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P 09 New Synthetic approaches and Strategies P 10

STEREOSELECTIVE SYNTHESIS OF ASPARTYL PEPTIDES VIA C-N-BOND FORMATION

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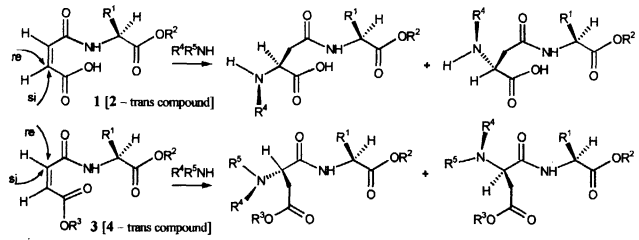
We have developed a convenient way to receive ß- and α-aspartyl peptides by the addition of N-nucleophils to the double bond of N-(cis-/trans-ß-carboxyacryloyl)-amino acid esters **1/2** and N-(cis-/trans-ß-alkoxycarbonylacryloyl)-amino acid esters **3/4**. In earlier investigations we have considered the question of regioselectivity in addition reactions of benzylamine by changing the reactivity of the vinylogous maleyl system by introducing a ß-ester group into 1-Phe-OMe [1].
In this contribution the results of the stereoselective formation of aspartyl peptides by 1,4-addition of different amines or metal amides to a variety of prochiral α,ß-unsaturated amino acid esters **1-4** will be presented. Especially the N-nucleophil and the temperature play an important role for diastereoselection. A highly diastereoselective synthesis of aspartyl peptide succeeded when using chiral amines or metal amides at low temperatures. Furthermore, it will be illustrated for a model peptide that the synthesis of the SS- or RS-α-aspartyl peptide products can be controlled by the choice of the nucleophil, which attacks either the si- or re-face of the unsaturated amino acid ester.



The stereoselectivity was monitored qualitatively and quantitatively by RP-HPLC and NMR spectroscopy and will be discussed in combination with the results of semiempirical calculations.

[Supported by DFG]

[1] C. Griehl, D. Ströhl, H. Jeschkeit, E. Kleinpeter, Monatshefte für Chemie 123 (1992) 647

NAPHTHALENE AMIDE LINKER (NAL), A NEW BACKBONE AMIDE HANDLE FOR SOLID-PHASE SYNTHESIS

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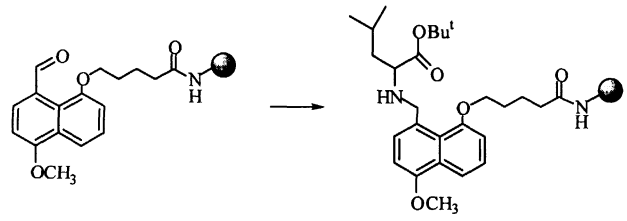
[b]Department Of Organic Chemistry, Technical University of Denmark, Lyngby, Denmark

A new handle for backbone amide anchoring, based on di(alkoxy)naphthalenecarboxaldehyde (NALdehyde) was synthesized in five convenient steps from 1,5-dihydroxynaphthalene, a key step being a highly regioselective mono-demethylation of 1,5-dimethoxynaphthalenecarboxaldehyde under chelation control. NALdehyde was coupled to an aminomethyl polystyrene support, the first monomer attached by an efficient reductive amination, and the secondary amine acylated to form a naphthalene amide linker (NAL) anchoring. After several on-resin synthesis steps cleavages were effected with TFA-H_2O (19:1) or TFA-DCM (1:1). The properties of the NAL handle were evaluated in the solid-phase synthesis of several peptides.



P 11 P 12

CHEMOSELECTIVE ACYLATION OF HYDRAZINOPEPTIDES : A NOVEL AND MILD METHOD FOR THE DERIVATIZATION OF PEPTIDES WITH SENSITIVE FATTY ACIDS

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The modification of peptides by hydrophobic moieties, such as fatty acids, is now widely recognized as a means of enhancing their transport across biological membranes.[1] Among other properties, lipopeptides have found widespread use in both the targeting of intracellular receptors[2] and for the development of synthetic vaccines.[3] However, most of the available synthetic methods for lipopeptides do not allow the modification of peptides by unsaturated fatty acids or cholesterol derivatives. This point is crucial since the nature of the fatty acid is known to have a profound effect upon the interaction with the cell membrane and its alteration. Thus, a novel method was developed for the synthesis of peptides substituted by unsaturated, polyunsaturated or sensitive fatty acid, based upon the following criteria : 1) The reaction of a fully deprotected and purified peptide with an activated fatty acids. 2) The necessity of very mild and chemoselective experimental conditions, compatible with sensitive fatty acids. 3) The stability of the linkage during the purification steps and *in vivo*. We will describe that the reaction of α-hydrazinoacetylpeptides $H_2NNHCH_2CO\text{-}AA_1\text{---}AA_n$ with activated fatty acids (*N*-hydroxysuccinimidyl esters) fulfills these specifications, and allows the synthesis of hydrazides $RCO\text{-}HNNHCH_2CO\text{-}AA_1\text{---}AA_n$ in good yield and high purity.[4] The acylation is chemoselective and was applied to the sequence 95-132 of the murine interferon-γ to give agonists of the cytokine.

1. Loing, E. ; Delanoye, A. ; Sergheraert, C. ; Tartar, A. ; Gras-Masse, H. *Pept. Res.* 1996, *9*, 229-232.
2. Thiam, K. ; Loing, E. ; Verwaerde, C. ; Auriault, C. ; Gras-Masse, H. *J. Med. Chem.* 1999, 1999, 42, 3732-3736.
3. a) Déprez, B. ; Sauzet, J.-P. ; Boutillon, C. ; Martinon, F. ; Tartar, A. ; Sergheraert, C. ; Guillet, J.-G. ; Gomard, E. ; Gras-Masse, H. *Vaccine* 1996, *14*, 375-. b) Zeng, W. ; Jackson, D.C. ; Rose, K. *J. Pep. Science* 1996, *2*, 66-72.
4 Bonnet, D. ; Gras-Masse, H. ; Melnyk, O. FR 99 10626 (08/19/1999). Bonnet, D. ; Rommens, C. ; Gras-Masse, H. ; Melnyk, O. Tetrahedron letters 2000, 41, 45-48.

SYNTHESIS OF HCV AND HIV B-CELL EPITOPES USING POLYSTYRENE SUPPORTS WITH NEW CROSS-LINKING AGENT

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The great influence of polymer matrix structure on swelling properties and kinetic of coupling reaction in solid phase peptide synthesis (SPPS) is well documented. Recently «Advanced Chemtech» has demonstrated the superior characteristics of their new ParaMax Merrifield resin with regular positions of chloromethyl groups.

Here we present the preliminary study of regular cross-linking agent influence on the utility of polymeric support in SPPS. By using the suspension copolymerization of styrene with bis-vinylphenyl ether (BVPE), the polymer beads with average size 150-300 mesh have been obtained. The extent and regularity of matrix cross-linking were checked during the study of its relaxation properties by NMR. Then the polystyrene core was modified by standard methods produce Merrifield and MBHA resins.

In order to estimate the utility of these resins in SPPS we have performed the synthesis of acyl carrier protein fragment 65-74, antigenic determinant of HCV NS4 protein (21 amino acids) and HIV-2 gp36 epitope (16 amino acids) with «difficult» sequences using new polymers, standard Merrifield resin («Sigma») and MBHA resin («Neosystem Laboratoire») in parallel. In all three cases the HPLC picture of crude peptides was similar or somewhat better for polystyrene - BVPE support. The application of new polymer permits us to obtain HCV NS4 antigenic determinant in reasonable yield without double coupling synthetic protocol. It should be mentioned that during the synthesis we have observed the known side reaction of glycine residue transfer to the N-terminal amino group of peptide chain via preliminary acylation of histidine imidazole ring. This process may be efficiently suppressed by additional piperidine treatment.

The present results suggest that the resins based on polystyrene crosslinked by BVPE may produce at least equal results in the synthesis of peptides with «difficult» sequences as compared to standard polystyrene - divinylbenzene resins.

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FULLY SOLID-PHASE SYNTHESIS AND ANTI-MICROBIAL PROPERTIES OF A CYCLIC ANALOG OF PYRRHOCORICIN

Marc Cudic, Philippe Bulet[a], John D. Wade[b], Goran Kragol, Barry A. Condie and Laszlo Otvos, Jr., The Wistar Institute, Philadelphia, PA, USA; [a]Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France; [b]Howard Florey Institute, Melbourne, Australia

Inducible antibacterial peptides represent a field of study where contemporary biochemistry, immunology and drug design converge. The peptides isolated from insects are resistant to proteolytic degradation in hemolymph, but quickly decompose in mammalian sera making them unsuitable for drug development. In our efforts to stabilize pyrrhocoricin, a peptide originally isolated from *P. apterus*, we prepared a 29-mer cyclic analog, *cyclo*[VDKGSYLPRPTPPRPIYNRNRPPTPRPLK], which showed high efficacy and broad activity spectrum against model bacterial strains in our preliminary studies. When the synthesis of this cyclopeptide was repeated in a larger scale using a commercially available Fmoc-Asp(resin)-OAll support, the peptide assembly failed due to insufficient removal of the α-carboxyl-protecting allyl group. However, the cyclopeptide could be easily prepared on a new support, in which the allyl group was replaced with the hydrazine cleavable 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}-benzyl ester (Dmab). When the cyclopeptide was submitted to stability studies in human and mouse serum. it decomposed unexpectedly fast, with the main endopeptidase cleavage site located between Asn18 and Arg19 (numbering according to unmodified pyrrhocoricin). In detailed *in vitro* efficacy studies the cyclopeptide was found to kill the Gram-negative strains *E. coli, A. tumefaciens* and *S. typhimurium* in low micromolar concentrations. It also killed the Gram-positive *M. luteus, A. viridens* and *B. megaterium* albeit with somewhat lower efficacy. No activity was detected against *P. aeruginosa, E. caratovora, S. aureus, S. pyogenes*, the yeast strain *C. albicans* or the filamentous fungus *A. fumigatus*. The increased antibacterial activity compared to pyrrhocoricin and its linear analogs is likely due to stabilization of the bioactive turn conformation at the termini, as well as to the repetition of the central octapeptide segment. In support, another pyrrhocoricin analog, which contained two copies of the peptide on a C-terminal diaminopropionic acid scaffold, exhibited activities similar to the lead cyclic compound.

