Poster presentations

Topic A

A1 - New synthetic approaches and strategies
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A1 - New synthetic approaches and strategies

P A1 - A synthetic strategy toward constrained head-to-tail cyclopeptides

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Cyclic peptides are interesting tools in medicinal chemistry for epitope/pharmacophore studies because they provide a predictable conformation of diverse functionalities around a core whose flexibility depends on the size of the ring. Moreover, cyclopeptides exhibit improved metabolic stability and increased potency, receptor selectivity and bioavailability. We recently set up the synthesis of His containing cyclotetra-, and hexapeptides [1], by a solid phase head-to-tail cyclization strategy, based on anchoring trifunctional amino acids side-chains on a trityl resin. Following this strategy, we succeeded the synthesis of the difficult constrained cyclotetrapeptide c(His-Gly)$_2$, usually prone to cyclodimerization, using Fmoc-His(Trt-resin)-OAl as a three-dimensional orthogonal protected building block. In order, to extend this cyclization approach we described the synthesis of diketopiperazine and RGD containing cyclotetrapeptides using Fmoc-Asp(Trt-resin)-OAl [2]. Herein we report a systematic approach to generalize the solid phase cyclization methodology of constrained homodetic head-to-tail cylopeptides bearing in the peptide sequence at least a tri-functional amino acid to be anchored by its side chain to a trityl resin. Starting from Fmoc-Asp(Trt-resin)-OAl, we synthesized the series of RGD cyclotetrapeptides c(Xaa-Arg-Gly-Asp), Xaa=Ala, Phe, Phg, D-Ala, D-Phe, D-Phg, to evaluate the effect of the steric hindrance of the amino acids side chains involved in the ring closure. We found that the racemization of the Xaa amino acid, and the reactivity strongly depends on the steric hindrance of the side chain. Subsequently, we decided to investigate the effect on the reactivity, oligomerization and epimerization induced by different coupling reagents (TBTU and PyBop), and bases (DIPEA and collidine). In order to verify the dependence of the cyclization step on the amino acids involved in the ring closure, we extended the applicability of this strategy, synthesizing Fmoc-Trp(Trt-resin)OAl. The Trp and the His building blocks were used for two different approaches to the synthesis of the cyclopeptide c(His-Gly-Gly-Gly-Trp), a possible model of the prion protein. Moreover, a series of cyclopenta-, hexa- and heptapeptides already described in the literature was synthesized to compare yield and cyclooligomerization of the different cyclization approaches.

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P A2 - Controlling the cysteine framework of N to C cyclic analogues of α-Conotoxin ImI

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α-Conotoxins are a class of peptide neurotoxins isolated from the venom of predatory marine snails. They specifically target nicotinic acetyl choline receptors, hence are important for the study of neurotransmission. They also are potenial drug leads in the treatment of neurological diseases such Tourettes syndrome and schizophrenia [1]. α-Conotoxins are characterised by two disulfide bonds in a 1-3, 2-4 arrangement, which facilitates a highly rigid structure.
α-Conotoxin ImI is the first known antagonist of the α7 subunit of the nicotinic acetylcholine receptor. Previous structural studies in our laboratory show that the N and C terminal are in close proximity and that residues important for binding appear on one face of the molecule [2]. By insertion of an optimised spacer unit between the N and C terminal through backbone cyclisation, we expect to increase the bioavailability of ImI, with minimal disruption to the native 3D structure.
We have synthesised a series of N → C cyclic analogues of α-conotoxin ImI: cImI, cImI-A cImI-βA, cImI-AG and cImI-AGG. The strategy employed an intramolecular native chemical ligation reaction to form the cyclic amide backbone [3]. Under the conditions used to rapidly effect cyclisation of the linear thioester precursors, disulfide bonds are also formed. When all four cysteine residues remain unprotected, varying ratios of disulfide bonds isomers are obtained, depending on the size of the spacer and reaction conditions. This has implications in synthetic peptide chemistry, since it may offer another means to control folding in synthetic peptides. Futhermore, we have also been able to direct disulfide bond formation to the desired arrangement using various methods, including orthogonal cysteine protection [4],[5] and the use of isomorphic selenocysteine [6]. We have also elucidated the 3D n.m.r structure of the cyclic analogues together with biological stability data and binding affinity to the α7 subunit of the nicotinic acetylcholine receptor.

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P A3 - Conformational studies of novel macrocyclic hairpin mimetics of the cationic antimicrobial peptide protegrin I

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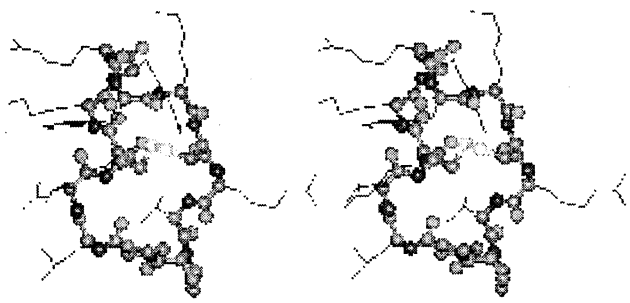
The problems associated with increasing microbial antibiotic resistance has stimulated great interest in newly discovered families of naturally occurring cationic antimicrobial peptides. These include protegrin I, tachyplesin and RTD-1, which adopt ß-hairpin-like structures. We report here an approach to novel peptidomimetics patterned on these natural products. The mimetics were designed by transplanting the cationic and hydrophobic residues onto a ß-hairpin-inducing D-Pro-L-Pro template. The family of 12 residue mimetics showed good antimicrobial activity against a range of gram-positive and gram-negative bacteria (MIC ~ 12-50 μg/mL). The preferred conformations of two of these mimetics in aqueous solution was studied by NMR. The $^3J_{HN\alpha}$ values and NOEs for mimetic-1 suggest an absence of stable loop structure in aqueous solution. In contrast, the ^{1}H NMR spectra of mimetic-2 in water showed $^3J_{HN\alpha}$ values for several residues in the range 2-2.5Hz, characteristic of a helical conformation, and for others in the range 8.5-11 Hz typical of ß-structure. Several long range cross-strand NOEs were observed, as well as a strong NOE between the C(α)H atoms of Val^{12} and D-Pro^{13}, indicating a (100%) *cis* Val^{12}-D-Pro^{13} peptide bond. The average solution structures, calculated for mimetic-2 using NOE and angle constraints are well defined, include a *cis* Val^{12}-D-Pro^{13} peptide bond, and reveal a relatively well-defined backbone conformation, which includes unexpectedly a short stretch of 3_{10}-helix between residues 1 and 4 (see Figure).



