2 to 7 residues, using the three-dimensional orthogonal Fmoc/*t*Bu/OAl strategy on a trityl resin. We used Fmoc-Xaa(Trt-resin)-OAl (Xaa = Lys, Glu, Ser, Cys, Trp, Tyr) to obtain a series of cyclotetra-, penta- and hexapeptides containing the RGD sequence (Arg-Gly-Asp). This is a well-known tripeptide sequence able to bind to the glycoprotein GPIIb/IIIa, a membrane protein that mediates the aggregation of platelets. Different synthetic pathways for some cyclic RGD-containing peptides, interesting for their potential antithrombotic effects, are described in the literature. With the aim of verifying the validity of our cyclization strategy we also synthesized cyclo(-Arg-Gly-Asp-Phg-). The difficulty of this synthesis is widely demonstrated by the different results obtained *via* various approaches up to now described [Nishino *et al.*, *Tetrahedron Lett.* 33 (1992) 1479-1482; Richter *et al.*, *Tetrahedron Lett.* 35 (1994) 5547-5550].
Finally, libraries of cyclopenta- and hexapeptides of general sequences cyclo(-Xaa-Xaa-Arg-Gly-Asp-) and cyclo(-Xaa-Xaa-Xaa-Arg-Gly-Asp-) can be built following our synthetic methodology.

P 59 P 60

COUPLING REAGENTS: ON THE USE OF $Cu(OBt)_2$, $Cu(OAt)_2$ AND HOBt-Cl IN SOLID-PHASE PEPTIDE SYNTHESIS

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Stereochemical control is an essential requirement for the successful assembly of peptides in both solution and solid phase methods. The loss of configuration of an amino acid residue is one of the most important side-reactions that occurs in peptide synthesis and gives rise to considerable problems regarding purification and evaluation of the desired compounds. The use of potent activation reagents for performing the coupling steps often increase the occurrence of these racemic species.

The present communication introduces the use in the solid-phase strategy of Cu(II)-based complexes [$Cu(OBt)_2$ and $Cu(OAt)_2$], as well as HOBt-Cl as suppressers of racemization. Cu(II)-based complexes have been recently developed by Blodgett *et al.*[1] and shown to reduce racemisation in solution peptide segment coupling involving DIPCDI. HOBt-Cl was first introduced by König and Geiger[2] at the same time that HOBt, but it has not received much attention.

The usefulness of these compounds will be demonstrated and evaluated through different known models frequently applied in literature, which are known to be prawn to induce racemization. In special, $Cu(OBt)_2$ has showed very efficient in the suppression of racemization in the solid-phase peptide assembly in the reverse *N*→*C* direction, using a Cl-Trityl resin and allyl ester as a temporary protecting groups.

We thank to Yoav Luxembourg for his interest in this work.

[1]J.K. Blodgett, N.M. Brammeier, J.C. Califano, C. Devin and J. Tolle, in *16th American Peptide Symposium*, Minneapolis (MN), Juny 26-July 1 (1999), poster # 039; [2]W. König and R. Geiger. Chem. Ber. 103, 788 (1970).

SYNTHESIS OF A BOC-PROTECTED ALDEHYDE USING AN FMOC-BASED STRATEGY

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In this work, an Fmoc-based strategy was developed useful for the synthesis of Boc-protected aldehydes. Application of this protocol for the synthesis of Boc-Ala-Glu-Val-Asp-H will be described in detail.
In the first step, the protected alcohol derivative was obtained after Fmoc-solid phase synthesis in the presence of benzyl ester protected carboxy groups on our polymeric diphenyldiazomethane(PDDM)-resin. In the next step the protected alcohol derivative was cleaved from the resin and subsequently oxidized to the corresponding aldehyde. Finally, selective deprotection of the ester functionalities furnished the desired product in good yield and excellent purity.

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Synthesis of orthogonally protected α-alkyl amino glycines (A3G)

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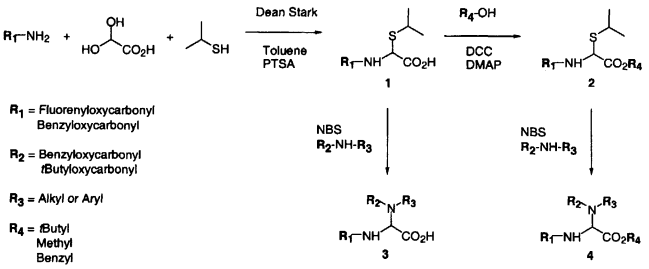
We have developed a three step synthesis of orthogonally protected A3G. α-alkylthioglycines were first obtained in a three component reaction using simple and cheap chemicals commercially available [1].
Following N-Bromosuccinimide (NBS) treatment, these new N-acyliminium ion precursors (**1** or **2**) react with N-substituted carbamates to afford the corresponding protected A3G (**3** or **4**) as a mixture of enantiomers.
The yield of this reaction depends on the type of carbamate $\mathbf{R_2}$ and on the nature of the nitrogen substituent $\mathbf{R_3}$.



