No 86

Application of Benzhydryl Halides and Amines Resins in Solid Phase Synthesis

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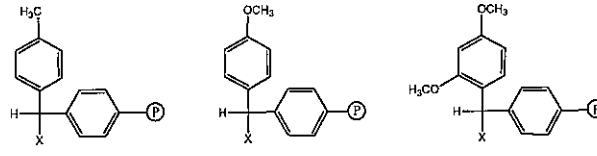
4-Methylbenzhydryl- *(1)*, 4-Methoxybenzhydryl- *(2)*, and 2,4-Dimethoxybenzhydryl- *(3)*, bromide, chloride and amine resins were studied for their application in solid phase synthesis. Suitably protected amino acids, primary and secondary aminoalcohols, primary amines, diamines, phenols, aminothiols, and mercapto acids were loaded onto the bromide and chloride resins. The obtained derivatives were applied in the solid phase synthesis of peptides and organic compounds. 2,4-Dimethoxybenzhydryl amine resin was found to be quite sensitive against acids and therefore well suited for the preparation of peptide amides using the Fmoc/tBu-amino acids. In contrary the 4-Methylbenzhydryl- and 4-Methoxybenzydryl- amine resins were proved stable against treatment with TFA and therefore suitable for the preparation of N-alkyl amides usig Boc/benzyl-amino acids The corresponding N-alkyl amides of all resins were proved to be much more acid sensitive.



1 2 3

X = Cl, Br, NH_2

No 87

A Structure-Activity Relationship Study of Thyrotropin Releasing Hormone (TRH)

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Thyrotropin Releasing Hormone (TRH) is secreted in pulses from the hypothalamus and stimulates the pituitary to effect the secretion of the Thyroid Stimulating Hormone (TSH). The last stimulates the secretion of Thyroxine (T_4) and Triidothyroxine (T_3) by the thyroid gland. Both T_4 and T_3 have a feedback effect on the hypothalamus and the pituitary that reduces TRH and TSH secretion. TRH is a peptide hormone consisted by three amino acids. Both the N and C terminals of TRH are not "free", that is, they exist neither as a free acid nor as a base. The structure of TRH is L-pGlu-L-His-L-Pro-NH_2.

The goal of this research is the synthesis of TRH analogues, with prolonged activity, for possible application in cases of malignant tumors of the thyroid gland. For the achievement of this purpose, analogues of TRH were synthesized by substituting the amino acids Histidine and Proline with the non-natural imino acids nipecotic [Nip], isonipecotic [Inp] and pipecolic [Pip], the non-natural amino acid 2-amino-isobutyric acid [Aib], and 1,2,3,4-tetrahydro-D-isoquinolic-3-carboxylic [Tic] acid, and also a number of TRH analogues (tetrapeptides) with the introduction of the moieties mentioned above between Histidine and Pyroglutamic acid in order to: (a) obtain a high-affinity binding between the TRH–receptor and the hormone and (b) protect the later against enzymatic degradation. Pyroglutamic acid (pGlu) of

TRH structurally keeps its position in the hormone, as the most necessary TRH section for the binding with the TRH-receptor. These TRH analogues were synthesized in liquide phase and by Fmoc/tBu methodology utilizing a 2-chlorotrityl-chloride resin as solid support with a Rink Bernatowitz linker. The synthesized analogues were puriefed by gel filtration (Sephadex G-10 and 15% solution of AcOH) and semipreparative HPLC. ES-MS was in agreement with the expected results.The biological activity of the new analogues is under investigation.

No 88

Studies in the Synthesis of Peptide Thioesters with Applications to the Chemical Synthesis of Proteins

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Peptide C- terminal thioacids and thioesters are key intermediates in a variety of applications, most notably the recently developed native chemical ligation methods for the total synthesis of proteins. So far, they have been prepared mainly by the use of the least prevalent Boc / Bzl / solid phase method, owing to the stability of the thioester bond to strong nucleophiles, such a piperidine, normally used in Fmoc solid-phase method.

Our present research efforts have been focused on the Fmoc-based preparation of peptide thioacids and thioesters by using trityl-type resins and the non-nucleophilic base 1,7-diazubicyclo [5,4,0] entec-7-ene (DBU). The synthesis route we studied (fig.1) involves the transformation and Trt-aminoacids to the corresponding thioacids and subsequent attachment on the 2-chlorotrityl, trityl and 4-methoxy-trityl resins. The stability of the thioester bond was studied during the peptide chain elongation either by the stepwise or the fragment condensation method.