P A4 - Synthesis by metathesis reaction and crystal structure of bicyclic bridged hexapeptide

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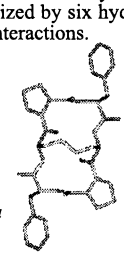
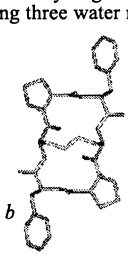
In this study, we have investigated the synthesis of molecules able to induce cytokine receptors dimerisation which has been established as a general mecanism of signal transduction. Dimeric molecules seem to be good candidates for mediating dimerisation of these type of receptors. On the other hand, β-turn playing a key role in many biological molecular recognition events including interaction between peptide hormones and their receptors, we focused our effort in the synthesis of β-turn inducing dipeptides dimeric compounds. It has been shown that bicyclic structures, which are not well described compared to cyclic structures, represent templates for the design of rigid β-turned structures. We synthesized conformationally constrained bicyclic hexapeptides of general structure: **cyclo(AA1-Allgly-AA2-AA1-Allgly-AA2)cyclo(2-5)**.
These molecules can be considered as a dimeric form of AA2-AA1-AllGly and we expected that the AA2-AA1 sequence occupies the 2 and 3 positions of two β- turns. The crystal structure of cyclo(Phe-Allgly-Pro-Phe-Allgly-Pro)cyclo(2-5) has been solved by X-ray diffraction. The molecule adopts a binary axis perpendicular to the ethylene bond. The ethylene bridge adopts two conformations, a and b, corresponding to values of 83°(a) and 46°(b) for the χ^1 angles (Allgly side-chain) and of –94°(a) and 92°(b) for the χ^2 angles (Allyl side-chain) respectively (see figures below). The conformational ϕ and ψ angles are of –65° and 117° for Pro, 77° and 10° for Phe and –149° and 172° for Allgly, respectively. As expected, Pro and Phe occupy the 2 and 3 positions of a βII-turn stabilized by an i+3 ◄– i intramolecular hydrogen bond. The crystal packing is stabilized by six hydrogen bonds including three water molecules and by Van der Waals interactions.



Conformation a



Conformation b

P A5 - Novel approach to enzymatic synthesis of peptides containing chromogenic and fluorogenic moieties

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Enzymatic peptide synthesis is a promising technique for production of optically pure biologically active peptide and their analogs, especially in organic media.
We developed a new approach for enzymatic peptide synthesis using as catalysts proteases which are covalently immobilized on macroporous carrier, poly(vinyl alcohol) (PVA) cryogel. Immobilized subtilisin 72, thermolysin, a-chymotrypsin, trypsin were prepared by chemical coupling of corresponding enzyme to the carrier and their synthetic efficiency in nonaqueous media was examined. The immobilized enzymes were able to catalyze peptide bond formation with high yield in DMF-MeCN mixtures with low water content. The series of N-protected p-nitroanilides of tetrapeptides with general formula of Z-Ala-Ala-Xaa-Yaa-pNA (Xaa = Leu, Lys, Glu; Yaa = Phe, Asp) was synthesized in 70 - 98% yield using immobilized subtilisin as a biocatalyst in a DMF-MeCN (6/4) mixture. Synthesis of intramolecularly quenched fluorogenic substrate for pepsin, Abz-Ala-Ala-Phe-Phe-Ala-Ala-DeD, was carried out in DMF-MeCN mixture with equimolar amounts of amino- and acylating components and at [E]/[S] molar ratio of 1:800. The yield of the product (by HPLC) was 88% after 24 h. Immobilized thermolysin and a-chymotrypsin catalyzed the formation of chromogenic subtilisin substrate, Z-Ala-Ala-Leu-pNA in the DMF-MeCN mixture to yield 90% (1h) and 60% (24 h), respectively. Synthesis of Z-Phe-Arg-Leu-pNA from Z-Phe-Arg-OMe and Leu-pNA, with unprotected guanido group of arginine, was catalyzed by trypsin-cryoPVAG in MeCN yielding 60% of tripeptide in 24h. Our studies have demonstrated high potential of cryoPVAG-immobilized proteinases as catalysts of synthetic reactions in organic media with low water content. The main advantage of our novel biocatalysts is that they can be used repeatedly without essential loss of activity and might be easily removed from the reaction mixture.

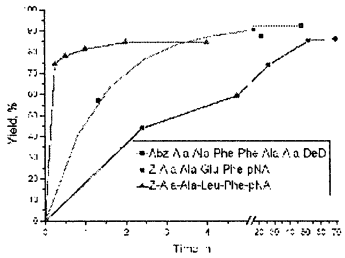

Fig. 1 - The time dependence of the peptide yield in synthesis, catalyzed by subtilisin-cryoPVAG

Aknowledgements
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P A6 - The synthesis of histidine containing cyclic peptides

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The synthesis of histidine containing cyclic peptides via SPPS is quite troublesome. Not only the permanent risk of epimerisation during the coupling of the activated histidine derivatives is problematic but also undesired side reactions occur during the cyclisation of these peptides in solution. Due to the acid lability of N-trityl protected histidine derivatives, which is the protection method of choice in Fmoc/*t*Bu peptide synthesis, it is nearly impossible to get fully protected peptides without a larger amount of peptides deprotected at histidine. Even very mild cleavage conditions (0,5 % TFA or 20 % HFIP/DCM) of acid sensitive linkers (2-chlorotrityl chloride) and neutralization with pyridine are accompanied to some extent by loss of the imidazole protecting group. The partially deprotected peptides form a by-product guanylated at histidine during the cyclisation reaction, when coupling reagents of the uronium type are used. Hence it is necessary to purify the crude product with RP-HPLC using eluents containing 0.1 % pyridine in order to avoid further deprotection of His(Trt).
These problems lead us to the strategy of on-resin cyclisation. The attachment of an -carboxy ODmab [1] protected aminoacid is achieved by anchoring of the side chain functionality to the resin. After assembly of the peptide sequence according to Fmoc/*t*Bu tactics the Dmab protection group is cleaved with 2% hydrazine in DMF. Cyclisation with HATU/HOAt or DIC/HOAt gives the desired cyclic product in good yields.
Undesired N^{im}-guanylation of unprotected histidine let us to investigate a new way to obtain histidine containing cyclic peptides. Due to the fact that the guanidinium group is cleaved with hydrazine from the imidazole side chain it is possible that these group acts as a protection group in Fmoc-histidine chemistry.

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P A7 - Synthesis of new analogues of plant mitogenic peptide phytosulfokine-α and their biological evaluation

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Phytosulphokine-α (PSK-α), a sulfated growth factor universally found in both monocotyledons and dicotyledons, strongly promotes proliferation of plant cells in culture. It is similar to animal polypeptide hormones in that it is processed from a larger precursor, preprophytosulfokine (PP-PSK-α). The active phytosulfokine- is a pentapeptide fragment H-Tyr(4-OSO_3H)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (I), located between residues 80-84 of PPPSKα. The aim of these investigation was evaluation of the role of N-terminal Tyr(4-OSO_3H) on the mitogenic activity in plants. Because the -OH group in position 4 of the N-terminal Tyr residue of PSK-α is esterificated by sulfuric acid, and sulfur atom is connected to aromatic ring by oxygen atom. Basing on the above, we performed the synthesis of PSK-α analogues modified in position 1 by series of non-protein aromatic amino acid residues, the sulphonic group was connected to the C-atom or by nitrogen atom to aromatic system. The subject of our investigation was synthesis of PSK-α and a series of following analogues:
H-D-Tyr(4-OSO_3H)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (II),
H-Phe(4-SO_3H)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (III),
H-D-Phe(4-SO_3H)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (IV),
H-Phg(4-SO_3H)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (V),
H-D-Phg(4-SO_3H)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (VI),
H-Phe(4-$NHSO_2CH_3$)-Jle-Tyr(4-OSO_3H)-Thr-Gln-OH (VII),
H-Phe(4-NO_2)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (VIII),
H-D-Phe(4-NO_2)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (IX),
H-Phg(4-NO_2)-Ile-Tyr(4-OSO_3H)-Thr-Gln-OH (X),
H-D-Phg(4-NO_2)-Ile- Tyr(4-OSO_3H)-Thr-Gln-OH (XI).
N^{α}-Boc-derivatives of the following amino acids: D-Tyr(4-OSO_3H), Phe(4-SO_3H), D-Phe(4-SO_3H), Phg(4-SO_3H), D-Phg(4-SO_3H) and Phe(4-$NHSO_2Me$) were synthesized and used in this form for modification of PSK-α.