The application of A3G in peptide synthesis will be discussed.

1. D. QASMI, L. RENE and B. BADET. *Tetrahedron. Lett.*, **1993**, *34*, 3861-3862.

Posters: topic A2

P 62 Peptides Conjugates: Lipo-, Glyco-peptides and Chimeras P 63

A NEW SYNTHETIC STRATEGY FOR THE PREPARATION OF GLYCO-PEPTIDE CONJUGATES

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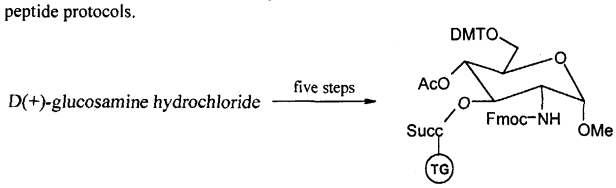
Glycoconjugates are the most functionally and structurally diverse molecules in nature and it is now well established that protein- and lipid-bound saccharides play essential roles in many molecular processes impacting eukaryotic biology and disease. Glycosylation of peptides and other potential therapeutic agents is a promising approach in rational drug design. The presence of a sugar moiety is believed to influence the transport of the drug through biological membranes as well as to increase its resistance to hydrolytic enzymes. The synthesis and modification of oligosaccharide units and their coupling with appropriate lipids, phospholipids and proteins are essential to extend the knowledge on the molecular mode of action of glycoconjugates and, furthermore, to derive new principles of physiological activity. A large number of solution studies for the formation of the O-glycosidic linkage between the sugar and the amino acid has been reported. On the contrary, very few data are available in the literature for the more appealing solid phase methods. We here describe a fully automated synthesis of peptide-glycoconjugates. The proposed synthetic strategy exploits a new Tentagel support derivatized with the first amino sugar unit functionalized to allow standard Fmoc peptide protocols.



DMT = 4,4'-dimethoxytriphenylmethyl, Fmoc = fluorenylmethoxycarbonyl, Ac = acetyl, Succ = succinyl, TG = Tentagel

[Supported by CNR and MURST]

NEW PEPTIDE CONJUGATES AS LIGANDS FOR CHOLECYSTOKININ RECEPTOR.

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Several human tumors overexpress receptors for small regulatory peptides, an observation which has led to a number of clinical applications in the diagnosis and treatment of tumors. Among the receptors, the cholecystokinin type A and type B receptors have been extensively studied in order to have molecular probes for the diagnosis of several human tumors.
Based on the structural NMR characterization[1] of the interaction between the type A cholecystokinin receptor and the endogenous CCK-8 ligand, we synthesized new peptide conjugates able to bind radiometal such as ^{111}In for the development of new diagnostics in nuclear medicine and paramagnetic Gd(III) ions for the obtainment of specific and selective contrast agents in magnetic resonance imaging (MRI).
In the first case a porphyrin macrocycle was covalently bound to the N-terminus of CCK-8 through a lysine residue. This macrocycle allowed the coordination of stable indium for preliminary structural evaluation of the compound by NMR techniques. The preserved structure of the In(III)-porphyrin CCK-8 conjugate, as compared to the native CCK-8 peptide, will serve as a basis for NMR characterization of receptor-ligand interaction.
In a similar way CCK-8 peptide was functionalized with the DO3A macrocycle and the compound was able to coordinate Gadolinium ion. Actually, we are testing the ability of paramagnetic molecular complex to enhance the water proton relaxation rate as a function of pH.

[1] M. Pellegrini and D. F. Mierke *Biochemistry* 1999, *38*, 14775-14783.

P 64 P 65

ANTIMICROBIAL INSECT GLYCOPEPTIDES: SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF DROSOCIN AND APIDAECIN ANALOGUES