NEW SYNTHESIS OF α-AMINO ALDEHYDE

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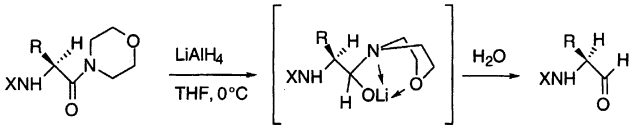
A new method for the synthesis of N-protected α-amino aldehydes was developped. N-protected α-amino amides of morpholine were easily prepared and then reduced with $LiAlH_4$ to produce clean N-protected α-amino aldehydes. This new scheme of synthesis can be used with Boc, Z and Fmoc protected amino acids.



X = Fmoc, Boc, Z

This method of preparation of N-protected α-amino aldehyde is comparable with that of Weinreb (1) and provides an interesting alternative. The high cost of N,O-dimethylhydroxamate hydrochloride makes this new approach attractive since morpholine amides can easily be prepared at low cost. We have also shown the compatibility of the reduction conditions with most of the commonly used N-protecting groups. The use of morpholine amide as protecting group at the C-terminus allowed peptide synthesis and its reduction at the final step leading to peptide aldehydes. This group is also stable in the presence of bulky hydrides such as $AlLiH(OtBu)_3$, providing interesting alternatives.

1. Nahm S. & Weinreb S. *Tetrahedron Letters*, **1981**, *39*, 3815-3818

P 15 — P 16

POST-SYNTHESIS MODIFICATION OF ASPARTYL OR GLUTAMYL SIDE-CHAINS ON SOLID SUPPORT

Céline Douat[1], Marielle Paris[1], Annie Heitz[2], William Gibbons[1], Jean Martinez[1] and Jean-Alain Fehrentz[1]

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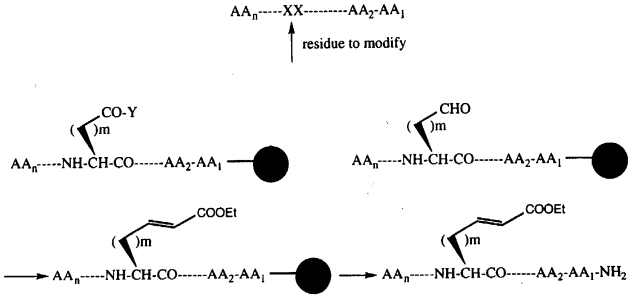
A methodology to modify aspartyl or glutamyl residue side-chains after their incorporation on solid phase synthesis in a peptide sequence was developed. This strategy using the concept of Weinreb amide on the side chain of aspartyl or glutamyl residues allowed reduction of the amide side-chain into aldehyde and the reaction of different groups with this aldehyde function on solid support. The usefulness of this method was proved by the synthesis of H-Phe-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-leucine amide and is under study in our laboratory.



This methodology has been applied to the synthesis of chiral allyl- and homoallyl-glycine derivatives. Recent developments will be presented.

TRIS(PIPERIDINO)PHOSPHONIUM SALTS AS NEW COUPLING REAGENTS AND THEIR APPLICATIONS IN SPPS

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Abteilung für Physikalische Biochemie des Physiologisch-chemischen Instituts der Universität Tübingen, Hoppe-Seyler-Str. 4, D-72076 Tübingen, Germany.

One of the most common methods for the formation of the peptide bond involves the presence of 1-hydroxybenzotriazole (HOBt). HOBt is used either in conjunction with carbodiimides, or as a constituent of *in situ* active ester generating phosphonium (BOP, PyBOP®) or uronium (HBTU, TBTU, HAMTU, etc.) salts. The PyBOP® reagent represents a tris(pyrrolidino)phosphonium salt, but to our knowledge nothing is known about tris(piperidino)phosphonium salts as coupling reagents.

Our presentation focusses on the syntheses and applications of new tris(piperidino)phosphonium salts in combination with 1-hydroxybenzo-triazole, pentafluorophenol, 1-hydroxy-7-azabenzotriazole 3-hydroxy-4-oxo-2,4-dihydro-1,2,3-benzotriazine and the mercapto compounds 2-mer-captobenzoxazole, 1-mercaptobenzotriazole and 1-mercapto-2,4-dinitro-benzene for the *in situ* thioester activation [1, 2].

The syntheses of these new coupling reagents will be given. To test the usefulness of these compounds for SPPS a series of peptides has been successfully synthesized in combination with different resins and conditions, e.g.: Thymopoetin II (29-41), some Troponin T sequences, Diptericin, Thymosin β_{10} (1-15), etc.

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2. Echner, H., Voelter, W., in *Innovation and Perspectives in Solid Phase Synthesis and Combinatorial Chemical Libraries, 6th International Symposium*, (R. Epton, ed.), in press.

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CONTROL OF THE EXTENSION OF THE IODINATION ON TYR RESIDUES / IODINATION OF PHE RESIDUES WITH THE REAGENT IPy_2BF_4

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Previous work with the reagent IPy_2BF_4 proved the direct electrophilic aromatic iodination of Tyr residues, both in solution and on the solid phase. The selective iodination of phenol groups on the solid phase in molecules where several phenol groups are present was also achieved.

Iodinated derivatives have various uses in organic synthesis, nuclear medicine and radioimmunoassay . Although the monoiodinated product is mainly required, most methods of iodination yield a high percentage of diiodinated derivative on substrates showing aromatic activated residues. In this concern, the control of the extension of the reaction with IPy_2BF_4 was studied. By using a slight molar excess of the iodinating reagent in an acid containing medium, the activated aromatic residues on Tyr derivatives and peptides selectively react yielding, as major products, the monoiododerivatives. These conditions are compatible with the presence of non-activated Phe residues, disulfide bridges and post-translational modifications, such as in glyco- and phosphopeptides. The reaction proceeds in organic solvents as well as in aqueous systems.

One step further on the application of this reagent in peptide chemistry was to investigate this iodination reaction upon non-activated aromatic rings. Thus, conditions for the direct iodination of Phe were found by combining an acidic medium with the reagent IPy_2BF_4. The method was assayed on several peptides, including aspartame and a chemotactic peptide. The procedure is a very straighforward method which allows the direct iodination of Phe residues in one step reaction, while other procedures require activation of the aromatic moeity by metallation.

DE NOVO SYNTHESIZED PROTEINS WITH METALLO-PORPHYRIN COFACTORS

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The aim of protein *de novo* design is to construct minimal functional units of complex natural enzymes [1,2]. Based on the structural motif of a four-helix-bundle a small polypeptide module was built via chemical peptide synthesis. The hydrophobic interior of this four-helix bundle contains one binding pocket with two histidine residues which ligate metalloporphyrins. In this work porphyrins containing Fe^{3+}, Co^{3+}, or Zn^{2+} as the central metal ion were successfully bound to the polypeptide modules.
The assembly of the polypeptide module was studied by circular dichroism and tryptophan fluorescence. In accordance with the design concept a high helical content and hydrophobic shielding of tryptophan residues located in the interior of the four-helix bundle was observed. The stability of the *de novo* synthesized proteins towards unfolding with the denaturant guanidinium hydrochloride was monitored with circular dichroism (loss of helical secondary structure), tryptophan fluorescence (loss of hydrophobic shielding) und UV/VIS absorption spectroscopy (loss of cofactor binding).
EPR techniques have been applied to characterize the binding situation of the paramagnetic Fe(III)-porphyrin [3] and the triplet state of the Zn(II)porphyrin which was generated by *in situ* light irradiation. The redox chemistry of Fe(III)- and Co(III)-porphyrin incorporated in *de novo* synthesized proteins was studied. It was shown that the Zn(II)-porphyrin polypeptide module can act as electron donor in light induced electron transfer to a quinone.
These *de novo* designed proteins are well suited to study the influence of the amino acid surrounding on the functional properties of the cofactors. The limitations and possibilities to develop these small polypeptide modules into more complex systems, for example artificial protein models for photosynthesis and other catalytic processes are discussed.

[1] Robertson et al., *Nature* (1994) *368*, 425-432.
[2] H.K. Rau, W. Haehnel, *J. Am. Chem. Soc.* (1998) *120*, 468-476.
[3] M. Fahnenschmidt, H.K.Rau, R. Bittl, W. Haehnel, W. Lubitz, *Chem. Eur. J.* (1999) *5*, 2327-2334.

