Chemoenzymatic synthesis of nucleopeptides

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Acid- and base-labile multifunctional nucleopeptides have been selectively constructed under mild conditions by means of enzymatic protecting group techniques.

Nucleoproteins are naturally occuring biopolymers in which the hydroxy group of a serine, a threonine or a tyrosine is linked *via* a phosphodiester group to the 3′- or 5′-end of DNA or RNA.[1] These protein conjugates play decisive roles in important biological processes; in particular, they may hold a central position in the process of viral replication *via* nucleoprotein-primed elongation of the oligonucleotide strand.[2] For the study of the biological phenomena in which nucleoproteins are involved, nucleopeptides embodying the characteristic linkage between the peptide chain and the oligonucleotide of their parent nucleoproteins may serve as powerful tools. In particular, the possibility of delineating the details of the above-mentioned mechanism of viral replication and developing a new class of antiviral agents based thereupon calls for the development of efficient methods for the construction of these peptide conjugates. However, their synthesis poses two major challenges: (i) the multifunctionality of the peptide-nucleotide conjugates makes the application of a variety of orthogonally stable amino, carboxy-, phosphate- and hydroxy-blocking groups necessary, and (ii) fully protected serine/threonine nucleopeptides are acid-labile (under acidic conditions the purine nucleotides may be depurinated[3]) and base-labile (under basic conditions, *i.e.* pH ⩾ 8, the entire oligonucleotide part may be split off *via* β-elimination[4]).

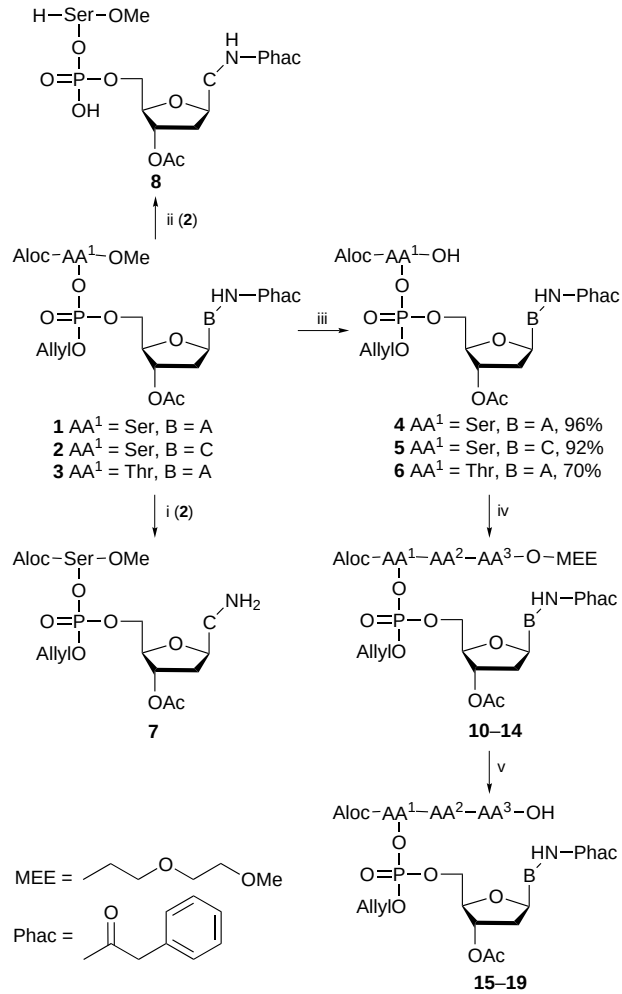
Consequently, in nucleopeptide chemistry not only is a variety of orthogonally stable blocking functions needed, they all must be removable selectively under mild, preferably neutral conditions. In the light of these seemingly contradictory demands it is not surprising that only a few reports on the successful construction of nucleopeptides have appeared.[4,5]

We have now found that enzymatic protecting group techniques[6] are efficient methods for the selective deprotection of the carboxylic acids and nucleobases of nucleopeptides.

In order to develop an enzymatic protecting group strategy for nucleopeptide synthesis, the protected nucleoamino acids **1**–**3** were constructed by means of established methods of oligonucleotide chemistry.† In these compounds, the nucleobases were masked with enzyme-labile phenylacetamide groups[7] and the carboxy groups of the amino acids were protected as methyl esters.

