Chemoenzymatic Synthesis of Nucleopeptides

Stefanie Flohr, Volker Jungmann, and Herbert Waldmann*[a]

Abstract: Nucleoproteins, in which the hydroxy group of a serine, a threonine, or a tyrosine, is linked through a phosphodiester group to the 3′- or 5′-end of DNA or RNA, play decisive roles in important biological processes. They may even have a major part in the process of viral replication by nucleoprotein-primed elongation of the oligonucleotide strand. For the study of the biological phenomena, in which nucleoproteins are involved, nucleopeptides with the characteristic linkage between the peptide chain and the oligonucleotide of their parent nucleoproteins may serve as powerful tools. However, the synthesis of these compounds is complicated by their pronounced acid- and base-lability, as well as their multifunctionality. As a result, protecting groups, which can be removed under the mildest conditions, are required. For the construction of such peptide conjugates using a flexible building block strategy, a combination of enzyme-labile and chemical protecting groups was developed. The C-terminal blocking function can be removed selectively from fully protected nucleoamino acid methyl, 2-methoxyethyl (ME), and methoxyethoxyethyl (MEE) esters by saponification of the esters. After elongation of the peptide chain with amino acid or peptide methyl, ME, MEE, and choline esters, the C-terminal ester blocking group can again be removed easily. The methyl, ME, and MEE esters are cleaved off with lipase, and the choline ester group is selectively attacked by butyrylcholine esterase. The nucleoamino acids and peptides formed may be fully deprotected. To this end, the enzyme-labile *N*-phenylacetyl (PhAc) group, which was employed to mask the amino functions of the nucleobases, was removed. The *O*-acetate in the deoxyribose was saponified, and the allyl protecting groups present were cleaved by Pd^0-mediated allyl transfer. By combination of these techniques, a nucleopeptide was produced, which represents the characteristic linkage region of the nucleoprotein of adenovions 2. The conditions, under which the enzymatic deprotections proceed, are so mild that no undesired side reaction is observed, that is no depurination or β elimination of the nucleosides occurs. In addition, the specificity of the biocatalysts ensures that the peptide bonds and the other protecting groups present are not attacked either.

Keywords: bioorganic chemistry · enzyme catalysis · nucleopeptides · nucleotides · protecting groups

Introduction

Nucleoproteins are naturally occurring biopolymers, in which the hydroxy group of a serine, a threonine, or a tyrosine is linked, through a phosphodiester group to the 3′- or 5′-end of DNA or RNA.[1] They play decisive roles in important biological processes, for example, DNA topoisomerases and DNA gyrases employ nucleopeptide bonds while carrying out their biological functions,[2] and the DNA is bound to the nuclear matrix as a nucleoprotein.[1] Particularly important is the finding that these protein conjugates may hold a central position in the process of viral replication. For instance, the single-stranded RNA of the polio virus and the double-stranded DNA of the *Bacillus subtilis* phage Φ 29 and hepatitis B virus are nucleoproteins.[3] The replication of these organisms starts with the covalent attachment of a nucleotide to a protein primer, the 'terminal protein', and then proceeds by elongation of the oligonucleotide strand (Scheme 1).[3]

For the study of the biological processes in which nucleoproteins are involved, nucleopeptides with the characteristic linkage between the peptide chain and the oligonucleotide of their parent nucleoproteins may serve as powerful tools. In particular, the possibility of describing the mechanism of viral replication in detail and the possible development of a new class of antiviral agents based thereupon (for example, against hepatitis B virus) call for the development of efficient methods for the construction of these peptide conjugates. However, the synthesis of nucleopeptides poses two major challenges (Scheme 2):

1) The multifunctionality of the peptide–nucleotide conjugates requires the application of a variety of orthogonally stable amino, carboxy, phosphate, and hydroxy blocking groups.
2) Fully protected serine/threonine nucleopeptides are acid-labile (under acidic conditions the purine nucleotides may

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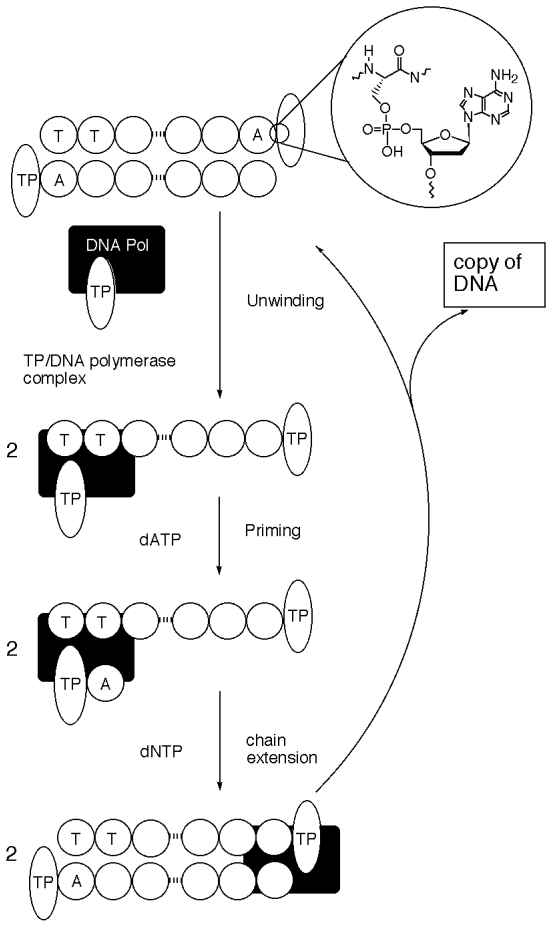
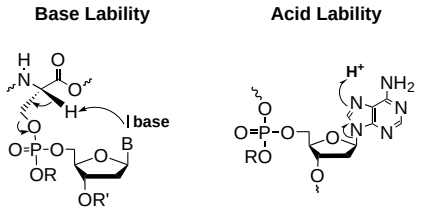


Scheme 1. Priming of viral replication by a nucleoprotein. TP: terminal protein, dNTP: deoxyribonucleotide triphosphate.

be depurinated),[4] and base-labile (under basic conditions, that is at pH ≥ 8, the entire oligonucleotide part may be split off by β elimination) (Scheme 2).



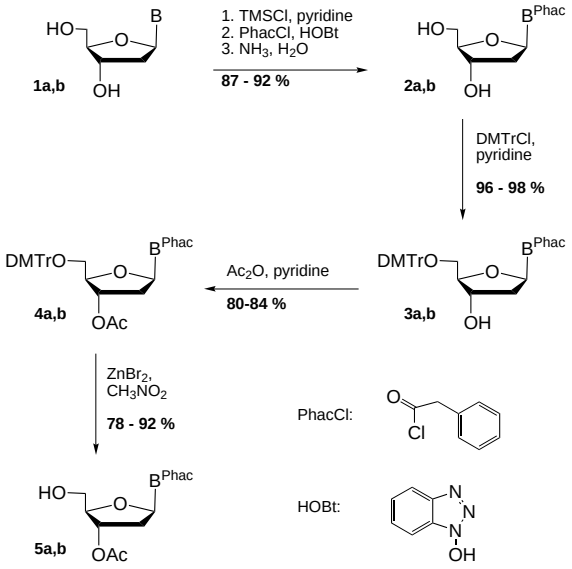
Scheme 2. Acid- and base-lability of nucleopeptides. B = adenine, cytosine, guanine, thymine.

Consequently, in nucleopeptide chemistry not only a variety of orthogonally stable blocking functions is needed, but also 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.[5, 6] In the most successful of these investigations, either an oligonucleotide is built up first, which is then coupled with an activated peptide, or alternatively, an oligopeptide is synthesized, and in a subsequent series of reactions the oligonucleotide chain is constructed at the peptide backbone. We reasoned that for the development of a highly flexible and efficient strategy for nucleopeptide synthesis, the use of nucleoamino acid building blocks, which can be selectively deprotected at their amino acid or nucleotide part, would be particularly advantageous. Such building blocks would have to have several orthogonally stable blocking groups, which on the one hand must tolerate the conditions of peptide and nucleotide synthesis, and on the other hand must be removable selectively and without attacking the peptide oligonucleotide linkage. Thus, the reaction takes place under the mildest conditions.

Enzymatic protecting group techniques[7] offer viable alternatives to classical chemical methods. As a result of the mild conditions under which they can be split off (pH 6–8, room temperature), enzyme-labile blocking functions have served as efficient tools in the synthesis of lipo-,[8] glyco-,[9] and phosphopeptides.[10] This paper presents an investigation into the use of enzyme-labile protecting groups and their use as the key method for the construction of complex nucleopeptides.[11]

Results and Discussion

In order to develop a general building block strategy for nucleopeptide synthesis, the nucleoamino acids **8** were built up (Schemes 3 and 4). To this end, the exocyclic amino groups of the nucleosides **1** were masked with the enzyme-labile phenylacetamido (PhAc) protecting group[12] by silylation, followed by treatment of the resulting persilylated intermediate with phenylacetyl chloride and HOBt. The N-protected nucleosides (**2**) obtained were then converted into the 5′-dimethoxytrityl (DMTr) ethers[13] **3**, and the 3′-OH groups were masked as acetates.



Scheme 3. Synthesis of selectively protected nucleosides **5**. **a**: B = adenine; **b**: B = cytosine.

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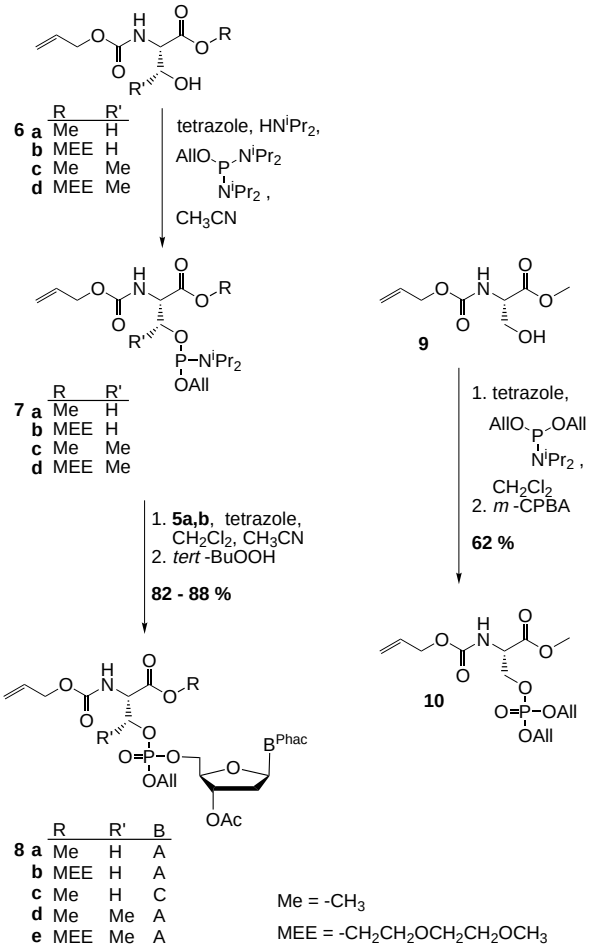
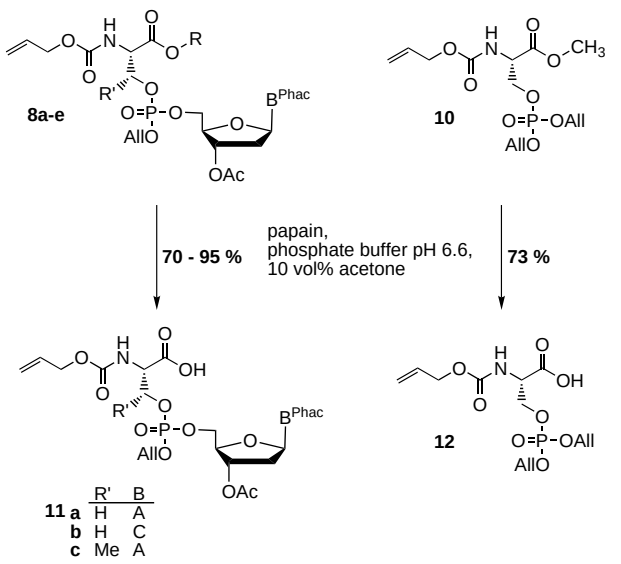
The DMTr group was selectively removed from the fully deprotected nucleotides **4** by treatment with $ZnBr_2$.[14] The selectively unmasked nucleosides **5** were then coupled with serine and threonine derived phosphoramidites **7** in the presence of tetrazole, and this reaction yielded, after subsequent oxidation with *tert*-butylhydroperoxide, fully masked nucleoamino acids **8** (Scheme 4). The amino acid derived phosphoramidites **7** were built up from the N-protected amino acid esters **6**, according to the method developed by Bannwarth et al.[15] By analogy, serine methyl ester **9** was converted into the phosphorylated serine derivative **10**, which is of interest as an intermediate for the chemoenzymatic synthesis of phosphopeptides.



Scheme 4. Synthesis of the fully protected nucleoamino acids **8**.

In order to determine if the nucleoamino acid derivatives **8** are viable building blocks for nucleopeptide synthesis, the orthogonal stability of the blocking groups present was investigated. Both the C-terminal methyl (Me)[16] and the methoxyethoxylethyl (MEE)[17] ester protecting groups can be selectively removed from the amino acid esters **8** and **10** by treatment with lipase from *Aspergillus niger* at pH 7 and 37 °C. However, much better results were obtained if the protease papain from *Carica papaya* was used at pH 6.6 and 37 °C (Scheme 5, Table 1). The lipase only accepts the serine derivatives as substrates, and the threonine esters are not



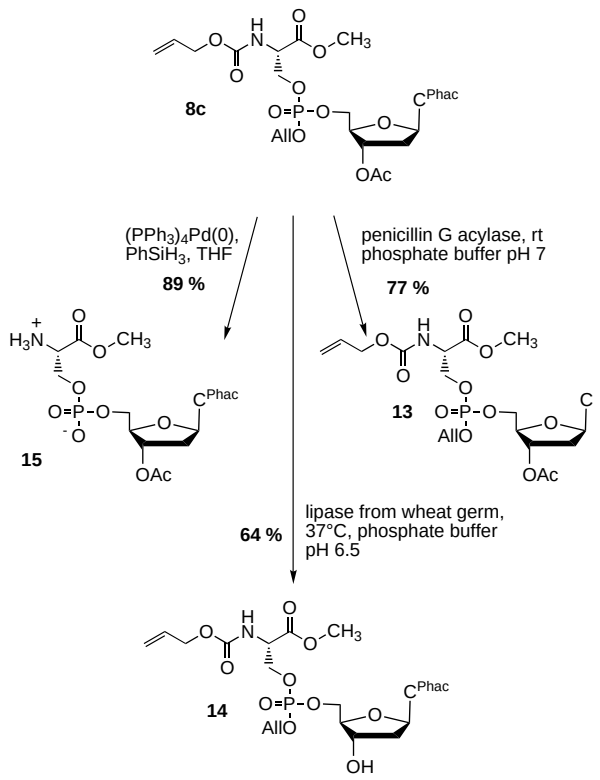
Scheme 5. Selective C-terminal deprotection of the nucleoamino acids **8** and the phosphoamino acids **10** by enzymatic ester hydrolysis.

Table 1. C-terminal deprotection of nucleoamino acids **8a–e** to the acids **11a–c**.

Entry	No	R′	B	R	No.	Deprotection with Lipase from *Aspergillus niger* yield [%]	Deprotection with Papain from *Carica papaya* yield [%]
1	**8a**	H	A	Me	**11a**	35	96
2	**8b**	H	A	MEE	**11a**	42	92
3	**8c**	H	C	Me	**11b**	39	91
4	**8d**	Me	A	Me	**11c**	0	70
5	**8e**	Me	A	MEE	**11c**	0	51

attacked. This observation is in line with findings that lipases can not or can only just accommodate substrates in their active sites, which have a branching point in β position to the ester C=O group.[18] Papain does not have this disadvantage, transformations proceed smoothly, yielding the desired selectively unmasked nucleoamino acids **11** and the phosphoamino acid **12** in much higher yield (Table 1). The conditions of the enzymatic transformations are so mild that no undesired side reaction is observed; the acid-labile purine nucleosides remain intact, and a β elimination of the nucleotide, which readily occurs under weakly basic conditions (vide supra), does not occur. The protease tolerates the presence of both purine and pyrimidine bases and does not attack the PhAc group, the acetate, and the phosphate at all.