P 19 P 20

PREPARATION OF BETA-AMYLOID PEPTIDES WITH FLUORESCENT LABELING

Lívia Fülöp, Botond Penke, Márta Zarándi, University of Szeged, Department of Medical Chemistry, Hungary

The undecapeptide [25-35] was found to be the active center of beta-amyloid peptides (Aβ) found in senile plaques in Alzheimer's disease. Earlier studies in our laboratory showed antagonistic effects of a tetrapeptide analog of Aβ [25-35] both *in vitro*[1] and *in vivo*[2]. The fast and high performance testing of new potentially antagonistic derivatives of this peptide has not been resolved yet. Possible effective techniques like fluorescence microscopy or flow cytometry require a fluorophore built into the molecule avoiding the change of biological activity. Attempts were done on the selective liquid phase labeling of peptides containing lysine residues[3]. Despite of the controlled conditions of the reactions, polylabeled derivatives were formed. The aim of our work was to develop a method for the selective fluoresceination at the N-terminus of Aβ undecapeptide. Aβ[25-35] was synthesized by solid phase methodology with the use of Boc-chemistry, where the side chain functional group of lysine was protected by Fmoc-group. The peptide was cleaved off the resin with HF, and the crude peptide was further reacted with fluorescein isothiocyanate (FITC) or carboxyfluorescein succinimidyl ester (FAM). Finally the Fmoc-group was removed from the lysine in one fast step allowing the N-terminus labeling intact. After purification, the purity of the labeled peptides was >95%. The synthesis was optimized for both reactants considering the hydrophobic character and aggregation ability of Aβ[25-35]. Based on our findings, extension of the method for labeling of Aβ[1-40] is in progress.

[1] Laskay, G.; Zarándi, M.; Varga, J.; Jost, K.; Fónagy, A.; Torday, Cs.; Latzkovits, L.; Penke, B.; Biochemical and Biophysical Research Com. 235,479-481 (1997).
[2] Harkány, T.; Ábrahám, I.; Laskay, G.; Timmerman, W.; Jost, K.; Zarándi, M.; Penke, B.; Nyakas, C.; Luiten, P.G.M.; NeuroReport 10, 1693-1698 (1999).
[3] Chersi, A.; Sezzi, M.L.; Romano, T.F.; Evangelista, M.; Nista, A.; Biochimica et Biophysica Acta 1034, 333-336 (1990).

POTENT AND SELECTIVE INHIBITION OF ZINC AMINOPEPTIDASE A BY GLUTAMYL AMINOPHOSPHINIC PEPTIDES

Dimitris Georgiadis,[a] Gilles Vazeux,[b] Catherine Llorens-Cortes,[c] Anastasios Makaritis,[a] Magdalini Matziari,[a] Vincent Dive[b] and Athanasios Yiotakis[a]
[a]Department of Chemistry, Laboratory of Organic Chemistry, University of Athens, Panepistimiopolis Zografou, 15771, Athens, Greece
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[c]INSERM Unité 36, Collège de France, 3 rue d'Ulm, 75005 Paris, France

Aminopeptidase A (glutamyl aminopeptidase, APA, EC 3.4.11.7), a zinc metallopeptidase was recently shown to be involved in the *in vivo* formation of angiotensin III by cleaving the N-terminal aspartyl residue of angiotensin II. Due to the major role of angiotensin III on the brain renin angiotensin system, APA is considered as a novel target for the development of potent inhibitors able to elicit central antihypertensive effects. Through the development of a new chemical strategy, aminophosphinic peptides containing a pseudoglutamyl residue, GluΨ[PO_2CH_2]LeuXaa, in the N-terminal position were synthesized and evaluated as inhibitors of APA. The most poent inhibitor developed in this study, GluΨ[PO_2CH_2]LeuAla, displayed a K_i value of 0.8 nM for APA, but was much less effective in blocking aminopeptidase N (APN) (K_i=31 μM). The critical role of the glutamyl residue in the phosphinic peptide, both in potency and selectivity, is exemplified by the P_1 position analogue, AlaΨ[PO_2CH_2]LeuAla, which exhibited a K_i value of 0.9 nM toward APA, but behaved as a rather potent inhibitor of APN (K_i=25 nM).

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DIMERIZATION OF PEPTIDE AND NON-PEPTIDE MOIETIES HAVING A C-TERMINAL GLYCINE

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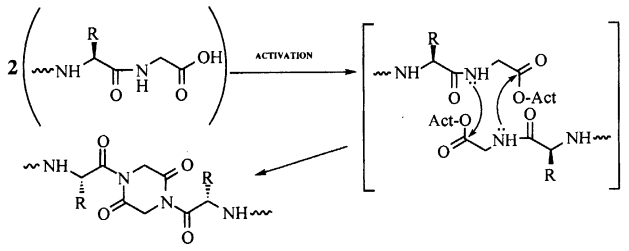
Dimerisation of an active compound often results in enhanced binding and improved pharmacological properties. This potency can be attributed to higher concentration of pharmacophores in proximity of recognition sites. Usually, this bivalent ligant approach implies the use of a symmetrical bifunctionnal linker X to anchor two substrates P, giving rise to the general structure P-X-P. In this study, we have shown that it was possible to build different dimeric structure, involving a Gly-Gly diketopiperazine scaffold, by activating C-terminal glycine peptides.



We have dimerized the minimal active CCK sequence (Ac-Trp-Leu-Asp-Phe-Gly-OH) as an example from its C-terminal glycine extended form and we have obtained the corresponding diketopiperazine dimer derivative, whose biological activity was evaluated. This strategy also applied to other peptides having other amino acids than glycine at their C-terminus, as well as for dimerization of non-peptide units.

DERIVATIZATION OF AMINO ACIDS WITH AN AZO DYE

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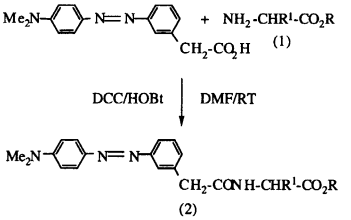
Coloured amino acid derivatives find applications as a markers in biological assays. We have been engaged in the derivatisation of amino acids at their amino group with an azo dye obtained either from 3-aminobenzoic acid and *N,N*-dimethylaniline or from 3- or 4-aminobenzoic acid and β-naphthol [1]. We wish now to present the results of the acylation of the amine function of amino acid methyl and ethyl esters (1) with another carboxyl azo dye obtained from 3-aminophenylacetic acid and *N,N*-dimethylaniline.



(a) R = Me, R¹ = H
(b) R = Me, R¹ = CH_3
(c) R = Me, R¹ = $CH(CH_3)_2$
(d) R = Me, R¹ = $CH(CH_3)(CH_2CH_3)$
(e) R = Me, R¹ = $CH_2CH(CH_3)_2$
(f) R = Me, NH_2-CHR^1-CO_2R = H-Pro-OR
(g) R = Me, R¹ = $CH_2CH_2SCH_3$
(h) R = Et, R¹ = Ph-CH_2

The amino acid esters (1a-h) were acylated with the dye by a DCC/HOBt coupling [2]. The coloured acylamino acids were obtained in yields of 53-91% and their structures confirmed by the usual analytical techniques. The stability of these compounds under electrochemical reductive conditions is now being evaluated.

[1] M.S.T. Gonçalves and H.L.S. Maia, 3rd National Meeting of Organic Chemistry, University of Beira Interior, Covilhã, Portugal, 1999; M.S.T. Gonçalves and H.L.S. Maia, 7th Iberic Peptide Symposium, University of Valencia, Valencia, Spain, 2000.
[2] M. Bodanszky, A. Bodanszky, "The Practice of Peptide Synthesis", Springer-Verlag, Berlin, 1984.

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SYNTHESIS AND SPECTROSCOPICAL STUDIES OF NEW UNSATURATED AMINO ACID DERIVATIVES AS PRECURSORS FOR STEREOSELECTIVE PEPTIDE SYNTHESIS

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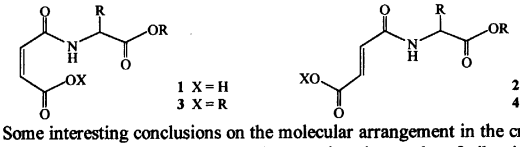
[a]Anhalt University of Applied Sciences, Dept. of Biotechnology, Köthen, Germany; [b]University of Halle, Dept. of Chemistry, Germany

Previously, we have suggested the use of N-(cis-ß-benzyloxycarbonylacryloyl)-phenylalanine methyl ester as precursor for a new aspartam synthesis [1]. In this contribution the results of the synthesis and spectroscopical studies of new N-(cis-/trans-ß-carboxyacryloyl)-amino acid esters **1/2** and N-(cis-/trans-ß-alkoxycarbonylacryloyl)-amino acid esters **3/4** as efficient starting compounds for a convenient synthesis of ß- and α-aspartyl peptides will be presented. The synthesis of the cis compound **1** was provided in quantitative yields by using standard acylation procedure with maleic anhydride. A suitable way to **3** we found in the reaction of **1**-caesium salts with alkylhalogenids. Other esterification methods give undesirable side reactions. The synthesis of the **3**-pro derivative is more successful via DCC-DMAP activation of **1**-pro with alkyl alcohol. The trans compounds **2** and **4** have been obtained by cis/trans isomerization of the corresponding cis derivatives **1** in high-boiling solvents and **3** with piperidine in ether.



1 X = H
3 X = R

2 X = H
4 X = R

Some interesting conclusions on the molecular arrangement in the crystals as well as in the dissolved state may be drawn using the results of vibrational spectroscopy based on the existence of groups possessing high potential to form hydrogen bonds, namely NH, OH and COOH. In the substances with an esterificated carboxyl group no dominant molecular interactions may be detected, but in the other compounds, bearing a free COOH group, OH···N interactions are the main features determining the structures. Nearly all molecular arrangements will be described in terms of vibrational spectroscopy.

