REVIEW OF CLINICAL PHARMACOLOGY AND
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Polyamines Bind in Close Proximity to RRNA Residues Implicated in the Interaction of Ribosomes with Antibiotics

Alexandros D. Petropoulos, Maria A. Xaplanteri and Dimitrios L. Kalpaxis

Laboratory of Biochemistry, School of Medicine, University of Patras, GR-26500 Patras, Hellas

Key words: Polyamines, spermine, RRNA residues, ribosomes-antibiotics Interaction

Spermine, one of the naturally occurring polyamines, increases the chloramphenicol and blasticidine S inhibitory effect on protein synthesis by facilitating the drug accommodation to ribosomes (1,2). In contrast, spermine reduces the potency of spiramycin, a macrolide antibiotic (2). To unveil the molecular basis of this polyamine effect, the locations of spermine bound to rRNA were characterized by a photoaffinity labeling technique in combination with RNase H digestion and primer extension analysis. Cross-linking sites of ABA-spermine, a photoreactive analogue of spermine (Fig. 1), were identified in helices H18, H27 and H44 of 16S rRNA, all implicated in the interaction of ribosomes with several antibiotics, such as tetracycline and aminoglycosides. On the other hand, cross-linking was found in helices H35, H42-H44, H89, H90, H95 and the central loop of domain V of 23S rRNA, also involved in the binding of antibiotics which inhibit important ribosomal functions.[3] Ribosomes labeled by ABA-spermine exhibited a behavior towards antibiotics similar to that of native ribosomes interacting with spermine free in solution. Our results suggest that polyamines bound at the vicinity of antibiotic binding pockets modulate diversely the interaction of these drugs with ribosomes.

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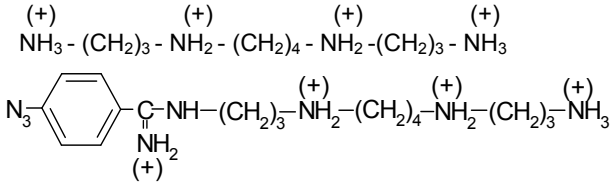

Figure1. Chemical structures of spermine and N^1-azidobenzamidino (ABA)-spermine

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Synthesis of Cyclodextrin–Crown Ether Biomimetic Systems

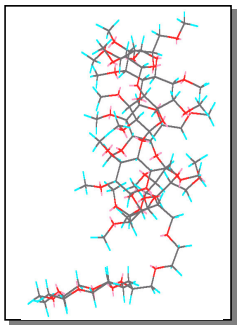
N. Kyritsis[1], E. Petridou[1] and G. Tsivgoulis[1]

[1]Laboratory of Organic Chemistry, Department of Chemistry, University of Patras, Patra GR26500, Hellas

Key words: β-Cyclodextrin, crown ether, biomimetic systems, synthesis

Cyclodextrins (CD) are toroidal shaped molecules that show excellent complexation properties as a result of their unique structure. Thus, cyclodextrins have been used extensively in the construction of supramolecular systems and many examples where cyclodextrin derivatives have served as biomimetic systems in catalysis and/or transport have been already described. Crown ethers, also, represent an important category of supramolecular compounds because of their ability to complex cations.

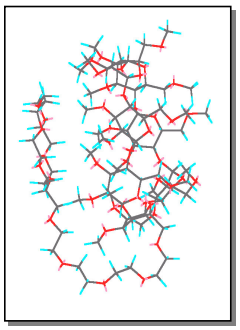
The aim of the present study is to synthesize supramolecular multireceptors and study their ability to complex charged organic molecules such as benzoic and picric acid salts and amino acids. Particularly, molecules that combine permethylated β-cyclodextrin and 18-crown-6 will be presented. The main difference between these molecules is the length of the spacer that connects the cyclodextrin with the crown ether moiety. A short spacer (compound **A** in Figure) does not permit a parallel accommodation of the crown ether with the large rim of the cyclodextrin while a long spacer (compound **B** in Figure) permits such an accommodation. This variation is expected to significantly differentiate the complexing properties of the two compounds.



Ένωση Α

Η επαφή του αιθέρα στέμματος με το μεγάλο άνοιγμα της κυκλοδεξτρίνης δεν είναι δυνατή.

Compound A
The contact of crown ether with the big opening of β-cyclodextrin is not feasible



Ένωση Β

Η επαφή του αιθέρα στέμματος με το μεγάλο άνοιγμα της κυκλοδεξτρίνης είναι δυνατή.