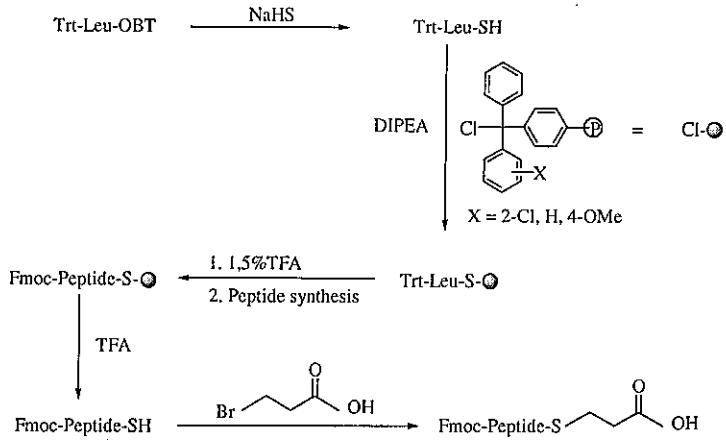

Figure 1. Generation of thioacid and thio-esterification to different resins

No 89

Appropriate Monomers for PNA Synthesis and PEGylation

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Peptide Nucleic Acids (PNAs), perhaps the most successful DNA mimics, are oligonucleotide analogues, where the sugar-phosphate backbone has been substituted by a pseudopeptide back-

bone consisting of N-(2-aminoethyl)glycine units. The natural occuring nucleobases are attached to this backbone via a methylene-carbonyl linker. PNAs have been shown to recognize and bind strongly to complementary DNA and RNA sequences with remarkable affinity and selectivity and are resistant to nucleases and proteases. These properties make them potentially extremely useful as antisense therapeutics and as diagnostic tools in molecular biology.

PEGylation, on the other hand, is a procedure of growing interest for enhancing the therapeutic and biotechnological potential of peptides, proteins and other bioactive substances like lipids, liposomes and low molecular weight drugs. The method uses polyethylene glycol (PEG) derivatives for the chemical modification of biomolecules and biomaterials by conjugation. PEG conveys to molecules its physicochemical properties and therefore modifies also their biodistribution, solubility and pharmacokinetics.

In this study, our aim was to develop synthetic routes leading to the preparation of appropriate monomers for PNA synthesis (*1,2*) and PEGylation (*3-5*). Some of our synthetic attempts are depicted in figure 1.

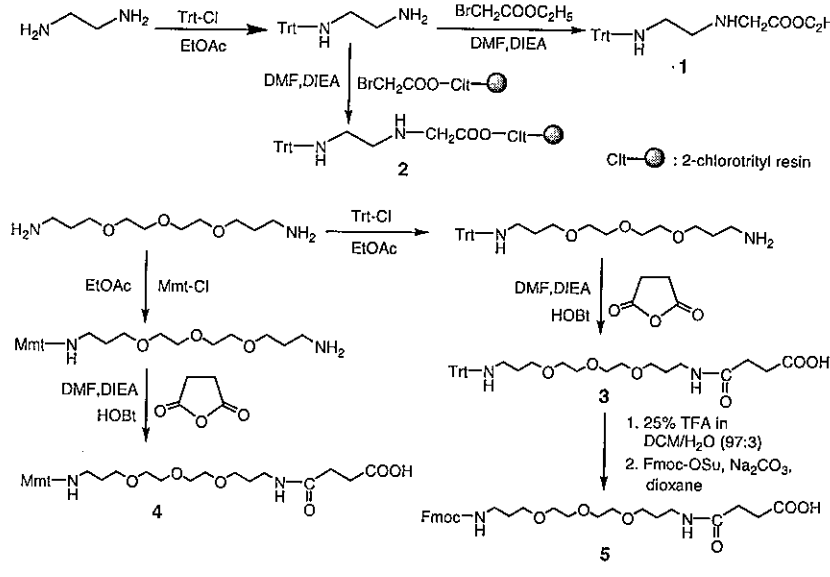

Figure 1

No 90

Synthesis and Characterization of Biologically Active Glycopeptides

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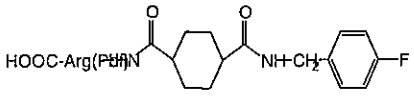
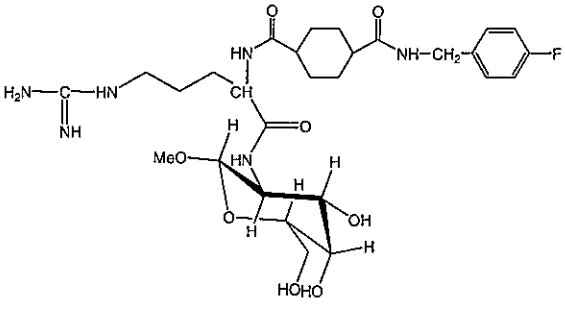
Biologically relevant glycopeptides are used as antithrombotic, antibacterial and anticancer agents and as antibiotics. These glycopeptides can be synthesized from aminosugars (glycosamine, galactosamine etc.) using several chemical pathways. The amino-group of glycosamine or galactosamine is initially protected using the Fmoc-protecting group. The anomeric carboatom is then methylated using hydrochloric acid in methanol. The methylated Fmoc-Aminosugar is coupled on the 4-methoxy-diphenyl-methyl-resin (MDMR) and the Fmoc-group is removed using piperidine solution. The next step is the binding of a Thrombine mimetic analogue (DABF4). After

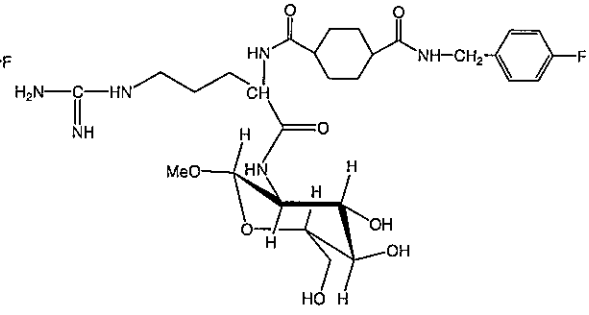
the cleavage from the resin, follows the removal of the Pbf-group, so as to afford the (methylated Aminosugar)-DABF5 product. The final product is purified by semi-preparative HPLC-analysis and characterized by Mass Spectrometry and NMR-Spectroscopy. Biological activity will be studied in order to define its role on prevention of Angiogenesis.