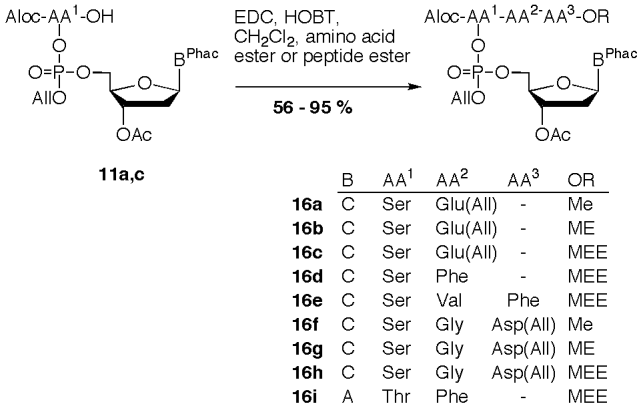
In order to demonstrate the orthogonal stability of the enzyme-labile ester groups to the other blocking functions present, the phenylacetamide, the *O*-acetate, and the allyl-type protecting groups were removed selectively from the nucleoamino acid **8c** (Scheme 6). Thus, upon treatment of **8c** with penicillin G acylase from *E. coli*[12] under neutral conditions, the corresponding amine was liberated smoothly and in high yield. The partially deblocked nucleoamino acid derivative **13** obtained may, for instance, serve as a versatile intermediate for the construction of artificial nucleopeptide analogues carrying, for example, a fluorescent label.[19] The acetate blocking group in the 3′-position was cleaved off by



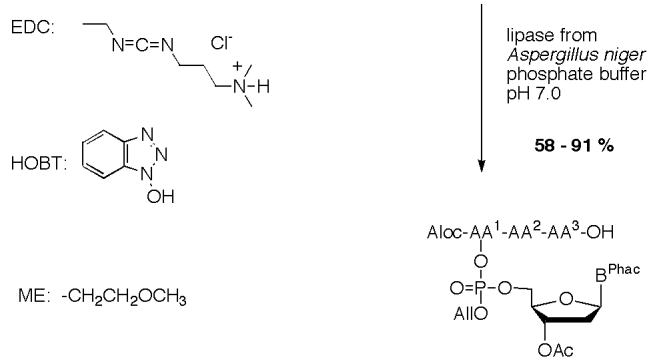
Scheme 6. Selective deprotection of the nucleoamino acid **8c** by different methods.

employing lipase from wheat germ as a biocatalyst (Scheme 6).[20] The best results were obtained at pH 6.5 and 37 °C in the presence of 10–20 vol% of ethylene glycol as solubilizing cosolvent. In this process, the deoxyribose derivative is obtained, without the C-terminal methyl ester being attacked. Thus, by appropriate choice of the lipase, nucleoamino acid derivatives like **8c** can be regioselectively deprotected at the carbohydrate or the amino acid part of the molecule. Finally, the *N*-terminal allyloxycarbonyl urethane and the allyl phosphate present in **8c** were cleaved chemoselectively by Pd^0-mediated allyl transfer to an accepting nucleophile. The nucleophile of choice in this case was phenylsilane as recommended by Guibé and Loffet et al.[21] The use of formic acid/*n*-butylamine mixtures introduced by Noyori et al. for the unmasking of oligonucleotides[22] was found to be less efficient. In all these deprotection reactions, the blocking groups were completely orthogonally stable to each other, and a β elimination or hydrolysis of the *N*-glycosidic bond could not be observed.

Further construction of nucleopeptides employing the C-terminally deprotected building blocks **11** was then attempted. The amino acid chain of the nucleoamino acids could be elongated by condensing the carboxylic acids **11** with N-terminally deblocked amino acid and dipeptide esters in the presence of *N*-ethyl-*N*′-dimethylaminopropyl carbodiimide hydrochloride (EDC) and *N*-hydroxybenzotriazole (HOBt). This reaction gave the fully protected nucleopeptides **16** in moderate to high yields (Scheme 7, Table 2). The



	B	AA^1	AA^2	AA^3	OR
16a	C	Ser	Glu(All)	-	Me
16b	C	Ser	Glu(All)	-	ME
16c	C	Ser	Glu(All)	-	MEE
16d	C	Ser	Phe	-	MEE
16e	C	Ser	Val	Phe	MEE
16f	C	Ser	Gly	Asp(All)	Me
16g	C	Ser	Gly	Asp(All)	ME
16h	C	Ser	Gly	Asp(All)	MEE
16i	A	Thr	Phe	-	MEE



	B	AA^1	AA^2	AA^3
17a	C	Ser	Glu(All)	-
17b	C	Ser	Phe	-
17c	C	Ser	Val	Phe
17d	C	Ser	Gly	Asp(All)
17e	A	Thr	Phe	-

Scheme 7. Synthesis and selective enzymatic deprotection of the nucleopeptide methyl, ME, and MEE esters **16**.

Table 2. Synthesis of the nucleopeptides **16a–i** and selective C-terminal deprotection with lipase from *Aspergillus niger* to the acids **17a–e**.

						Coupling		Deprotection	
Entry	B	AA^1	AA^2	AA^3	R	No.	yield [%]	No	yield [%]
1	C	Ser	Glu(OAll)	-	Me	**16a**	95	**17a**	78
2	C	Ser	Glu(OAll)	-	ME	**16b**	90	**17a**	89
3	C	Ser	Glu(OAll)	-	MEE	**16c**	62	**17a**	58
4	C	Ser	Phe	-	MEE	**16d**	75	**17b**	70
5	C	Ser	Val	Phe	MEE	**16e**	69	**17c**	59
6	C	Ser	Gly	Asp(OAll)	Me	**16f**	60	**17d**	81
7	C	Ser	Gly	Asp(OAll)	ME	**16g**	56	**17d**	91
8	C	Ser	Gly	Asp(OAll)	MEE	**16h**	75	**17d**	70
9	A	Thr	Phe	-	MEE	**16i**	71	**17e**	63

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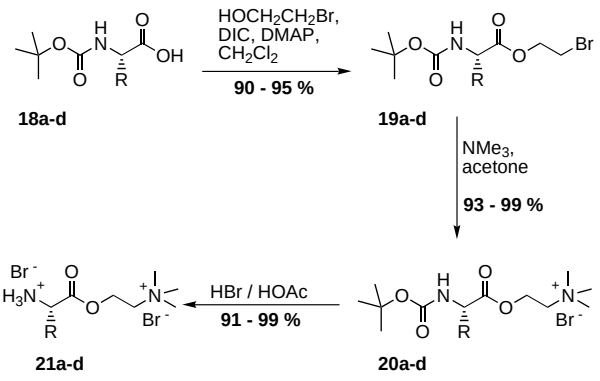
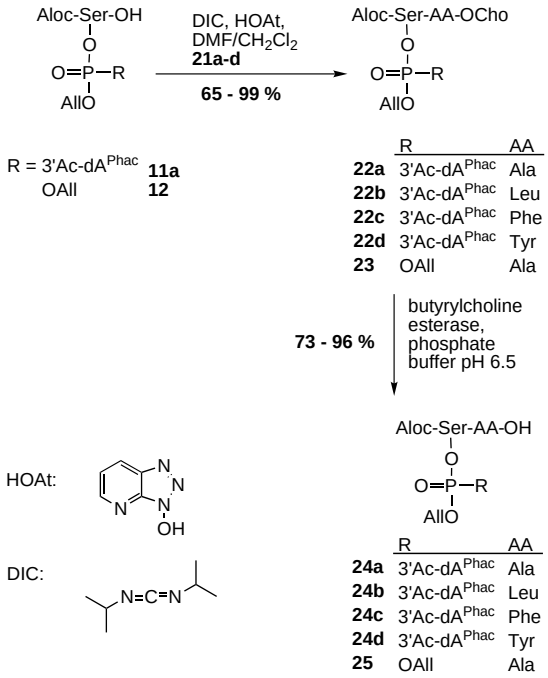
methyl and MEE esters mentioned above, were again used as C-terminal protecting groups. The use of methoxyethyl (ME) esters was found to work best, as this ester group is more polar than the methyl ester and therefore has already pronounced solubility enhancing properties. However, this group is not quite as hydrophilic as the MEE ester, so the nucleopeptide ME esters are easier to isolate than the corresponding MEE esters. Furthermore, through the series methyl to the ME, and finally the MEE ester, the size and the polarity of the enzyme-labile protecting group increase. By varying this part of the substrates, their properties may be finely adjusted to the steric and electronic requirements of the active site of the particular biocatalyst used in the deprotection. The protease papain was not used for the removal of the C-terminal protecting group from the nucleopeptide esters **16**, since in the presence of this enzyme an undesired attack on the peptide bonds was feared. However, if the peptide conjugates **16** are treated with lipase from *Aspergillus niger*, the C-terminally deprotected nucleopeptides **17** are obtained without any undesired attack on the peptide bonds (Scheme 7, Table 2). Once more the conditions of the enzymatic reactions were so mild that no undesired side reaction was observed, and, in addition, the substrate specificity of the enzyme guaranteed that the allyl esters present in **16a–c** and **16f–h** remained intact, too. The nature of the alcohol blocking function has a marked influence on the efficiency of the enzyme-mediated hydrolysis. Thus for **16a**, **16b**, and **16c**, which only differ in the nature of the C-terminal protecting group, as well as for **16f**, **16g**, and **16h**, for which the same is true, the highest yields were recorded for the deblocking of the methoxyethyl (ME) esters (Table 2; compare entries 1, 2, and 3 as well as entries 6, 7, and 8). All selectively unmasked nucleopeptides **17a–17e** were obtained in high yields.

As an alternative to the methyl, ME, and MEE esters, the choline ester protecting group[24] was investigated. This group has already proven to be an advantageous enzyme-labile blocking function in lipopeptide chemistry.[8] The charged choline esters have pronounced solubility enhancing properties and should be particularly advantageous for enzymatic transformations of hydrophobic substrates, which otherwise would be only sparingly soluble in the aqueous media needed for optimal performance of the biocatalysts. The amino acid choline esters employed were synthesized according to published procedures[24], as shown in Scheme 8. Thus, Boc-protected amino acids **18** were converted into their 2-bromoethyl esters **19**.



Scheme 8. Synthesis of the amino acid choline esters **21**.

These were then treated with trimethylamine to give the choline esters **20**, from which the Boc group was cleaved off by treatment with a solution of HBr in acetic acid, forming the desired esters **21** in high yield. The choline esters **21** were then coupled with the nucleoamino acid **11a** and the phosphoamino acid **12** in the presence of diisopropylcarbodiimide (DIC) and *N*-hydroxy-azabenzotriazole (HOAt)[25] to give the nucleopeptide choline esters **22** and the phosphopeptide **23** in high yield (Scheme 9, Table 3). Butyrylcholine esterase



Scheme 9. Synthesis and selective enzymatic deprotection of the nucleopeptide choline esters **22** and the phosphopeptide choline ester **23**.

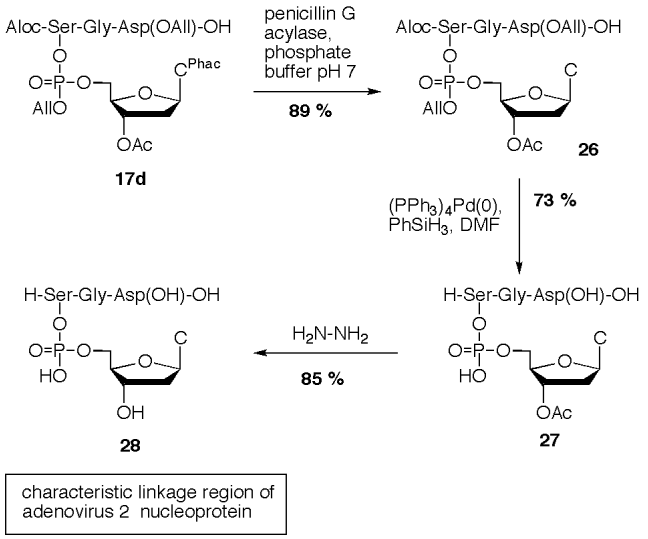
Table 3. Synthesis of the nucleo- and phosphopeptide choline esters **22a–d, 23** and selective C-terminal deprotection with butyrylcholine esterase to the acids **24a–d, 25** (AA = amino acid, A = 3′-Ac-dA^{Phac}, P = PO(OAll)$_2$).

			Coupling		Deprotection	
Entry	AA	R	no.	yield [%]	no.	yield [%]
1	Ala	A	**22a**	99	**24a**	96
2	Leu	A	**22b**	91	**24b**	78
3	Phe	A	**22c**	96	**24c**	61
4	Tyr	A	**22d**	65	**24d**	86
5	Ala	P	**23**	85	**25**	73

(9 units mg^{-1}) was utilized for the enzymatic removal of the protecting group. This enzyme was isolated from horse serum by a combination of anion-exchange chromatography and affinity chromatography on a procainamide column, according to a published procedure.[26] Upon treatment of an aqueous solution of the choline esters **22** and **23** (due to solubilizing properties of the choline ester group, addition of an organic cosolvent was unnecessary) with this enzyme at pH 6.5, the C-terminal ester was cleaved off smoothly and without any undesired side reaction. The selectively un-

masked nucleodipeptides **24** and the phosphodipeptide **25** were obtained in high yields. These results demonstrate that the choline ester can also be employed advantageously for the construction of nucleo- and phosphopeptides.

Finally, to prove that the combination of the enzyme-labile and the classical chemical blocking functions also allows for the synthesis of completely deprotected nucleopeptides, all protecting groups were removed from the nucleotripeptide **17d** (Scheme 10). To this end, the phenylacetamido protecting



Scheme 10. Complete deprotection of the nucleopeptide **17d**.

function was removed from the nucleobase first, to provide the modified peptide **26** in high yield. Next, the three allyl blocking groups present in **26** were removed by Pd^0-catalyzed allyl transfer with phenylsilane as the accepting nucleophile. The reaction yielded the nucleotripeptide **27**. Finally, **27** was deacetylated by treatment with hydrazine in methanol.[27] The completely deprotected nucleopeptide **28** obtained by this reaction sequence represents the characteristic linkage region of the nucleoprotein of adenovirus 2.[28]

Conclusion

We have demonstrated that sensitive and multifunctional nucleopeptides can be built up efficiently by means of a flexible building block strategy. This strategy makes use of a set of orthogonally stable enzyme-labile and classical chemical protecting groups. In particular, the conditions of the enzyme-mediated selective deprotection of the nucleopeptides are so mild that no undesired side reaction (that is β elimination of the nucleotide or depurination) occurs. Furthermore, no unwanted attack on the peptide bonds or the other blocking groups present is observed either. The ready accessibility and application of nucleopeptides may serve to develop new reagents and tools for research at the interface between chemistry and biology, that is in the study of viral propagation.

Experimental Section

General procedures: ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 250, AM 400, and DRX 500. Mass spectra were measured on a Finnigan MAT MS 70 spectrometer. Analytical chromatography was performed on E. Merck silica gel 60 F_{254} plates. Flash chromatography was performed on Baker silica gel (40–64 μm). Solvents were dried according standard procedures. All reactions except the enzymatic transformations were carried out under nitrogen. Penicillin G acylase was immobilized on Eupergit C, lipase was purchased from Amano, papain from Fluka.

***N*-Phenylacetyl-2′-deoxynucleosides (2a–c)**: A solution of the nucleoside **1** (4 mmol) and TMSCl (3 mL, 25 mmol) in pyridine (20 mL) was stirred for 30 min at 0 °C, and a solution of HOBt (1.08 g, 8.0 mmol) and phenylacetyl chloride (1.65 mL, 12 mmol) in a mixture of CH_3CN (1.8 mL) and pyridine (0.9 mL) was added. After 12 h, water (5 mL) and concentrated aqueous NH_3 (7.4 mL) were added, the solution was stirred for 10 min, concentrated, and the residue was taken up in hot acetone and filtered. The filtrate was concentrated, and the crude oil was purified by chromatography on silica gel (chloroform/ethanol 9:1).