[Supported by DFG]

[1] C. Griehl, D. Ströhl, H. Jeschkeit, E. Kleinpeter, Monatshefte für Chemie 123 (1992) 647

RESIN BOUND *N*-TERMINAL PEPTIDE ALDEHYDES: FORMATION AND THEIR APPLICATION AS ELECTROPHILES IN THE COMBINATORIAL SYNTHESES OF PEPTIDOMIMETICS

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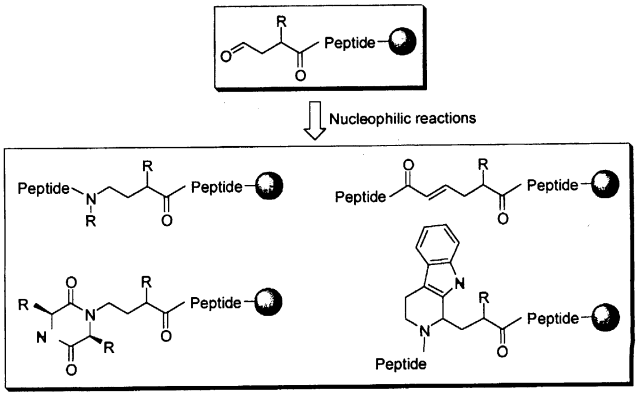
Positioning aldehydes on the *N*-terminal position of peptides is a rather unexplored, yet very promising concept. Similar to *C*-terminal peptide aldehydes, this class of compounds may act as potent inhibitors against various enzymes such as cysteine proteases. Furthermore, the retention of these compounds on solid support permits the possibility of on-bead assaying techniques.

In addition, *N*-terminal peptide aldehydes can be treated with various nucleophiles in order to produce a range of diverse peptidomimetics intended for enzyme inhibition. Reactions such as reductive amination, Horner-Wadsworth-Emmons, and Pictet-Spengler have been investigated. Some representative products are shown below.



Posters: topic A1

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SYNTHESIS AND SEPARATION OF TOPOLOGICAL ISOMERS OF GUANYLATE CYCLASE C ACTIVATING PEPTIDE (GCAP II)

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The systematic isolation of circulating regulatory peptides which generate cyclic guanosine-5'-monophosphate (cGMP) as a second messenger resulted in the identification of a novel member of the guanylin family of peptides, GCAP-II. It is an acidic peptide containing 24 amino acids (AAs) with the following sequence: FKTLRTIANDDCELCVNVACTGCL, and two disulfide bonds. A very interesting feature of this peptide hormone is the exhibition of topological isomerism with the same disulfide connectivity. GCAP-II stimulates chloride secretion in isolated human intestinal mucosa mediated by intracellular cGMP increase. It activates the specific guanylate cyclase receptor (GC-C) of cultured human colon carcinoma. The 16 C-terminal residues of this peptide are identical to uroguanylin, isolated from human urine. Both peptides have the same precursor containing 112 AAs as deduced from its deoxyribonucleic acid (DNA) sequence. Guanylin and related peptides share sequence similarity with the bacterial heat-stable enterotoxin (STa and STp).

Synthesis of GCAP-II was achieved by the continuous-flow solid phase peptide synthesis (CF-SPPS) using a peptide synthesizer and applying standard 9-fluorenylmethoxycarbonyl (Fmoc) strategy using 4-fold protected AAs and the coupling reagent. The couplings were achieved using *O*-(7-azabenzotriazolyl-tetramethyl uronium hexafluorophosphate (HATU). Deprotection of the Fmoc groups was affected with diazabicyclo[5.4.0]-undec-7-ene/dimethylformamide (DBU/DMF). After removal of the finished peptide from the resin the first disulfide bond was formed selectively between cysteine^{12} (Cys) and cysteine^{20} followed by the high performance liquid chromatographic (HPLC) purification to obtain the partially protected [acetamidomethyl-Cys^{15}, acetamidomethyl-Cys^{23}]-GCAP II as a single compound. After the second selective disulfide bond formation two different products were obtained which corresponded to the topological isomers of GCAP-II with the same AA sequence, disulfide connectivity and molecular mass. These isomers, however, were separable by HPLC and could be isolated.

• R. Hess et al. FEBS Lett. **1995**, *374*, 34-38.

NEW CHEMOTACTIC PEPTIDES

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A large number of synthetic peptides with different composition and sequence has been reported to have chemotactic character for different living cells [1]. Ciliated protozoa (e.g., *Tetrahymena sp.*) serve as established models for cell receptor research [2], especially for the analysis of chemotaxis [3]. We have studied the chemotactic properties of some peptides with SXWS or WSXWS sequence by capillary method [4]. We found that depending on the identity of X, some peptides exhibited pronounced chemoattractant character (for example, SEWS did it at 10^{-12} M). Based on this observation we have synthesized further members belonging to these two peptide families by Fmoc-technique (X = one of 19 proteinogen amino acids; cysteine was omitted). Two types of fluorescent derivatives of these peptides were also prepared for confocal laser scanning microscopy. For N-terminal fluorescent labelling we have used 4-[7-hydroxycoumaryl]acetic acid [5] or 4-ethoxymethylene-2-[1]-naphthyl-5(4H)-oxazolone [6].

[1] Kőhidai L., Kovács P., Csaba G.: *Cell Biol. Int.* 18, 119-122 (1994).
[2] Csaba G.: *Int. Rev. Cytol.* 95, 327-377 (1985).
[3] Csaba G., Kőhidai L.: *Bioscience Reports* 15, 185-190 (1995).
[4] Kőhidai L., Lemberkovits É., Csaba G.: *Acta Protozool.* 34, 181-185 (1995).
[5] Kele P., Illyés E., Mező G., Dóda M., Mák M., Kaposi A.D., Sebestyén F.: *Lett. Peptide Sci.* 6, 235-238 (1999).
[6] Kóczán G., Csík G., Balog E., Bősze S., Hudecz F.: submitted to *Tetrahedron* (2000).

Supported by grants from the Hungarian Ministry of Education (N° FKFP 0101/97 and 0229/99) and from the Hungarian Research Fund (OTKA) N° W 15598 and T 032533.

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CATECHOL AS A NUCLEOPHILIC CATALYST OF THE PEPTIDE BOND FORMATION

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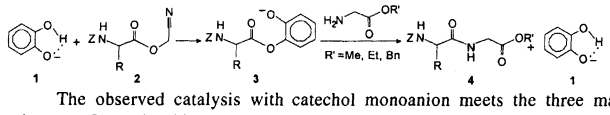
We found that catechol oxyanion **1** accelerates the peptide bond synthesis by transesterification of the mildly activated aminoacid esters **2** followed by aminolysis of the resulted catechol ester **3**.



1 2 3 4 1

The observed catalysis with catechol monoanion meets the three main requirements for nucleophilic covalent catalysis:

(i) the catechol monoanion **1** reacts faster with cyanomethyl esters **2** than the latter does with the amino component H-Gly-OR' since an accumulation of the intermediate **3** occurs.

(ii) the intermediate catechol ester **3** is capable of faster aminolysis than the parent ester **2**.

(iii) the concentration of intermediate **3** is kinetically controlled suggesting that the product **4** is thermodynamically more stable than the intermediate catechol ester.

The observed catechol-catalyzed peptide synthesis is a congruent reaction to the ribosomal peptide synthesis the catechol being a transfer RNA mimic. The 3'-terminal of the t RNA *cis*-2',3'-diol reacts with an activated amino acid to yield the covalent intermediate amino acyl-t-RNA that undergoes aminolysis on the ribozome by subsequent amino acid t-RNA. On the other hand, this methodologically new catalytic peptide bond synthesis is an enzymomimetic alternative of enzymic peptide bond synthesis that avoids limitations such as specificity of and aqueous medium, required by biocatalysts.

The interaction of the catecholate **1** with N-protected amino acid and peptide cyanomethyl esters **2** provides an easy access to catechol esters **3** known to aminolyse abnormally rapidly and racemization-free (Jones & Young, Chem. Comm. (1967) 35-36 and Kemp & Chien, J. Amer. Chem. Soc. 89 (1967) 2743-2745).

THE INFLUENCE OF THE RESIN, C-TERMINAL AMINO ACID AND TEMPERATURE UPON THE YIELD OF PEPTIDYL-RESIN CLEAVAGE BY THE TFMSA/TFA/THIOANISOLE METHOD.

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[a]Dept. of Biophysics, Universidade Federal de São Paulo, São Paulo, Brazil, [b]Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Kobe, Japan.

We have recently proposed a rule for resin selection used in Boc-chemistry based upon the study of the stability of the peptidyl-resin linkage towards both HF cleavage and TFA deprotection steps [*Chem. Pharm. Bull.* (1999) *47*, 1560]. Following this approach, the present report evaluated the TFMSA/TFA/thioanisole/m-cresol (9:74:12:5, v/v) cleavage method varying parameters such as the temperature, resin (benzhydrylamine-resin, BHAR; methylbenzhydrylamine-resin, MBHAR and 4-(oxymethyl)-phenylacetamidomethyl-resin, PAMR) and the C-terminal amino acid (Gly and Phe). The angiotensin II (AII) bearing Phe or Gly at C-terminal position linked to those resins were submitted comparatively to a time course study towards this cleavage method. At 0°C, Gly^8-AII sequence was completely removed in 1, 2 and 6 h from PAMR, MBHAR and BHAR, respectively, whereas the more stable Phe^8-AII-resin linkage was not cleaved quantitatively from MBHAR or BHAR even after 6 h. At 0°C, the apparent first-order rate constants measured in TFMSA-containing cleavage mixture were smaller than those obtained in anhydrous HF. At 25°C, it was possible to cleave completely Phe^8-AII from MBHAR but not from BHAR (up to 6 h). Noticeably, in contrast to the observation in HF, peptidyl-resin linkage seems to be more labile in PAMR than in MBHAR during the TFMSA/TFA/thioanisole reaction. These findings suggested that the cleavage stability follows the order: BHAR > MBHAR > PAMR and that the TFMSA/TFA/thioanisole might be substituted for the HF method but with some restrictions. PAMR is suitable for this alternative cleavage method regardless the protocol but the feasibility of using MBHAR or BHAR is temperature and peptide C-terminal residue-dependent. At 0°C, the use of both resins is only valid when the resin-bound sequence contains hydrophilic amino acid at its C-terminal portion. With hydrophobic residue attached to the solid support, the increase in the temperature up to 25°C is needed for peptide removal from MBHAR but still not sufficient when BHAR is used. Supported by FAPESP, CNPq and Capes.