P A8 - Exploring difficult Fmoc deprotections

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A previous report has documented that "difficult" Fmoc-deprotections during SPPS, as observed by the Applied Biosystems 433A Peptide Synthesizer with UV monitoring, can be converted into "easy" deprotections by changes in the preceding amino acid sequence [1]. This study was carried out by substituting the corresponding "difficult" and "easy" strings from the chemokines MCP-1 and Rantes, respectively, into one another. Although the two chemokines have similar 3D structures in solution [2], their UV deprotection patterns are distinctly different, starting at MCP-1's residue 34 (from the C-terminus). It appears that "difficult" and "easy" deprotections in the two chemokines are not an inherent property of the particular residue-strings themselves, but rather that the preceding residues play the critical role in determining the ease/difficulty of deprotections, i.e. structure in a growing peptide chain controls the accessibility of the Fmoc-group. The development of secondary structure has long been viewed as responsible for the onset of coupling problems up to approximately 15 residues after the start of tBoc SPPS [3]. A similar pattern of difficulties 7-15 residues after the start of Fmoc syntheses has been observed via UV monitoring of deprotections [4]. The development of tertiary structure has been advanced as a factor in the Fmoc deprotection problems observed long after the start of a synthesis (greater than 30 residues) [5]. The corresponding "difficult" and "easy" residues from MCP-1 and Rantes have been inserted into other natural and designed sequences. Deprotection troubles arise only where peptide structure appears to arise. In particular, varying the C-terminal length of the MCP-1 sequence (prior to residue 34) alters the difficulty of the deprotections. These observations point to structural changes in the peptide as chain-length varies and require more than secondary structure as an explanation. The 433A with UV monitoring is providing a tool to investigate structure in the peptide-resin environment.

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P A9 - Synthesis and applications of N-tetrachlorophthaloyl protected amino acids

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The use of the TCP (tetrachlorophthaloyl) cyclic imide protecting group was introduced by Debeham and Fraser-Reid in carbohydrate chemistry. Recently, we have reported its use for the protection of amino acids and studied their synthetic applications. *N*-TCP protected amino acids may be obtained with good yields and optical purity from amino acids either refluxing with tetrachlorophthalic anhydride followed by Ac_2O cyclization or by microwave irradiation (MWI) [1]. *N*-TCP amino acids are stable to Fmoc removal conditions (piperidine:DMF, 1:4) and show a particular λ_{max} UV absorption at 335nm which allows their specific detection.
N-TCP amino acids can be used for the solid-phase synthesis (PAL-PS-PEG resins) of peptidyl amides following a TCP/*t*Bu synthetic protocol as shown in Figure 1 [2]. Coupling of *N*-TCP protected α-amino acids with classical DIC-HOBt or DIC-HOAt methods work well, treatement with coupling reagents that require the presence of a tertiary base may lead to a significant level of racemization (5-15%). The optimal deprotection conditions for *N*-TCP peptidyl resins include treatment with hidrazyne-DMF (3:17) for 2 h at 40° C or ethylenediamine-DMF 1:49 at 25-30° C. The same synthetic approach has been used to prepare PNA oligomers from TCP/Z protected monomers. Boc-PNA monomers can be deprotected with TFA followed by TCP protection with TCPO at room temperature followed by Ac_2O cyclization

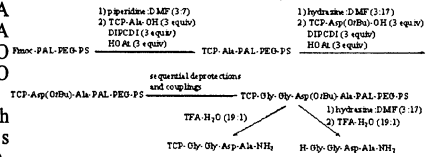

Fig. 1 - Solid-phase synthesis of peptide amides from N-TCP amino acids.

Treatment of *N*-TCP amino acids with aminoresins or diamines (ethylenediamine, propanediamine) under mild conditions may lead to the corresponding N,N'-disubstituted tetrachlorophthalamides. Moreover, starting with TCP-Glu-OAl this synthetic approach can be applied to the obtention macrocyclic peptide-arene hybrids, as shown in Figure 2. In summary, *N*-TCP amino acids may be considered a new kind of synthetic building blocks with different applications from the conventional protected amino acids.

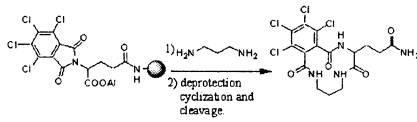

Fig. 2 - Solid-phase synthesis of macrocyclic peptide-arene hybrids from N-TCP amino acids.

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P A10 - Chimeric peptide-PEG-oligonucleotides

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Oligonucleotides represent a class of potential therapeutics, but further improvements in certain properties may be required to optimise the pharmacological use since their low biomembrane permeability and relative rapid degradation make them of limited therapeutic value. Proteins have been successfully used as enhancers of the oligonucleotide properties, but the large size and complex structure of proteins hamper this approach. Cell-specific delivery, cellular uptake efficiency and intracellular distribution may be improved by forming stable complexes with specific peptides.[1] As recently demonstrated, the conjugation of bioactive oligonucleotides with proper peptide sequences can improve their properties, as binding to complementary DNA, hybridization speed, binding to RNA and proteins and nuclease resistance.[2] In addition, the conjugation of polymeric moieties to oligonucleotides can be used to increase their biovailibility. Many cationic polymers were able to promote the internalisation of the oligonucleotides, however some cytotoxicity is due to positively charged polymers. Neutral polymers have also been proposed as carrier moieties to assure a sustained release of the conjugated molecules in the long term. Among the different polymers, the polyethylenglycol (PEG) has been successfully used also in peptide [3] and oligonucleotide [4] synthesis due to its advantageous chemical features. Moreover, its lack of toxicity and immunogenicity gives an higher value to the biotechnological applications. The liquid-phase synthetic methods that employs large PEG moieties as soluble supports allowed to set up a new procedure for the stable conjugation of high-molecular weight PEGs to oligonucleotides [5]. This approach overcomes any difficulty given by the post-synthetic conjugation of the large molecular polymers due to their low reactivity. On the bases of our previous investigations on supported synthesis of stable PEG-conjugated oligonucleotides we have explored the preparation of chimeric peptide-PEG-oligonucleotides. This synthesis represents an interesting challenge because the standard protection and activation procedures used in peptide and oligonucleotide synthesis are not fully compatible with each other. The peptide and oligonucleotide can be synthesized sequentially on automatic synthesizers, but this in-line synthesis lead to a restricted choice of sequences to be prepared. Alternatively, the two biopolymers can be obtained separately and then joined together. On this case some limitation occurs due to the low solubility and lack of reactivity during the coupling reaction and overall yields are usually quite low. To this aim, a first mixed conjugate oligonucleotide-PEG-peptide has been successfully synthesized by a liquid-phase, PEG-supported, synthetic procedure, purified, and fully characterized, and different synthetic strategies have been evaluated.

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P A11 - Native chemical ligation through nucleofile-stable thioester precursor

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A novel strategy to generate C-terminal thioester peptide compatible with Fmoc chemistry is presented.
Three model peptides bearing Gly, Lys and Phe at the C-terminus have been synthesized with Fmoc chemistry using standard 20% piperidine deprotection, condition incompatible with the thioester functionality. The peptides have been synthesized with standard Fmoc protocols and no additional manipulation or modification of the resin is required after completion of the desired sequence.
The peptides after standard TFA cleavage were purified with RP-HPLC. Then each of the purified segments was dissolved at pH 7 in standard condition for Native Chemical Ligation with a peptide fragment bearing a N-Terminal Cys. In all cases the ligation was completed in high yield in few hours, in accordance to the standards of NCL.
To demonstrate the practical usefulness of our invention the total synthesis of NNY-Rantes (1-67 a.a. residues) will be presented.
The ligation of the unprotected peptide fragment of NNY-Rantes (1-32) made with our strategy via Fmoc Chemistry with the N-Terminal Cys (33-67) peptide is then compared with the "Classical" NCL where the fragment 1-32 is directly obtain as thioester after the HF cleavage of the peptide.