6-*N*-Phenylacetyl-2′-deoxyadenosine (2a): Yield: 87 %; white solid; m.p. 169 °C; $R_f = 0.53$ (ethyl acetate/methanol 2:1); ^{1}H NMR (250 MHz, [D$_6$]DMSO): $\delta = 10.97$ (s, 1H), 8.68 (s, 1H), 8.65 (s, 1H), 7.40–7.25 (m, 5H), 6.45 (t, $J = 7$ Hz, 1H), 5.35 (d, $J = 5.1$ Hz, 1H), 4.98 (t, $J = 6.5$ Hz, 1H), 4.44 (s, 1H), 3.71 (m, 3H, 4′H), 3.64–3.43 (m, 2H), 2.82–2.71 (m, 1H), 2.36–2.32 (ddd, $J_{2'a,3'} = 3.8$ Hz, $J_{2'a,1'} = 6.5$ Hz, $J_{2'a,2'b} = 13.5$ Hz, 1H); $[\alpha]_D^{20} = -15.1$ ($c = 1$, $CHCl_3$); elemental analysis for $C_{18}H_{19}N_5O_4$(%): calcd C 58.53, H 5.18, N 18.96; found C 58.49, H 5.14, N 18.83.

4-*N*-Phenylacetyl-2′-deoxycytidine (2b): Yield: 92 %; m.p. 75 °C; $R_f = 0.57$ (ethyl acetate/methanol 2:1); ^{1}H NMR (250 MHz, [D$_6$]DMSO): $\delta = 8.34$ (d, $J = 7.0$ Hz, 1H), 7.94 (s, 1H), 7.35–7.24 (m, 5H), 7.19 (d, $J = 7.0$ Hz, 1H), 6.10 (t, $J = 6.3$ Hz, 1H), 5.27 (d, $J = 4.2$ Hz, 1H), 5.05 (t, $J = 5.2$ Hz, 1H), 4.22–4.19 (m, 1H), 3.87–3.84 (m, 1H), 3.73 (s, 2H), 3.65–3.53 (m, 2H), 2.29 (m, 1H), 2.01 (m, 1H); $[\alpha]_D^{20} = 64.1$ ($c = 1$, DMSO); elemental analysis for $C_{17}H_{19}N_3O_5$(%): calcd C 59.12, H 5.55, N 12.17; found C 58.99, H 5.28, N 11.99.

2-*N*-Phenylacetyl-2′-deoxyguanosine (2c): Yield: 89 %; m.p. 165 °C (decomp); $R_f = 0.42$ (ethyl acetate/methanol 2:1); ^{1}H NMR (250 MHz, [D$_6$]DMSO): $\delta = 11.97$ (s, 1H), 8.24 (s, 1H), 7.35–7.25 (m, 5H), 6.21 (t, $J = 7$ Hz, 1H), 5.31 (d, $J = 3.9$ Hz, 1H), 4.95 (t, $J = 5.4$ Hz, 1H), 4.37 (m, 1H), 3.84 (m, 1H), 3.80 (s, 2H), 3.54–3.43 (m, 2H), 2.56 (m, 1H), 2.27 (m, 1H); $[\alpha]_D^{20} = -5.1$ ($c = 1$, DMSO); elemental analysis for $C_{18}H_{19}N_5O_5$: calcd C 56.10, H 4.97, N 18.17; found C 55.95, H 5.00, N 18.00.

5′-*O*-Dimethoxytrityl-*N*-phenylacetyl-2′-deoxynucleosides (3a–c): A solution of **2** (1 mmol) and DMTrCl (407 mg, 1.2 mmol) in pyridine (10 mL) was stirred for 3 h at room temperature. A saturated solution of aqueous $NaHCO_3$ (30 mL) was added, and the mixture was extracted with CH_2Cl_2 (30 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residual yellow foam was purified by chromatography on silica gel (chloroform/ethanol 30:1).

5′-*O*-Dimethoxytrityl-6-*N*-phenylacetyl-2′-deoxyadenosine (3a): Yield: 98 %; white foam; m.p. 97 °C; $R_f = 0.66$ (ethyl acetate/methanol 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.88$ (s, 1H), 8.61 (s, 1H), 8.10 (s, 1H), 7.37–6.76 (m, 18H), 6.43 (t, $J = 6.4$ Hz, 1H), 4.68 (m, 1H), 4.19–4.14 (m, 3H, 4′H), 3.74 (s, 6H), 3.39 (m, 2H), 2.81 (m, 1H), 2.52 (m, 1H)); $[\alpha]_D^{20} = -4.0$ ($c = 1$, $CHCl_3$); elemental analysis for $C_{39}H_{37}N_5O_6$: calcd C 69.73, H 5.55, N 10.43; found C 69.85, H 5.51, N 10.33.

5′-*O*-Dimethoxytrityl-4-*N*-phenylacetyl-2′-deoxycytidine (3b): Yield: 96 %; white foam; m.p. 102 °C; $R_f = 0.68$ (ethyl acetate–methanol 2:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.86$ (s, 1H), 8.22 (s, 1H), 7.40–6.82 (m, 19H), 6.27 (t, $J = 5.9$ Hz, 1H), 4.48 (m, 1H), 4.14 (m, 1H), 3.77 (s, 6H), 3.74 (s, 2H), 3.47–3.37 (m, 2H), 2.77 (m, 1H), 2.19 (m, 1H); $[\alpha]_D^{20} = -33.4$ ($c = 1$, $CHCl_3$); elemental analysis for $C_{38}H_{37}N_3O_7$: calcd C 70.46, H 5.76, N 6.49; found C 70.73, H 5.81, N 6.40.

5′-*O*-Dimethoxytrityl-2-*N*-phenylacetyl-2′-deoxyguanosine (3c): Yield: 93 %; white foam; m.p. 118 °C; $R_f = 0.61$ (ethyl acetate–methanol 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 12.20$ (s, 1H), 10.69 (s, 1H), 7.79 (s, 1H), 7.34–6.68 (m, 18H), 6.12 (t, $J = 6.4$ Hz, 1H), 4.57 (s, 1H), 4.14 (d, $J = 2.7$ Hz, 1H), 3.71 (s, 2H), 3.65 (s, 6H), 3.25 (m, 2H), 2.52–2.43 (m, 1H),

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2.38–2.36 (m, 1H)); $[\alpha]_D^{20} = -3.3$ ($c = 1$, $CHCl_3$); elemental analysis for $C_{39}H_{37}N_5O_7$: calcd C 68.11, H 5.42, N 10.18; found C 68.13, H 5.40, N 10.10.

3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-*N*-phenylacetyl-2′-deoxynucleosides (4a,b): A solution of **3** (0.5 mmol) and acetic anhydride (0.26 mL, 2.5 mmol) in pyridine (8 mL) was stirred for 4 h. A sat. solution of aqueous $NaHCO_3$ (12 mL) was added, and the mixture was extracted with $CHCl_3$ (20 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residual foam was purified by chromatography on silica gel (chloroform–triethylamine 100:1).

3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-6-*N*-phenylacetyl-2′-deoxyadenosine (4a): Yield: 80%; white foam; m.p. 58°C; $R_f = 0.22$ (chloroform–ethanol 20:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 8.72$ (s, 1H), 8.65 (s, 1H), 8.12 (s, 1H), 7.45–7.15 (m, 14H), 6.85–6.72 (m, 4H), 6.47 (dd, $J_{1',2'a} = 5.2$ Hz, $J_{1',2'b} = 8.0$ Hz, 1H), 5.51 (d, $J = 6.2$ Hz, 1H), 4.33–4.25 (m, 1H), 4.20 (s, 2H), 3.75 (s, 6H), 3.50–3.37 (m, 2H), 2.87–3.03 (m, 1H), 2.56–2.68 (m, 1H), 2.12 (s, 3H); $[\alpha]_D^{20} = 5.4$ ($c = 0.5$, $CHCl_3$); MS EI: m/z: 713.3 ([M]) (calcd for $C_{41}H_{39}N_5O_7$ 713.3); elemental analysis for sesquihydrate: calcd C 66.47, H 5.71, N 9.45; found C 66.21, H 5.68, N 9.34.

3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-4-*N*-phenylacetyl-2′-deoxycytidine (4b): Yield: 84%; white foam; m.p. 95°C; $R_f = 0.75$ (chloroform/ethanol 9:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.13$ (s, 1H), 8.07 (d, $J = 7.6$ Hz, 1H), 7.38–7.21 (m, 14H), 7.16 (d, $J = 7.6$ Hz, 1H), 6.85–6.81 (m, 4H), 6.25 (t, $J = 5.9$ Hz, 1H), 5.35 (m, 1H), 4.22 (m, 1H), 3.78 (s, 6H), 3.74 (s, 2H), 3.46–3.40 (m, 2H), 2.76–2.72 (m, 1H), 2.28–2.22 (m, 1H), 2.05 (s, 3H); $[\alpha]_D^{20} = 67.3$ ($c = 1$, $CHCl_3$); elemental analysis for $C_{40}H_{39}N_3O_8$, hemihydrate: calcd C 68.75, H 5.77, N 6.01; found C 68.58, H 5.68, N 6.39.

3′-*O*-Acetyl-*N*-phenylacetyl-2′-deoxynucleosides (5a,b): A suspension of **4** (3.33 mmol) and $ZnBr_2$ (3 g, 13.33 mmol) in CH_3NO_2 (30 mL) was stirred for 15 min at room temprature. A solution of NH_4OAc (1M, 50 mL) was added, and the mixture was extracted with chloroform. The organic phase was dried over $MgSO_4$ and concentrated. The residual oil was purified by chromatography on silica gel (chloroform–ethanol 19:1).

3′-*O*-Acetyl-6-*N*-phenylacetyl-2′-deoxyadenosine (5a): Yield: 78%; white solid; m.p. 77°C; $R_f = 0.14$ (ethyl acetate–methanol 50:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.90$ (s, 1H), 8.70 (s, 1H), 8.09 (s, 1H), 7.26–7.40 (m, 5H), 6.31 (dd, $J_{1',2'a} = 6.2$ Hz, $J_{1',2'b} = 9$ Hz, 1H), 5.75 (br, 1H), 5.56 (d, $J = 6.2$ Hz, 1H), 4.27 (m, 1H), 4.23 (s, 2H), 3.91 (m, 2H), 3.09–3.18 (m, 1H), 2.40–2.46 (m, 1H), 2.13 (s, 3H); $[\alpha]_D^{20} = -21.3$ ($c = 0.4$, CH_3OH); elemental analysis for $C_{20}H_{21}N_5O_5$, hemihydrate: calcd C 57.14, H 5.27, N 16.66; found C 57.14, H 5.27, N 16.36.

3′-*O*-Acetyl-4-*N*-phenylacetyl-2′-deoxycytidine (5b): Yield: 92%; white solid; m.p. 187°C; $R_f = 0.53$ (chloroform–ethanol 9:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.28$ (d, $J = 7.5$ Hz), 7.37 (d, $J = 7.5$ Hz, 1H), 7.29–7.20 (m, 5H), 6.18 (t, $J = 5.8$ Hz, 1H), 5.23 (m, 1H), 4.10 (m, 1H), 3.81–3.72 (m, 2H), 3.66 (s, 2H), 2.59–2.55 (m, 1H), 2.20–2.13 (m, 1H), 2.03 (s, 3H); $[\alpha]_D^{20} = 21.5$ ($c = 0.4$, CH_3OH); elemental analysis for $C_{19}H_{21}N_3O_6$, hemihydrate: calcd C 58.90, H 5.46, N 10.84; found C 58.52, H 5.46, N 10.70.

Synthesis of the phosphoramidites (7a–d): The phosphoramidites **7a–d** were synthesized according to a published procedure.[29] A solution of **6a–d** (3.32 mmol, 1.5 h, room temperature) in methylene chloride (4 mL) was added to a solution of allyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (1.3 mL, 4 mmol), diisopropylamine (0.23 mL, 1.65 mmol), and tetrazole (115 mg, 1.65 mmol) in methylene chloride (9 mL). After 2 h, the reaction mixture was taken up in a saturated aqueous solution of $NaHCO_3$ (15 mL). The mixture was extracted four times with methylene chloride. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The residual oil was filtered with silica gel using hexane/acetic acid ethyl ester mixtures as eluent. The crude products were concentrated and immediately used for subsequent steps without further purification.

Synthesis of the nucleoamino acids (8a–e): A solution of tetrazole (58 mg, 0.82 mmol) in acetonitrile (3 mL) at room temperature was added dropwise to a solution of **5a,b** (0.49 mmol) and **6a–d** (0.8 mmol) in CH_2Cl_2 (5 mL). After completion of the reaction (18 h), *t*BuOOH (80%, 3.4 mL) was added at 0°C. After 10 min, the reaction was taken up in H_2O (100 mL), and the solution was extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$ and concentrated, and the residual oil purified by chromatography on silica gel (chloroform/ethanol 40:1).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-serine-methyl ester (8a)**: Yield: 72%; white foam; $R_f = 0.29$ ($CHCl_3$–EtOH 20:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.75$ (s, 1H), 8.72 (s, 1H), 8.46 (m, 1H), 7.37–7.22 (m, 5H), 6.57–6.46 (m, 2H), 5.91–5.81 (m, 2H), 5.48 (m, 1H), 5.33–5.15 (m, 4H), 4.63–4.10 (m, 12H), 3.72 (m), 2.98 (m, 1H), 2.65 (m, 1H), 2.13 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 170.45$, 169.43, 169.38, 155.83, 152.37, 151.16, 149.34, 141.77, 134.26, 132.46, 131.88, 129.51, 128.51, 126.99, 118.93, 117.76, 84.29, 83.22, 74.19, 68.74, 67.55, 66.88, 65.90, 54.26, 52.84, 44.27, 37.02, 20.85; $[\alpha]_D^{20} = -1.25$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{31}H_{37}O_{12}N_6P$: calcd C 51.96, H 5.20, N 11.73; found C 51.95, H 5.17, N 11.65.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-serine-methoxyethoxyethyl ester (8b)**: Yield: 78%; white hygroscopic foam; $R_f = 0.23$ ($CHCl_3$–EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.45$ (m, 1H), 8.73 (m, 1H), 8.41 (d, 1H), 7.38–7.25 (m, 5H), 6.55–6.44 (m, 2H), 5.89–5.82 (m, 2H), 5.48 (m, 1H), 5.35–5.09 (m, 4H), 4.65–4.09 (m, 12H), 3.70 (m, 2H), 3.62 (m, 2H), 3.52 (m, 2H), 3.35 (s, 3H), 2.94 (m, 1H), 2.66 (m, 1H), 2.14 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 170.47$, 168.97, 168.92, 155.84, 152.48, 151.18, 149.32, 141.59, 134.22, 132.53, 131.93, 129.56, 128.64, 127.14, 119.04, 117.81, 84.32, 83.34, 74.31, 71.79, 70.43, 68.84, 68.68, 67.64, 66.94, 65.05, 58.95, 54.39, 44.39, 37.24, 20.89; $[\alpha]_D^{20} = -2.2$ ($c = 0.5$, CH_2Cl_2); elemental analysis for $C_{35}H_{45}O_{14}N_6P$: calcd C 52.24, H 5.64, N 10.44; found C 52.18, H 5.57, N 10.30.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-serine-methyl ester (8c)**: Yield: 87%; white wax; $R_f = 0.32$ ($CHCl_3$–EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.07$ (s, 1H), 7.99 (m, 1H), 7.38 (m, 1H), 7.21–7.16 (m, 5H), 6.22 (m, 2H), 5.83–5.79 (m, 2H), 5.30–5.09 (m, 5H), 4.52–4.19 (m, 10H), 3.76 (m, 2H), 3.67 (m, 3H), 2.89 (m, 1H), 2.00 (m, 3H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 171.44$, 170.20, 169.14, 162.65, 155.53, 154.77, 143.70, 133.63, 132.27, 131.67, 129.08, 128.45, 127.02, 118.85, 117.50, 96.75, 86.66, 83.17, 73.87, 68.61, 67.28, 66.91, 65.65, 54.07, 52.59, 43.95, 38.28, 20.55; $[\alpha]_D^{20} = -40.2$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{30}H_{37}O_{13}N_4P$): calcd C 52.02, H 5.38, N 8.09; found C 51.95, H 5.40, N 7.96.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-threonine-methyl ester (8d)**: Yield: 68%; white wax; $R_f = 0.32$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.31$ (s, 0.5H), 9.27 (s, 0.5H), 8.72 (s, 1H), 8.39 (m, 1H), 7.46–7.21 (m, 5H), 6.68–6.47 (m, 2H), 5.96–5.80 (m, 2H), 5.50 (m, 1H), 5.47–5.02 (m, 5H), 4.67–4.07 (m, 10H), 3.74 (m, 3H), 2.92 (m, 1H), 2.68 (m, 1H), 2.14 (s, 3H), 1.41 (m, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 170.45$, 169.75, 169.48, 156.83, 152.39, 151.14, 149.28, 141.70, 134.26, 132.46, 131.88, 129.51, 128.59, 127.10, 118.94, 117.73, 84.21, 83.25, 76.10, 74.30, 68.72, 66.32, 65.51, 58.39, 52.92, 44.29, 37.68, 20.85, 18.95; $[\alpha]_D^{20} = -1.5$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{32}H_{39}O_{12}N_6P \cdot H_2O$: calcd C 51.34, H 5.51, N 11.23; found C 51.70, H 5.46, N 11.04.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-threonine-methoxyethoxyethyl ester (8e)**: Yield: 75%; white wax; $R_f = 0.25$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.08$ (s, 0.5H), 9.03 (s, 0.5H), 8.77 (s, 0.5H), 8.71 (s, 0.5H), 8.37 (m, 1H), 7.46–7.25 (m, 5H), 6.68–6.52 (m, 2H), 5.94–5.81 (m, 2H), 5.51–5.45 (m, 1H), 5.32–5.04 (m, 5H), 4.68–4.07 (m, 12H), 3.70 (m, 2H), 3.62 (m, 2H), 3.53 (m, 2H), 3.36 (s, 1H), 2.92 (m, 1H), 2.68 (m, 2H), 2.14 (s, 3H), 1.41 (m, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 170.44$, 169.81, 169.57, 156.65, 152.42, 151.13, 149.20, 141.65, 134.14, 132.24, 131.92, 129.57, 128.69, 127.19, 118.95, 117.70, 84.17, 83.32, 76.06, 74.47, 71.81, 70.51, 70.42, 68.71, 66.29, 65.05, 65.03, 58.99, 58.40, 44.44, 37.71, 20.87, 18.53; $[\alpha]_D^{20} = -0.75$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{36}H_{47}O_{14}N_6P$: calcd C 52.81, H 5.79, N 10.26; found C 52.74, H 5.47, N 10.55.