Posters: topic A1

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AN ECONOMIC SYNTHESIS OF A PEPTIDE ALCOHOL USING A NOVEL LINKER ON POLYSTYRENE RESIN

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To synthesize a peptide containing "C" terminal amino acid in the alcohol form by solid phase method is a challenge. Several synthesis schemes are possible, which require use of specialized acid labile resins such as expensive Rink resin or Chlorotrityl resin and orthogonal protection such as FMOC group to produce the protected peptide having the entire sequence except for the last amino acid with the reduced carboxyl group (alcohol). The last amino acid alcohol in the protected form is then coupled to this protected peptide using special coupling conditions to reduce racemization. Despite this some possibility of racemization exists during the last coupling, since the carboxyl group of the peptide is activated. We have previously published[1] reports on using a novel resin linker, Cysteinyl-S-thiopropionylamido p-methyl benzhydrylamine polystyrene resin. We postulated that this resin would allow one to avoid all these problems, since the attachment of the peptide to the resin would be through the side chain of Cysteine residue. To test this hypothesis, we chose to synthesize D-Ala-Cys-Ala-D-Trp-Lys-Ala-Cys-L-Threonin-ol. This sequence is very challenging. The synthesis is economical since it uses conventional resin p-methyl benzhydrylamine polystyrene and BOC chemistry. It also allowed us to cleave cyclic peptide off the resin. The successful results will be presentedI. We are confident that one can use this linker to synthesize "Octreotide-". It is an octapeptide with a "C" terminal Threonine alcohol. Its acetate is used in the long acting form called Sandostatin LAR- Depot for the treatment of acromegalic patients. Its sequence D-Phe-Cys- Phe-D-Trp-Lys-Thr-Cys-L-Threonin-ol, is also very challenging.

1: S. Kalbag, D. Narindray, D. Slavazza and E. Rinderknecht, "Synthesis of Cyclic S-S Containing Peptides Using BOC Chemistry and a Novel Linker on a Polystyrene resin." Peptides 1998, 100-101 (Akade'miai Kiado' Publishers, 1998; Sa'ndor Bajusz and Ferenc Hudecz, editors).

SYNTHESIS OF OPTICALLY PURE PEPTIDES FROM RACEMIC AMINO-ACIDS BY MEANS OF ENANTIODIFFERENTIATING COUPLING REAGENTS PREPARED IN SITU FROM TRIAZINE CONDENSING REAGENT AND CHIRAL TERTIARY AMINES. THE NEW GENERATION OF COUPLING REAGENTS

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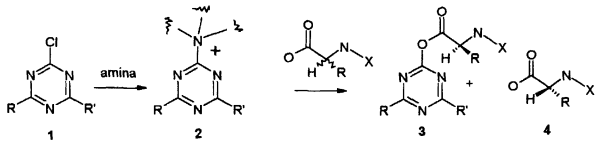
We developed the synthetic procedure for preparation of optically pure peptide directly from racemic amino acids. According to our concept, the chiral coupling reagent consist of a binary system, obtained *in situ* from the two readily accessible components – achiral and already known triazine coupling reagent and the chiral auxiliary – tertiary amine, which could be recovered after enantiospecific activation of carboxylic function.

The reaction product – being chiral quarternary triazinium salt **2** bearing chiral nitrogen atom - exchanges rapidly counterion with racemic carboxylate anione and than, *via* the substitution of chiral ammonium leaving group inside of the two diastereomeric transition states leads to pair of enantiomeric triazine esters **3.**



Even if only twofold excess of racemic carboxylic acid is used, 80-90% yield of the chromatographically homogeneous and optically active (usually, ee 65-100%) products of enantioselective condensation are obtained.

Broad range of common amines was found useful as a chiral auxiliary including strychnine, brucine, and sparteine etc., as well as the variety of easily accessible triazine condensing reagents.

The study was supported by the Polish State Committee for Scientific Research. Grant 3 T09A 029 16.

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PHOSPHORYLATED POLYPEPTIDE SYNTHESIS USING AN EXPRESSED PEPTIDE AS A BUILDING BLOCK VIA THE THIOESTER METHOD

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An expressed peptide was proved to be useful as a building block in the synthesis of a polypeptide *via* a thioester method[1]. Based upon this strategy, phosphorylated p21Max(1—101), **MpS**DNDDIEVE**pS**DA↓ DKRAHHNALERKRRDHIKDSFHS LRDSVPSLQGEKASRAQILDKATEYIQYMRRKNHTHQQDIDDLKRQNALLQ QVRALEKARSSAQ, was synthesized, in which **pS** represents a phosphoserine residue and the arrow, ↓, indicates the coupling site. A polypeptide, p21Max(13—101), which was obtained by expression using *E. coli*, was converted to the N^{α}–α-oxoacyl derivative of p21Max(14—101) *via* transamination of the *N*-terminal amino group by treatment with glyoxylic acid (0.5 M) and nickel(II) sulfate (5 mM) in an acetate buffer (2 M acetic acid and 1 M sodium acetate)[2]. To this intermediate, Boc groups were introduced to the side chain amino groups, followed by treatment with *o*-phenylenediamine to obtain a partially protected peptide segment, [Lys(Boc)^{15,25,31,48,57,68,80,95}]-Max(14—101). This peptide was condensed with a chemically synthesized phosphorylated peptide thioester, Boc-[Ser$(PO_3H_2)^{2,11}$]-p21Max(1—13)-SCH_2CH_2CO-Leu-OH, in the presence of AgCl, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and *N,N*-diisopropylethylamine in DMSO for 24 h to give [Ser$(PO_3H_2)^{2,11}$]- p21Max(1—101). The overall yield was ca. 10% based on p21Max(13—101).

1. (a) Hojo, H.; Aimoto, S. *Bull. Chem. Soc. Jpn.*, **64**, 111–117 (1991). (b) Aimoto, S., Biopolymers (Peptide science), **51**, 247-256 (1999)
2. (a) Dixon, H. B. F.; Moret, V. *Biochem. J.*, **94**, 463–469 (1965). (b) Dixon, H. B. F. *Biochem. J.*, **103**, 38P (1967). (c) Dixon, H. B. F.; Fields, R. *Methods Enzymol.*, **25**, 409–419 (1972)

AMINE RELEASING DUAL LINKER ANALYTICAL CONSTRUCTS FOR FACILE MONITORING OF SOLID PHASE CHEMISTRY.

Corinne Kay*, Geoffrey Willams*, Peter John Murray*, Miles S Congreve*, Jan J. Scici_ski*, Stephen C. McKeown,† Stephen P. Watson,† and Robin A. E. Carr†
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Solid phase chemistry offers many practical advantages over conventional solution phase methods. However, effective monitoring of reactions conducted on solid phase remains problematic as products cleaved from small samples of resins during the synthesis are too often invisible to commonly used analytical techniques. The use of amine releasing dual linker analytical constructs to monitor reactions by high throughput electrospray Mass Spectroscopy will be described. The method enables the user to choose between conventional cleavage, releasing substrate molecules or cleaving at an analytical linker to release a fragment substrate, which is sensitised by possessing an amino group, guaranteeing ionisation and detection by mass spectrometry. The use of a hallmark (MS peak splitter) in the construct will also be described in which the user can distinguish a compound originating from resin from extraneous material and background noise. The methodology is ideally suited to solid phase reaction optimisation, and has also been successfully applied to the development of a novel solid phase linker. Application to monitoring synthesis of short peptide sequences will also be discussed.

TOTAL SYNTHESIS OF CHOLECYSTOKININ-58 BY A THIOESTER SEGMENT CONDENSATION APPROACH

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Many synthetic methods for Tyr(SO_3H)-containing peptides have been reported to date including the Fmoc-solid-phase approach developed by us[1]; however, a general method for the synthesis of long sulfated peptides (> 50 amino acid residues) has not yet been established. In this report, we examined an applicability of a thioester segment condensation approach[2] to the synthesis of cholecystokinin (CCK)-58.
The C-terminal sulfated peptide **[I]** was prepared by our method[1] in 18% yield after deprotection with 90% aq.TFA (0°C, 12 h) followed by HPLC purification. Partially protected thioester segments having C-terminal Pro, **[II]** and **[III]**, were prepared by the following manipulations developed by Futaki *et al.*[3]; 1) assembly of the peptide chain on a Pro-2-chlorotrityl resin by Fmoc-chemistry, 2) cleavage of the fully protected peptide from the resin, 3) thioesterification with HS-$(CH_2)_2$-COOEt using WSCDI-HOBt as a coupling reagent, 4) deprotection of the protecting groups with TFA followed by HPLC purification, and 5) reintroduction of the Boc group to the amino functions.
We conducted two successive segment condensations to construct the full length peptide; first between the segment **[I]** and the Fmoc-protected segment **[II]** to give Fmoc-**[II–I]** (67% yield after HPLC purification), then between the Fmoc-deprotected **[II–I]** and the Boc-protected segment **[III]** to give $(Boc)_3$-**[III–II–I]** (69% yield). Each segment condensation was conducted in DMSO in the presence of $AgNO_3$ (3 eq)-HOOBt (30 eq)-DIEA (20 eq). Final treatment of $(Boc)_3$-**[III–II–I]** with 90% aq.TFA (0 °C, 1.5 h) completed the total synthesis of CCK-58. In MALDI-TOFMS, this product exhibited the $[MH]^+$ ion at *m/z* 6725.7 which coincided with the expected molecular mass (M= 6724.6) for CCK-58.