DABF4



1-MeO-GalN-DABF5



1-MeO-GlcN-DABF5

No 91

Correlation of Thrombin Receptor Presence with Angiogenic and Metastatic Phenotype of Prostate and Breast Tumor Cells

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We proposed earlier that the tumor-promoting effect of thrombin / thrombosis may be related to our finding that thrombin is a potent promoter of angiogenesis, a process essential for tumor growth and metastasis. In addition, results showing that metastatic ability of human breast cancer cells is related to the presence of thrombin receptor (PAR-1) on these cells, supported the role of thrombin receptors in tumor progression and metastasis.

The aim of this study was to further investigate the role of the thrombin receptor, in human prostate (PC-3 and LNCaP) and breast (MDA-231 and MCF-7) tumor cell lines. We correlated the presence of thrombin receptor with the angiogenic (Vascular Endotheleum Growth Factor, VEGF) and the metastatic ($\alpha_v\beta_3$-integrin and Metalloprotease-9) phenotype of these cells.

We employed a sensitive quantitative RT-PCR technique to examine gene expression of PAR-1, VEGF, MMP-9 and $\alpha_v\beta_3$-integrin.

MDA-231 and PC-3 cells, which exhibit rapid growth and high metastatic activity, expressed high levels of PAR-1. In contract, MCF-7 and LNCaP cells, which display low growth and metastatic activity, expressed low levels of PAR-1. Although, all cell lines expressed and released comparable levels of VEGF, when MDA-231 and

PC-3 cells exposed to thrombin the level of VEGF was significantly increased. Furthermore, $\alpha_v\beta_3$-integrin and MMP-9, which are involved in tumor metastasis, did not expressed or expressed in lower levels in MCF-7 and LNCaP cells compared with MDA-231 and PC-3 cells.

Taken together these results, it seems that thrombin can support the angiogenic phenotype of tumor cells and thrombin receptor maybe plays a crucial role in tumor metastasis.

No 92

Synthetic Studies Towards the Preparation of Conformationally Restricted Polyamine Analogues and Polyamine Conjugates of Minoxidil

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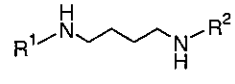
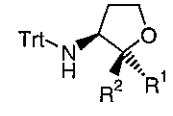
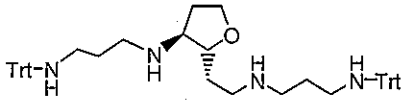
Two chiral, conformationally restricted, analogues (*IIIa* and *IIIb*) of spermine *(Ia)* were synthesized using acid *IIa* and methyl ester *IIb* as starting material, respectively. Attempts to synthesize the branched polyamine analogue *V* failed at the stage of $LiAlH_4$-mediated reduction of the precursor bisamide *IV*. In addition, two minoxidil *(VI)* conjugates (*VII* and *VIII*) from minoxidil and the appropriately *N*-trityl protected polyamines putrescine (*Ib*) and spermidine (*Ic*) were synthesized. Finally, attempts to synthesize the minoxidil conjugate *IX* failed.



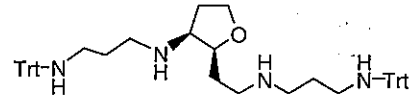
Ia : $R^1=R^2=(CH_2)_3NH_2$
Ib : R^1=Trt, R^2=H
Ic : R^1=Trt, $R^2=(CH_2)_3NHTrt$



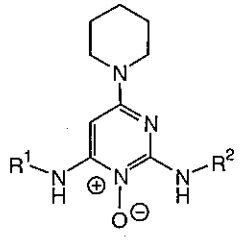
IIa : $R^1=CH_2CO_2H$, R^2=H
IIb : R^1=H, $R^2=CH_2CO_2Me$,



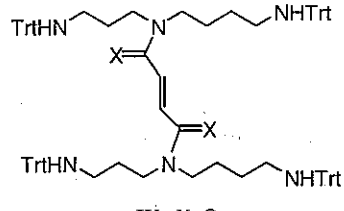
(IIIa)



(IIIb)



VI : $R^1=R^2$=H
VII : R^1=H, $R^2=CONH(CH_2)_3NHTrt$
VIII : R^1=H, $R^2=CON(CH_2)_3NHTrt$
$(CH_2)_4NHTrt$
IX : $R^1=R^2=CONH(CH_2)_3NHTrt$



IV : X=O
V : $X=H_2$