***N*-Allyloxycarbonyl-(*O*-diallylphosphato)-L-serine-methyl ester (10)**: Compound **9** (1.01 g, 4.14 mmol) was added dropwise at 0°C to a solution of **8** (700 mg, 3.45 mmol) and tetrazole (290 mg, 4.14 mmol) in CH_2Cl_2 (10 mL). After completion of the reaction, *m*CPBA (80%, 1.53 g, 6.9 mmol) was added. After 30 min, Et_2O (200 mL) was added, and the solution was extracted with aqueous $NaHSO_3$ (10%, 50 mL) and saturated $NaHCO_3$ (50 mL). The organic phase was dried over $MgSO_4$, concentrated, and the residual oil was purified by chromatography on silica gel (hexane/ethyl acetate 2:1).

yield: 62%; colorless oil; $R_f = 0.21$ (hexane/ethyl acetate 1:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 6.03–5.87$ (m, 3H), 5.81 (d, $J = 6.7$ Hz, 1H), 5.41 (d, $J_{trans} = 17.1$ Hz, 3H), 5.21 (d, $J_{cis} = 10.4$ Hz, 3H), 4.56–4.53 (m, 6H), 4.49 (br, 1H), 4.38 (br, 1H), 4.19–4.13 (m, 1H), 3.72 (s, 3H); ^{13}C NMR

(62.8 MHz, $CDCl_3$): $\delta = 168.9$, 155.9, 132.5, 132.2 (2 C), 118.6, 117.9 (2 C), 68.5 ($^2J_{C,P} = 5.6$ Hz, 2 C), 66.7 (d, $^2J_{C,P} = 5.3$ Hz), 66.5, 54.8 (d, $^3J_{C,P} = 7.1$ Hz), 52.59; $[\alpha]_D^{20} = -2.7$ ($c = 1$, CH_3OH); HRMS (EI): m/z: 363.1094 ([M]) (calcd for $C_{14}H_{22}NO_8P$: 363.1083); elemental analysis: calcd C 46.28, H 6.10, N 3.86; found C 46.34, H 6.08, N 3.86.

Cleavage of the C-terminal ester of 8a–e with lipase from *Apergillus niger* (Method A): A solution of **8a–e** (0.15 μmol) in acetone (0.2 mL) was added dropwise to a solution of lipase from *Apergillus niger* (20 mg) and deoxytaurocholic acid (0.2 mg) in phosphate buffer (1.8 mL, 0.2 M, pH 7). The mixture was shaken at 37 °C until completion of the reaction (18–72 h), and then the pH was adjusted to 3. The resulting suspension was extracted eight times with chloroform (3 mL), and the chloroform layers were dried over $MgSO_4$. The solution was concentrated, and the residual oil was purified by chromatography on silica gel (chloroform/ethanol 40:1–10:1).

Cleavage of the C-terminal ester of 8a–e and 10 with papain (Method B): A solution of **8a–e** or **10** (0.22 mmol) in acetone (2.4 mL) was added dropwise to a suspension of papain (120 mg) and L-cysteine (95 mg) in phosphate buffer (pH 6.6, 0.07 M, 21 mL). The mixture was shaken at 37 °C until completion of the reaction (6–18 h). After filtration, NaCl was added, the solution was extracted eight times with chloroform (4 mL), and the chloroform layer was dried over $MgSO_4$. The solution was concentrated, and the residual oil was purified by chromatography on silica gel (chloroform/ethanol 10:1).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-serine (11a)**: Yield: 92–95% (Method B), 35–42% (Method A); white foam; m.p. 61 °C; $R_f = 0.26$ (ethyl acetate–methanol 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.67$ (s, 1H), 8.44 (m, 1H), 7.38–7.21 (m, 5H), 6.56–6.27 (m, 2H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.58–4.10 (m, 12H), 2.79–2.60 (m, 2H), 2.12 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 172.51$, 170.59, 170.55, 156.00, 152.65, 151.03, 149.09, 141.65, 134.19, 132.58, 131.87, 129.57, 128.67, 127.20, 119.26, 117.94, 84.51, 83.55, 74.21, 69.07, 68.29, 67.16, 65.99, 54.80, 44.33, 37.99, 20.95; $[\alpha]_D^{20} = 0.5$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{30}H_{35}O_{12}N_6P \cdot 2H_2O$): calcd C 48.98, H 5.32, N 11.38; found C 48.90, H 5.31, N 11.42.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-serine (11b)**: Yield: 91% (Method B); white solid; m.p. 71 °C; $R_f = 0.28$ (ethyl acetate/methanol 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.19$ (d, $J = 7.6$ Hz, 0.5H), 8.12 (d, $J = 7.6$ Hz, 0.5H), 7.55 (d, $J = 7.6$ Hz, 0.5H), 7.50 (d, $J = 7.6$ Hz, 0.5H), 7.38–7.23 (m, 5H), 6.31 (m, 0.5H), 6.23 (m, 0.5H), 6.00–5.85 (m, 2.5H), 5.73 (d, $J = 7.1$ Hz, 0.5H), 5.44–5.09 (m, 5H), 4.65–4.18 (m, 10H), 3.70 (m, 2H), 2.82 (m, 1H), 2.30–2.00 (m, 3H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 172.20$, 171.94, 170.85, 162.30, 155.76, 153.44, 145.82, 133.31, 132.39, 131.72, 129.55, 128.65, 127.40, 119.57, 117.92, 96.40, 86.74, 83.91, 74.93, 69.18, 67.99, 66.94, 66.04, 54.17, 44.12, 38.55, 20.94; $[\alpha]_D^{20} = 50.25$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{29}H_{35}O_{13}N_4P$: calcd C 51.33, H 5.20, N 8.26; found C 51.33, H 5.31, N 8.00.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-threonine (11c)**: Yield: 51–70% (Method B); white solid, m.p. 80 °C; $R_f = 0.30$ (ethyl acetate/methanol 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.68$ (s, 0.5H), 8.64 (s, 0.5H), 8.45 (m, 0.5H), 8.42 (m, 0.5H), 7.41–7.23 (m, 5H), 6.46–6.04 (m, 2H), 5.93–5.72 (m, 2H), 5.38–5.07 (m, 6H), 4.59–4.07 (m, 10H), 2.65–2.57 (m, 2H), 2.10 (m, 3H), 1.47 (m, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 172.50$, 170.73, 170.47, 156.67, 152.62, 150.89, 149.20, 141.68, 134.18, 132.51, 131.83, 129.52, 128.64, 127.16, 118.98, 117.95, 84.29, 83.46, 74.23, 74.13, 68.67, 66.89, 66.13, 58.47, 44.34, 37.95, 20.90, 18.65; $[\alpha]_D^{20} = 3.4$ ($c = 0.3$, CH_2Cl_2); elemental analysis for $C_{37}H_{49}O_{12}N_6P \cdot 2H_2O$: calcd C 49.40, H 5.61, N 11.15; found C 49.71, H 5.62, N 10.95.

***N*-Allyloxycarbonyl-*O*-diallylphosphato-L-serine (12)**: Yield: 73% (Method B); yellow oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 6.01–5.90$ (m, 3H), 5.41–5.37 (m, 2H), 5.37–5.34 (m, 1H), 5.28–5.25 (m, 2H), 5.19 (d, 1H, $J_{cis} = 10.5$ Hz), 4.58–4.48 (m, 6H), 4.42–4.40 (m, 1H), 4.38–4.33 (m, 2H); ^{13}C NMR (62.8 MHz, CD_3OD): $\delta = 171.9$, 158.1, 134.1, 133.7 (2 C), 118.9 (2 C), 117.7, 69.8 (2 C), 68.5, 66.7, 55.6; $[\alpha]_D^{20} = 27.2$ ($c = 0.5$, CH_2Cl_2); HRMS (FAB) (glycerol): m/z: 349.0976 ([M]) (calcd for $C_{13}H_{20}NO_8P$ 349.0926).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-2′-deoxycytidine-allyl-phosphato)-L-serine-methyl ester (13)**: A solution of **8c** (50 mg, 72 mmol) in CH_3OH (4 mL) was added dropwise to a suspension of immobilized penicillin G acylase (500 mg, 60 units) in phosphate buffer (15 mL, 0.07 M, pH 7) at 25 °C. After 18 h, the mixture was filtered and extracted six times with chloroform (20 mL). The organic phase was dried with $MgSO_4$, concentrated, and the residue was purified by chromatography on silica gel (chloroform/ethanol 40:1–10:1).

yield: 77%; white solid; m.p. 90 °C; $R_f = 0.53$ (ethyl acetate/methanol 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.73$ (m, 1H), 6.35–5.78 (m, 5H), 5.41–5.17 (m, 5H), 4.71–4.15 (m, 10H), 3.85 (m, 3H), 2.60 (m, 1H), 2.23–2.04 (m, 3H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 170.72$, 170.32, 169.38, 164.88, 155.79, 154.33, 140.89, 132.44, 131.90, 119.29, 118.12, 95.14, 86.09, 82.98, 74.21, 68.98, 67.57, 67.42, 66.12, 54.36, 53.01, 38.11, 20.91; $[\alpha]_D^{20} = 4.4$ ($c = 0.5$, CH_2Cl_2); elemental analysis for $C_{22}H_{31}O_{12}N_4P \cdot 1H_2O$: calcd C 44.60, H 5.61, N 9.46; found C 44.47, H 5.53, N 9.70.

Alcohol (14): A suspension of wheat germ lipase (WGL) (4 U) and **7b** (20 mg, 29 μmol) in a mixture of phosphate buffer (pH 6.5, 50 mM, 8 mL) and ethylene glycol (2 mL) was shaken for 24 h in a polyethylene flask at 37 °C. After completion of the reaction, the solution was lyophilized, the residue was taken up in CH_3OH, and the product was isolated by chromatography on silica gel (choroform/ethanol 19:1).

yield: 64%; colorless oil; $R_f = 0.35$ (chloroform/ethanol 9:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.99$ (m, 1H), 7.38 (m, 1H), 7.21–7.16 (m, 5H), 6.12 (m, 1H), 5.83–5.79 (m, 3H), 5.30–5.09 (m, 5H), 4.52–4.40 (m, 6H), 4.40–4.31 (m, 2H), 4.31–4.19 (m, 2H), 4.04 (m, 1H), 3.83 (s, 3H), 3.76 (s, 2H), 2.55 (m, 1H), 2.14 (m, 1H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 171.44$, 170.20, 162.65, 155.53, 154.77, 143.70, 133.63, 132.27, 131.67, 129.08, 128.45, 127.02, 118.85, 117.50, 96.75, 86.66, 83.17, 73.87, 68.61, 67.28, 66.91, 65.65, 54.07, 52.59, 43.95, 38.28; $[\alpha]_D^{20} = 18.0$ ($c = 1$, CH_3OH); HRMS (EI): m/z: 651.2189 ([M+H]) (calcd for $C_{28}H_{36}N_4O_{12}P$ 651.2067).

***O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-serine-methyl ester (15)**: Tetrakis(triphenylphosphane)palladium(0) (10 mg, 8 mmol) and phenylsilane (37 mL, 0.3 mmol) at room temperature were added to a solution of **8c** (52 mg, 75 mmol) in CH_2Cl_2 (4 mL). After 10 min, the solution was concentrated and extracted with acetone. The remaining residue was purified by chromatography on silica gel (chloroform/ethanol 20:1–3:1).

yield: 89%; white solid; m.p. 140 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 8.34 (d, $J = 7.5$ Hz, 1H), 7.47 (d, $J = 7.5$ Hz, 1H), 7.31–7.22 (m, 5H), 6.26 (dd, $J_{1',2'a} = 5.7$ Hz, $J_{1',2'b} = 8.1$ Hz, 1H), 5.33 (d, $J = 6$ Hz, 1H), 4.30–4.10 (m, 6H), 3.79 (s, 3H), 3.73 (m, 2H), 2.60 (m, 1H), 2.29 (m, 1H), 2.09 (s, 3H); ^{13}C NMR (125.75 MHz, CD_3OD): $\delta = 173.51$, 172.09, 168.72, 164.35, 157.74, 146.14, 135.61, 130.45, 129.58, 128.14, 98.48, 86.66, 85.74, 76.35, 66.67, 64.23, 54.87, 54.00, 44.71, 39.53, 20.93; $[\alpha]_D^{20} = 15.0°$ ($c = 0.4$, MeOH); HRMS (FAB) (glycerol): m/z: 569.1649 ([M+H]) (calcd for $C_{23}H_{30}O_{10}N_4P$: 569.1745).