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The remarkable resistence of insects to bacterial infection is at least in part explained by their ability to rapidly synthesizing a battery of small sized cationic peptides. Over 50 inducible insect antibacterial peptides have been characterized. They can be grouped in at least four families, namely: a) cecropins, b) insect defensins, c) glycine-rich peptides and d) proline-rich peptides. Remarkably some of the inducible antibacterial proline-rich peptides are glycosylated. **Drosocin**, a 19-residue Pro-Arg rich peptide identified in the hemolymph of infected larvae or adults of *Drosophyla*, exists in two glycoforms bearing either a disaccharide or a monosaccharide α-O glycosydically linked to the Thr[11] residue. Previous studies have shown that glycosylation is very important for maximal antimicrobal activity. A significant sequence homology with drosocin is shown by ***apidaecins***, a family of 18-residue peptides first isolated from honey bees and, like drosocin, predominantly active against Gram-negative bacteria. Apidaecins contain up to 33% of proline, with characteristic Pro-Arg-Pro and Pro-His-Pro motifs but they lack the O-glycosylated threonine residue. For both peptides a similar antibacterial mechanism requiring stereospecific recognition of target molecules has been proposed. In order to study how modifications of the carbohydrate mojety can affect the biological activiy of these antimicrobial peptides, we synthesized some drosocin analogues differing from the parent compound either by the sugar residue or by the type of glycosidic linkage. Moreoever, to mimic the drosocin features and to test the effect of glycosylation on the apidaecin antibacterial activity, we prepared an analogue containing an extra residue, either glycosylated or not, in the middle of the sequence. Minimal inhibitory concentration (MIC) *in vitro* assays indicate that the spectrum of activity of the glycosylated analogues of drosocin, against several Gram-negative strains, is similar to that of the natural monoglycosylated peptide. The apidaecin antimicrobial activity is totally abolished by the insertion of the extra Thr residue but glycosylation re-establishes, at least partially, the activity and the [*endo* GalNAcαO-Thr[13a]]-apidaecin possesses some activity against Gram-negative bacteria.

BIOTIN 2-NITRO-4-SULPHOPHENYL ESTER SODIUM SALT - USEFUL REAGENT FOR PEPTIDES AND BIOPOLYMERS BIOTINYLATION

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N-succinimidyl and 4-nitrophenyl esters of biotin are widely used for peptides and biopolymers biotinylation. However, a number of problems arise while application due to their poor solubility in water solutions. At the same time usage of organic solvents causes degradation of biopolimers structures (irreversible denaturation of proteins, for example). We propose to use the 2-nitro-4-sulphophenyl ester of biotin (BtONsp), which allows to introduce biotin into peptides and biopolymers in water buffer systems at pH 6-10. BtONsp is stable to hydrolysis at this pH range. Moreover, its usage allows to control a degree of biotin introduction by spectrometric measurement of the liberated 2-nitro-4-sulphophenol. Our experiments demonstrated that biotinylation by BtONsp has the same effectiveness as by N-succinimidyl ester of biotinyl-6-aminocaproic acid. Immunoglobulin conjugates, obtained with BtONsp, have low baseline in enzyme immune assay.

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P 66 Peptides Conjugates: Lipo-, Glyco-peptides and Chimeras P 67

SYNTHESIS, CONFORMATION AND MEMBRANE ACTIVITY OF PEPTAIBOLINS

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Peptaibolin (**1**), a new peptide antibiotic isolated from *Sepedonium* fungal strains, is unusual in that its sequence is characterized by only four amino acids. Nevertheless, **1** shows antimicrobial activity against Gram-positive bacteria and yeasts, although to a moderate extent.

1 Ac-L-Leu-Aib-L-Leu-Aib-L-Fol

2 *n*Oct-L-Leu-Aib-L-Leu-Aib-L-Fol

3 Ac-L-Leu-Aib-L-Leu-Aib-L-Phe-OMe

4 *n*Oct-L-Leu-Aib-L-Leu-Aib-L-Phe-OMe

(where *n*Oct is *n*-octanoyl, Aib is α-aminoisobutyric acid, and Fol is phenylalaninol)

We have synthesized by solution methods peptaibolin (**1**) and its three analogues **2-4** and we have investigated their preferred conformation by FT-IR absorption and ^{1}H NMR. The X-ray diffraction structure of **4** has been solved. Four analogues, each characterized by a double Aib replacement with L-Iva (isovaline), were also prepared and their 3D-structure compared to that of parent compounds **1-4**. All eight peptides are folded in a well developed 3_{10}-helical conformation in structure-supporting environments. However, only the N-octanoylated peptides exhibit significant membrane-modifying properties.