CCK-58
VSQRTDGESRAHLGALLARYIQQARKAP **[III]** SGRMSIVKNLQNLDP **[II]** SHRISDRDY(SO_3H)MGWMDF-NH_2 **[I]**

1) Kitagawa, K., *et al.*, (1997) *Tetrahedron Lett.*, **38**, 599–602.
2) Hojo, H. and Aimoto, S., (1992) *Bull. Chem. Soc. Jpn.*, **65**, 3055–3063.
3) Futaki, S., *et al.*, (1997) *Tetrahedron Lett.*, **38**, 6237–6240.

NEW METHOD FOR THE SOLID-PHASE SYNTHESIS OF PHOSPHOSERINE CONTAINING PEPTIDES

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Protein phosphorylation has been recognized as one of the most important element of cell regulation and signal transduction. For studying the role of the phosphorylation / dephosphorylation event in the biological function or investigating its conformational consequence the isolation of the appropriate phosphorylated protein usually is not feasible, therefore the efficient chemical synthesis of the releated phosphopeptide is a valuable alternative. There are two major possibilities for the preparation of phosphopeptides: the synthon and the global approach. The synthon method (both in liquid and solid phase) requires appropriately protected phosphorylated derivatives - these derivatives are often commercially not available and their syntheses are multistep, complicated and expensive. For phosphitylation, usually symmetrical phosphoramidite derivatives are used which produce symmetrically diprotected phosphate derivatives. However, while this method can be applied with good yield in the case of phosphotyrosine containing peptides, for phosphoserine and phosphothreonine containing peptides the treatment with piperidine during the standard deprotection cycle leads to β-elimination with loss of the phosphate moiety and formation of the corresponding dehydropeptide . The removing of the protecting group from the phosphate moiety would hinder this reaction, but the resulting free acidic hydroxyl functions can cause other sidereactions such as pyrophosphate formation. The monoprotected derivatives of these hydroxyl group containing aminoacid phosphate esters seem to be the optimal choice, but to date, only the synthon method has been applied for this purpose utilizing Fmoc-Ser(PO(OBzl)OH)-OH and Fmoc-Thr(PO(OBzl)OH)-OH monomers. The global approach, being applicable for any hydroxyl group-containing residue, would offer a more universal method. However, the application of the asymmetrically protected phosphoramidite reagent needed for this has not yet been reported. Here we describe the synthesis of a novel asymmetrical phosphoramidite reagent and its application for the synthesis of a phosphoserine/phosphothreonine containing peptide by the global approach.

New Chromogenic Substrates of Bovine β-Trypsin Based on Sequence of Binding Loop of Trypsin Inhibitor CMTI-III: Design, Chemical Synthesis and Kinetic Investigation.

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A series of trypsin chromogenic substrates with general formula: X1-Ala-X2-Abu-Pro-X3-pNA, where X1 = Ac, H; X2 = Gly, Ala, Abu, Val, Ile, Phe, Ser, Glu; X3 = Orn, Lys, Arg and pNA = paranitroanilide moiety was synthesised. They were designed based on the sequence of the binding loop one of the smallest trypsin inhibitor CMTI-III (*Cucurbita maxima* trypsin inhibitor). All substrates were synthesised by the solid phase method using Fmoc chemistry. To evaluate the influence of the introduced modifications on the substrate-enzyme interactions, kinetic parameters (k_{cat}, K_m and k_{cat}/K_m) against bovine β-trypsin were determinated.
The positively charged amino group in position X1 destabilised the complex between substrate and enzyme whereas acetylation of group increased the substrate specificity (k_{cat}/K_m). The character of amino acid residue introduced in position X2 also significantly influenced this parameter. The k_{cat}/K_m increased in order: Gly, Ala, Abu to achieve the highest value for Val (1.1×10^6 $s^{-1}M^{-1}$). The introduction of more polar amino acid residues (Glu, Ser) considerably reduced the value of this parameter. In the case of substrates modified in position X3 (corresponds to position P_1 of substrate or inhibitor in the contact with enzyme) the k_{cat}/K_m values for such modified substrates increasing in order: Orn, Lys, Arg. These results presented here correspond well with those obtain for trypsin inhibitor CMTI-III modified in discussed positions.
Acknowledgements: This work was supported by the Polish State Committee for the Scientific Research (KBN) research grant No 3 T09 158 18 and BW-8000-5-0269-9.

Synthesis of the Tentoxin various conformational states.

Nicolas Loiseau[#], Jean-Marie Gomis[‡], Jean-Pierre Noël[‡], Marcel Delaforge[#§], François André[§*].
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For several years, the cyclopeptides have been of strong interest because they are a significant source of structure to high biological activity, such as the cyclosporine, or HC-toxins. They are also often used for the studies of the relation structure /activity of various bioactive linear peptides. Tentoxin is a cyclic tetrapeptide produced by the fungus *Alternaria-Tenuis*. This cyclotetrapeptide, found in 1960 by Fulton, presents a specific phytotoxic activity. One a long time hoped to make of it a weedkiller but its industrial range production was a limiting factor. This cyclotetrapeptide is the only inhibitor known of chloroplastic ATPase F0F1, which made of it a molecule of choice for this protein study. This compound exhibit an atypical comportment since after inhibition observed at micromolar concentrations, an two or five times reactivation is observed at millimolar concentration.
In 1995, Eric PINET et al. showed, by NMR (500 MHz), the presence of a multiconformational equilibrium of the tentoxin in aqueous solution. From these experiments, we developed by molecular modeling a conformational predictive model for various tentoxin analogs. We thus conceive and synthesize different cyclic tetrapeptides having a blocked structure, most of time by insertion of an intermolecular bridge, to imitate each conformational state of the tentoxin. These new analogs have been tested in comparison to tentoxin on biological target: the ATPase CF0CF1, in particular by the determination of the bioactive conformation of the tentoxine. These compounds have been metabolised by hepatic cytochrome P450 to generate various metabolites having particular activation toward ATPase.

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KAHALALIDE F: SYNTHESIS AND STRUCTURAL DETERMINATION

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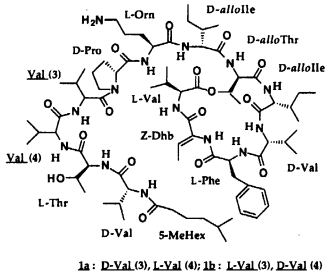
We have developed an efficient solid-phase synthetic approach for the preparation of Kahalalide F, which is the only member of the family of peptides called Kahalalides, isolated from the Sacoglossan mollusc *Elysia rufescens* and the green alga *Bryopsis* sp., with noteworthy bioactivity. Thus, it is presently in clinical trials as a very promising drug for treatment of prostate cancer.



1a: D-Val (3), L-Val (4); **1b**: L-Val (3), D-Val (4)

Besides to be a cyclic compound, the Kahalalide F presents several synthetic difficulties: (i) an ester bond between two β-branched and sterically hindered amino acids (Val and D-*allo*Thr); (ii) the didehydroamino acid (Dhb); (iii) a rather hydrophobic sequence with two fragments containing several β-branched amino acids in a row, and one of them terminated with the saturated aliphatic acid 5-MeHex. The main cornerstones of our strategy are:
(i) use of a quasi orthogonal protecting system with concourse of allyl, *t*-butyl, fluorenyl, and trityl based groups; (ii) use of azabenzotriazole coupling reagents; (iii) formation of the didehydroamino acid residue on solid-phase; and (iv) cyclization and final purification in solution.
HPLC, high field NMR, and biological activity studies have allowed to solve the controversy about the absolute stereochemistry of the natural compound and to conclude that the correct stereochemistry is the one proposed by Rinehart *et al.* (**1a**)[1] whereas the stereochemistry proposed by Scheuer *et al.* (**1b**)[2] correspond in fact to a biologically inactive diastereoisomer of Kahalalide F.

We thank Pharma Mar, s.a. (Madrid, Spain) for supporting this work.

[1] Rinehart, K. personal communication.
[2] Goetz, G., Yoshida, W. Y., Scheuer, P.J. *Tetrahedron* **1999**, *55*, 7739-7746.

SOLID-PHASE SYNTHESIS OF PEPTIDES CONTAINING α,β-DIDEHYDROAMINO ACIDS

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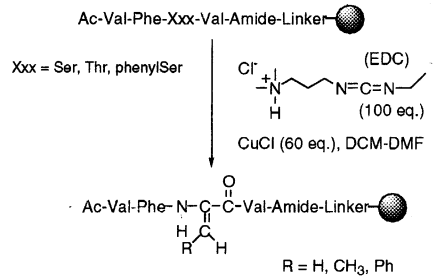
α,β-Didehydroamino acids are frequently encountered in natural peptides with important biological activity. The presence of these residues in peptides confers increased resistance to enzymatic degradation and restricts the conformational flexibility of both, the backbone and the side chain of the didehydroresidue. The incorporation of DDAA into normal bioactive peptides has become an interesting objective, and therefore the search of feasible and suitable methods for the synthesis of peptides containing DDAA (α,β-didehydropeptides (DDP)) has increased considerably in the last years.



Herein, we report a mild and convenient method for preparation of peptides containing α,β-didehydroamino acids, where the solid-phase techniques are used for both the elongation of the peptide chain and the formation of the double bond. This is formed through a β-elimination reaction, using water soluble carbodiimide as activating reagent of the hydroxil function, catalyzed by CuCl.