From the nucleoamino acid esters **1**–**3** the C-terminal protecting groups could be selectively removed by saponifying the methyl esters with the protease papain from *Carica papaya* at pH 6.6 and 37 °C (Scheme 1).[8] The conditions of the enzymatic transformations were so mild that the selectively unmasked nucleoamino acids **4**–**6** were obtained in high yield and without any undesired side reactions, *i.e.* the acid labile purine nucleosides remained intact and a β-elimination of the nucleotide, which readily takes place under weakly basic conditions (*vide supra*), was not observed. The protease did not attack the *O*-acetates. On the other hand, the nucleobase of *e.g.* **2** could be selectively N-deprotected by means of penicillin acylase-catalysed removal of the phenylacetamido base protecting group[7] at pH 7 and room temp. in 77% isolated yield (Scheme 1). In addition, the allyl-type blocking groups present in **2** were cleaved off without undesired side reaction by Pd^0-mediated allyl transfer to $PhSiH_3$ as accepting nucleophile[9] at room temp. in 89% isolated yield (Scheme 1).

Whereas the protease papain could advantageously be used to unmask the amino acid esters **1**–**3** its application to the deprotection of nucleopeptides might result in an undesired attack of the enzyme on the formed peptide bonds.[10] Therefore, for the selective C-terminal deprotection of nucleopeptides the lipase-mediated cleavage of the 2-(2-methoxyethoxy)ethyl (MEE) esters[11] was investigated, since lipases do not display any protease activity. To this end, the selectively deprotected nucleoamino acids **4**–**6** were coupled with different amino acid or dipeptide MEE esters **9** to give the fully masked nucleopeptides **10**–**14** in high yields (Scheme 1, Table 1). Upon treatment of the nucleopeptide acid MEE esters **10**–**14** with lipase from



Scheme 1 *Reagents and conditions*: i, Penicillin acylae, phosphate buffer pH 7)–MeOH, 80 : 20; ii, $Pd(PPh_3)_4$, $PhSiH_3$; iii, papain from *Carica papaya*, cysteine buffer, pH 6.6; iv, H–AA^2–AA^3–OMEE **9**, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole; v, lipase from *Aspergillus niger*, phosphate buffer (pH 7)–acetone, 90 : 10

Table 1 Synthesis of the nucleopeptide esters **8**–**12** and their selective deprotection with lipase from *Aspergillus niger* (AA = amino acid, B = nucleobase)

					Coupling		Deprotection	
Entry	AA[1]	AA[2]	AA[3]	B	Yield (%)		Yield (%)	
1	Ser	Gly	Asp (All)	C	**10**	61	**15**	70
2	Ser	Glu (All)	—	C	**11**	62	**16**	58
3	Ser	Phe	—	C	**12**	75	**17**	70
4	Ser	Val	Phe	C	**13**	69	**18**	59
5	Thr	Phe	—	A	**14**	71	**19**	63

Aspergillus niger at pH 7 and 37 °C the C-terminal carboxylic acid was smoothly deprotected (Scheme 1, Table 1). In the case of these multifunctional, complex peptide conjugates, too, the enzymatic transformations occured without any undesired side reaction, *i.e.* neither the acetate, N-terminal urethane, allyl phosphate, phenylacetamide and peptide bonds, nor the acid-labile purine nucleoside and the extremely base-labile serine phosphates were attacked. The biocatalyst again tolerated the presence of purine and pyrimidine bases and different amino acids and amino acid sequences in the nucleopeptides. Its substrate specificity guaranteed that the allyl esters which were employed for masking of the amino acid side chain functions in **10** and **11** were not removed either. By means of this enzymatic protecting group technique the desired selectively unmasked nucleopeptides **15**–**19** were obtained in high yield. Nucleopeptide **15** represents the characteristic linkage region of the nucleoprotein of adenovirus 2,[12] and **16** is derived from the nucleoprotein of bacteriophage Ø 29.[13]

Overall, the enzymatic protecting group strategy and the set of orthogonally stable blocking groups described here open a route to the construction of complex and sensitive nucleopeptides by means of a flexible building block strategy. The ready accessibility of these multifunctional peptide conjugates may serve to develop new tools for research at the interface between chemistry and biology.

This work was supported by the Bundesministerium für Bildung und Forschung, the Boehringer Mannheim GmbH and the Fonds der Chemischen Industrie.

Footnote and References

† Synthesis of **1**–**3**: i, Me_3SiCl (6 equiv.), pyridine, room temp., then $PhCH_2COCl$ (3 equiv.), 1-hydroxybenzotriazole (2 equiv.), MeCN–pyridine (2 : 1), room temp., then conc. NH_3, 0 °C, 87–92%; ii, 4,4′-dimethoxytrityl chloride (1.2 equiv.), pyridine, room temp., then Ac_2O (6 equiv.), DMAP, 81–89%; iii, $ZnBr_2$, $MeNO_2$, room temp., 10 min, 71–74%; iv, *N*-allyloxycarbonyl amino acid methyl ester phosphoroamidate, tetrazole, room temp., then Bu^tOOH, 0 °C, 68–82%.

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Received in Glasgow, UK, 16th June 1997; 7/04215I