Compound B
The contact of crown ether with the big opening of β-cyclodextrin is feasible

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Thrombin-Angiotensin Mimetics: Design of Double Action Mimetics (Talps: Thrombin Ang II like Peptides)

Ioannis Papanastasiou, Panagiota Roumelioti, Maria-Eleni Androutsou and John Matsoukas

Department of Chemistry, Section of Organic Chemistry, Biochemistry and Natural Products, University of Patras, GR-26500, Patra, Hellas

Key words: Thrombin-angiotensin mimetics, double action, design

The results of human thrombin amino acid sequence revealed that the segment 84-135 of thrombin contains two Ang II-like sites. The identified 84-97 and 114-126 sequences are called the *T*hrombin *A*ng II-*L*ike *P*eptides, *TALP*s (TALP I and TALP II, respectively). In this study, we have synthesized a number of non-peptide mimetics, which are designed to carry the pharmacophoric features of the active pentapeptide SFLLR (Phe and Arg) and the crucial pharmacophoric groups of octapeptide Ang II (Phe, His and Arg). The nipecotic acid, the isonipecotic acid (piperidine-4-carboxylic acid) and the cyclohexane-1,4-dicarboxylic acid were chosen as templates upon which the pharmacophoric groups are mounted. Also, all the synthesized compounds will be tested in the rat aorta relaxation assay, in platelet aggregation studies and on the CAM system.

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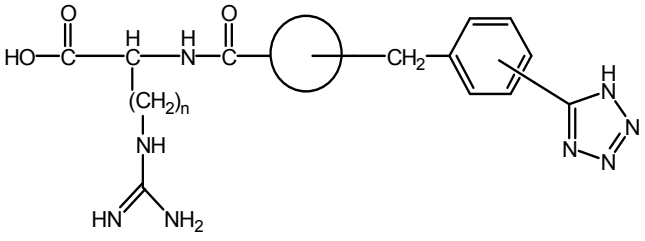

Γενική μορφή υβριδικού μορίου με πιθανή συνδυασμένη δράση

General form of hybrid molecule with likely combined action

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Interlaboratory Validation of the In-House Developed Method for the Analysis of the Spasmolytic Hyoscine-N-Butylbromide

Vasiliki Levendakou, Konstantinos Prousalis, Christos Kaltsonoudis and Theodore Tsegenidis

Laboratory of Organic Chemistry, Biochemistry and Natural products, Department of Chemistry, University of Patras, GR-26500 Patra, Hellas

Key words: Spasmolytic Hyoscine-N-butylbromide, analysis, in-house method, validation

According to the legislative frame of the European Union, laboratories must be accredited in order to ensure the quality of analytical results (1). Analytical chemists are allowed to use either internally (in–house) developed methods or significantly modify standard methods (2). In both cases method validation is the process of proving that an analytical method is acceptable for its intended purpose (3). During the development of a new analytical method, the analysts determine the important performance characteristics such as specificity, linearity, precision, measurement range, detection and quantitation limit and robustness (4). Validation must include studies on these characteristics in order to find out whether the method is appropriate or not. Following this framework, validation studies were performed for the determination method of hyoscine–N-boutylbromide in pharmaceutical products employing high performance liquid chromatography. The analysis was conducted using a Supercosil C18 column at 254 nm. The performance characteristics studies demonstrated that the method is scientifically appropriate.

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REVIEW OF CLINICAL PHARMACOLOGY AND
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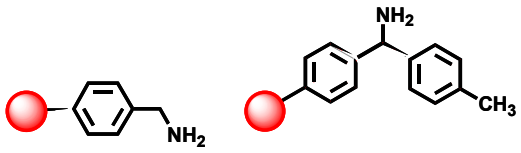
Preparation and Applıcation on Synthesis Hydrophilic Resins

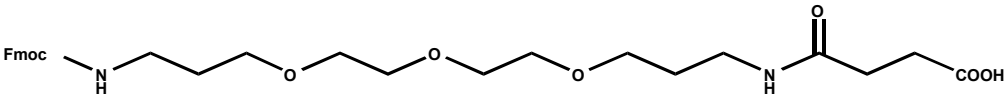
A. Saravanos and K. Barlos

Laboratory of Organic Synthesis, Department of Chemistry, University of Patras, GR-26500 Patras, Hellas

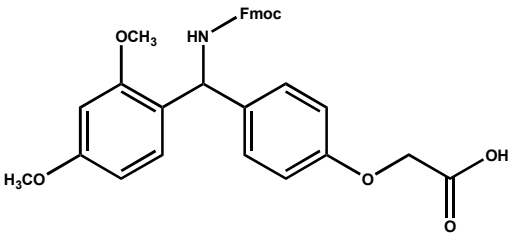
Key words: Hydrophilic resins, preparation, application