Synthesis of the nucleo-di- and tripeptides (16a–i): A solution of **11a** or **c** (0.13 mmol), HOBt (35 mg, 0.26 mmol), and EDC (50 mg, 0.26 mmol) in dry CH_2Cl_2 (3.5 mL) was stirred for 30 min at 0 °C. Then, a solution of an amino acid ester or dipeptide ester hydrosalt (0.29 mmol), and diisopropylethylamine (34 μmol, 0.2 mmol) in dry CH_2Cl_2 (1 mL) was added. After stirring for 15 h at room temperature, the solution was extracted with 0.1N HCl and saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated, and the residue was purified by chromatography on silica gel (chloroform/ethanol 50:1–20:1).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glutamic acid-γ-allyl ester-methyl ester (16a)**: Yield: 95%; white wax; $R_f = 0.22$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.81$ (s, 1H), 8.27 (m, 0.5H), 8.10 (m, 1H), 8.03 (d, $J = 7.1$ Hz, 0.5H), 7.46 (m, 1H), 7.33 (m, 5H), 6.24–6.13 (m, 2H), 5.94–5.83 (m, 3H), 5.38–5.34 (m, 1H), 5.30–5.18 (m, 6H), 4.99 (s, 0.5H), 4.89 (s, 0.5H), 4.60–4.24 (m, 13H), 3.88 (m, 2H), 3.72 (m, 2H), 2.63 (m, 1H), 2.44 (m, 2H), 2.23–2.03 (m, 6H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 172.34$, 171.89, 171.86, 170.50, 168.65, 162.54, 156.00, 155.32, 144.20, 133.62, 132.44, 132.35, 132.01, 129.51, 128.88, 127.49, 119.33, 118.41, 117.96, 97.21, 86.84, 83.45, 73.83, 68.97, 67.57, 66.12, 65.33, 58.92, 54.39, 51.96, 44.34, 38.72, 30.22, 26.81, 20.81; $[\alpha]_D^{20} = 15.3$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{38}H_{48}O_{16}N_5P \cdot 2.5H_2O$: calcd C 50.33, H 5.89, N 7.12; found C 50.56, H 5.95, N 7.23.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glutamic acid-γ-allyl ester-methoxyethyl ester (16b)**: Yield: 90%; slightly yellow wax; $R_f = 0.21$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.71$ (s, 1H), 8.56 (s, 0.5H), 8.38 (d, 0.5H),

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8.07 (d, $J = 7.4$ Hz, 0.5H), 7.98 (d, $J = 7.4$ Hz, 0.5H), 7.42 (d, $J = 7.5$ Hz, 0.5H), 7.37 (d, $J = 7.5$ Hz, 0.5H), 7.28–7.26 (m, 5H), 6.39–6.21 (m, 2H), 5.92–5.78 (m, 3H), 5.37–5.06 (m, 8H), 4.65–4.24 (m, 14H), 3.81 (m, 2H), 3.54 (m, 2H), 3.32 (s, 3H), 2.57 (m, 1H), 2.41 (m, 2H), 2.17–2.06 (m, 6H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 172.31$, 171.64, 171.52, 170.43, 168.69, 162.65, 156.01, 155.48, 143.95, 133.87, 133.80, 132.33, 132.01, 129.43, 128.78, 127.37, 119.26, 118.29, 117.84, 97.18, 86.51, 83.28, 73.69, 70.11, 68.85, 67.73, 66.47, 66.01, 65.26, 64.25, 58.87, 54.25, 51.97, 44.12, 38.59, 30.12, 26.73, 20.85; $[\alpha]_D^{20} = 15.6$ ($c = 0.45$, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 906.3174 ([M+H]) (calcd for $C_{40}H_{53}O_{17}N_5P$ 906.3062).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glutamic acid-γ-allyl ester-methoxyethoxyethyl ester (16c)**: Yield: 62%; yellow wax; $R_f = 0.30$ ($CHCl_3$–EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.42$ (s, 1H), 8.50 (d, $J = 5.3$ Hz, 0.5H), 8.29 (d, $J = 6$ Hz, 0.5H), 8.11 (d, $J = 7.5$ Hz, 0.5H), 8.01 (d, $J = 7.5$ Hz, 0.5H), 7.45 (d, $J = 7.5$ Hz, 0.5H), 7.40 (d, $J = 7.5$ Hz, 0.5H), 7.35–7.27 (m, 5H), 6.33–6.21 (m, 2H), 5.97–5.84 (m, 3H), 5.40–5.35 (m, 1H), 5.30–5.17 (m, 6H), 5.04 (s, 1H), 4.65–4.24 (m, 14H), 3.84 (m, 2H), 3.71 (m, 2H), 3.63 (m, 2H), 3.54 (m, 2H), 3.38 (s, 1H), 2.62 (m, 1H), 2.45 (m, 2H), 2.24–2.06 (m, 6H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 172.29$, 171.46, 171.37, 170.45, 168.69, 162.63, 156.10, 155.34, 144.02, 133.79, 132.35, 132.01, 131.96, 129.48, 128.86, 127.53, 119.03, 118.37, 117.92, 97.13, 86.88, 83.31, 73.68, 71.87, 70.49, 68.93, 68.89, 67.65, 66.10, 65.27, 65.25, 64.42, 59.03, 54.31, 51.99, 44.32, 38.72, 30.19, 26.84, 20.91; $[\alpha]_D^{20} = 13.0$ ($c = 0.4$, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 950.3436 ([M+H]) (calcd for $C_{42}H_{67}O_{17}N_5P$: 950.3306); elemental analysis for $C_{42}H_{56}O_{18}N_5P \cdot 0.5H_2O$ (%): calcd C 52.61, H 5.99, N 7.30; found C 52.61, H 5.97, N 6.86.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-phenylalanine-methoxyethoxyethyl ester (16d)**: Yield: 75%; slightly yellow wax; $R_f = 0.20$ ($CHCl_3$–EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.32$ (s, 1H), 8.06 (d, $J = 7.5$ Hz, 0.5H), 8.01 (d, $J = 7.5$ Hz, 0.5H), 7.72 (s, 0.5H), 7.65 (d, $J = 6.8$ Hz, 0.5H), 7.43 (d, $J = 7.6$ Hz, 0.5H), 7.41 (d, $J = 7.6$ Hz, 0.5H), 7.35–7.12 (m, 10H), 6.26–6.19 (m, 1.5H), 6.14 (d, $J = 7.1$ Hz, 0.5H), 5.95–5.82 (m, 2H), 5.38–5.15 (m, 5H), 4.84 (m, 1H), 4.75 (m, 1H), 4.55 (m, 4H), 4.44 (m, 2H), 4.40–4.16 (m, 5H), 3.78 (m, 2H), 3.64 (m, 2H), 3.60 (m, 2H), 3.54 (m, 2H), 3.37 (s, 1H), 3.13 (m, 2H), 2.64 (m, 1H), 2.12–2.04 (m, 4H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 171.36$, 171.14, 170.51, 168.24, 162.49, 156.08, 155.22, 144.06, 136.02, 133.50, 132.37, 131.94, 129.47, 129.32, 129.01, 128.47, 127.61, 126.98, 119.34, 117.96, 96.89, 87.02, 83.42, 73.95, 71.86, 70.47, 68.89, 68.75, 67.24, 66.86, 66.12, 64.47, 59.04, 54.42, 53.72, 44.50, 38.67, 37.60, 20.88; $[\alpha]_D^{20} = 25.0$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{43}H_{64}O_{17}N_5P$ (%): calcd C 55.66, H 5.87, N 7.55; found C 55.38, H 5.76, N 7.21.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-valyl-L-phenylalanine-methoxyethoxyethyl ester (16e)**: Yield: 69%; white wax; $R_f = 0.16$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.87$ (s, 1H), 8.06 (d, $J = 7.5$ Hz, 0.5H), 7.99 (d, $J = 7.5$ Hz, 0.5H), 7.92 (s, 0.5H), 7.87 (s, 0.5H), 7.47 (d, $J = 7.6$ Hz, 0.5H), 7.38 (d, $J = 7.6$ Hz, 0.5H), 7.35–7.13 (m, 11H), 6.56 (m, 1H), 6.24 (m, 1H), 5.94–5.80 (m, 2H), 5.36–5.10 (m, 5H), 4.85 (m, 1H), 4.74 (m, 1H), 4.58–4.11 (m, 12H), 3.89 (m, 2H), 3.77 (m, 2H), 3.73 (m, 2H), 3.61 (m, 2H), 3.36 (s, 1H), 3.11 (m, 2H), 2.62 (m, 1H), 2.17–2.04 (m, 4H), 0.89 (m, 6H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 171.79$, 171.16, 171.04, 170.54, 168.58, 162.70, 156.07, 155.40, 144.33, 135.89, 133.71, 132.45, 132.00, 129.43, 129.37, 128.94, 128.54, 127.52, 127.06, 119.21, 118.00, 97.30, 87.26, 83.30, 73.91, 71.86, 70.44, 68.90, 68.75, 67.66, 66.82, 66.08, 64.40, 59.05, 56.07, 54.87, 53.41, 44.36, 38.41, 37.66, 30.53, 20.87, 19.18; $[\alpha]_D^{20} = 0.75$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{48}H_{64}O_{17}N_6P$ (%): calcd C 56.08, H 6.27, N 8.17; found C 55.93, H 6.45, N 7.83.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glycyl-L-aspartic acid-γ-allyl ester-methyl ester (16f)**: Yield: 60%; white wax; $R_f = 0.27$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.88$ (s, 0.5H), 9.78 (s, 0.5H), 8.34 (s, 0.5H), 8.18 (s, 0.5H), 8.06 (d, $J = 7.5$ Hz, 0.5H), 8.02 (d, $J = 7.5$ Hz, 0.5H), 7.89 (s, 0.5H), 7.66 (d, $J = 7.3$ Hz, 0.5H), 7.46 (m, 1H), 7.32–7.27 (m, 5H), 6.69 (d, $J = 7.0$ Hz, 0.5H), 6.37 (s, 0.5H), 6.26–6.19 (m, 1H), 5.95–5.82 (m, 3H), 5.37 (m, 0.5H), 5.34 (m, 0.5H), 5.30–5.17 (m, 6H), 4.87–4.84 (m, 1.5H), 4.74 (s, 0.5H), 4.55–4.25 (m, 11H), 4.12–3.96 (m, 2H), 3.83 (m, 2H), 3.70 (s, 1H), 2.97–2.86 (m, 2H), 2.64 (m, 1H), 2.17–2.04 (m, 4H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 170.89$, 170.52, 170.45, 170.16, 169.11, 169.01, 162.67, 156.19, 155.52, 144.25, 133.80, 132.33, 131.94, 131.70, 129.40, 128.73, 127.35, 119.14, 118.51, 117.90, 97.24, 87.01, 83.41, 73.87, 69.01, 68.97, 68.87, 66.11, 65.57, 54.58, 52.66, 48.66, 44.19, 43.29, 38.44, 36.03, 20.85; $[\alpha]_D^{20} = 37.8$ ($c = 0.4$, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 905.2970 ([M+H]) (calcd for $C_{39}H_{50}O_{17}N_6P$: 905.2839).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glycyl-L-aspartic acid-γ-allyl ester-methoxyethyl ester (16g)**: Yield: 56%; slightly yellow wax; $R_f = 0.24$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.87$ (s, 0.5H), 9.81 (s, 0.5H), 8.23 (s, 0.5H), 8.13 (s, 0.5H), 8.05 (m, 1H), 7.75 (d, $J = 7.4$ Hz, 0.5H), 7.62 (d, $J = 8.1$ Hz, 0.5H), 7.46 (m, 1H), 7.33–7.28 (m, 5H), 6.60 (d, $J = 7.7$ Hz, 0.5H), 6.35 (d, $J = 7.8$ Hz, 0.5H), 6.25–6.18 (m, 1H), 5.94–5.82 (m, 3H), 5.37 (m, 0.5H), 5.34 (m, 0.5H), 5.31–5.17 (m, 6H), 4.89–4.82 (m, 1.5H), 4.74 (s, 0.5H), 4.57–4.24 (m, 13H), 4.20–3.97 (m, 2H), 3.82 (m, 2H), 3.55 (m, 2H), 3.33 (s, 1H), 3.01–2.87 (m, 2H), 2.66 (m, 1H), 2.19–2.09 (m, 4H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 170.53$, 170.39, 170.21, 170.15, 169.05, 168.98, 162.64, 156.16, 155.48, 144.20, 133.71, 132.34, 131.89, 131.71, 129.44, 128.77, 127.34, 119.21, 118.50, 117.94, 97.13, 87.06, 83.37, 73.87, 69.99, 68.98, 68.92, 68.87, 67.64, 66.95, 64.55, 58.78, 54.62, 48.75, 44.20, 43.22, 38.39, 36.06, 20.84; $[\alpha]_D^{20} = 32.5$ ($c = 0.4$, CH_2Cl_2); elemental analysis for $C_{41}H_{53}O_{18}N_6P \cdot H_2O$ (%): calcd C 50.93, H 5.73, N 8.69; found C 50.91, H 5.89, N 8.56.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glycyl-L-aspartic acid-γ-allyl ester-methoxyethoxyethyl ester (16h)**: Yield: 61%; white wax; $R_f = 0.09$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.78$ (s, 0.5H), 9.68 (s, 0.5H), 8.39 (s, 0.5H), 8.22 (s, 0.5H), 8.07 (d, $J = 7.5$ Hz, 0.5H), 8.02 (d, $J = 7.5$ Hz, 0.5H), 7.80 (d, $J = 6.9$ Hz, 0.5H), 7.62 (d, $J = 8.0$ Hz, 0.5H), 7.47 (d, $J = 7.7$ Hz, 0.5H), 7.45 (d, $J = 7.8$ Hz, 0.5H), 7.35–7.26 (m, 5H), 6.64 (d, $J = 7.5$ Hz, 0.5H), 6.37 (d, $J = 7.8$ Hz, 0.5H), 6.26–6.20 (m, 1H), 5.93–5.82 (m, 3H), 5.37 (m, 0.5H), 5.34 (m, 0.5H), 5.30–5.17 (m, 6H), 4.89–4.78 (m, 2H), 4.55–4.25 (m, 13H), 4.20–3.97 (m, 2H), 3.82 (m, 2H), 3.66 (m, 2H), 3.59 (m, 2H), 3.52 (m, 2H), 3.36 (s, 1H), 2.99–2.87 (m, 2H), 2.63 (m, 1H), 2.17–2.10 (m, 4H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 170.56$, 170.50, 170.40, 170.16, 169.18, 169.08, 162.69, 156.23, 155.60, 144.26, 133.78, 132.39, 131.80, 131.77, 129.46, 128.84, 127.42, 119.17, 118.55, 117.95, 97.13, 87.13, 83.39, 73.92, 71.83, 70.39, 69.03, 68.99, 68.91, 68.76, 67.10, 66.14, 65.62, 58.98, 54.59, 48.80, 44.25, 43.36, 38.41, 36.08, 20.90; $[\alpha]_D^{20} = 20.0$ ($c = 0.4$ in CH_2Cl_2); elemental analysis for $C_{43}H_{57}O_{19}N_6P \cdot 0.5H_2O$ (%): calcd C 51.55, H 5.85, N 8.39, found C 51.76, H 6.25, N 8.12.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-threonyl-L-phenylalanine-methoxyethoxyethyl ester (16i)**: Yield: 71%; white wax; $R_f = 0.27$ ($CHCl_3$/EtOH 20:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.78$ (s, 0.5H), 8.44 (s, 0.5H), 7.75 (m, 2H), 7.55 (m, 0.5H), 7.44–7.00 (m, 10.5H), 6.54 (m, 1H), 6.17 (d, $J = 8$ Hz, 0.5H), 6.10 (d, $J = 8$ Hz, 0.5H), 5.89–5.81 (m, 2H), 5.46 (m, 1H), 5.32–5.18 (m, 4H), 5.10–4.83 (m, 2H), 4.66–4.08 (m, 12H), 3.58 (m, 4H), 3.52 (m, 2H), 3.35 (s, 1H), 3.11 (m, 2H), 2.90 (m, 1H), 2.64 (m, 1H), 2.13 (m, 3H), 1.33 (m, 3H); ^{13}C NMR (125.75 MHz, $CDCl_3$): $\delta = 171.08$, 170.42, 168.95, 168.04, 156.18, 152.56, 151.23, 149.00, 135.81, 133.93, 132.40, 131.96, 129.59, 129.31, 128.71, 128.54, 127.03, 126.45, 119.02, 117.08, 84.32, 83.53, 75.11, 74.39, 71.84, 70.41, 68.88, 68.70, 66.99, 66.15, 64.50, 59.05, 58.31, 53.71, 44.48, 37.77, 37.65, 20.90; $[\alpha]_D^{20} = 0.14$ ($c = 0.15$, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 966.3650 ([M+H]) (calcd for $C_{45}H_{67}O_{16}N_7P$: 966.3483); elemental analysis for $C_{45}H_{66}O_{16}N_7P$ (%): calcd C 55.90, H 5.94, N 10.14; found C 55.79, H 5.79, N 8.59.