P A12 - Synthesis of cyclic pseudo-peptides containing a reduced amide bond isostere using the safety catch linker approach.

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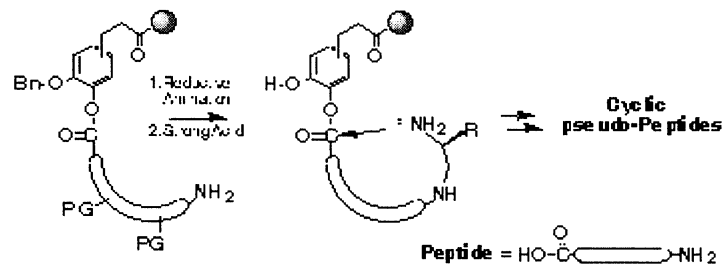
Recently we have been interested the rapid synthesis of cyclic peptide libraries using a novel 'safety catch linker'.[1] We present an extension of this work to accommodate reduced amide moieties within the peptide backbone. The strategy comprises (i) linking the linker to resin, (ii) solid phase peptide synthesis incorporating 'in situ' neutralisation, (iii) reductive amination using freshly prepared Boc-amino aldehydes, (iv) solid-phase activation using strong acid or HF, and (v) final cleavage under mild base treatment to afford the pseudo-cyclic pentapeptides. To illustrate this approach several pseudo- pentapeptides were prepared in excellent yields and purity.



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P A13 - Synthesis of combinatorial neoglycopeptide arrays

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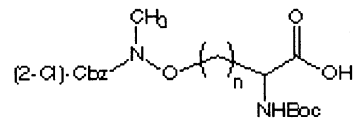
1. Santa Clara University - U.S.A

The chemoselective reaction of completely unprotected carbohydrates and peptides offers an attractive route for the synthesis of glycopeptides. Successful strategies have focused on oxime formation between aminooxy-containing peptides and native reducing sugars. However, a major drawback has been that the resulting glycoconjugates place the sugars at large distances from the peptide backbone and fail to maintain the sugars in their cyclic conformations. To solve these problems, we have designed and synthesized the novel N'-methyl-aminooxy amino acids 1 and 2 for use in Boc chemistry-based solid phase peptide synthesis (SPPS). These derivatives have been successfully incorporated into peptides using standard SPPS procedures to yield N'-methyl-aminooxy-containing peptides. Reaction of these peptides with reducing sugars in aqueous buffers produces the desired neoglycopeptides, whose attached sugars are close to the peptide backbone and are predicted to exist in their cyclic conformations. Importantly, the nature of the method allows individual peptides to be reacted with a wide variety of sugars and facilitates the synthesis of combinatorial arrays of neoglycopeptides. The results of the various syntheses and structural characterizations will be presented.



1 : n = 1
2 : n = 2

Fig. 1 - N'-methyl-aminooxy amino acid derivatives

P A14 - Mass spectrometric elucidation of an amyloid plaque-specific epitope: molecular basis for probing the proteolytic degradation mechanism of Alzheimer's Amyloid Precursor Protein APP

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2. University of Toronto, Centre for Research in Neurodegenerative Diseases - Canada

The accumulation of extracellular plaques containing the neurotoxic β-amyloid peptide fragment Aβ42 of the β-amyloid precursor protein (APP) as the major product, is one of the characteristics of Alzheimer's disease (AD). Although APP and presenilin(s) (PS; γ-secretase) have been recognised as key molecules, the molecular pathophysiological degradation pathways of APP are still unclear. In this study the proteolytic reactions of APP have been probed chiefly by identification of Aβ-specific epitopes using new methods of mass spectrometry, particularly Fourier transform ion-cyclotron resonance mass spectrometry (FT-ICR-MS) as a bioanalytical tool of ultrahigh sensitivity and resolution. This molecular approach has been based on the recent mass spectrometric elucidation of a plaque-specific Aβ epitope recognised by therapeutically active antisera from transgenic AD mice, which is independent of the Aβ42 structure, aggregation and toxicity (patent applications; *Nature,* submitted for publication). Mass spectrometric epitope mapping studies (epitope excision on intact antigen-antibody complexes) have been performed to identify epitopes recognised in Aβ-polypeptides, N-terminal APP ectodomains, and synthetic polypeptides spanning the Aβ-transmembrane sequence. The recognition specificity revealed in these studies will enable the use of the epitope as a new probe for molecular AD diagnostic applications.

P A15 - Synthesis of glycopeptide with a partial structure of a tumor associated mucin.

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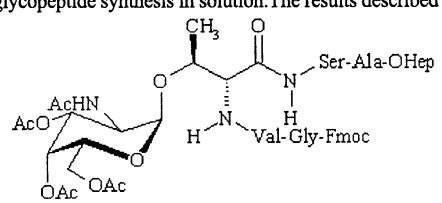
2. J.Gutenberg-University Mainz/Inst. for Org. Chemistry - Germany

Selective chemical synthesis of complex oligosaccharides and glycoconjugates is now accessible as a result of methodical improvements in the field of chemical synthesis. Many complex glycopeptides have been synthesized and investigated in terms of their preferred conformation and their role in biological recognition processes on cell membranes. The recognition phenomena are important for the communication between cells as well as in the interaction of receptors with enzymes, hormones and virus. In this context the synthesis of a glycopeptide with a partial structure of tumor associated mucin is reported. The synthesis of the targeted glycopeptide structure has been performed in solution. The carbohydrate component of this structure is T_N antigen (N-acetylgalactosamine) in accord with the Thomsen-Friedensreich's definition. It was linked to the aminoacidic counterpart (Fmoc-Thr(OH)-OAll) via a Koenigs-Knorr's type reaction. Specifically, the fully protected sugar moiety has been armed as the glycosyl iodide. This reactive intermediate was prepared from the precursor unblocked at the anomeric position using a polymer bound triphenylphosphine-iodine complex in presence of imidazole. The intermediate quaternary phosphonium salt obtained from triphenylphosphine with iodine is capable to interact with the anomeric hydroxy group and induces its substitution by the iodide anion. Conditions applied to activate the sugar are very mild. Therefore, the described method is useful for the formation of reactive glycosyl donors (glycosyl iodides) of sensitive carbohydrates. Moreover, the polymer bound triphenylphosphine-oxide, which is the main by-product, is separated simply by filtration. An convenient work up and purification procedure is the result. Evaporation of the solvent affords the product. The obtained Fmoc/OAll protected glycosyl threonine has been introduced into standard glycopeptide synthesis in solution.The results described herein show the possibility to obtain a reactive glycosyl species via a novel mild procedure. Glycosyl iodides synthesized under these mild conditions open up an efficient way to prepare complex glycoconjugate structures, glycopeptides and oligosaccharides, which are of interest as immunogens for the induction of monoclonal antibodies .



Fmoc-Gly-Val-Thr(Ac_3GalNAc)-Ser-Ala-OHep

Fig 1: Full protected synthesized glycopeptide structure

P A16 - Application of the DMSO/TFA oxidation to the one–pot regioselective formation of multiple disulfide bonds in peptides: the synthesis of oxytocin, RGD-4C and heat stable enterotoxin STa 1-18.