A NOVEL CHEMICAL APPROACH TO GLYCOSYLATED SULFOPEPTIDES

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Tyrosine O-sulfation and N- or O- glycosylation are two important posttranslational modifications found in mature proteins, involved in diverse biological functions such as modulation of protein folding, intra- and intercellular trafficking, receptor binding and cell signaling. Recently, chemoenzymatic glycosulfopeptide assembly has become increasingly important [1] although chemical synthesis is still preferred when larger amounts of material are required for biochemical studies. In the search of potential inhibitors for the first step in the cell adhesion cascade, a novel strategy was developed for the chemical synthesis of glycosylated sulfopeptides resembling a region of P-selectin glycoprotein ligand-1 (PSGL-1), a dimeric membrane mucin involved in selectin mediated cell adhesion. Evidence suggests that the N-terminus of the extracellular mature PSGL-1 is important for high affinity binding to P-selectin with a pivotal role in recognition of at least one tyrosine sulfate residue either at position 46, 48 or 51 and a glycosylated threonine within that segment. Here, we present a straightforward approach to glycosulfopeptides based on a combination of acid labile protecting groups and resin and the chemoselective oxime bond formation for the 'post-synthetic' introduction of the carbohydrate moiety. The sulfated N-terminal peptide sequence was assembled using Fmoc-Tyr(SO_3Na)-ONa as building block [2] and functionalized selectively at ε-NH_2 lysine with an aminooxy acetic acid moiety for the subsequent ligation of a reducing sugar in aqueous solution [3]. In contrast to the inherent heterogeneity of post-translational modifications of recombinant proteins and the intrinsically low quantities of glycosylated sulfopeptides obtained in vitro by the chemoenzymatic approach applying sulfo- and glycosyltransferases, this chemical strategy offers the advantage of (i) insertion of Tyr sulfate residues and glycans at defined positions, (ii) rendering complex protection/deprotection schemes superfluous and (iii) generation of sugar libraries for a systematic investigation of the role of glycans in molecular recognition processes.

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P 68 P 69

SYNTHESIS OF NEW MDP OR nor-MDP CONJUGATES WITH BATRACYLIN DERIVATIVES AS POTENTIAL ANTITUMOR AGENTS

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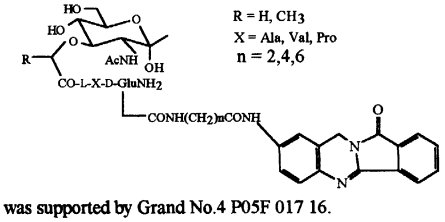
The batracylin synthesized by Bayer's undergoes preclinical and toxicological testing in NCI (Bethesda, USA) as an antitumor agent. This compouds administered to mouse exhibites high activity against such tumor as leukemia P388, colon 38 and adenocarcinoma 38. Up to now several batracylin analogues have been synthesized with modification in position C8 (Cl, Br, NO_2, CH_3, NH_2) and C7 (Cl). However these analogues are sparely water soluble. In the previouse papers we showed that coupling of some antitumor agents to muramyl dipeptide (MDP) increased their antitimor activity. We have prepared conjugates of batracylin derivatives with MDP or nor-MDP (nor-muramyl dipeptide) by forming a covalent amide linking at the C-terminal isoglutamine (Fig.1). The modification caused its better water solubility and we hope also positiv influence to the antitumor activity. The acylation reaction was performed in DMF in the presence of DPPA (diphenyl azidophosphate) and TEA (triethylamine). Batracylin was sythesized with small modification according to the method propouse Rservear's and Wilshire's. The procedure started from p-nitroaniline and *N*-hydroxy-methylftalimide. The final products were purifed using radial chromatography. The amino acid composition of the conjugates was confirmed by an elemental analysis, NMR (500 MHz) spectra and by the TLC qualitative amino acid analysis. Our new analogues will be send to NCI (Bethesda) for in vitro screening on cytotoxic activity.

Fig.1



This work was supported by Grand No.4 P05F 017 16.

SYNTHESIS OF C-LINKED SUGAR-LIPOAMINO ACID CONJUGATES

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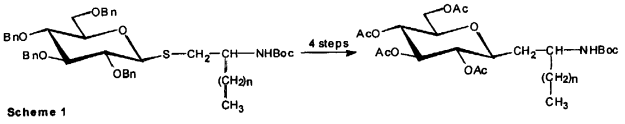
Many drug molecules are too hydrophilic to cross biological membranes and suffer from chemical and/or enzymatic degradation within the gastrointestinal tract. One approach to this problem is to increase the lipophilicity of such compounds by conjugating them to a hydrophobic moiety. We have been interested in the use of lipoamino acids to enhance the membrane transport of poorly absorbed drugs and peptides. Whilst this approach has been shown to enhance oral uptake, solubility in aqueous media may be compromised. This can often be overcome by incorporating one or more sugar units to the molecule, thus forming glycolipids. We now report the synthesis of a series of C-linked glycolipids, using thioglycosides as precursors. Thioglycosides were synthesised from β-1-thiosugars and lipoamino alcohols using a Mitsunobu reaction in excellent yields. After protecting group manipulation, these conjugates were converted to the corresponding sulphone which can then be transformed to an alkene by a Ramberg-Backlund rearrangement. Once reduced and re-protected, the target C-glycosides were produced with β-configuration in very good yields.



