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An Enzymatic Protecting Group Strategy for the Synthesis of Nucleopeptides

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Abstract: Enzymatic protecting group techniques are used for the selective synthesis of acid- and base labile multifunctional nucleopeptides under mild conditions.

Nucleoproteins are naturally occurring bioconjugates 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 key roles in important biological processes; in particular they hold a central position in the process of the nucleoprotein-primed viral replication.[2] Synthetic nucleopeptides embodying the characteristic linkage between the peptide chain and the oligonucleotide part of their parent nucleoproteins may serve as powerful tools for the study of the biological phenomena in which nucleoproteins are involved. Particularly, the possibility to investigate the details of the above-mentioned mechanism of viral replication and the perspective to develop a new class of antiviral agents based thereupon call for the development of efficient methods for the construction of these peptide conjugates. However, their synthesis poses two major challenges: 1) the numerous amino-, carboxy-, phosphate- and hydroxy-functions in nucleopeptides require the application of a variety of orthogonally stable protecting groups, and 2) fully protected serine- or threonine-nucleopeptides are both base labile (they lose their entire oligonucleotide part via a β-elimination mechanism[3] under basic conditions) and acid labile (under acidic conditions the purine nucleotides may be depurinated[4]).

Thus, in nucleopeptide chemistry a variety of orthogonally stable protecting groups is needed, which must all be removable selectively under mild, preferably neutral, conditions. In the light of these apparently contradictory demands it is not surprising that only a few successful syntheses of these conjugates have been published so far.[3,5] We now report that nucleopeptides can be built up efficiently by employing different enzymatic protecting group techniques[6] for the selective deprotection of the carboxylic acids, the nucleobases and the hydroxy groups.[5h]

In order to devise an enzymatic protecting group strategy for the synthesis of nucleopeptides, the protected nucleoserine conjugates **2a,b** were built up from the corresponding deoxynucleosides **1a,b** by established methods of oligonucleotide and peptide chemistry. In these compounds, the exocyclic amino functions of the nucleobases were masked with the enzyme labile phenylacet(PhAc)amide[7] and the carboxy groups of the amino acids were protected as methyl esters.

From the nucleoserine esters **2a,b**, the C-terminal methyl esters could be selectively removed by saponification

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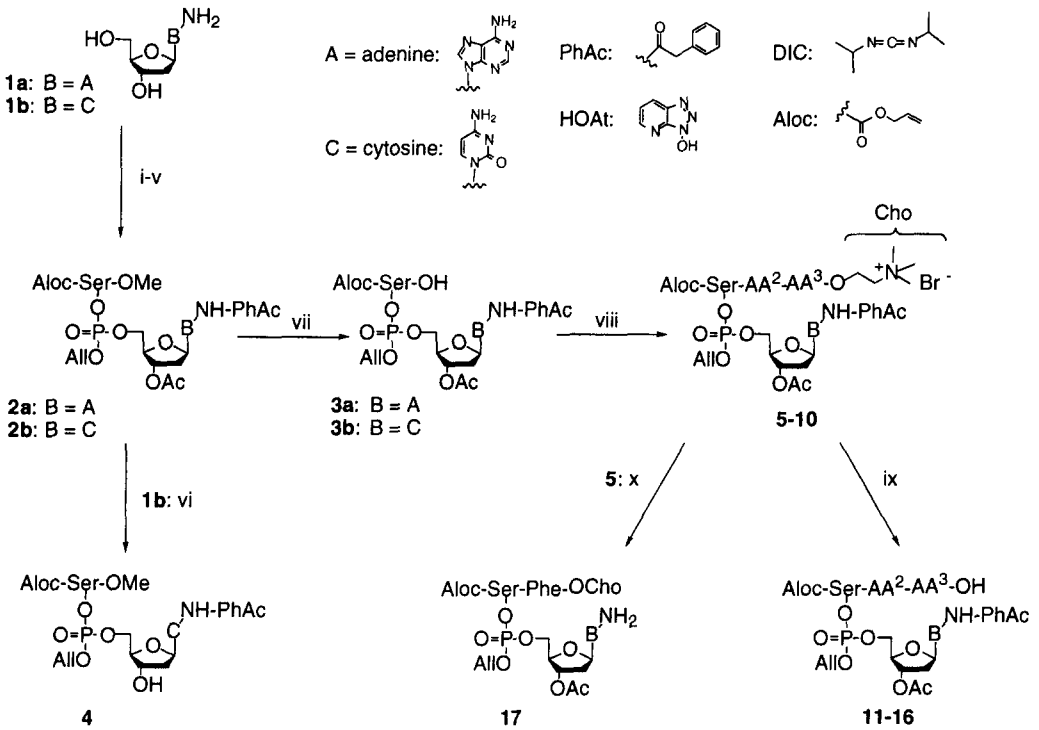
with the protease papain from *Carica papaya* at pH 6.6 and 37°C (Scheme 1).[8] Under these mild conditions, the selectively C-terminally deprotected nucleoserines **3a,b** were obtained in high yields and without observation of undesired side reactions like depurinations or β-eliminations. In addition, the 3'-hydroxy function of **2b** could be selectively deprotected by means of wheat germ lipase catalysed removal of the acetyl group at pH 6.5 and 37°C to give the alcohol **4** in 64% yield (Scheme 1).