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The DMSO/TFA oxidation provides a rapid and convenient method for the generation of disulfide bonds in peptides possessing either free thiol groups or in some cases directly from S-protected precursors [1]. In a further extension of this procedure we have recently reported on studies utilising temperature-controlled orthogonality for the regioselective one-pot formation of multiple disulfide bonds in peptides [2, 3]. The cysteine derivative t-butyl is stable to the standard acidolytic cleavage of peptides from the solid-support but is rapidly removed with simultaneous oxidation to the disulfide in DMSO/TFA mixtures. In contrast however the 4-methylbenzyl (4-MeBn) protecting groups are stable in DMSO/TFA becoming sufficiently labile only when the temperature of the solution is raised. These derivatives are therefore orthogonal to one another under identical chemical conditions, the selectivity being a function of temperature.

The aim of this study was to further expand on our understanding of the underlying kinetics and specificity of the S-S bond forming reaction. Firstly, we chose oxytocin as a model peptide comparing, by HPLC analysis, the rate of product formation in DMSO/TFA as a function of temperature. The t-butyl protected precursor was completely converted to product in 15 minutes at room temperature compared to <5% conversion for the 4-MeBn precursor. Only after 6 hours incubation at 60 °C was the 4-MeBn partially protected peptide converted fully to oxytocin. No decomposition of the peptide was observed by analytical HPLC.

Secondly, in a further extension of utility we investigated the one-pot folding of the 2 disulfide peptide RGD-4C [4]. Once again the t-butyl groups were rapidly removed with oxidation complete within 10 minutes at room temperature. Subsequent heating of the peptide solution at 60 °C for 2 hours was carried out in order to effect conversion to the fully-folded product. In a third example the 3 disulfide bonds of enterotoxin STa 1-18 were regioselectively introduced once again using a onepot protocol. The inclusion of Trityl protected cysteine into the synthetic route allowing for full orthogonality.

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P A17 - Facile synthesis of a homo-peptide containing seven consecutive sterically hindered residues of C-alfa-methyl valine via the azido/bromide method

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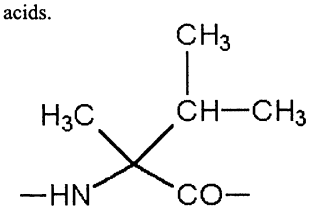
Homo-peptides based on optically active C^{α}-tetrasubstituted α-amino acid residues are very interesting structures, because they can form unique fully developed 3_{10}-helices in sequences longer than tetramer (rigid molecular rulers). [1] However, their synthesis is quite challenging. Here, we present the application of the azido/bromide method to the synthesis of a peptide containing seven consecutive residues of the β-branched (αMe)Val, which is known to be one of the most sterically demanding C^{α}-tetrasubstituted α-amino acids.



(αMe)Va

N^{α}-Protected α-amino acid bromides, pioneered in our laboratories,[2] proved to be superior reagents for the solution synthesis of peptides containing extremely hindered amino acids. Amino acid bromides, generated *in situ* under mild conditions, can acylate hindered amino functions in a short time and with high yields. The azido group, as a precursor of the NH_2 function, proved to be stable in the presence of these overactivated halides. Moreover, the occurrence of the N_3 group at the N-terminus endows peptides with favourable solubility properties during the assembly of long chains. IR absorption and ^1H NMR conformational studies of the new oligomers will be presented. Extension of the azido/ bromide coupling method to solid-phase syntheses is underway.

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P A18 - A new family of β-hairpin mimetics based on a trypsin inhibitor from sunflower seeds

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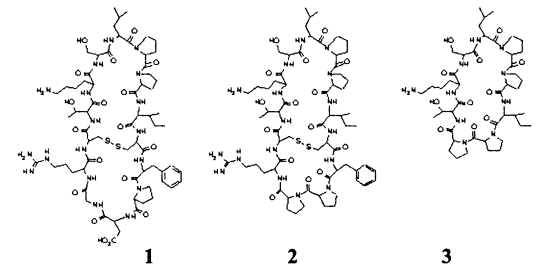
Serine proteases are involved in many biological processes such as defense and cell regulation. Inhibitors of serine proteases are, therefore, interesting targets in drug design. The work we present here is based on the naturally occurring serine protease inhibitor 1 isolated recently from sunflower seeds 1. This 14 residue cyclic peptide, which inhibits trypsin in the nanomolar range, is one of the shortest Bowman-Birk-like serine protease inhibitors known. This high potency seems to arise from its considerable structural rigidity and it has been shown that this inhibitor adopts a stable β-hairpin conformation when bound at the active site of bovine β-trypsin. Based on these interesting features, we have designed a new family of inhibitors by transplanting the -hairpin from the naturally occurring peptide 1 onto a hairpin inducing template. We present here the synthesis of two mimetics (2 and 3) using a L-Pro-D-Pro Template 2. NMR studies showed that the -hairpin structure is maintained in these two molecules. The results of trypsin inhibitory assays indicated that the mimetics 2 and 3 retained an activity similar to 1. A library of cyclic peptides based on the 9-residue peptide 3 was then designed and produced by parallel synthesis. An alanine scan was performed in order to determine the importance of each residue. Attempts were also made to change the specificity of inhibitor 3 by mutating the P1 residue. Some of these 9-mer mutant peptides proved to be potent inhibitors against chymotrypsin.



1 2 3

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P A19 - Dendribodies design

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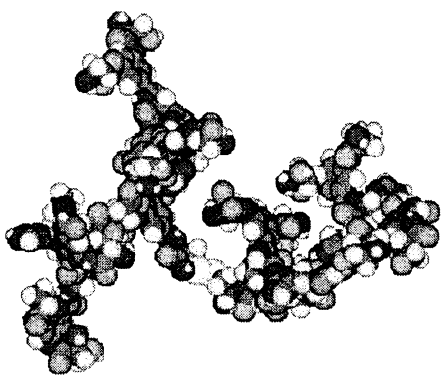
De novo enzyme design is one of the most difficult and fascinating challenges in the chemistry, nowadays. Designing proteins made from linear peptide sequences as nature does is difficult because the linear peptide sequence that one starts with first has to fold to adopt a defined globular shape. Dendrimers offer an interesting and simple alternative to protein topology in that folding is not required to obtain a globular structure[1].
We explored the combinatorial synthesis of dendrimers based on solid phase peptide synthesis. Each dendrimer contains a cysteine residue on which is built the peptide. The synthesis alternates the standard aminoacids with branching diaminoacid building blocks (x), which allow dendrimers to grow. A terminating group (T) is used at the end of the chain to confer homogeneus physicochemical properties to the dendrimers. Most of the diversity arises from the pairing of two chains.
The combinatorial approach for the synthesis of a 30 products library will be presented: a fluorophore terminus was used, in order to study, with fluorescence tests, catalytic and molecular recognition properties



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P A20 - The segment condensation of peptides containing multifunctional amino acid residues catalyzed by modified subtilisins in nonaqueous media

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2. Institute of Organoelement Compounds, Russian Academy of Sciences - Russian Federation

Previously we reported the use of subtilisin either in the complex with sodium dodecyl sulfate (SDS) or covalently attached to the poly(vinyl alcohol)–cryogel (cryoPVAG) as a catalyst for peptide bond formation between segments with mostly hydrophobic amino acid residues in the nearly anhydrous polar organic solvents [1,2]. In the present work we studied the synthetic possibilities of the SDS-subtilisin complex and subtilisin immobilized on cryoPVAG in the following general reaction: Z-Ala-Ala-P_1-OH + H-P_1'-pNA Z-Ala-Ala-P_1-P_1'-pNA,
where P_1 = Lys, or Glu; P_1'= Arg, Glu, Asp; pNA – p-nitroanilide. A series of N-acylated p-nitroanilides of tetrapeptides containing basic or acidic amino acid residues both in the P_1 and P_1'-positions using modified subtilisins as biocatalysts were synthesized in good yields without activation and protection of the ionogenic groups of multifunctional amino acid. (Table). For example, Z-Ala-Ala-Lys-Asp-pNA was obtained in preparative scale with 98% within 20h and isolated with 93% yield.

