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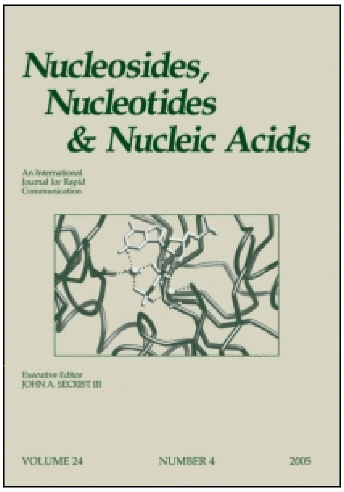

Nucleosides, Nucleotides and Nucleic Acids

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Stepwise Solid-Phase Synthesis of Serine-, Tyrosine- and Homoserine-nucleopeptides

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STEPWISE SOLID-PHASE SYNTHESIS OF SERINE-, TYROSINE- AND HOMOSERINE-NUCLEOPEPTIDES.

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Abstract. Hydroxylated amino acids can be introduced in nucleopeptides using the acetyl group for their side chain protection. Base-stable nucleopeptide analogues are obtained if homoserine is used as the linking residue.

Nucleopeptides, as peptide-oligonucleotide hybrids covalently linked through a phosphodiester bond, can be used to mimic the bond that is established during the replication of the genetic material of some viruses[1,2] or in the topoisomerase-mediated relaxation of DNA[3], as models to study the interactions between peptides and oligonucleotides, or as drugs in antisense therapy.

So far, in the syntheses of nucleopeptides described both in our laboratory and in others, the only trifunctional aminoacids present have been acidic aminoacid residues (aspartic and glutamic acids). In order to broaden the range of molecules available synthetically, we should ideally be able to introduce any amino acid or nucleoside residue in the peptide-oligonucleotide hybrid. We now report on the synthesis of nucleopeptides containing other hydroxylated aminoacids besides the linking residue and on the use of homoserine to establish the covalent linkage between the peptide and the oligonucleotide.

During the synthesis of an N-acylphosphoramidate-linked nucleopeptide analogue[4], the side chain hydroxyl group of the serine residue was protected as an acetate, which can be cleaved under fairly mild conditions (overnight treatment with concentrated aqueous ammonia/dioxane 1:1 at room temperature, or 4 h at 55 °C), so we inferred that the acetyl group would be suitable for the protection of the side chains of the hydroxylated amino acids when they are present in nucleopeptides but not as the linking residue.

Following the synthetic protocol developed in our laboratory for the stepwise solid-phase assembly of nucleopeptides[5,6], we obtained the protected nucleopeptides Phac-Ser($p^{3'}$dTATAT)-Ala-Ser(Ac)-Val-resin (Phac=phenylacetyl), Phac-Tyr($p^{3'}$dTATAT)-Ala-

Ser(Ac)-Val-resin and Phac-Tyr($p^{3'}$dTATAT)-Ala-Tyr(Ac)-Val-resin. A 1:1 conc. aq. ammonia/dioxane treatment afforded the desired products (Phac-Ser($p^{3'}$dTATAT)-Ala-Ser-Val-OH, Phac-Tyr($p^{3'}$dTATAT)-Ala-Ser-Val-OH and Phac-Tyr($p^{3'}$dTATAT)-Ala-Tyr-Val-OH), which were characterized by electrospray mass spectrometry and from amino acid and nucleoside composition after acid hydrolysis and enzymatic digestion, respectively.

Peptide-oligonucleotide hybrids need not necessarily be covalently linked through a phosphodiester bond, as in nucleopeptides, but the idea of keeping an amino acid as the anchoring point for the oligonucleotide seems attractive because the amino acid can be placed anywhere along the peptide sequence without restricting the union (as other linkers do) to the ends of the two molecules. The simplest modification, on the basis of the structure of a nucleopeptide, is to replace the naturally occurring linking amino acid (serine, threonine or tyrosine) by a different hydroxylated residue such as homoserine (Hse). The presence of an additional methylene group with respect to the serine residue should render nucleopeptides with a homoserine-nucleoside phosphodiester linkage stable to bases.

We synthesized three homoserine derivatives, Boc-Hse(DMT)-O^-, Fmoc-Hse(MMT)-O^- and Phac-Hse(DMT)-O^-, which allow the preparation of nucleopeptide analogues with the linking amino acid either in the middle of the peptide sequence or at the N-terminus, and with a free or a permanently blocked amino group, respectively. Finally, the HPLC analysis of the reaction crudes after the treatment of nucleopeptides H-Ser($p^{5'}$dG)-Gly-OH and H-Hse($p^{5'}$dG)-Gly-OH with 1 M NaOH showed that the serine-nucleopeptide had been partially degraded to by β-elimination $p^{5'}$dG (4 % after 2 h at room temperature and 57 % after 30 min at 55 °C), whereas, as expected, this nucleotide was not detected in the case of the homoserine-nucleopeptide.

In conclusion, the use of homoserine as the linking residue affords base-stable nucleopeptide analogues and considerably simplifies the overall synthetic protocol, since a wider choice of final deprotection conditions is available.

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