Scheme 1. Synthesis and selective deprotection of the nucleoamino acids **2a,b** and the nucleopeptides **5-10**. *Reagents and conditions:* i. a) 6 eq. TMSCl, pyridine, r.t.; b) 3 eq. phenylacetyl chloride, 2 eq. HOBt, CH_3CN/pyridine 2:1, 0°C; 3. conc. NH_3, O°C, yields: 82-87%; ii. DMTrCl, pyridine, r.t., yields: 96-98%; iii. 3 eq. Ac_2O, DMAP, pyridine, yields: 80-84%; iv. $ZnBr_2$, CH_3NO_2, r.t., 10 min., yields: 78-85%; v. a) N-Aloc-serine methyl ester-O-phosphoramidite, tetrazole, r.t.; b) *t*BuOOH, 0°C. yields: 82-88%; vi. wheat germ lipase, phosphate buffer, pH 6.5, yield: 64%; vii. papain from *Carica papaya*, cysteine buffer, pH 6.6, yields: 74-80%; viii. $Br^-H_3N^+$-AA^2-AA^3-OCho, 1 eq. NBu_3, 1.1 eq. HOAt, 1.1 eq. DIC, DMF/CH_2Cl_2 1:1, yields: 65-99%; ix. butyrylcholine esterase, phosphate buffer, pH 6.5, yields: 61-96%; x. penicillin acylase, phosphate buffer, pH 7.0, yield: 82%

Unfortunately, the use of the protease papain for the unmasking of methyl esters is limited to the amino acid esters **2a,b**, as its application to the deprotection of nucleopeptides might result in an undesired attack of the enzyme on the formed peptide bonds.[9] In general, the limitation to the use of proteases for the unmasking of

peptide substrates can be overcome by employing esterases which are devoid of any protease activity and which selectively recognize a particular ester group.[6] For instance, the enzyme butyrylcholine esterase from horse serum could advantageously be employed in the construction of sensitive lipidated peptides.[10] Therefore, the butyrylcholine esterase mediated cleavage of choline esters was investigated for the selective C-terminal deprotection of nucleopeptides. To this end, the selectively deprotected nucleoamino acids **3a,b** were coupled with different amino acid or dipeptide choline esters to give the fully protected nucleopeptides **5-10** in high yields (Scheme 1, Table 1). Upon treatment of the nucleopeptide choline esters **5-10** with butyrylcholine esterase at pH 6.5 and 37°C, the C terminal carboxylic acid was smoothly deprotected (Scheme 1, Table 1). Even on these multifunctional, complex peptide conjugates, the mild enzymatic transformations occurred without any undesired side reaction. Neither the acetate, the N-terminal urethane, the allyl phosphate, the phenylacetamide and the peptide bonds, nor the acid and base labile purine nucleosides and serine phosphates were attacked. The biocatalyst tolerated different amino acids in di- or tripeptide sequences in the nucleopeptides. Through this enzymatic deprotection technique the selectively unmasked nucleopeptides **11-16** were obtained in high yields.

Table 1: Results of the synthesis of the nucleopeptide esters **5-10** and their selective C-terminal deprotection with butyrylcholine esterase (AA = amino acid, B = nucleobase).

					Coupling		Deprotection	
entry	AA^1	AA^2	AA^3	B	No	yield[%]	No	yield[%]
1	Ser	Phe	-	A	**5**	96	**11**	61
2	Ser	Ala	-	A	**6**	99	**12**	96
3	Ser	Tyr	-	A	**7**	65	**13**	86
4	Ser	Leu	-	A	**8**	91	**14**	78
5	Ser	Ala	Leu	A	**9**	88	**15**	73
6	Ser	Ala	-	C	**10**	87	**16**	71

On the other hand, the exocyclic amino function of the nucleobase in **5** could be selectively N-deprotected by means of penicillin acylase catalysed removal of the phenylacetamido base protecting group[7] at pH 7.0 and r.t. to give the amine **17** in 82% yield (Scheme 1).

Overall, this enzymatic protecting group strategy and the applied set of orthogonally stable protecting groups described in this article open up a new and mild route to the synthesis of complex and sensitive nucleopeptides. The application of these multifunctional peptide conjugates may serve to develop new tools for research at the interface between chemistry and biology, i.e. in the study of viral propagation.

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