Table. Synthesis of p-nitroanilides of tetrapeptides containing multifunctional amino acid residues catalyzed by cryoPVAG-immobilized subtilisin and SDS-subtilisin

Enzyme	Product	Time, h	Yield, %
cryoPVAG immobilized subtilisin[1]	Z-Ala-Ala-Lys?Asp-pNA	2	98
	Z-Ala-Ala-Glu?Asp-pNA	4	74
SDS-subtilisin[2]	Z-Ala-Ala-Glu?Arg-pNA	19	52
	Z-Ala-Ala-Glu?Arg-pNA	120	92
	Z-Ala-Ala-Lys?Glu-pNA	21	38
	Z-Ala-Ala-Lys?Glu-pNA	120	83

[1] DMF/MeCN=60/40 (v/v); [S]:[E]=1200:1) [2] DMF/ethanol (30/70 v/v); [S]:[E]=5000:1)

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Acknowledgements
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P A21 - Novel method for the synthesis of cyclic peptides via azo bridge formation

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A novel method for peptide cyclization in solution: the azo cyclization is presented herein. Ring closure by forming an azo bridge was achieved in situ by connecting the corresponding side chains of para amino phenylalanine (Pap) residues with those of tyrosine or histidine residues present in the corresponding linear precursors. The reaction was performed using an initial step of diazotization in acidic media followed by an intramolecular azo cyclization in a mild basic media. This new method of cyclization is facile, applicable to various sequences and results in high yield of pure products and hence is suggested as an additional method for peptide cyclization. Here we report the successful utilization of this method for the synthesis of ten new cyclic azo peptides, derived from RGD, GnRH, Tuftsin, VIP, and SV40 NLS.

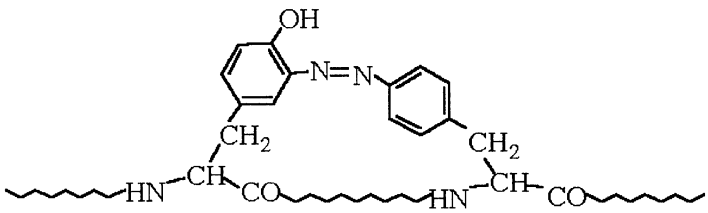

Fig. 1 - Schematic description of a cyclic azo peptide

P A22 - Convergent synthesis of peptide amides. A comparative study

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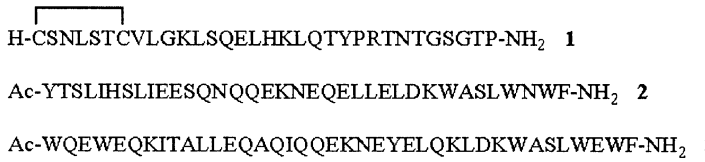
Salmon Calcitonin (**1**) and the viral fusion inhibitors T-20 (**2**) and T-1249 (**3**) were synthesized by the convergent methodology, using the 2,4-dimethoxybenzhydrylamin and the Rink amide-2-chlorotrityl resins. Syntheses were performed by sequencial condensation of 2 or 3 protected peptide fragments, prepered on the 2-chlorotrityl resin, on the resin-bound C-terminal fragments. Results concerning the influence of the solid suport, the protection scheme and the applied fragment excess on the final peptide purity and yield will be presented.



P A23 - Amino acid and peptide labeling with an azo dye

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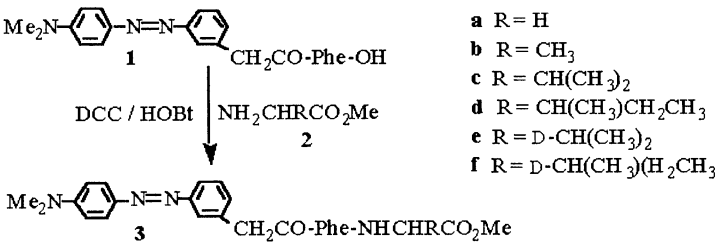
In the last few years a great progress could be observed in new areas of dye chemistry, especially those dealing with biomedical applications. This is illustrated by the use of dyes in many diagnostic fields often to allow qualitative and quantitative measurements [1]. Following our preliminary work [2] concerning labeling of amino acids by derivatisation with azo dyes [3], we now present the results of our investigation of the amount of epimerisation that occurs during coupling of labelled phenylalanine (**1**) with amino acid esters.



a R = H
b R = CH_3
c R = $CH(CH_3)_2$
d R = $CH(CH_3)CH_2CH_3$
e R = D-$CH(CH_3)_2$
f R = D-$CH(CH_3)(H_2CH_3$

N-labeled phenylalanine **1** was reacted with L and D amino acid methyl esters **2a-d** and **2e-f** by a DCC/HOBt coupling. The coloured diastereomeric pure *N*-acyldipeptides were obtained in yields within the range 50-84% and their structures were confirmed by the usual analytical techniques. Epimerisation was evaluated by 1HRMN and it was found that coupling with the L-amino acids leads to an enanteomeric excess (ee) of at least 90%, while an ee of 60% was obtained in the reactions with the corresponding D derivatives .

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P A24 - New strategies for the asymmetric synthesis of peptides via conjugate addition of N-nucleophiles

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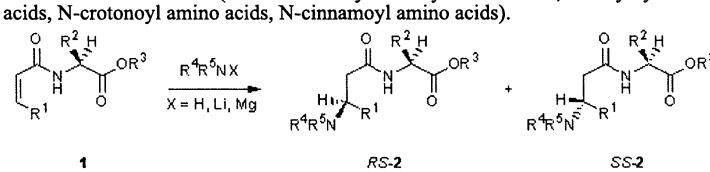
The incorporation of non-natural amino acids into peptides is a field of rapidly growing interest. For this purpose, it is necessary to develop novel and efficient methods for the stereoselective synthesis of non-natural amino acids and peptides. Recently, we have introduced the use of N-(*cis/trans*-ß-benzyloxycar-bonylacryloyl)-amino acid esters as precursors for the asymmetric synthesis of -aspartyl peptides [1]. In this contribution the synthesis and molecular modelling studies of new prochiral amino acid derivatives as efficient starting compounds for the stereoselective synthesis of peptides via conjugate addition of N-nucleophiles (amines, metal amides) will be presented.
Various peptides **2** with N-terminal ß-amino acids (ß-Glu, ß-Ala, ß-Abu, γ-Phenyl-ß-Abu) can be obtained by varying the substituent R^1 at the double bond of unsaturated amino acid derivatives **1** (N-*cis*-ß-carboxycrotonoyl amino acids, N-acryloyl amino acids, N-crotonoyl amino acids, N-cinnamoyl amino acids).