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NEW METHOD OF MICROCYSTIN SYNTHESIS

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Microcystins are toxic heptapeptides produced by cyanobacteria. They cause the death of animals and are the health risk to human beings. Structure of majority of microcystins is cyclo(-D-Ala-X-D-*erythro*-β-methylAsp-Y-D-(-Glu-Z-), where X, Y and Z are variable amino acids. The synthesis of a unique amino acid, Adda, [(2S,3S,8S,9S,4E,6E)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid] is the most difficult task in the syntheses of microcystins. Our new synthesis of Adda was relied on the coupling of two fragments: C_1-C_4 and C_5-C_{10}. The fragment C_1-C_4 was synthesised from Boc-D-Asp(OMe)-OBzl through stereoselective C_β-methylation followed by the transformation of α-carboxyl group into the appropriate aldehyde. For controlling the desired chirality during the synthesis of C_5-C_{10} Adda fragment we used Oppolzer's bornane-10,2-sultam as a starting material. Acylation provided N-propionyl-sultam, which was transformed through aldol condensation with phenylacetaldehyde into desired chiral alkohol. O-Methylation of this compound was followed by the hydrolysis of sultam group. The free carboxyl group in the obtained intermediate was transformed into aldehyde, which underwent in a next step the Wittig reaction giving the appropriate ester of C_5-C_{10} fragment. The Boc-Adda was obtained by the condensation of both fragments using known procedures. The work on syntheses of several hexapeptides found in microcystins in order to cyclize them with Adda residue into microcystins are in progress.

This work was supported by Polish Scientific Research Committee (KBN).

A GENERAL METHOD FOR THE SYNTHESIS OF ENANTIOPURE ω-SUBSTITUTED α-AMINO ACIDS

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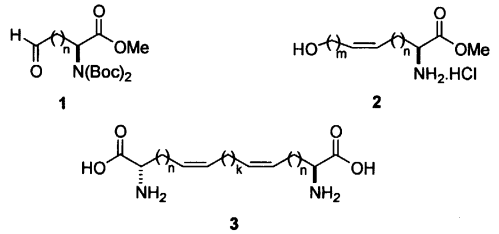
New approaches to the synthesis of non-natural α-amino acids are of current interest, because these compounds represent useful building blocks for the synthesis of analogues of bioactive peptides. Furthermore, α-amino acids bearing a side chain functional group are versatile starting materials for combinatorial chemistry. Continuing our research on the asymmetric synthesis of unsaturated lipidic α-amino acids (G. Kokotos, et al. *J. Org. Chem.* **1998**, *63*, 3741-3744), we present here a general method for the synthesis of enantiopure ω-substituted α-amino acids starting from natural dicarboxylic amino acids. Our strategy is based on the chiral key-intermediate aldehydes **1**, which are obtained from Glu or Asp after suitable protection and selective reduction of γ-methyl ester group by DIBAL. The introduction of the second Boc group is critical, since reduction of the mono Boc derivative led to a mixture of products. A number of ylides containing masked functional groups [$Ph_3P{=}CH(CH_2)_mCN$, $Ph_3P{=}CH(CH_2)_mN_3$, $Ph_3P{=}CH(CH_2)_mOTrt$] were prepared starting from α,ω-alkanediols. Their reaction with the aldehydes **1** was studied under various conditions. As an example, Wittig reaction of **1** with $Ph_3P{=}CH(CH_2)_mOTrt$, followed by deprotection led to ω-hydroxy-α-amino acids **2**. Bis amino acids **3** were prepared by treatment of aldehydes **1** with symmetrical bis(phosphonium ylides) $Ph_3P{=}CH(CH_2)_kCH{=}PPh_3$.



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SYNTHESIS OF NEW CARRIER MOLECULES BASED ON REPEATED TUFTSIN SEQUENCES

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The combination of macromolecules and small bioactive molecules has a wide range of application, particularly in the development of polymer therapeutics. In the field of immunology, polypeptide carriers are frequently applied to induce immune response against covalently attached low molecular weight peptide epitopes. To construct effective synthetic vaccines usually two different epitope peptides are attached to a carrier molecule representing B- and T-cell epitope sequences. We have designed and synthesized oligopeptides as new carrier molecules based on a tuftsin like pentapeptide (Thr-Lys-Pro-Lys-Gly)$_n$ (n=4, 6, 8). Tuftsin (Thr-Lys-Pro-Arg) and its analogue present in dogs (Thr-Lys-Pro-Lys) have various advantageous properties in the living organism (eg. immunostimulation, chemotactic, bactericidal and tumoricidal activities, phagocytosis stimulation). The oligopeptides were synthesized by stepwise synthesis on solid support using Boc/Bzl strategy or by fragment condensation using orthogonal side chain protection (cyclohexyloxycarbonyl and chloroacetyl) of lysine. The stepwise removal of lysine ε-amino protection makes possible selective conjugation (amide and thioether bond formation) of bioactive small molecules. GFLG tetrapeptide as enzyme labile spacer sequence was also connected to the ε-amino group of lysine residues. Solution conformation of oligomer peptides was studied by CD-spectroscopy. The preliminary biological data suggested, that some of the nontoxic (on mouse spleen cells) carrier molecules show effective immunostimulation (sheep red blood cells specific humoral immune response) and chemotactic activity (*Tetrahymena pyriformis* cells).

These studies are supported by Hungarian Research Fund OTKA (T 32425)

METHANOLYSIS AND HYDROLYSIS ON KAISER'S OXIME RESIN ASSISTED BY METAL IONS

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Recently, we have demonstrated that Ca^{2+} or Eu^{3+} are able to assist methanolysis and aminolysis of peptidyl-Kaiser's resin (KOR) with high yields[1, 2]. In order to extend the application of this new procedure to the preparation of protected peptides derived from cholecystokinin, we investigated the following possibilities: 1- preparation of Ac-Ile-Ser(Bzl)-Asp(OcHex)-OMe (**I**) through methanolysis of the corresponding peptidyl-KOR assisted by Ca^{2+}, Eu^{3+} or Tb^{3+}. 2- preparation of Ac-Ile-Ser(Bzl)-Asp(OBzl)-Arg(Mtr)-OMe (**II**) through methanolysis of the corresponding peptidyl-KOR assisted by Ca^{2+}; 3- preparation of Ac-Ile-Ser(Bzl)-Asp(OcHex)-OH (**III**) through hydrolysis of the corresponding peptidyl-KOR assisted by Ca^{2+}. While in DCM/MeOH **I** was selectively obtained with yields in the range of 60-100%, in DMSO/MeOH the desired peptide ester was produced only in the presence of Ca^{2+} (100% yield). In the absence of the metal ions no product was formed in the reaction media. **II** was also selectively obtained in DCM/MeOH (84% yield), indicating that the present procedure can also be applicable to peptidyl-KOR containing arginine as the C-terminal amino acid. **III** was selectively obtained with 36% yield in THF/water (in the absence of Ca^{2+} the yield was only 15%). Temperature enhancement and solvent change allowed us to optimize the production of **I** and **III** in the presence of Ca^{2+}: hydrolysis and methanolysis yields were increased to 70 and 100%, respectively. Measurements of peptidyl-KOR swelling in DCM, DMSO, $CHCl_3$, DMF, THF, DCM/MeOH, DMSO/MeOH, $CHCl_3$/MeOH, DMF/MeOH, DMF/water (6:1, v/v) and THF/water indicated that this parameter is not critical for the efficiency of these reactions. As far we know, this is the first report on the hydrolysis of peptidyl-KOR assisted by Ca^{2+} at neutral pH.

[Supported by FAPESP and CNPq]

Moraes, C.M. et al., [1] Peptides 1998, 230-231, 1999; [2] J. Pept. Res., 2000, in press

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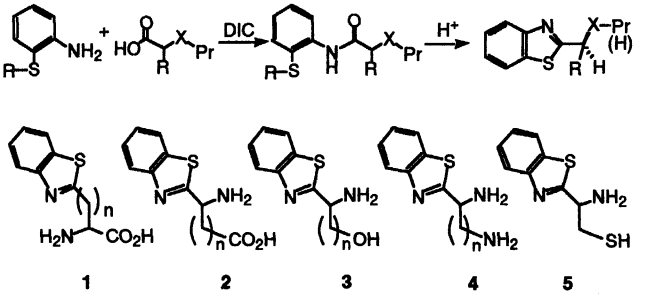
SOLID- AND LIQUID-PHASE SYNTHESIS OF 2-BENZOTHIAZOLYL-COMPOUNDS AND THEIR APPLICATION IN PEPTIDE SYNTHESIS

Spyros Mourtas, Manolis Karavoltsos, Dimitrios Gatos and Kleomenis Barlos from Department of Chemistry, University of Patras, Patras, Greece.

2-Aminobenzenethiol was protected at the thiol function with a group of the trityl-type or alternatively attached onto a resin of the same type. Coupling of these derivatives with Fmoc-, Boc-, Trt-aminoacids and various other acids, followed by acidic treatment provided the corresponding 2-benzothiazolyl-compounds (Scheme) in high purity. Among others, the derivatives 1-5 were successfully applied in solid and liquid phase peptide synthesis.



X = O, S, NH; Pr = Trt, Boc, Fmoc.- 1- 3, n = 1, 2; 4, n = 1-4

REEVALUATION OF THE CONBINED SOLID-PHASE AND SOLUTION APPROACH FOR PROTEIN SYNTHESIS

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The segment condensation method in solution employing a maximum protection strategy with Boc chemistry is ideal for the synthesis of large peptides or proteins. To synthesize the fully protected segments to be used with this method, we recently introduced a standard solid-phase peptide synthesis [1] on an *N*-[9-(hydroxymethyl)-2-fluorenyl]succinamic acid (HMFS) linker developed by Rabanal *et. al.* [2]. The HMFS linker is an Fmoc-type protecting group that is designed to be cleaved by nucleophiles such as piperidine and morpholine. To apply the above strategy, high compatibility is needed between the side-chain protecting groups of the segment and anchoring groups. For this purpose, we employed the cyclohexyloxycarbonyl (Hoc) and 3-pentyl groups for the Trp and Tyr residues, respectively, as base-resistant protecting groups. However, closer examination of the products by TLC, HPLC and ESI MS revealed that side reactions had occurred during the elongation of peptide chains on the resin and the HF deprotection; amino acid deletion at the N-terminus of particular amino acid residues and modification of the Trp residues associated with use of the Hoc group, respectively. Amino acid deletion products generated at the N-terminus of the His(Bom) residues resulted more or less from incomplete deprotection of the Boc group even with high concentrations of TFA. As for the modification of the Trp residues, using thiol as an additive (e.g. thiophenol) during the HF reaction was found to significantly reduce the extent of the side reaction with Trp(Hoc) although the Hoc group could be removed without resorting to the use of thiols.