C-Terminal deprotection of the nucleopeptides 16a–i by lipase from *Apergillus niger*: A solution of the nucleopeptide **16a–i** (0.15 μmol) in acetone (0.2 mL) was added dropwise to a solution of lipase from *Apergillus niger* (20 mg) and deoxytaurocholic acid (0.2 mg) in phosphate buffer (1.8 mL, 0.2 M, pH 7). The mixture was shaken at 37 °C until completion of the reaction (4–72 h), and then the pH was adjusted to 3. The resulting suspension was extracted eight times with chloroform (4 mL), and the chloroform layers were dried with $MgSO_4$. The solution was concentrated, and the residual oil was purified by chromatography on silica gel (chloroform/ethanol 40:1–10:1).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glutamic acid-γ-allyl ester (17a)**: Yield: 58–89%; slightly yellow wax; $R_f = 0.47$ (ethyl acetate/methanol 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 9.94$ (s, 1H), 8.04 (m, 1.5H), 8.99 (d, $J = 7.5$ Hz, 0.5H), 7.45 (d, $J = 7.5$ Hz, 0.5H), 7.42 (d, $J = 7.5$ Hz, 0.5H), 7.34–7.25 (m, 5H), 6.30–6.09 (m, 2H), 5.93–5.83 (m, 3H), 5.36–5.17 (m, 7H), 4.91 (s,

0.5 H), 4.82 (s, 0.5 H), 4.63 – 4.22 (m, 12 H), 3.75 (m, 2 H), 2.61 (m, 1 H), 2.45 (m, 2 H), 2.26 – 2.05 (m, 6 H); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 172.60, 172.01, 171.57, 170.44, 168.52, 162.63, 156.03, 155.06, 144.73, 133.58, 132.39, 132.06, 131.84, 129.48, 128.85, 127.49, 119.28, 118.37, 117.91, 97.06, 87.27, 83.43, 73.78, 69.08, 67.73, 66.53, 66.07, 65.33, 54.18, 52.21, 44.35, 38.45, 30.35, 26.60, 20.88; $[\alpha]_D^{20}$ = 21.0 (c = 0.4, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 848.2634 ([M+H]) (calcd for $C_{37}H_{47}O_{16}N_5P$: 848.2755).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-phenylalanine (17b)**: Yield: 70 %; white wax; R_f = 0.58 (ethyl acetate/methanol 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.05 (d, J = 7.5 Hz, 0.5 H), 7.98 (d, J = 7.5 Hz, 0.5 H), 7.43 (d, J = 7.6 Hz, 0.5 H), 7.41 (d, J = 7.6 Hz, 0.5 H), 7.33 – 7.14 (m, 10 H), 6.15 (m, 1.5 H), 5.95 – 5.83 (m, 2.5 H), 5.37 – 5.19 (m, 5 H), 4.84 (m, 1 H), 4.60 (m, 1 H), 4.56 – 4.50 (m, 4 H), 4.33 – 4.21 (m, 5 H), 3.72 (m, 2 H), 3.20 – 3.04 (m, 2 H), 2.64 (m, 1 H), 2.19 – 2.08 (m, 4 H); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 171.60, 171.49, 170.64, 168.28, 162.42, 156.12, 155.85, 144.97, 136.15, 133.28, 132.35, 131.83, 129.51, 129.44, 128.95, 128.49, 127.62, 126.96, 119.46, 118.01, 96.91, 87.71, 83.59, 73.86, 69.12, 67.48, 66.79, 66.10, 54.25, 53.92, 44.42, 38.43, 37.37, 20.91; $[\alpha]_D^{20}$ = 16.4 (c = 0.28, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 826.2701 ([M+H]) (calcd for $C_{38}H_{45}O_{14}N_5P$: 826.2593).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-valyl-L-phenylalanine (17c)**: Yield: 59 %; white wax; R_f = 0.60 (ethyl acetate/methanol 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.14 (d, J = 7.5 Hz, 0.5 H), 8.09 (d, J = 7.5 Hz, 0.5 H), 7.59 – 7.44 (m, 2 H), 7.34 – 7.16 (m, 10 H), 6.98 (m, 1 H), 6.30 – 6.07 (m, 2 H), 5.94 – 5.82 (m, 2 H), 5.37 – 5.17 (m, 5 H), 4.74 – 4.19 (m, 12 H), 3.75 (m, 2 H), 3.25 – 3.07 (m, 2 H), 2.62 (m, 1 H), 2.37 – 2.04 (m, 4 H), 0.85 (m, 6 H); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 171.54, 171.16, 171.00, 170.79, 168.69, 162.57, 155.95, 154.88, 144.33, 135.89, 133.71, 132.33, 131.85, 129.46, 129.27, 128.94, 128.55, 127.58, 126.98, 119.36, 118.06, 96.09, 87.26, 83.62, 73.99, 69.08, 67.38, 66.40, 66.16, 59.58, 54.02, 53.85, 44.42, 38.05, 37.17, 30.38, 20.91, 19.12; $[\alpha]_D^{20}$ = 4.0 (c = 0.2, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 925.3385 ([M+H]) (calcd for $C_{43}H_{54}O_{15}N_6P$: 925.3564).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glycyl-L-aspartic acid-γ-allyl ester (17d)**: Yield: 70 – 91 %; slightly yellow wax; R_f = 0.17 (ethyl acetate/methanol 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.98 (s, 1 H), 8.11 (m, 1 H), 8.10 (m, 1 H), 7.90 (m, 1 H), 7.47 (m, 1 H), 7.39 – 7.14 (m, 5 H), 6.64 (s, 1 H), 6.31 (s, 1 H), 6.18 (m, 1 H), 5.93 – 5.82 (m, 3 H), 5.36 – 5.17 (m, 7 H), 4.84 – 4.61 (m, 2 H), 4.60 – 4.25 (m, 11 H), 3.89 (m, 2 H), 3.78 (m, 2 H), 2.99 – 2.88 (m, 2 H), 2.65 (m, 1 H), 2.18 – 2.08 (m, 4 H); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 171.92, 171.85, 170.80, 170.73, 169.35, 169.13, 162.74, 155.40, 154.69, 144.90, 133.61, 132.36, 131.74, 131.20, 129.49, 128.82, 127.58, 119.38, 118.55, 118.10, 97.08, 87.59, 83.59, 74.06, 69.12, 68.07, 67.16, 66.26, 65.67, 54.89, 48.84, 44.31, 43.136, 38.42, 35.94, 20.91; $[\alpha]_D^{20}$ = 15.0 (c = 0.4, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 891.2814 ([M+H]) (calcd for $C_{38}H_{48}O_{17}N_6P$: 891.3037).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-threonyl-L-phenylalanine (17e)**: Yield: 63 %; white wax; R_f = 0.3 (ethyl acetate/methanol 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.77 – 8.56 (m, 2 H), 7.42 – 7.06 (m, 10 H), 6.57 (m, 1.5 H), 6.05 (m, 0.5 H), 5.94 – 5.87 (m, 2 H), 5.49 (m, 1 H), 5.35 – 5.18 (m, 4 H), 4.95 – 4.76 (m, 2 H), 4.68 – 4.10 (m, 10 H), 3.21 – 3.11 (m, 2 H), 2.67 (m, 1 H), 2.64 (m, 1 H), 2.15 (m, 3 H), 1.26 (m, 3 H); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ = 171.11, 170.49, 168.90, 168.24, 156.28, 152.50, 151.21, 149.14, 135.91, 133.97, 132.46, 131.95, 129.79, 129.38, 128.78, 128.54, 127.23, 126.55, 119.32, 117.07, 84.39, 83.57, 75.14, 74.29, 68.80, 66.87, 66.13, 58.34, 53.79, 44.48, 37.67, 37.62, 20.89; $[\alpha]_D^{20}$ = 8.9 (c = 0.19, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 864.2969 ([M+H]) (calcd for $C_{40}H_{48}O_{13}N_7P$: 864.2833).

Boc-amino acid-2-bromoethyl esters (19a – d): A solution of DIC (5.34 g, 26 mmol) in CH_2Cl_2 (40 mL) was added slowly at 0 °C to a solution of BOC amino acid **18a – d** (21.7 mmol), DMAP (269 mg, 2.2 mmol), and 2-bromoethanol (1.86 mL, 26 mmol) in CH_2Cl_2 (80 mL). After stirring for 12 h, the solution was filtered, extracted with dilute aqueous HCl, conc. aqueous $NaHCO_3$, and water. The organic phase was dried with $MgSO_4$, concentrated, and the crude product was purified by chromatography on silica gel (hexane/ethyl acetate 2:1).

Boc-L-alanine-2-bromoethyl ester (19a): Yield: 95 %; colorless oil; R_f = 0.80 (hexane/ethyl acetate 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.17 (d, J_{vic} = 6.7 Hz, 1 H), 4.48 (ddt, $J_{1-1'}$ = 6.0 Hz, $J_{2-1'}$ = 5.9 Hz, 2 H), 4.34 (t, J_{vic} = 7.3 Hz, 1 H), 3.53 (t, J_{vic} = 6.1 Hz, 2 H), 1.45 (s, 9 H), 1.43 (d, J_{vic} = 7.3 Hz, 3 H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 172.95, 155.08, 79.85, 64.30, 49.17, 28.36, 28.27 (3 C), 18.45; $[\alpha]_D^{20}$ = – 12.6 (c = 1, $CHCl_3$); elemental analysis for $C_{10}H_{18}BrNO_4$ (%): calcd C 40.56, H 6.12, N 4.73; found C 40.41, H 6.04, N 4.84.

Boc-L-leucine-2-bromoethyl ester (19b): Yield: 90 %; colorless oil; R_f = 0.56 (hexane/ethyl acetate 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.99 (d, J_{vic} = 8.2 Hz, 1 H), 4.43 (ddt, $J_{1-1'}$ = 6.0 Hz, $J_{2-1'}$ = 6.1 Hz, J_{1-2} = 14.9 Hz, 2 H), 4.33 (q, J_{vic} = 5.3 Hz, 1 H), 3.52 (t, J_{vic} = 6.1 Hz, 2 H), 1.73 (t, J_{vic} = 6.9 Hz), 1.64 (m, 1 H), 1.44 (s, 9 H), 0.94 (d, J_{vic} = 6.5 Hz, 6 H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 173.02, 155.36, 79.81, 64.24, 52.07, 41.54, 34.86, 28.26 (3 C), 24.25, 21.83, 22.78; $[\alpha]_D^{20}$ = – 27.2 (c = 2, CH_3OH); HRMS (EI): m/z: 337.0911 (calcd for $C_{13}H_{24}BrNO_4$: 337.0889); elemental analysis (%): calcd C 46.16, H 7.15, N 4.14; found C 46.31, H 7.00, N 4.12.

Boc-L-phenylalanine-2-bromoethyl ester (19c): Yield: 90 %; slightly yellow oil; R_f = 0.56 (hexane/ethyl acetate 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.25 – 7.32 (m, 3 H), 7.17 (d, J_{vic} = 7.0 Hz, 2 H), 4.98 (d, J_{vic} = 7.6 Hz, 1 H), 4.61 (q, J_{vic} = 6.7 Hz, 1 H), 4.42 (t, J_{vic} = 3.2 Hz, 2 H), 3.45 (t, J_{vic} = 6.2 Hz, 2 H), 3.11 (t, J_{vic} = 5.2 Hz, 2 H), 1.42 (s, 9 H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 171.49, 155.04, 135.80, 129.29 (2 C), 128.59 (2 C), 127.10, 80.02, 64.44, 54.39, 38.23, 28.27 (3 C), 28.06; $[\alpha]_D^{20}$ = 21.2 (c = 1, $CHCl_3$); elemental analysis for $C_{16}H_{22}BrNO_4$(%): calcd C 51.62, H 5.96, N 3.76; found C 51.69, H 5.92, N 4.09.

Boc-L-tyrosine-2-bromoethyl ester (19d): Yield: 91 %; colorless oil; R_f = 0.19 (hexane/ethyl acetate 3:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.97 (m, 2 H), 6.75 (d, J_{vic} = 7.9 Hz, 2 H), 6.23 (br, 1 H), 4.55 (q, J_{vic} = 6.7 Hz, 1 H), 4.36 (m, 2 H), 3.45 (m, 2 H), 3.04 (m, 2 H), 1.45 (s, 9 H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 171.49, 155.04, 130.43, 130.38 (2 C), 127.28 (2 C), 115.60, 80.02, 64.44, 54.39, 38.23, 28.27 (3 C), 28.06; $[\alpha]_D^{20}$ = – 0.7 (c = 1, CH_3OH); HRMS (EI): m/z: 387.0694 ([M]) (calcd for $C_{16}H_{22}NO_5Br$: 387.0682).

Boc-amino acid choline ester bromides (20a – d): At – 78 °C, liquid trimethylamine (5 mL, 109 mmol) was added to a solution of the bromoethyl ester **19a – d** (17.5 mmol) in acetone (50 mL). The solution was stirred for three days at room temperature, and the solvent and the excess trimethylamine were removed under reduced pressure.

Boc-L-alanine choline ester bromide (20a): Yield: 98 %; very hygroscopic white solid; ^{1}H NMR (400 MHz, CD_3OD): δ = 4.76 (d, J_{vic} = 13.93 Hz, 1 H), 4.61 (d, J_{vic} = 14.2 Hz, 1 H), 4.25 (t, J_{vic} = 7.3 Hz, 1 H), 4.20 (s, 2 H), 3.55 (s, 9 H), 1.43 (d, J_{vic} = 8.6 Hz, 3 H), 1.42 (s, 9 H); ^{13}C NMR (100.5 MHz, CD_3OD): δ = 172.75, 155.45, 80.11, 64.84, 58.65, 54.40 (3 C), 49.31, 28.34 (3 C), 17.54; $[\alpha]_D^{20}$ = – 23.5 (c = 1, $CHCl_3$); HRMS (FAB) (glycerol): m/z: 275.1935 ([M]$^+$) (calcd for $C_{13}H_{27}BrN_2O_4$: 275.1971); elemental analysis for the hydrate (%): calcd C 42.86, H 7.75, N 7.69; found C 43.05, H 7.70, N 7.73.

Boc-L-leucine choline ester bromide (20b): Yield: 99 %; very hygroscopic white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.12 (d, J_{vic} = 7.5 Hz, 1 H), 4.81 (d, J_{vic} = 14.5 Hz, 1 H), 4.52 (d, J_{vic} = 14.7 Hz, 1 H), 4.18 (m, 3 H), 3.55 (s, 9 H), 1.70 (m, J_{vic} = 6.6 Hz, 1 H), 1.59 (t, J_{vic} = 7.5 Hz, 2 H), 1.42 (s, 9 H), 0.95 (dd, J_1 = 6.6 Hz, J_2 = 6.7 Hz, 6 H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 172.96, 155.78, 80.31, 64.92, 58.51, 54.50 (3 C), 52.36, 40.32, 28.31 (3 C), 24.76, 21.63, 22.87; $[\alpha]_D^{20}$ = – 23.7 (c = 1, CH_3OH); HRMS (FAB) (glycerol): m/z: 317.2389 ([M – Br]) (calcd for $C_{16}H_{33}N_2O_4$: 317.2440); elemental analysis for $C_{16}H_{33}N_2O_4Br$, hemihydrate (%): calcd C 47.29, H 8.43, N 6.89; found C 47.38, H 8.45, N 7.01.