Scheme 1

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P 70 Peptides Conjugates: Lipo-, Glyco-peptides and Chimeras P 71

LOLIGOMERS AS PEPTIDE-BASED DELIVERY AGENTS

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Peptide scaffolds incorporating drugs and cell localization signals offer novel solutions to the challenge of improving the efficiency of drug delivery to cells. In particular, a polylysine scaffold can be rapidly assembled on a solid phase support and provides a practical starting point for designing delivery vehicles. Using this strategy, we have constructed branched peptides known as loligomers that serve as multi-tasking, peptide-based shuttles able to penetrate cells and self-localize into distinct cellular compartments. Each branch of a loligomer carries peptide signals that guide their import and localization into cells. Microscopy and flow cytometry studies performed on cell lines have confirmed the vectorial delivery of nucleus-directed loligomers into cells. Examples of the potential of loligomers to act as carriers for "cargos" such as cytotoxic groups, peptides or macromolecules have also been recently reported. The uptake by loligomers of large molecular entities such as plasmids was demonstrated using vectors harboring reporter genes suggesting that such constructs can act as non-viral transfection agents. The photodynamic probe chlorin e_6, a low-molecular weight agent, was also introduced into a nucleus-directed loligomer during synthesis, resulting in a construct with a 40- to 400-fold molar enhancement in potency as a light-activated cytotoxic agent. We have now designed short peptide motifs able to "home" loligomers to breast cancer cells. Alternate assembly scaffolds are also being envisioned to introduce more complex functional signals. Future challenges lie in identifying signals that will optimize cellular routing mechanisms and cell targeting events. [Funded by NCIC and PENCE]

E.coli 0157:H7 - A Combinatorial Approach to Glycopeptide Therapeutics

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E. coli 0157:H7 produces shiga-like toxins (SLT's) which are comprised of AB_5 subunits. The enzymatically active A-subunit (~30 kDa) has N-glycanase activity towards ribosomal RNA and is responsible for inhibition of protein synthesis. There are also five noncovalently associated receptor binding B-subunits (7.5 kDa per unit). All cells that are susceptible to SLT's express αGal-(1-4)-βGal-(1-4)-βGlc-ceramide (**1**) on their surface, with which the B-subunit of SLT's interact. The minimum component for interaction of sugar and protein is αGal-(1-4)-βGal-OMe, hence the synthesis of mimics (**2**) should provide potential inhibitors of the SLT cell adhesion. Two possible sites of modification have been identified from the crystal structure of the toxin-saccharide complex, which indicates specific channels on the protein surface near to the carbohydrate binding sites. Two of these channels correlate to the positions indicated on the modified reducing terminal galactose of galabiose(**2**).

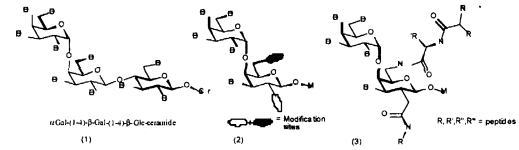

Figure 1 : Gb_3 (**1**), Modification sites on galabiose (**2**), Glycopeptides (**3**).

Our approach involves the synthesis of template (**2**) and its modification at two sites with short peptides in a combinatorial fashion.

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P 72 P 73

SUGAR AMINO ACIDS: SYNTHESIS AND NMR-STRUCTURAL ANALYSIS OF LINEAR AND CYCLIC HOMOOLIGOMERS

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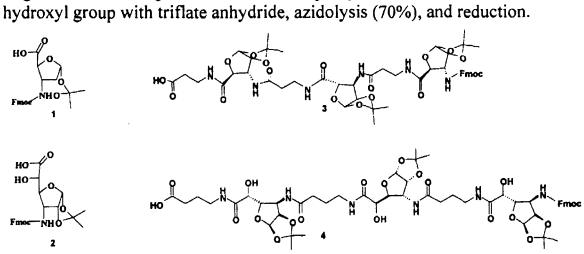
Sugar Amino Acids (**SAAs**) are sugar moieties containing at least one amino as well as at least one carboxyl group. In this work we studied them mainly as structural templates in respect to their ability to induce new, potentially useful structures for peptidomimetic drug design. They can be used as substitutes for conventional amino acids or peptide fragments [1]. Sugar amino acid monomeres **1**, **2** were synthesised starting from diacetoneglucose. The amino group was introduced via activation of the hydroxyl group with triflate anhydride, azidolysis (70%), and reduction.



Using standard solid phase coupling procedures **1** and **2** were alternatively coupled with β-alanine or GABA to form trimers up to hexamers (**3**, **4**). The solution structure in DMSO and pyridine were investigated by 2-D NMR techniques as well as by CD-spectroscopy.