In the present study, we reviewed the removability of the Boc group on amino acid derivatives and found that this group on the His(Bom) residue was much more resistant under the deprotecting conditions than we had expected. We also characterized the structure of the modified Trp residues obtained by treating Trp(Hoc)-containing peptides with HF in the absence of thiols.

[1] Nishiuchi, Y., Nishio, H., Inui, T., Bódi, J. and Kimura, T. (2000) *J. Pept. Sci.*, in press.
[2] Rabanal, F., Giralt, E. and Albericio, F. (1995) *Tetrahedron*, **51**, 1449-1458.

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SOLID PHASE MODIFICATION OF A NATURAL CYCLO-DEPSIPEPTIDE

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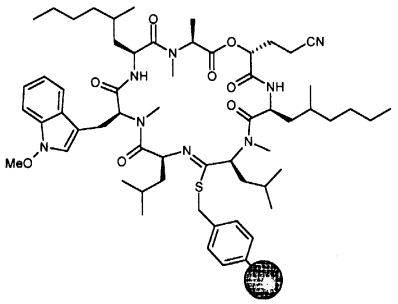
Introduction: Despite the enormous impact on medicinal chemistry, solid-phase synthesis and combinatorial methods are rarely used in natural product chemistry. To demonstrate the value of solid-phase methodology for modification of peptidic natural products, we describe the selective exchange of a single amino acid in a resin bound cyclodepsipeptide.

Discussion: A single amide of the fungal cycloheptadepsipeptide is selectively transformed into the thioamide by reaction with Lawesson's reagent. The introduced sulfur atom now provides a useful attachment site to a solid support. Reacting the thioamide with Merrifield resin with KI in DMF at 80°C leads to the formation of a resin-bound *S*-linked thioimidate. At this stage the bound cyclodepsipeptide can be released unchanged from the resin by silver-promoted hydrolysis of the reactive benzlythioimidate with aqueous $AgNO_3$ in *t*-BuOH. This is a novel example of a "traceless" linker using a peptide bond as attachment site.



Through the conversion into the reactive resin-bound benzlythioimidate the former MeLeu[3]-Leu[4] amide bond is now susceptible to acid hydrolysis and is selectively cleaved to an open-chain depsipeptide that is linked to the support via a thioester group. The free N-terminus is now ready for Edman degradation and amino acid coupling using standard peptide synthesis protocols. Both, Fmoc- and Boc-strategies are compatible with the thioester linkage. After deprotection, ring-closing cleavage from the support is accomplished by activating the thioester with silver ions in the presence of collidine.

USE OF DIISOPROPYLCARBODIIMIDE IN THE SOLID-PHASE SYNTHESIS OF PHOSPHORYLATED PEPTIDES BY THE PRE-FORMED PHOSPHOAMINO ACID BUILDING BLOCK APPROACH

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Today, phosphorylated peptides are easily prepared by stepwise Fmoc-based solid-phase peptide synthesis using building blocks such as Fmoc-Xaa(PO_3H_2)-OH or Fmoc-Xaa(PO(OBzl)OH)-OH (Xaa = Ser, Thr, Tyr). Methods for the introduction of these building blocks have been investigated and optmized.[1] However, difficulties have often been noticed for the introduction of residues following the phosphorylated amino acid, as shown, for instance, by the use of large excess of reagents or by the duplication of coupling reactions. In the course of the synthesis of phosphorylated oligopeptides mimicking the *C*-terminus of α subunit of GTP-binding protein Gq, we suspected, as other investigators,[2] these difficulties to be related to the presence of secondary amine retained on the resin after the prior removal of Fmoc protecting group. Thus, the concentration of activated amino acids may be depleted by reaction with residual secondary amine linked to the resin by ionic interaction with phosphate group.
We report on results identifying clearly that piperidine salt with phosphate is responsible for the difficulty of coupling reactions and that usual coupling conditions are efficient after exchange of piperidinium counter-ion for unreactive tertiary ammonium. In particular, no special difficulties are associated with the activation by diisopropylcarbodiimide in the presence of hydroxybenzotriazole.

[1] J. W. Perich, N. J. Ede, S. Eagle, A. M. Bray, *Lett. Pept. Sci.* 1999, 6, 91-97.
[2] C. Krog-Jensen, M. K. Christensen, M. Meldal, *Lett. Pept. Sci.* 1999, 6, 193-197.

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SYNTHESIS OF PROTECTED PEPTIDES ON *N*-[(9-HYDROXY-METHYL)-2-FLUORENYL] SUCCINAMIC ACID (HMFS) DERIVATISED RESINS: ASPARTIMIDE SIDE REACTION SUPPRESSION

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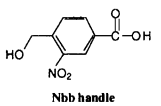
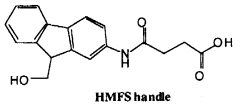
Formation of aspartimides is a severe side reaction that still remains unresolved in the chemical synthesis of peptides. In the course of a synthetic preparation of the B domain of *Staphylococcus aureus* protein A, we encountered this sidereaction during the synthesis of the *C*-terminal protected fragment 227-233: Boc-Asn-Asp(OcHex)-Ala-Gln-Ala-Pro-Lys(ClZ)-COOH. The heptapeptide was initially assembled using a Boc/Bzl protection scheme on a 4-hydroxymethyl-3-nitrobenzamide derivatised PEGA resin in order to obtain the protected peptide by nucleophilic displacement. Treatment of the peptide resin with a hydroxide ion source [LiOH in DMF:H_2O (1:1) or TBAF•$3H_2O$ in DMF] proved to be totally unsuccessful. Analysis of the crudes obtained by HPLC and HPLC-ESMS revealed the only presence of byproducts lacking a cyclohexyl group (m/z = M-100, and m/z = M-100+18 a hydrated form). The target peptide, though, was not detected.


Nbb handle


HMFS handle

We decided then, to use the bifunctional linker *N*-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid (HMFS).[1] The main advantage of this handle lies in its *fine tuned* lability that enables the release of protected peptides from the solid support with a mild base treatment. In this case, cleavage with morpholine in DMF (1:4, 1h) afforded the desired protected peptide in excellent yields and without any trace of aspartimide related byproducts. The extent of this methodology will be discussed.

1.- F. Rabanal ; E. Giralt, F. Albericio, *Tetrahedron Lett.* **1992**, *33*, 1775–1778; *Tetrahedron* **1995**, *51*, 1449–1458

BLOCK-INVERSE SYNTHESIS OF ANTIMICROBIAL PEPTIDE TEMPORIN F IN SOLUTION

Maxim G. Ryadnov and Yuri V. Mitin, Institute of Protein Research, RAS, 142292 Pushchino, Moscow Region, Russia

Earlier we have found conditions allowing to synthesize racemization-free peptides in the N→C direction ('inverse peptide synthesis') with high yields using free amino acids as amino components in solution [1]. This became possible thanks to an effective aprotic water-free system to dissolve amino acids in their NH_2^{α}-, $COOH^{\alpha}$-unprotected form (free amino acids) [2] and an unique ability of some cupric salts to suppress epimerization (D-epimer, <0.1%) [1,3]. Since during synthesis no deprotection stage of an N^{α}-amino group is required, the inverse peptide synthesis with the use of free amino acids as amino components is most advantageous for peptide chain elongation as regards the number of medium stages. We have already tested the conditions found in the inverse synthesis of several peptides [4]. It is reasonable to use a stepwise peptide chain building in the N→C direction in solution for the synthesis of relatively short peptide chains (5-7 amino acids). Peptide chains of a greater length can be synthesized using the coupling of peptide blocks. There are not many problems in the case of peptides containing glycine or proline to choose a site for block condensation with such amino acids as the end ones of the carboxyl component [4]. But because of epimerization the situation with the other amino acids is completely different. There have been no reports so far concerning peptide-fragment coupling in conjunction with the use of cupric (II) salts as effective epimerization suppressants. So, mainly the block-inverse synthesis of antimicrobial peptide temporin F (FLPLIGKVLSGIL-NH_2) is reported here as a convenient model to demonstrate the approach. Temporin F was divided into four (FL, PLIG, KVLS, GIL-NH_2) and three (FLPLIG, KVLS, GIL-NH_2) blocks, each being synthesized in the N→C direction in solution using free amino acids as amino components in the presence of cupric (II) ions by the N-(dimethylaminopropyl)-N'-ethylcarbodiimide – hydroxybenzotriazole coupling method. The peptide was synthesized in the C→N direction as well to compare the main characteristics of both products that proved identical.

1). Ryadnov M. G. et al. (1999) *J. Pept. Res.*, *53*, 322-328.
2). Ryadnov M. G. et al. (1999) *Bioorg. Khim.*, *25*, 323-328.
3). Miyazawa T. et al. (1984) *Tetrahedron Lett.*, *25*, 771-772.
4). Mitin Yu. V. & Ryadnov M. G. (1999) *Prot. Pept. Lett.*, *6*, 87-90.