Boc-L-phenylalanine choline ester bromide (20c): Yield: 94 %; very hygroscopic white solid; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.29 – 7.37 (m, 3 H), 7.21 (d, J_{vic} = 7.3 Hz, 2 H), 5.20 (d, J_{vic} = 7.1 Hz, 1 H), 4.67 (dd, J_{vic} = 6.6 Hz, J_{gem} = 14.4 Hz, 1 H), 4.43 (q, J_{vic} = 7.3 Hz, 2 H), 4.11 (dd, J_{vic} = 6.7 Hz, J_{gem} = 13.8 Hz, 1 H), 3.96 (dd, J_{vic} = 6.6 Hz, J_{gem} = 13.9 Hz, 1 H), 3.03 – 3.14 (m, 2 H), 3.38 (s, 9 H), 1.41 (s, 9 H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 171.68, 155.33, 129.25 (2 C), 128.92 (2 C), 127.41 (2 C), 80.55, 64.78, 58.47, 55.20, 54.36 (3 C), 38.14, 28.30 (3 C); $[\alpha]_D^{20}$ = – 2.3 (c = 1, CH_3OH); elemental analysis for $C_{19}H_{31}BrN_2O_4$, hemihydrate (%): calcd C 51.82, H 7.32, N 6.36; found C 51.39, H 7.25, N 6.31.

Boc-L-tyrosine choline ester bromide (20d): Yield: 93 %; very hygroscopic white solid; ^{1}H NMR (500 MHz, CD_3OD): δ = 7.05 (m, 2 H), 6.73 (m, 2 H, J_{vic} = 7.9 Hz), 4.54 (m, 1 H), 4.42 (m, 1 H), 4.27 (t, J_{vic} = 7.7 Hz, 1 H), 3.70 (dd, J_{vic} = 6.7 Hz, J_{gem} = 13.8 Hz, 1 H), 3.55 (dd, J_{vic} = 6.6 Hz, J_{gem} = 13.9 Hz, 1 H), 3.38 (s, 9 H), 2.94 (m, 2 H), 1.38 (s, 9 H); $[\alpha]_D^{20}$ = 2.7 (c = 2, CH_3OH); HRMS (FAB) (glycerol): m/z: 367.2293 ([M – Br]) (calcd for $C_{19}H_{31}N_2O_5$:

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367.2233); elemental analysis for $C_{19}H_{31}N_2O_5Br$ (%): calcd C 51.01, H 6.98, N 6.26; found C 50.92, H 7.18, N 6.04.

Amino acid choline ester bromide hydrobromides (21a–d): A solution of the choline esters **20a–d** (8.2 mmol) in HBr in glacial acetic acid (45%, 25 mL) was stirred for 30 min at room temperature. After addition of Et_2O (150 mL), the precipitate was filtered, dissolved in CH_3OH (3 mL), and the product reprecipitated with Et_2O.

L-Alanine choline ester bromide hydrobromide (21a): Yield: 96%; slightly yellow, hygroscopic solid; m.p. 215 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 4.75 (t, $J_{vic} = 2.5$ Hz, 2H), 4.30 (q, $J_{vic} = 7.2$ Hz, 1H), 3.93 (t, $J_{vic} = 4.7$ Hz, 2H), 3.32 (s, 9H), 1.61 (d, $J_{vic} = 7.3$ Hz, 2H); ^{13}C NMR (100.5 MHz, CD_3OD): $\delta = 170.38$, 65.72, 60.84, 54.59 (3 C), 49.93, 16.07; $[\alpha]_D^{20} = 2.8$ ($c =$ 1, CH_3OH); elemental analysis for $C_8H_{20}Br_2N_2O_2$ (%): calcd C 28.59, H 6.00, N 8.34; found C 28.53, H 5.94, N 8.30.

L-Leucine choline ester bromide hydrobromide (21b): Yield: 99%; slightly yellow, hygroscopic solid; m.p. 210 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 4.82 (d, $J_{vic} = 14.2$ Hz, 1H), 4.73 (d, $J_{vic} = 14.4$ Hz, 1H), 4.23 (q, $J_{vic} = 6.9$ Hz, 1H), 3.97 (t, $J_{vic} = 4.7$ Hz, 2H), 3.35 (s, 9H), 1.75–1.92 (m, 3H), 1.02 (t, $J_{vic} = 6.2$ Hz, 6H); ^{13}C NMR (100.5 MHz, CD_3OD): $\delta = 170.35$, 65.66, 60.87, 54.60 (3 C), 52.59, 40.26, 25.57, 22.70, 22.26; $[\alpha]_D^{20} = 13.9$ ($c = 2$, CH_3OH); HRMS (FAB) (glycerol): m/z: 217.1892 ($[M-2Br-H]$) (calcd for $C_{11}H_{25}N_2O_2$: 217.1916); elemental analysis for $C_{11}H_{26}Br_2N_2O_2$ (%): calcd C 34.94, H 6.93, N 7.41; found C 35.10, H 6.77, N 7.45.

L-Phenylalanine choline ester bromide hydrobromide (21c): Yield: 99%; slightly yellow, hygroscopic solid; m.p. 130 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 7.33–7.44 (m, 5H), 4.75 (dddd, $J_{2-1'} = 2.4$ Hz, $J_{2-2'} = 6.8$ Hz, $J_{2-1} = 14.1$ Hz, 1H), 4.60 (dddd, $J_{1-1'} = 2.5$ Hz, $J_{1-2'} = 6.8$ Hz, $J_{1-2} = 14.2$ Hz, 1H), 4.52 (t, $J_{vic} = 7.3$ Hz, 1H), 3.83 (dddd, $J_{2-1'} = 2.3$ Hz, $J_{2-2'} = 7.1$ Hz, $J_{2-1} =$ 14.7 Hz, 1H), 3.69 (dddd, $J_{1-1'} = 2.3$ Hz, $J_{1-2'} = 7.0$ Hz, $J_{1-2} = 14.7$ Hz, 1H), 3.29 (d, $J_{vic} = 7.3$ Hz, 2H), 3.14 (s, 9H); ^{13}C NMR (100.5 MHz, CD_3OD): $\delta = 169.62$, 135.62, 130.62 (2 C), 130.31 (2 C), 129.06 (*p*-CH), 65.60, 60.73, 55.28, 54.51 (3 C), 37.38; $[\alpha]_D^{20} = 16.6$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 251.1782 ($[M-2Br-H]$) (calcd for $C_{14}H_{23}N_2O_2$: 251.1760); elemental analysis for $C_{14}H_{24}Br_2N_2O_2$, hemihydrate (%): calcd C 39.93, H 5.98, N 6.65; found C 40.14, H 5.92, N 6.43.

L-Tyrosine choline ester bromide hydrobromide (21d): Yield: 91%; hygroscopic white solid; m.p. 181 °C; ^{1}H NMR (500 MHz, CD_3OD): $\delta =$ 7.18 (m, 2H), 6.83 (m, 2H), 4.74 (m, 1H), 4.62 (m, 1H), 4.41 (t, $J_{vic} = 7.7$ Hz, 1H), 3.85 (dd, $J_{vic} = 6.7$ Hz, $J_{gem} = 13.8$ Hz, 1H), 3.71 (dd, $J_{vic} = 6.6$ Hz, $J_{gem} = 13.9$ Hz, 1H), 3.38 (s, 9H), 2.94 (m, 2H); $[\alpha]_D^{20} = 12.1$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 267.1662 ($[M-2Br-H]$) (calcd for $C_{14}H_{23}N_2O_3$ 267.1709); elemental analysis for $C_{14}H_{24}Br_2N_2O_3$ (%): calcd C 39.27, H 5.65, N 6.54; found C 38.99, H 5.79, N 5.96.

Nucleo- and phospho-dipeptide choline ester bromides (22a–d, 23): A solution of **11a** or **12** (0.036 mmol), HOAt (5.3 μg, 0.039 mmol), and DIC (6.03 μg, 0.039 mmol) in dry CH_2Cl_2 (3 mL) was stirred for 30 min and then added to a solution of **21a–d** (0.036 mmol) and NBu_3 (8.47 μL, 0.036 mmol) in DMF (3 mL). After 14 h, the solution was concentrated under reduced pressure, and the product was precipitated by addition of dry Et_2O (10 mL). The precipitate was filtered, dissolved in dry CH_3OH (1 mL), and reprecipitated with dry Et_2O.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-seryl-L-alanine choline ester bromide (22a)**: Yield: 99%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.77$ (s, 1H), 8.44 (m, 1H), 7.38–7.21 (m, 5H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.75 (t, $J_{vic} = 2.5$ Hz, 2H), 4.58–4.10 (m, 13H), 3.93 (t, $J_{vic} =$ 4.7 Hz, 2H), 3.32 (s, 9H), 2.79–2.60 (m, 2H), 2.14 (s, 3H), 1.51 (d, $J_{vic} =$ 7.3 Hz, 2H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 172.51$, 170.59, 170.55, 170.38, 156.11, 152.65, 151.03, 149.09, 141.65, 134.19, 132.58, 131.87, 129.57, 128.67, 127.20, 119.26, 117.94, 84.51, 83.55, 74.21, 69.07, 68.29, 67.16, 65.99, 65.72, 60.84, 54.80, 54.59 (3 C), 49.93, 44.33, 37.99, 20.95, 16.07; $[\alpha]_D^{20} =$ -11.5 ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 859.3330 ($[M-Br]$) (calcd for $C_{38}H_{52}N_8O_{13}P$: 859.3391).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-seryl-L-leucine choline ester bromide (22b)**: Yield: 91%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.67$ (s, 1H), 8.54 (m, 1H), 7.38–7.21 (m, 5H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.82 (d, $J_{vic} = 14.2$ Hz, 1H), 4.73 (d, $J_{vic} = 14.4$ Hz, 1H), 4.58–4.10 (m, 13H), 3.97 (t, $J_{vic} = 4.7$ Hz, 2H), 3.35 (s, 9H), 2.79–2.60 (m, 2H), 2.13 (s, 3H), 1.75–1.92 (m, 3H), 0.92 (m, 6H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 172.53$, 170.79, 170.54, 170.35, 156.00, 152.65, 151.03, 149.09, 141.65, 134.19, 132.58, 131.87, 129.57, 128.67, 127.20, 119.26, 117.94, 84.51, 83.55, 74.21, 69.07, 68.29, 67.16, 65.99, 65.66, 60.87, 54.80, 54.60 (3 C), 52.59, 44.33, 40.26, 37.99, 25.57, 22.26, 22.70, 20.95; $[\alpha]_D^{20} = -11.5$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 901.4031 ($[M-Br]$) (calcd for $C_{41}H_{58}N_8O_{13}P$: 901.3861).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-seryl-L-phenylalanine choline ester bromide (22c)**: Yield: 96%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.64$ (s, 1H), 7.94 (m, 1H), 7.44–7.21 (m, 10H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.75–4.10 (m, 15H), 3.83 (m, 1H), 3.69 (m, 1H), 3.29 (d, $J_{vic} = 7.3$ Hz, 2H), 3.14 (s, 9H), 2.79–2.60 (m, 2H), 2.11 (s, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 172.31$, 170.58, 170.52, 169.52, 156.30, 152.65, 151.03, 149.09, 141.65, 135.62, 134.19, 132.58, 131.87, 130.62, 130.31, 129.57, 129.06, 128.67, 127.20, 119.26, 117.94, 84.51, 83.55, 74.21, 69.07, 68.29, 67.16, 65.99, 65.60, 60.73, 55.28, 54.80, 54.51 (3 C), 44.33, 37.99, 37.38, 20.95; $[\alpha]_D^{20} = -10.5$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 935.3771 ($[M-Br]$) (calcd for $C_{44}H_{56}N_8O_{13}P$: 935.3704).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-seryl-L-tyrosine choline ester bromide (22d)**: Yield: 99%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.65$ (s, 1H), 7.97 (m, 1H), 7.38–7.21 (m, 5H), 7.18 (m, 2H), 6.83 (m, 2H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H, 3′-H), 4.74 (m, 1H), 4.62 (m, 1H), 4.58–4.10 (m, 13H), 3.85–3.71 (m, 2H), 3.38 (s, 9H), 2.94 (m, 2H), 2.80–2.61 (m, 2H), 2.13 (s, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 173.48$, 171.26, 170.54, 170.51, 156.06, 152.65, 151.03, 149.09, 141.65, 134.19, 132.58, 131.87, 130.43, 130.38, 129.57, 128.67, 127.28, 127.20, 119.26, 117.94, 115.60, 84.51, 83.55, 74.21, 69.07, 68.29, 67.16, 66.76, 65.99, 60.54, 54.80, 54.55 (3 C), 54.39, 44.33, 38.23, 37.99, 20.95; $[\alpha]_D^{20} = -13.3$ ($c = 1.5$, CH_3OH); HRMS (FAB) (glycerol): m/z: 951.3318 ($[M-Br]$) (calcd for $C_{44}H_{56}N_8O_{14}P$ 951.3654).

***N*-Allyloxycarbonyl-*O*-diallylphosphato-L-seryl-L-alanine choline ester bromide (23)**: Yield: 85%; colorless oil; ^{1}H NMR (500 MHz, CDH_3OD): $\delta = 6.01–5.90$ (m, 3H), 5.41–5.37 (m, 2H), 5.37–5.34 (m, 1H), 5.28–5.25 (m, 2H), 5.19 (d, $J_{cis} = 10.5$ Hz, 1H), 4.75 (t, $J_{vic} = 2.5$ Hz, 2H), 4.58–4.48 (m, 6H), 4.42–4.30 (m, 4H), 3.93 (t, $J_{vic} = 4.7$ Hz, 2H), 3.32 (s, 9H), 1.45 (d, $J_{vic} = 7.3$ Hz, 2H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 171.9$, 170.4, 158.1, 134.1, 133.7 (2 C), 118.9 (2 C), 117.7, 69.8 (2 C), 68.5, 66.7, 65.7, 60.8, 55.6, 54.6 (3 C), 49.9, 16.1; $[\alpha]_D^{20} = -10.9$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 506.2206 ($[M-Br]$) (calcd for $C_{21}H_{37}N_3O_9P$: 506.2267).

C-Terminal deprotection of nucleo- and phosphopeptide choline esters by means of butyrylcholine esterase (24a–d, 25): A solution of **22a–d** or **23** (0.03 mmol) and butyrylcholine esterase (30 U) in phosphate buffer (pH 6.5, 50 mM, 20 mL) was shaken for 64 h at 37 °C. The solution was lyophilized, and the residue was treated sequentially with CH_2Cl_2, $CHCl_3$, and CH_3OH. The combined organic solutions were concentrated, and the product was isolated after chromatography on silica gel (chloroform/methanol 4:1).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosyl-allyl-phosphato)-L-seryl-L-alanine (24a)**: Yield: 96%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.65$ (s, 1H), 8.42 (m, 1H), 7.38–7.21 (m, 5H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.58–4.10 (m, 13H), 2.79–2.60 (m, 2H), 2.02 (s, 3H), 1.61 (d, $J_{vic} = 7.3$ Hz, 2H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 173.21$, 170.77, 170.57, 170.34, 155.88, 152.68, 151.00, 149.11, 141.65, 134.19, 132.58, 131.77, 129.57, 128.67, 127.23, 119.26, 117.94, 84.51, 83.55, 74.21, 69.07, 68.29, 67.16, 65.72, 54.86, 54.59, 49.93, 44.33, 38.11, 20.88, 16.12; $[\alpha]_D^{20} = 1.4$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 796.2228 ($[M+Na]$) (calcd for $C_{33}H_{40}N_7O_{13}PNa$: 796.2319).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosine-allyl-phosphato)-L-seryl-L-leucine (24b)**: Yield: 78%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.78$ (s, 0.5H), 8.66 (s, 0.5H), 8.64 (m, 0.5H), 8.52 (m, 0.5H, 7.38–7.21 (m, 5H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.58–4.10 (m, 13H), 2.79–2.60 (m, 2H), 2.13 (s, 3H), 1.75–1.92 (m, 3H), 0.90 (m, 6H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 171.99$, 170.48, 170.41, 170.25, 156.23, 152.65, 151.03, 149.09, 141.65, 134.03, 132.58, 131.87, 129.57 128.67, 127.20, 119.26, 117.86, 84.61, 83.66, 74.25, 69.07, 68.29, 67.16, 66.21, 54.80, 52.59, 44.38, 40.26, 37.92, 25.56, 22.26, 22.70, 20.99; $[\alpha]_D^{20} = -12.0$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 838.2690 ($[M+Na]$) (calcd for $C_{36}H_{46}N_7O_{13}PNa$: 838.2789).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosine-allyl-phosphato)-L-seryl-L-phenylalanine** (**24c**): Yield: 61%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.78$ (s, 1H), 8.63 (m, 1H), 7.44–7.21 (m, 10H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.75–4.10 (m, 13H), 3.29 (d, $J_{vic} = 7.3$ Hz, 2H), 2.79–2.60 (m, 2H), 2.11 (s, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 172.71$, 170.63, 170.48, 169.68, 155.85, 152.62, 151.31, 149.19, 141.86, 135.62, 134.19, 132.71, 131.76, 130.62, 130.31, 129.57, 129.06, 128.67, 127.20, 119.26, 117.94, 84.51, 83.55, 74.21, 69.07, 68.36, 67.19, 65.88, 55.28, 54.86, 44.38, 38.11, 37.38, 20.95; $[\alpha]_D^{20} = 3.8$ ($c = 1.5$, CH_3OH); HRMS (FAB) (glycerol): m/z: 872.2795 ([M+Na]) (calcd for $C_{39}H_{44}N_7O_{13}PNa$: 872.2632); elemental analysis for $C_{39}H_{44}N_7O_{13}P$, tetrahydrate (%): calcd C 50.81, H 5.69, N 10.85; found C 50.99, H 5.56, N 11.14.