AM-PS **MBHA-PS**



Fmoc-PEG-OH



Rink Amide Linker

Solid phase synthesis still possesses a pivotal place in Combinatorial Chemistry. Basic principle of solid phase synthesis is immobilization: a substrate is tightly attached on the resin in the form of a linker. Sometimes, other molecules (e.g. polyethylenoglycol known as PEG) interfere between the resin and the linker, in order to increase the swelling properties of the polymer in polar solvents. The desired chemical reactions take place in a different location of the linker. The selection of the resin we are going to use each time depends on the chemistry demanded for the synthesis of the desired product.

The purpose of this research is the preparation of resins, which demonstrate exceptional swelling properties both in polar and non-polar solvents as well as the applications

of these resins to Combinatorial and Organic chemistry.

Preparation and study of the following resins: (a) A.M –PEG_n-linker (b) A.M-Lys-$(PEG_2)_n$-$(linker)_2$ and (c) MBHA-PEG_n-linker, (linker = Rink Amide Linker, n = 1-10) was carried out concerning the swelling properties in various solvents, kinetic and substitution through synthesis and cleavage of selected peptides (e.g. calcitonin).

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Synthesis of Selected Fragments of the CRF-Related Diuretic Peptide of *Locusta Migratoria*

Kaskani Charoula and Constantine Poulos

Department of Chemistry, University of Patras, GR-26500 Patra, Hellas

Key words: *Locusta Migratoria*, corticotropin-releasing factor (CRF), CRF-related diuretic peptides, synthesis

Locusta Diuretic Hormone (Locmi-DP) is a neuropeptide that has been isolated from the locust Locusta migratoria. It belongs to the CRF (corticotropin-releasing factor)-related diuretic peptides and has the following amino acid sequence.

Locmi-DP stimulates fluid secretion and cyclic AMP production by Malpighian tubules *in vitro* and is released into the haemolymph from corpora cardiaca.

Our aim was to synthesize a series of fragments analogues of the Locmi-DP for structure-activity studies. These fragments are: the C-terminal Locmi-DP_{34-46}, Locmi-DP_{36-46}, Locmi-DP_{38-46} and also the N-terminal [$Hse(Me)^{1,3,13}$]-Locmi-DP_{1-30}, [$Hse(Me)^{13}$]-Locmi-DP_{6-30}, [$Hse(Me)^{13}$]-Locmi-DP_{13-30}.

The synthesis of above analogues was performed in solid phase on the 2-Chlorotrityl chloride Resin using fragments condensation strategy and the Fmoc/Bu^t methodology. The DIC/HOBt method was used for the coupling of amino acids and peptide fragments. The analogues were purified by HPLC and identified by ESI-MS.

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Locmi-DP sequence: MGMGPSLSIVNPMDVLRQRLLLEIARRRLRDAEEQIKANKDFLQQI-NH_2

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REVIEW OF CLINICAL PHARMACOLOGY AND
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The Combined Use of CBMN and Fish Methods as a Tool for Cytogenetic Analysis of the Effect of Alkylating Cytostatic Compounds in Human Lymphocytes Treated *in vitro*

M. Efthimiou[1], C. Andrianopoulos[1], G. Stephanou[1], N.A. Demopoulos[1] and S. Nikolaropoulos[2]

[1]Division of Genetics, Cell and Developmental Biology, Department of Biology and [2]Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, University of Patras, GR-26500 Patra, Hellas
Key words: Human lymphocytes, cytostatic compounds, Cytokinesis-Block Micronucleus (CBMN), fish methods, cytogenetc analysis

The *C*ytokinesis-*B*lock *M*icronucleus (*CBMN*) assay has been widely used in human lymphocyte cultures for the identification of the genetic damage induced by the exposure of cells to various pharmaceutical compounds. By this method Micronucleus frequency is estimated in binucleated cells due to the action of cytochalasin B an inhibitor of actin polymerization. Micronuclei contain acentric chromosome fragments and/or whole chromosomes, they can be recognized as distinct formations that exist in daughter cells separated from the main nucleus and are the result of chromosome breakage and/or chromosome loss (1). The CBMN assay in human lymphocytes in combination with *F*luorescence *I*n *S*itu *H*ybridization (*FISH*) with centromeric probes was proved valuable to identify both chromosome breakage as well as chromosome missegregation induced by various pharmaceutical compounds (2). In the present study we investigated the genetic activity of four cytostatic agents that belong to the category of nitrogen mustards, in human lymphocyte cultures *in vitro*. The studied-molecules are: the pharmaceutical compounds melphalan and chlorambucil, used in the chemotherapy of cancer, the p-mustard of phenylacetic acid, which is the active metabolite of chlorambucil and its steroidal ester ASE, which has been found to exert anticancer activity in experimental tumors (3). We evaluated the ability of the above-referred compounds to induce cytotoxicity and to enhance micronucleus frequency. We also investigated the segregation of chromosomes X and Y with the application of combined CBMN/FISH by using specific centromeric probes. Our results conducted to the conclusion that the studied nitrogen mustards display increased cytotoxicity and induce increased frequencies of micronuclei. In addition they affect the segregation of sex chromosomes, X and Y, increasing chromosome loss and chromosome non-disjunction frequencies. These results are in accordance with previous studies of our group (4).