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SYNTHESIS OF MUCIN-TYPE GLYCOPEPTIDE

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Glycoproteins play important roles in biological processes, such as cell-cell interaction, cell adhesion, immunogenic recognition, and so on. In order to study these functions of oligosaccharide moieties, synthesis of glycopeptides and their mimics are important. Recently we have reported the chemo-enzymatic synthesis of N-glycopeptides by the combined method of the solid-phase synthesis of N-glycopeptide having N-acetyl-D-glucosamine (GlcNAc) using the corresponding dimethylphosphinothioic mixed anhydrides (Mpt-MAs) and transglycosylation using endo-β-*N*-acetylglucosaminidase from *Mucor hiemalis* (Endo-M). In accordance with this synthetic strategy we synthesized eel calcitonins having several kinds of natural oligosaccharides on Asn residue [1]. In this paper we describe a new chemo-enzymatic synthetic methodology for mucin-type glycopeptide (O-glycopeptide) similar to those reported for N-glycopeptide. This synthetic strategy consists of three steps; 1) chemical synthesis of Fmoc-Thr(Gal-GalNAc)-OH, 2) solid-phase synthesis of glycopeptide having disaccharide (Gal-GalNAc) without protecting the hydroxyl functions of sugar moieties, and 3) enzymatic sialylation by use of sialyltransferase. First, we tried the synthesis of a key intermediate of mucin-type glycopeptide, Fmoc-Thr(Gal-GalNAc)-OH by the usual chemical manner. Next, we chose HIV protease dimerization inhibitor (Ac-Thr-Val-Ser-Phe-Asn-Phe-OH) [2] as a target model peptide, and synthesized the mucin-type glycopeptide analog having a disaccharide residue, Gal-GalNAc, by means of solid-phase manner (Fmoc chemistry) without protecting the hydroxyl functions of sugar moiety using dimethylphosphinothioic mixed anhydride method [3]. Finally, incorporation of sialic acid (*N*-acetylneuraminic acid) to the glycopeptide having Gal-GalNAc using sialyltransferase in the presence of CMP-NANA (cytidine 5'-monophospho-*N*-acetylneuraminic acid). Sialyltransferase is an enzyme to synthesize sialooligosaccharide from CMP-sialic acid. Purification by RP HPLC gave the desired mucin-type glycopeptide, which was containing 6 amino acids and 3 sugars, in 97 % yield (overall 32 % yield based on the amount of the Phe content of starting resin).

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Posters: topic A2

P 74 Peptides Conjugates: Lipo-, Glyco-peptides and Chimeras P 75

A NEW SYNTHESIS OF C-GLYCOSYL-AMINO ACIDS. INCORPORATION INTO PEPTIDES

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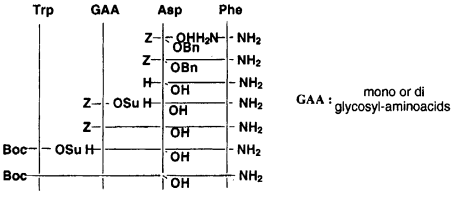
C-Glycosyl-amino acids are of high interest to biologists and organic chemists. They are more stable than natural O- and N-glycosyl-amino acids and they are interesting building blocks for stereoselective synthesis[1]. We propose a new approach to mono and di-glycosyl-aminoacids. Some of them were incorporated into the C-terminal tetrapeptide of cholecystokinin (CCK) replacing methionine. Biological activities if the resulting analogs at both CCK-1 and CCK-2 receptors are under investigation.



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CONJUGATES OF NEUROPEPTIDE Y AS CARRIER FOR TUMOR TREATMENT

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Among other compounds, peptides have an increasing potential for being used as carrier for tumor diagnosis and treatment. They bind to specific receptors and enter the cells by internalisation of the ligand-receptor complex. Furthermore, they are easily modified without significant loss of activity.
Neuropeptide Y (NPY) has been chosen as model peptide. NPY is a 36-amino acid peptide of the pancreatic polypeptide family. It is expressed in the peripheral and central nervous system, and is one of the most abundant neuropeptides in the brain. Furthermore, NPY receptors are produced in a number of neuroblastoma tumors and the thereof derived cell lines.
In the first approach, daunorubicin has been covalently linked to NPY. Daunorubicin is a widely used antineoplastic agent in tumor treatment. However, toxic dose-related side-effects limit its clinical application. In order to increase the therapeutic index of this drug, peptide conjugates of daunorubicin and NPY have been prepared using different spacers. Receptor affinity of the new compounds were determined in NPY displacement assays by using ^{3}H-propionyl-NPY and the human neuroblastoma cells SK-N-MC, which selectively express the Y_1 receptor subtype. Cytotoxic activity was tested by a XTT-based colorimetric cellular cytotoxicity assay. The effects of the prepared peptide conjugates and daunorubicin were compared. Confocal laser microscopy was used in order to gain further insights into the interaction of the peptides and the cells, and the potency of translocation.
In a second approach, peptides were used as a carrier for radiopharmaceuticals. Re was first used as a "cold" analogue of Tc, and these conjugates of NPY or derivatives were characterized. Depending on the structure of the peptide, different yields of the desired conjugate have been achieved by either direct or prelabelling methods.
By these two entirely different approaches it was shown that peptides can be a very interesting and useful tool in the huge field of tumor diagnosis and treatment, and can play an important role in further investigations.