R^1 = H, Me, CH_2COOH, Ph

The synthesis of **1** was achieved in quantitative yields by condensation of the corresponding carboxylic acid chlorides with amino acids. The novel amino acid derivatives were characterized by LC-MS/MS, NMR and vibrational spectroscopy. Molecular modelling studies were performed to get insight into the conformational and stereodynamic behaviour of **1**. Structural prerequisites for an optimum stereoselectivity in the addition of N-nucleophiles to **2** were determined taking the diastereofacial deshielding of the double bond into account.
Some significant conclusions about the control of the nucleophilic attack at the *si*- or *re*-face of the novel prochiral amino acid ester can be drawn. The usefulness of the strategy will be illustrated along with synthetic procedures. [Supported from LSA]

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P A25 - An efficient utilization of BTC for SMPS using the "tea bags" parallel synthesis: the "one day-one library" approach

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1. The Hebrew University of Jerusalem / Organic Chemistry - Israel

BTC is a novel coupling reagent for peptide synthesis which posses high efficiency for difficult couplings [1] .We describe a novel approach for the utilization of BTC as a coupling agent for the "tea bags" solid phase multiple synthesis. Two libraries of peptides consisting of 10-13 AA residues were prepared by the "tea bag" methodology. Coupling using BTC proceeded efficiently while the utilization of PyBroP failed to give the desired product even after repeated attempts for longer reaction periods then those used for BTC. We also demonstrate that, using BTC, enables us to reduce the coupling duration down to ten minutes of shaking the peptidyl-resin with the reaction mixture. This enables to finish the synthesis of this particular library in one day. The crude products were analyzed by HPLC/MS and found to possess high degree of purity.

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P A26 - Synthesis of β-peptides by chemical ligation

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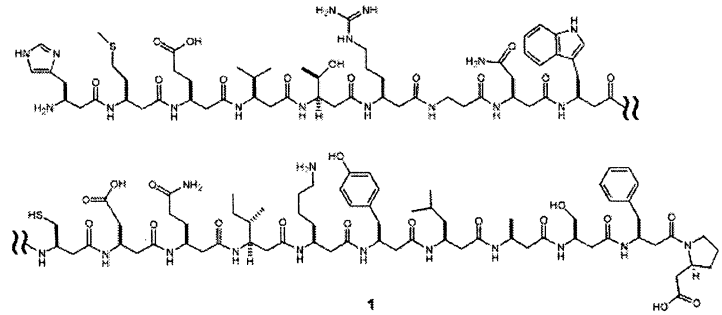
The chemical ligation techniques which use a chemoselective reaction that occurs between two unprotected peptide segments, each bearing a unique and mutually reactive group, have proven to be effective methods for the synthesis of larger peptides [1]. In the course of our investigation on β -peptides, we have applied the thiol ligation methodology to prepare β ^{3}-peptide **1**, containing all the 20 homologated proteinogenic amino acid .
This thiol ligation method involves the reaction of an *N*-terminal Cys fragment and a *C*-terminal thiolester fragment. Here we report the synthesis of a series of β ^{3}-peptides containing an *N*-terminal β ^{3}-HCys using the Fmoc strategy on a solid support [2], and the preparation of a series of β ^{3}-peptides containing a *C*-terminal thiolester by the Fmoc compatible solide-phase synthesis of peptide thioesters [3]. Furthermore, the synthesis β ^{3}-peptides via the thiol ligation of two fragments is presented.



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P A27 - Alpha-conotoxin [A10L] PnIA analogues as probes for nicotinic acetylcholine receptors

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Venom peptides make interesting pharmacological tools due to their action on ion channels and receptors. Conotoxins are small cysteine-rich peptides isolated from the venom of predatory marine snails. Moreover, they make excellent targets for drug design due to their high potency and selectivity in addition to their well-defined, highly constrained 3-dimensional structures.
Alpha-conotoxins which contain 2-3 disulfide bonds are specific antagonists of the nicotinic acetylcholine receptor (nAChR). Neuronal nAChRs have been associated with a number of nurological conditions, such as Parkinson's and Alzheimer's disease. There is great interest in the characterisation of these receptors for the development of selective therapeutic agents[1]. Alpha-Conotoxin PnIA is a 16 residue C terminal amide (GCCSLPPCAANNPDYC-NH_2) isolated from *Conus penaceous* which targets the 2 3 subtype nAChR. We and others have shown in recent studies[2,3] that a point mutation at position 10 of PnIA to form [A10L] PnIA (GCCSLPPCALNNPDYC-NH_2) changes its selectivity from the 32 nAChRs to the 7 subtype. It has also been found that [A10L] PnIA blocks the 7 receptor with an IC_{50} value of 1.4 nM, which is more potent than the parent peptide.
We have developed new parallel synthesis techniques based on Boc *in situ* neutralisation chemistry[4] to prepare synthetic libraries of [A10L] PnIA analogues. Libraries we have synthesised include an alanine scan of the 12 non-cysteine residues in the sequence and point mutations of position 10 using hydrophobic residues. The analogues were tested for binding affinity to the α7 subunit of the nAchR.

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P A28 - Efficient loading of sulfonamide "Safety-Catch" linkers by Fmoc amino acid fluorides

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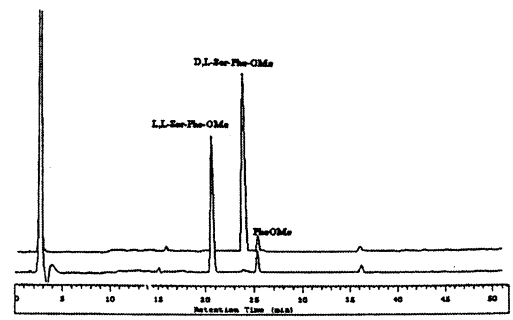
The efficient acylation of sulfonamide type linkers[1] by means of Fmoc protected amino acids with minimal epimerization at the chiral -carbon is critical for the successful Fmoc-based solid phase synthesis of thioesters by the safety-catch linker strategy[2]. Recently, it was reported that the reactivity of the sulfonamide function is comparable to that of an alcohol[3]. These results prompted a systematic study on the potential application of Fmoc protected amino acid fluorides[4] to the acylation of the sulfonamide SCL. Fmoc-Ser(tBu)-OH, known to readily racemize under commonly used solid phase peptide synthesis conditions was used as a sensitive model system. DCM was chosen as the solvent of choice and the efficiency of loading was examined in the presence of varying amounts of different bases commonly used in SPPS. Racemization was tested for all bases used, via assembly of the dipeptide L-Ser-Phe-OMe obtained from loading of the SCL with Fmoc-Ser(tBu)-F and subsequent nucleophilic displacement with NH_2-Phe-OMe (fig). In order to establish the general applicability of the method, all accessible amino acid fluorides were synthesized according to known procedures[4] and used for acylation of the SCL-PS resin. It was found that in most cases high loadings could be obtained in short reaction times (1 h). Loss of configuration was determined for selected cases (Cys, Leu, Glu) in addition to the standard Serine system by means of HPLC evaluation of the relative amount of the D,L- and L,L-diastereomers of the dipeptides Xxx-Phe-OMe. D,L-forms were found to be low in all cases.



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P A29 - Highly acid-labile backbone amide linkers: o-BAL and T-BAL

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The tris(alkoxy)benzyl Backbone Amide Linker (BAL) has found widespread application in solid-phase synthesis. Here we describe the efficient synthesis of the ortho regioisomer, *o*-BAL, the key step being a highly regioselective mono-demethylation of 2,4,6-trimethoxybenzaldehyde under chelation control followed by convenient steam distillation. Next, cleavage studies of peptides anchored through an *o*-BAL handle revealed an unexpected high acid-lability, as treatment of *o*-BAL anchored protected YGGFL with dilute TFA (TFA-CH_2Cl_2 1:99) released the peptide with retention of *tert*-butyl ether and ester groups. Finally, in an effort to develop new BAL type handles with improved properties, such as hyper acid-lability, we have prepared a novel bicyclic handle based on thiophene. Inexpensive 3,4-ethylenedioxy-thiophene (Edot) was transformed into the Thiophene Backbone Amide Linker (T-BAL) in 3 facile steps. Peptides assembled on T-BAL anchored to a solid support were released by treatment with dilute TFA.

