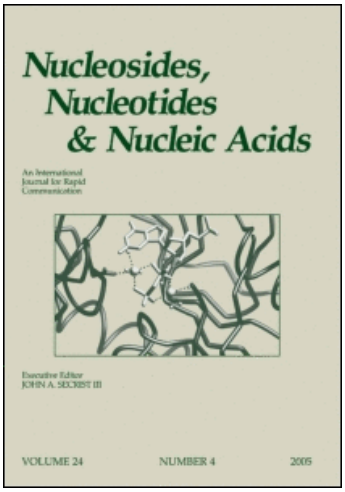

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The Stepwise Solid-Phase Synthesis Methodology is Suitable for the Preparation of a Great Variety of Nucleopeptides

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THE STEPWISE SOLID-PHASE SYNTHESIS METHODOLOGY IS SUITABLE FOR THE PREPARATION OF A GREAT VARIETY OF NUCLEOPEPTIDES

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ABSTRACT: The preparation of nucleopeptides containing tryptophan and basic residues (lysine, arginine) is described. Different solid supports and the necessity of primary carboxamide protection have also been evaluated.

So far, we have reported on the stepwise solid-phase synthesis of nucleopeptides containing, besides the linking residue, trifunctional amino acids such as aspartic acid[1], hydroxylated amino acids[2] (serine, threonine and tyrosine) and the histidine residue[3]. Here we summarize our results on the preparation of peptide-oligonucleotide hybrids containing tryptophan and basic lysine and arginine residues, and on the use of different solid supports. We have also evaluated whether the side chain of asparagine and glutamine must be protected to prevent phosphitylation of the primary carboxamides[4] during the oligonucleotide chain elongation.

Tryptophan-containing nucleopeptides. Boc-Trp-OH can be used to prepare nucleopeptides with both phosphodiester and phosphorothioate internucleoside linkages. The unprotected indole ring was shown to be unstable only to the iodine oxidizing reagent. Even though oxidation of phosphites to phosphates was carried out with *t*BuOOH, nucleopeptide H-Trp-Val-Hse(p$^{3'}$dACTAGT)-Gly-OH appeared to be much less contaminated when it was assembled from Boc-Trp(CHO)-OH. The formyl group was eliminated under the final ammonia deprotection conditions.

Lysine-containing nucleopeptides. Ac-Gly-Ala-Hse(p$^{3'}$dACTAGT)-Lys-Val-OH and Boc-Hse(p$^{3'}$dATATTTGTTACTCTGT)-Lys-Lys-Lys-Lys-Lys-OH were prepared using either Fmoc or trifluoroacetyl groups for the protection of the lysine ε-amine, with

no essential differences between the two protecting groups. On the basis of the controls carried out during the synthesis, the trifluoroacetyl group is more suitable if the lysine content is high, but, since its elimination requires more drastic conditions than those needed for the removal of Fmoc, the latter should be used when nucleopeptides with base-labile amino acid-nucleoside linkages are to be obtained.

Arginine-containing nucleopeptides. Boc-Arg(Fmoc)$_2$-OH was synthesized by temporary silylation of Boc-Arg-OH followed by reaction with Fmoc-Cl. This derivative was shown to be suitable for nucleopeptide assembly, but ornithine and a small quantity of arginine were found after acid hydrolysis of peptides containing Arg(Fmoc)$_2$. Oxidation of phosphites to phosphates with *t*BuOOH and elimination of the Fmoc groups prior to final deprotection facilitated the isolation of the target nucleopeptide, Phac-Ala-Hse(p$^{3'}$dACTAGT)-Gly-Arg-Val-OH.

Primary carboxamide protection. Asn/Gln-containing peptide-resins were submitted to 4-5 nucleotide incorporation cycles and the amount of dimethoxytrityl cations formed at each deprotection step was quantified. The highest level of phosphitylation of the side chain primary carboxamides was observed during the first nucleotide incorporation cycle (5-10 %). The carboxamide phosphitylation yield seems to be dependant on the peptide sequence and slightly higher on glutamine than on asparagine.

The choice of the solid support. Boc-Hse(p$^{3'}$dATATTTGTTACTCTGT)-Lys-Lys-Lys-Lys-Lys-OH was synthesized on three different solid supports, polystyrene-*co*-1%-divinylbenzene, CPG and polyethyleneglycol-polystyrene (PEG-PS or TentaGel resins). The last copolymer was abandoned in view of the difficulties found in coupling all lysine residues (trifluoroacetyl-protected) and since only some peptide could be detached from the resin. PAGE analysis showed a main band in the case of the polystyrene-assembled crude nucleopeptide, and two main bands for the CPG-assembled product (mass spectrometric analysis showed that the impurity lacked one lysine residue). Polystyrene-*co*-1%-divinylbenzene is thus the best solid support for stepwise nucleopeptide assembly.

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