P 76 P 77

SOLID PHASE SYNTHESIS OF C-TERMINAL GLYCOPEPTIDES

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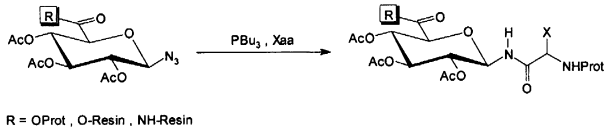
For a biologically active peptide to be of value as a drug substance, inherent problems such as poor absorption, poor delivery and biological instability need to be overcome to some extent. Glycosylated peptides, lipopeptides and peptide conjugates represent a very attractive target for drug development, either as active drugs in their own right or as a means of improving delivery, kinetics and/or targeting. Sugar conjugation can serve to not only improve physicochemical properties such as solubility, but can also allow the conjugate to utilise active transport uptake systems and can help target the compound. Here we report a facile, improved method for attachment of the first (*C*-terminal) amino acid of the peptide sequence to the resin-bound sugar azide via a modified Staudinger reaction. See Figure 1. This method is compatible with both Boc- and Fmoc- peptide synthesis strategies and avoids the need for two-stage reduction/coupling e.g. with propane-1,3-dithiol.



R = OProt , O-Resin , NH-Resin

Acknowledgements: This work was supported by an EC grant (PL 966087) for JM.

GLYCOTARGETING BY LYSINE-BASED OLIGOSACCHARIDYL CLUSTERS

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Nucleic acids (oligodeoxynucleotides and genes) are putative therapeutic agents. However their use is impaired by their poor ability to enter cells and by their lack of cell specificity. One possible way to improve both entry and selectivity is to use glycosylated nucleic acid carriers. This approach calls upon high-affinity ligands for cell specific receptors such as membrane lectins. High affinity ligands may be complex oligosaccharides of natural origin or synthetic clusters made of sugar units attached to a small peptide scaffold. As an example, Biessen *et al.* (*J. Biol. Chem., 271*, 28024-28034, 1996) synthesized lysine-based cluster mannosides, bearing phenylthio-carbamyl monosaccharides, with a high affinity for the human mannose receptor in a nanomolar concentration range.
We developed new sugar clusters based on a pentalysine core substituted with disaccharides through a peptidic linkage. Various new glycosynthons, glyco-amino acids and glyco-peptides, which upon hydrolysis release natural metabolites, have been prepared (Quétard *et al., Bioconjugate Chem.*, 9, 268-276, 1998). Here, we describe the synthesis of oligosaccharyl-pyroglutamyl-βalanyl derivatives and their coupling to different peptidic backbones, Lys_5-Ala-Cys-NH_2 , [Lys(Gly)]$_5$-Ala-Cys-NH_2 and (Lys-Ala)$_5$-Cys-NH_2 to obtain the related lactosyl and dimannosyl clusters. The thiol group of the cysteine residue enables conjugate tagging with iodoacetamido-biotine or -fluorescein, or coupling to oligonucleotides or to nucleic acid carriers. The affinity of the biotinylated sugar clusters for various plant lectins was determined by surface plasmon resonance. The specific binding and the intracellular localization of the fluorescent sugar clusters were determined for different cell lines by confocal microscopy and flow cytometry.

Posters: topic A2

P 78 Peptides Conjugates: Lipo-, Glyco-peptides and Chimeras P 79

Solid phase synthesis of peptide-dendrimer conjugates for the investigation of the multivalent effect in ligand integrin interactions.

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A solid-phase synthesis of symmetrical divergent dendrimers bearing up to eight peptide ligands (LDV) for the $\alpha_4\beta_1$ integrin receptor is reported. The solid-phase approach provides peptide-dendrimer conjugates of high purity for the investigation of the multivalent effect in ligand integrin interactions. An ELISA assay was used to screen the peptide-dendrimer conjugates for their ability to compete with a biotinylated peptide component of the CS1 region of fibronectin. When compared, the inhibition potential of the different generation peptide dendrimer conjugates waws found to increase with higher generations.

A METABOLIC ROUTE FOR THE SYNTHESIS AND HYDROLYSIS OF β-ASPARTYL PEPTIDES

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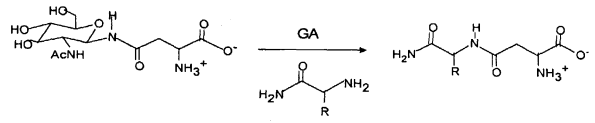
β-Aspartylpeptides are normal constituents of human urine and the central nervous system, but their formation and catabolism are poorly understood. We have now identified a new enzymatic system, which can account for the presence of β-aspartylpeptides in the body fluids and tissues of mammals.