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-6-*N*-phenylacetyl-2′-deoxyadenosine-allyl-phosphato)-L-seryl-L-tyrosine** (**24d**): Yield: 86%; colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.67$ (s, 1H), 8.44 (m, 1H), 7.38–7.21 (m, 5H), 6.56–6.27 (m, 1H), 5.93–5.81 (m, 2H), 5.41–5.16 (m, 5H), 4.58–4.10 (m, 12H), 2.79–2.60 (m, 2H), 2.12 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 173.32$, 170.75, 170.67, 155.87, 152.72, 151.01, 149.06, 141.87, 134.19, 132.67, 131.92, 129.57, 128.77, 127.24, 119.22, 117.83, 84.62, 83.58, 74.16, 69.09, 68.33, 67.18, 66.12, 54.88, 44.25, 37.88, 21.03; $[\alpha]_D^{20} = 0.8$ ($c = 1$, CH_3OH); HRMS (FAB) (glycerol): m/z: 888.2462 ([M+Na]) (calcd for $C_{39}H_{44}N_7O_{14}PNa$: 888.2581).

***N*-Allyloxycarbonyl-*O*-diallylphosphato-L-seryl-L-alanine** (**25**): Yield: 73%; colorless oil; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 6.01–5.90$ (m, 3H), 5.41–5.19 (m, 6H), 4.58–4.48 (m, 6H), 4.42–4.30 (m, 4H), 1.39 (d, $J_{vic} = 7.3$ Hz, 3H); $[\alpha]_D^{20} = -3.0$ ($c = 0.5$, CH_3OH); HRMS (FAB) (glycerol): m/z: 421.1439 ([M+H]) (calcd for $C_{16}H_{25}N_2O_9P$: 421.1376).

***N*-Allyloxycarbonyl-*O*-(3′-*O*-acetyl-2′-deoxycytidine-allyl-phosphato)-L-seryl-L-glycyl-L-aspartic acid-γ-allyl ester** (**26**): A solution of **17d** (29 mg, 32 μmol) in CH_3OH (4 mL) was added dropwise to a suspension of immobilized penicillin G acylase (500 mg, 60 units) in phosphate buffer (15 mL, 0.07 M, pH 7) at 25 °C. After 18 h, the mixture was filtered and extracted with chloroform (8 × 20 mL). The organic phase was dried with $MgSO_4$, concentrated, and the residue was purified by chromatography on silica gel (chloroform/ethanol 40:1–3:1). Yield: 91%; white solid; m.p. 113 °C; ^{1}H NMR (500 MHz, $CDCl_3$, MeOD): $\delta = 7.61$ (m, 1H), 7.28 (m, 1H), 6.24 (m, 1H), 5.99–5.86 (m, 3H), 5.37 (m, 0.5H), 5.33 (m, 0.5H), 5.29–5.14 (m, 6H), 4.54–3.71 (m, 15H), 2.87 (m, 2H), 2.48–2.04 (m, 5H); ^{13}C NMR (125.75 MHz, $CDCl_3$, MeOD): $\delta = 207.43$, 176.34, 172.30, 170.72, 169.34, 165.07, 156.36, 155.39, 140.82, 132.55, 132.53, 132.05, 119.06, 118.27, 117.98, 95.97, 86.28, 82.86, 74.43, 68.94, 67.57, 67.44, 66.91, 65.48, 54.84, 50.14, 42.83, 37.65, 36.79, 20.92; $[\alpha]_D^{20} = 5.3$ ($c = 0.15$, CH_2Cl_2); HRMS (FAB) (glycerol/TFA): m/z: 773.2395 ([M+H) (calcd for $C_{30}H_{42}O_{15}N_6P$: 773.2274).

***O*-(3′-*O*-acetyl-2′-deoxycytidine-phosphato)-L-seryl-L-glycyl-L-aspartic acid** (**27**): Tetrakis(triphenylphosphane)palladium(0) (5 mg, 4 μmol) and phenylsilane (15 μL, 0.1 mmol) at room temperature were added to a solution of **26** (14 mg, 18 μmol) in $CHCl_3$ (1.5 mL). After 5 h, the solution was concentrated, extracted with acetone, methanol, and water. The water layer was concentrated to give **27**. Yield: 73%; yellow solid; m.p. 192 °C (decomp); ^{1}H NMR (500 MHz, D_2O): $\delta = 7.76$ (d, $J = 7.7$ Hz, 1H), 6.17 (dd, $J_{1',2'a} = 6.2$ Hz, $J_{1',2'b} = 7.8$ Hz, 1H), 5.97 (d, $J = 7.1$ Hz, 1H), 5.20 (d, $J = 6.3$ Hz, 1H), 4.29–3.60 (m, 9H), 2.58–2.39 (m, 3H), 2.22 (m, 1H), 1.97 (s, 3H); ^{13}C NMR (125.75 MHz, D_2O): $\delta = 179.21$, 179.07, 174.79, 171.06, 168.52, 165.67, 156.53, 143.22, 97.53, 87.45, 84.53, 76.16, 67.59, 66.61, 54.71, 53.61, 43.83, 38.16, 37.53, 21.65; $[\alpha]_D^{20} = 4.0$ ($c = 0.05$, H_2O); HRMS (FAB) (glycerol/TFA): m/z: 609.1558 (calcd for $C_{20}H_{30}O_{14}N_6P$: 609.1440).

***O*-(2′-deoxycytidine-phosphato)-L-seryl-L-glycyl-L-aspartic acid** (**28**): A mixture of **27** (8 mg, 13 μmol) in hydrazine hydrate (300 mL) was stirred at room temperature, and after 2 h, acetone (3 mL) was added at 0 °C. The mixture was concentrated, and extracted with methanol. The remaining residue was dissolved in water, filtered through a Sep-Pak Cartridge from Waters, and concentrated to give **28**. Yield: 85%; white solid; m.p. 230 °C (decomp); ^{1}H NMR (500 MHz, D_2O): $\delta = 7.69$ (d, $J = 6.9$ Hz, 1H), 6.14 (s, 1H), 5.91 (s, 1H), 4.35–3.68 (m, 10H), 2.52–1.64 (m, 4H); ^{13}C NMR (125.75 MHz, D_2O): $\delta = 178.97$, 178.43, 174.71, 170.24, 166.13, 157.57, 141.41, 96.40, 85.99, 85.23, 70.69, 67.09, 64.91, 54.66, 52.92, 42.34, 39.46, 39.45; $[\alpha]_D^{20} = 9.0$ ($c = 0.1$, H_2O); HRMS (FAB) (glycerol/TFA): m/z: 565.1124 ([M – H]) (calcd for 565.1295).

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[1] a) B. A. Juodka, *Nucleosides Nucleotides* **1984**, *3*, 445–483; b) J. C. Wang, *Ann. Rev. Biochem.* **1985**, *54*, 665–697; c) A. B. Vartapetian, A. A. Bogdanov, *Prog. Nucleic Acid Res. Mol. Biol.* **1987**, *34*, 209–251; d) T. J. Kelly, M. S. Wold, J. Li, *Adv. Virus Res.* **1988**, *34*, 1–42; d) M. Salas in *The Viruses*, *Vol. 1 The Bacteriophages* (Ed.: R. Calender).

[2] K. A. Henningfeld, T. Arslan, S. M. Hecht, *J. Am. Chem. Soc.* **1996**, *118*, 11701–11714.

[3] a) Review: M. Salas, *Ann. Rev. Biochem.* **1991**, *160*, 39–71; b) Review: M. Salas, *Virus Strategies - Molecular Biology and Pathogenesis*, VCH, Weinheim, **1993**, pp. 1–23; c) L. Blanco, J. M. Lázaro, M. de Vega, A. Bonnin, M. Salas, *Proc. Natl. Acad. Sci.* **1994**, *91*, 12198.

[4] For a recent review on the synthesis of nucleotides, which also addresses the problems encountered in the removal of the blocking functions see: S. L. Beaucage, R. P. Iyer, *Tetrahedron* **1992**, *48*, 2223–2311.

[5] a) B. Juodka, V. Kirveliene, P. Povilionis, *Nucleosides & Nucleotides* **1982**, *1*, 497–508; b) E. Kuyl-Yeheskiely, P. A. M. van der Klein, G. M. Visser, G. A. van der Marel, J. H. van Boom, *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 69–70.

[6] a) E. Kuyl-Yeheskiely, C. M. Tromp, A. W. M. Lefeber, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1988**, *44*, 6515–6523; b) E. Kuyl-Yeheskiely, C. M. Dreef-Tromp, A. Geluk, G. A. van der Marel, J. H. van Boom, *Nucleic Acids Res.* **1989**, *17*, 2897–2905; c) C. M. Dreef-Tromp, J. C. M. van der Marel, H. van den Elst, G. A. van der Marel, J. H. van Boom, *Nucleic Acids Res.* **1992**, *20*, 4015–4020; d) Y. Ueno, R. Saito, T. Hata, *Nucleic Acids Res.* **1993**, *21*, 4451–4457; e) J. Robles, E. Pedroso, A. Grandas, *Tetrahedron Lett.* **1994**, *35*, 4449–4452; f) J. Robles, E. Pedroso, A. Grandas, *J. Org. Chem.* **1994**, *59*, 2482–2486.

[7] Reviews: a) H. Waldmann, M. Schelhaas, *Angew. Chem.* **1996**, *108*, 2192–2219; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2056; b) H. Waldmann, D. Sebastian, *Chem. Rev.* **1994**, *94*, 911–937.

[8] a) H. Waldmann, E. Nägele, *Angew. Chem.* **1995**, *107*, 2425–2428; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2259–2262; b) M. Schelhaas, S. Glomsda, M. Hänsler, H.-D. Jakubke, H. Waldmann, *Angew. Chem.* **1996**, *108*, 82–85; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 106–109; c) H. Waldmann, M. Schelhaas, E. Nägele, J. Kuhlmann, A. Wittighofer, H. Schroeder, J. R. Silvius, *Angew. Chem.* **1997**, *109*, 2334–2337; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2238–2241.

[9] a) T. Pohl, H. Waldmann, *Angew. Chem.* **1996**, *108*, 1829–1832; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1720–1723; b) P. Braun, H. Waldmann, H. Kunz, *Bioorg. Med. Chem.* **1993**, *1*, 197–207; T. Pohl, H. Waldmann, *J. Am. Chem. Soc.* **1997**, *119*, 6702–6710.

[10] a) H. Waldmann, A. Heuser, S. Schulze, *Tetrahedron Lett.* **1996**, *37*, 8725–8728; b) H. Waldmann, D. Sebastian, *Tetrahedron Lett.* **1997**, *38*, 2927–2930; c) D. Sebastian, A. Heuser, S. Schulze, H. Waldmann, *Synthesis* **1997**, 1098–1108.

[11] Parts of the results reported here were published in preliminary form: a) H. Waldmann, S. Gabold, *Chem. Comm.* **1997**, *19*, 1861–1862; b) V. Jungmann, H. Waldmann, *Tetrahedron Lett.* **1998**, *39*, 1139–1142.

[12] a) H. Waldmann, A. Reidel, *Angew. Chem.* **1997**, *109*, 642–644; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 647–649; b) M. A. Dineva, B. Galunsky, V. Kasche, D. D. Petkov, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2781–1784.

[13] a) K. L. Agarwal, A. Yamazaki, P. J. Cashion, H. G. Khorana, *Angew. Chem.* **1972**, *Angew. Chem. Int. Ed. Engl.* **1972**, *11*, 451–459; b) H. Schaller, G. Weinmann, B. Lerch, H. G. Khorana, *J. Am. Chem. Soc.* **1963**, *85*, 3821–3827.

[14] M. D. Matteucci, M. H. Caruthers, *Tetrahedron Lett.* **1980**, *21*, 3243–3246.

[15] W. Bannwarth, E. Küng, *Tetrahedron Lett.* **1989**, *30*, 4219–4222.

[16] For the enzymatic removal of methyl esters from glycosylated amino acids see: a) D. Cantacuzene, S. Attal, S. Bay, *Bioorg. Med. Chem. Lett.* **1991**, *1*, 197–200; b) S. Attal, S. Bay, D. Cantacuzene,

Tetrahedron **1992**, *48*, 9251–9260; c) H. Ishii, K. Unabashi, Y. Mimura, Y. Inoue, *Bull. Chem. Soc. Jpn.* **1990**, *63*, 3042–3043.
[17] For the enzymatic removal of MEE esters from glycosylated amino acids and glycopeptides see: J. Eberling, P. Braun, D. Kowalczyk, M. Schultz, H. Kunz, *J. Org. Chem.* **1996**, *61*, 2638–2646.
[18] E. P. Sonnet, M. W. Baillargeon, *Lipids* **1991**, *26*, 295–300.
[19] a) E. M. Kosower, B. Pazhenchevsky, *J. Am. Chem. Soc.* **1980**, *102*, 4983–4988; b) S. Shaninian, J. R. Silvius, *Biochem.* **1995**, *34*, 3812–3816; c) E. M. Kosower, B. Pazhenchevsky, H. Doduk, H. Klanety, D. Faust, *J. Org. Chem.* **1981**, *46*, 1666–1671.
[20] G. Braum, P. Braun, D. Kowalczyk, H. Kunz, *Tetrahedron Lett.* **1993**, *34*, 3111–3114.
[21] M. Dessolin, M.-G. Guillerez, N. Thieriet, F. Guibé, A. Loffet, *Tetrahedron Lett.* **1995**, *36*, 5741–5744.
[22] a) Y. Hayakawa, M. Hirose, R. Noyori, *J. Org. Chem.* **1993**, *58*, 5551–5555; b) Y. Hayakawa, M. Uchiyama, H. Kato, R. Noyori, *Tetrahedron Lett.* **1985**, *Vol. 26 No. 52*, 6505–6508.
[23] For the undesired attack of proteases on peptide bonds during enzymatic removal of blocking functions from peptides see the references given in ref. [7b], in particular a) E. Walton, J. O. Rodin, C. H. Stammer, F. W. Holly, *J. Org. Chem.* **1962**, *27*, 2255; b) G. Kloss, E. Schröder, *Hoppe-Seyler's Z. Physiol. Chem.* **1964**, *336*, 248.
[24] a) M. Buchholz, H. Kunz, *Liebigs Ann. Chem.* **1983**, 1859–1885; b) H. Kunz, M. Buchholz, *Chem. Ber.* **1979**, *112*, 2145–2157.
[25] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
[26] J. S. Ralston, A. R. Main, B. F. Kilpatrick, A. L. Chasson, *Biochem. J.* **1983**, *211*, 243–250.
[27] P. Schultheiss-Reimann, H. Kunz, *Angew. Chem.* **1983**, *95*, 64; *Angew. Chem. Int. Ed. Eng.* **1983**, *22*, 62.
[28] J. E. Smart, B. W. Stillmann, *J. Biol. Chem.* **1982**, *257*, 13499–13506.
[29] W. Bannwarth, E. Küng, *Tetrahedron Lett.* **1989**, *30*, 4219–4222.

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