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Determinants of the HIV-1 GP120 V3 – CCR5 N-Terminal Interaction at Peptide Level: An NMR Conformational Approach

Petros A. Galanakis[a], Nikolaos Kandias[a], Georgios A. Spyroulias[a], Maria Sioumpara[b], Dimitrios Morikis[c], Apostolos Rizos[d] and Elias Krambovitis[b]

[a]Department of Pharmacy, University of Patras, GR-26504, Patras, Hellas;
[b]Department of Applied Immunology and Biochemistry, Institute of Molecular Biology and Biotechnology, FORTH, GR-71110, Heraklion-Crete, Hellas;
[c]Department of Chemical and Environmental Engineering, University of California at Riverside, Riverside, CA 92521 USA;
[d]Department of Chemistry, University of Crete, GR-71409, Heraklion-Crete, Hellas

Key words: HIV-1 GP120 V3 – CCR5 N-terminal interaction, peptide level, NMR

Recent convincing evidence indicates that the majority of the cells that die due to HIV-1 are not actually infected by the virus. Instead, HIV-1 or its components lead these cells to programmed cell death by altering their physiological function (1) after the activation of apoptotic mechanisms. Ionic interactions between the variable V3 domain of the HIV-1 coat glycoprotein gp120 and the amino terminal of the chemokine receptor CCR5 (2) play a prominent role in this process. Standard multidimensional and multinuclear NMR spectroscopy was applied to probe the structural and physicochemical determinants of three representative peptides from V3 domain of the HIV-1 and a 22-residue peptide, representing the amino terminal of the chemokine receptor CCR5, in their free or interacting state. Titration of CCR5 peptide with V3-peptides was performed in NMR tube, at 286K. 1D 1H NMR spectra and 1H-15N HSQC were recorded after each addition of V3 peptides. Analysis of the NOESY maps, acquired for free and interacting peptides at 278K, suggests that the free CCR5 construct is structured, giving rise to numerous NOEs. Data analysis of HSQC and NOESY spectra verifies the interaction of the V3-CCR5 peptide constructs and suggests a remarkable role for the (i) 7-residue CCR5 Nt domain and particularly for Tyr3 and (ii) for the 7/9-residue V3 N-terminal peptide fragment, which especially in V3 LAI peptide is rich in basic residues (3).

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REVIEW OF CLINICAL PHARMACOLOGY AND
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Synthesis of Tetrazole Analogues of γ- and δ-Amino Acids

Maria Filippakou, Haralambos Sinanoglou, Panagiota Moutevelis-Minakakis, and George Kokotos

Department of Chemistry, University of Athens, Panepistimioupolis, Athens GR-15771, Hellas

Key words: γ- and δ-Amino acids, tetrazole analogues, synthesis

Non-natural amino acids play an important role in the design and synthesis of pharmacologically relevant molecules and peptide mimetics. The tetrazole group is considered isosteric to the carboxyl group and there are several examples in medicinal chemistry where the replacement of a carboxyl by tetrazole leads to products with improved biological properties. Here, we describe a general method for the synthesis of tetrazole, isosteric analogues of γ- and δ-amino acids and their methylated derivatives, isosteric analogues of aminoacid methylesters. Z-protected amino alcohols 1, easily prepared from α- and β-amino acids, were converted into aldehydes and directly reacted with (triphenylphosphoranylidene)acetonitrile. Treatment of nitriles 2 with NaN_3 and $ZnBr_2$ produced unsaturated tetrazoles 3, which were converted to compounds 4 by catalytic hydrogenation. Treatment of compounds 3 with CH_2N_2 produced the constitutional isomers 5,6 in ratio 1:2, which were converted to compounds 7,8 by catalytic hydrogenation. The constitutional isomers 5,6 and 7,8 can be easily distinguished by ^{1}H-NMR and ^{13}C-NMR.