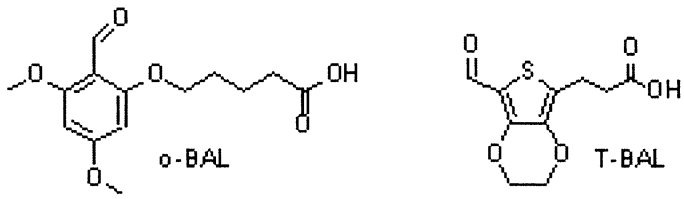

Fig. 1 - Structures of the o-BAL and T-BAL handles.

P A30 - Effect of the amino acid substituents on amide bond cleavage in N,α,α-trialkylglycine derivatives

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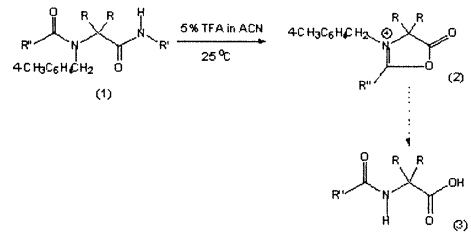
In previous work we have reported that the amide bond at the C-terminus of *N*,α,α-trialkylglycine derivatives is labile to acid and that for a series of such compounds (Deg, Dpg, D*i*bg and Db*n*g) the corresponding products **3** could be obtained by reaction with neat TFA [1]. We now report the synthesis of a set of derivatives of Aib and Db*n*g and the results of accurate kinetic studies concerning amide cleavage of their C-terminal amide bond, having in mind to establish structure-reactivity relationships allowing to understand the role of the various substituents on reactivity.



Cpd.	R	R'	R''
a	CH_3	C_6H_{11}	Ph
b	CH_3	C_6H_{11}	$PhCH_2$
c	CH_3	$4\text{-}CH_3C_6H_4CH_2$	Ph
d	CH_3	$4\text{-}CH_3C_6H_4CH_2$	$PhCH_2$
e	CH_2Ph	C_6H_{11}	Ph
f	CH_2Ph	C_6H_{11}	$PhCH_2$
g	CH_2Ph	$4\text{-}CH_3C_6H_4CH_2$	Ph
h	CH_2Ph	$4\text{-}CH_3C_6H_4CH_2$	$PhCH_2$

It was found that the reaction rate is very much affected by the bulk of the acyl substituent at the N-terminus, but when this is acetyl, reaction rates are very sensitive to the bulk of the alkyl substituents at the amino acid α-carbon atom. In other words, the lability of the C-terminal amide bond decreases when the bulk of the amino acid side chains increases, possibly by hindering the formation of the intermediate oxazolinonium derivative [2].

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P A31 - Solid-Phase Synthesis of Tentoxin. Synthesis of a Library of Analogues

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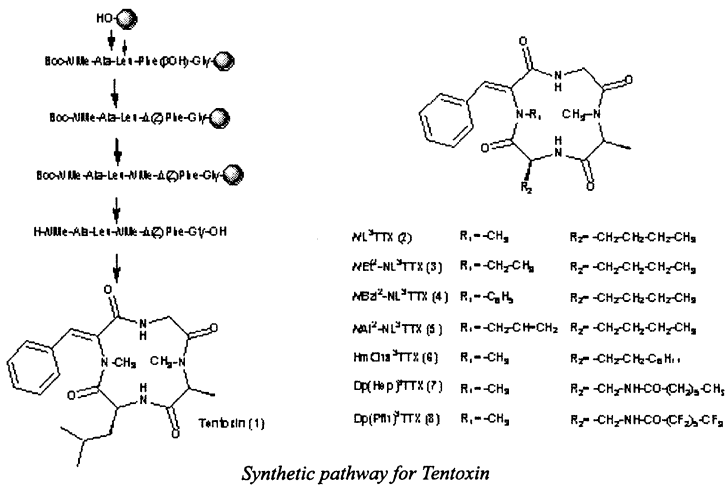
Interest in bioactive peptides has greatly expanded in recent years. Hence, the synthesis of new analogues in a systematic and fast way is crucial for the study and improvement of their biological action. An efficient solid-phase synthesis of the natural phytotoxin Tentoxin (1) and a library of analogues (2-8) is reported. The cyclic tetrapeptide Tentoxin, originally isolated as a metabolite of the phytopathogenic fungi *Alternaria Alternata*, has been shown to selectively induce chlorosis in some dicotiledone plants. Its species-specifity has raised interest in the potencial use of Tentoxin as a biogenic selective herbicide. Tentoxin exhibits structural features not commonly found in natural peptides: (i) the presence of a didehydroamino acid; (ii) the strained twelve membered ring system and (iii) the fact that two of the four residues are methylated, including the non-proteinogenic didehydroamino acid, that contributes to its challenge as a synthetic target.The scheme followed to achieve the synthesis of Tentoxin, as well as the seven new synthetic analogues is shown below. The elongation of the peptidic chain was carried out on solid-phase. The dehydration and subsequent alquilation reactions were also accomplished on solid-phase. Finally, the cyclization was performed in solution.



Synthetic pathway for Tentoxin

P A32 - Synthesis of efrapeptin C

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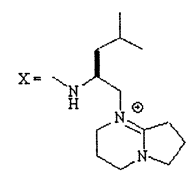
The efrapeptins are a class of peptide antibiotics produced by the fungus *Tolypocladium niveum*.[1] They are highly potent inhibitors of mitochondrial ATPase. The structure of the F1-ATPase/efrapeptin-complex has been determined X-ray crystallographically by Walker et al.[2] The efrapeptins are characterized by a high content of sterically hindered α,α-dialkylated amino acids (i.e. α-amino isobutyric acid Aib, Isovaline Iva). They also contain three pipecolic acid residues and one β-alanine residue. We decided to launch a project aiming at the synthesis of efrapeptin C and analogues thereof because we are interested in their biological and structural properties. The high content of Aib hampers the synthesis of these peptides. We assembled the complete molecule out of three fragments namely an N-terminal fragment (Pip_1 to Gly_8), a central fragment (Aib_9 to Gly_{13}) and a C-terminal fragment containing the residues Leu_{14}-Aib_{15} and the head group derived from leucinol. The C-terminal fragment was synthesized using solution phase methods. The other two fragments were synthesized using solid phase peptide chemistry following the Fmoc-strategy except for Aib. Aib-residues were introduced by a strategy first described by Meldal et al.,[3] i. e. acylation of the resin bound amino component with the highly reactive α-azido isobutyric acid chloride followed by reduction of the azide to an amine. The reduction of the azide was performed with Vilarrasa's reagent [Et_3NH][$Sn(SPh)_3$].[4] This reaction could be easily monitored by FT-IR-spectroscopy and was found to be complete in all cases after only a few minutes. With the fragments in hand the synthesis of the complete molecule was straightforward and allows us now to produce efrapeptin C routinely on a 10 mg-scale. The synthetic material was characterized using mass spectrometry, NMR- and CD-spectroscopy and is an inhibitor of *E. coli* F_1-ATPase.

Ac-Pip^1-Aib^2-Pip^3-Aib^4-Aib^5-Leu^6-βAla^7-Gly^8-Aib^9-Aib^{10}-Pip^{11}-Aib^{12}-Gly^{13}-Leu^{14}-Aib^{15}-X



Structure of Efrapeptin C

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