Glycosylasparaginase (EC 3.5.1.26, aspartylglucosaminidase, GA) is a lysosomal amidase involved in glycoprotein degradation by catalyzing the hydrolysis of the N-glycosidic linkage. Here we show that this enzyme is able to catalyze the hydrolysis of β-aspartyl peptides.The enzyme also effectively catalyzes the synthesis of β-aspartyl peptides by transferring the β-aspartyl moiety from β-aspartyl compounds such as aspartylglucosamine, β-aspartyl peptides and L-asparagine [H-Asp(GlcNAcNH)-OH, H-Asp(Phe-OMe)-OH and Asn-OH] to various amino acid amides and peptides:



The data presented here, suggest that the glycosylasparaginase is the enzyme involved in the metabolism of β-aspartyl peptides and glycopropteins. We propose the name glycosylasparaginase / β-aspartyltransferase for the multifunctional enzyme that catalyzes the enzymatic synthesis and degradation of β-aspartyl peptides , cleaves the N-glycosidic bond during degradation of glycoproteins and possesses L-asparaginase activity.

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LIPOSACCHARIDE BASED PEPTIDE DELIVERY SYSTEM FOR TUMOUR SELECTIVE SOMATOSTATIN ANALOGUES

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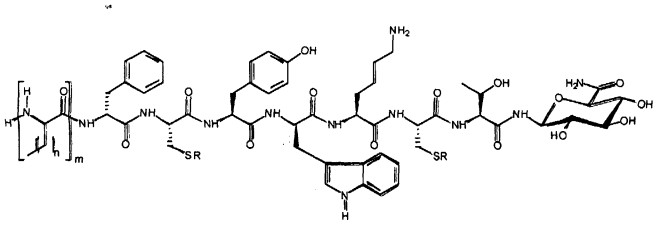
Inhibition of the incorrectly or over-expressed signals generated by oncogenes and growth factors have become established areas for anti-tumour research. The anti-tumour activity of somatostatin has long been known, however, clinical use is limited due to lack of specificity for cancer cells and additional problems of poor absorption, delivery and biological instability inherent to peptide drugs. Lipoamino acid and liposaccharide conjugates of a tumour-selective somatostatin analogue TT-232 [*Proc. Natl. Acad. Sci. USA* **93**, 12513 (1996)] were synthesised to modify the physicochemical properties of the parent peptide. Caco 2 cell uptake studies were carried out. Experiments *in vitro* clearly demonstrated that compounds modified at the *N*- with a lipoamino acids and sugars gained improved bioavailability. We will also report a method based on LC MS investigations to determine the P_{app} in the Caco 2 cell studies.



SYNTHETIC PEPTIDES REGULATORS OF IL-6 ACTION: DESIGN, SYNTHESIS AND CD CHARACTERIZATION

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Interleukin 6 (IL-6) is a multifunctional cytokine which is required, in cooperation and synergism with other growth factors, for inducing proliferation of hematopoietic progenitor cells, T and B cell growth and differentiation, neuronal and macrophage differentiation, Ig production and the acute phase response. Two transmembrane glycoproteins are necessary to form a functional IL-6 receptor complex: IL-6Rα and gp130. IL-6Rα specifically binds IL-6 at a relatively low affinity to form the active cytokine (1). The IL-6-IL-6Rα complex associates with gp130 at high affinity thus promoting a multimeric complex responsible for the signal transduction. In absence of crystallographic data of the IL-6 complex, three-dimensional molecular modeling studies and computational simulations (2) allowed the identification and the quantitative analysis of the molecular determinants for ligand-receptor recognition. It is of extreme interest the development of IL-6 inhibitor peptides in order to modulate its biological activity in those diseases where high serum levels of this cytokine contribute to their pathogenetic mechanisms. To this purpose, we have designed two helical peptides able to interact specifically with IL-6 and gp130, respectively. Each peptide mimics the receptor-cytokine binding interfaces in a complementary fashion to discourage the trimeric complex association. These peptides were investigated in solution by CD spectroscopy and the results obtained indicate an helical conformation for both peptides; moreover, as predicted, they interact each other increasing the total helical content. Finally, the IL-6 mimetic peptide structure analysis and the preliminary interaction results with gp130-mimetic peptide by ^{1}HNMR, confirm the structural predictions about the peptide complex made via molecular modelling.

[1] G. Paonessa, R. Graziani, R. Laufer, G. Ciliberto, G. Paonessa, *Embo J.* 1995, 14, 1942-1951

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