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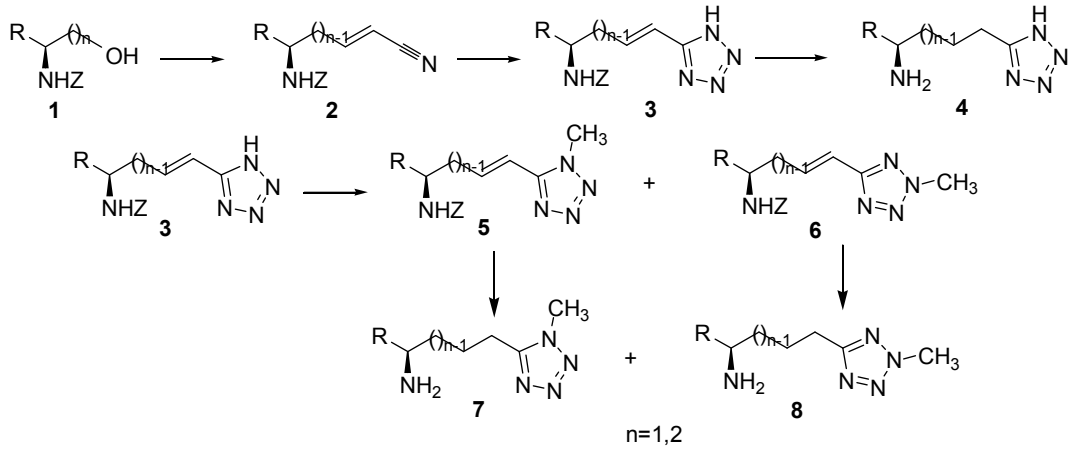

REVIEW OF CLINICAL PHARMACOLOGY AND
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Pepticom Hellas: Steps Forward Research and Innovation *Multidimensional Applications and Perspectives*

Charalampos Alexopoulos, Dimitrios Krikorian, Maria Sakarellos-Daitsiotis and Constantinos Sakarellos

Research Laboratory of Peptide Chemistry, Chemistry Department, University of Ioannina, GR-45110, Ioannina, Hellas

Key words: Pepticom Hellas, multidimensional applications and perspectives

Nowadays, the use of synthetic carriers as biochemical reagents and immunogens is entering to a new phase. The multimeric nature of these constructs, the unambiguous composition and the ease, reliability, versatility of their production make this type of carriers well suited to various biotechnological and biochemical applications for diagnostic purposes, protein mimetics, antiviral agents, vaccines, drug and gene delivery vehicles. We will be focused on an innovative type of multifunctional helicoid carrier-foldamer, named *SOC_n-I, II,* which was successfully developed in our laboratory. Our concept was to construct an artificial template with structural rigidity and regularity, so as the peptide epitopes/pharmacophore groups could be anchored without any conformational restriction and steric hindrance as demonstrated by conformational studies using ^{1}H-NMR, CD, FT-IR. SOC_n-I,II were used as antigenic substrates in developing immunoassays of high sensitivity, specificity and reproducibility, as well as potent immunogens to generate site-specific antibodies for therapeutic purposes. By exploiting the ability of macromolecules SOC_n-I,II to act as potent antigens, it would be possible to develop diagnostic tests (as for example an immunochromatographic lateral flow test), specific in screening and discrimination among various autoimmune diseases, which enable us to simultaneously detect various antigen and antibody combinations. In the case of massive analyses (as for hospitals, diagnostic centers) our macromolecules SOC_n-I,II could be served in developing a kit based on turbidimetry or nephelometry principles applicable in biochemical analytes.

Taking advantage of the SOC_n properties to generate site specific antibodies (potent immunogens), we have emphasized on the application of SOC_n carriers, covalently bearing a 'built-in' adjuvant moiety, for the preparation of totally synthetic peptide-based vaccine matrix for human use. Thus, according the selected epitope/pharmacophore group to be attached on this matrix, we could prepare multiple synthetic human vaccines for therapeutic potential.

On the other hand, the structural characteristics of SOC_n-I,II carriers, render them a useful tool for the study and comprehension of mechanism of interaction with membranes. To this direction, the SOC_n-I,II carriers could be tested not only as antimicrobial agents, but as tools for gene delivery as well.

Our increasing understanding of peptide/protein folding and assembly, raises possibilities for engineering novel self-assembling supramolecular structures and bio inspired materials. Potential applications of such assemblies, include the preparation of functionalized biomaterials of nano-dimension (nanomaterials), as well as scaffolds to recruit cells