Enzymatic Protecting Group Techniques for Glyco- and Phosphopeptide Chemistry: Synthesis of a Glycophosphopeptide from Human Serum Response Factor

Jörg Sander[b] **and Herbert Waldmann***[a]

Abstract: The covalent modification of proteins by phosphorylation and by glycosylation with GlcNAc residues are important regulatory processes which mediate biological signal transduction. For the study of such biological phenomena in molecular detail characteristic peptides which embody both types of modification may serve as efficient tools. However, their synthesis is complicated by their pronounced acid and base lability as well as their multifunctionality. For this purpose the enzyme-labile choline ester was developed. The choline ester can be removed selectively and in high yields from various GlcNAc-glycopeptides and phosphopeptides at pH 6.5 and 37 °C. The conditions under which the enzymatic deprotections proceed are so mild that no undesirable side reactions are observed (i.e., no cleavage or anomerization of the glycosidic bonds and no β-elimination of the phosphate or the carbohydrate occur). The specificity of the biocatalyst guarantees that neither the peptide bonds nor the other protecting groups present are being attacked. When this enzymatic protecting group technique was combined with the enzyme-labile 4-(phenylacetoxy)-benzyloxycarbonyl (PhAcOZ) urethane protecting group a complex glycophosphopeptide could be built up. The glycopeptide is equipped with a biotin label by which it can be traced in biological systems. This peptide represents a characteristic partial structure of a glycosylated and phosphorylated sequence from the transactivation domain of serum response factor (SRF), a widely occuring human transcription factor.

Keywords: cell signalling · enzyme catalysis · glycopeptides · protecting groups · protein phosphorylation

Introduction

Covalently modified proteins mediate the transduction of signals from the extracellular space across the plasma membrane into the interior of cells and ultimately to the cell nucleus.[1] Among the different types of covalent protein modifications, in particular, the dynamic phosphorylation and dephosphorylation of hydroxyamino acids mediate the intracellular response to a wide variety of extracellular stimuli.[2] In addition, the β-*O*-glycosidic attachment of single *N*-acetylglucosamine (*O*-GlcNAc) residues to serine and threonine units of intracellular proteins was discovered as a second type of modification.[3] *O*-GlcNAc is found in all eukaryotic cells on numerous proteins, for example on transcription factors,[4] oncogene products,[5] and enzymes[6] as well as on viral proteins.[7] The state of glycosylation and phosphorylation on individual proteins is often modified during the cell cycle.

An illustrative example is provided by mammalian serum response factor (SRF) (Figure 1). In response to various extracellular stimuli provided, for example by growth factors, this widely found transcription factor is phosphorylated at various serine and threonine residues.[3] It then translocates to the nucleus and binds to the serum response element (SRE), a 22 base pair long DNA sequence occuring in the promoter regions of numerous immediate early genes including, for example, the proto oncogene c-fos. After subsequent binding of further proteins also activated by growth promoting signals, like the closely related ternary complex factors Elk-1, SAP-1, and SAP-2, gene transcription is induced.[3] In the transactivation domain of SRF a cluster of hydroxyamino acids is found that are targets of several different protein serine kinases, namely casein-kinase II,[9] ribosomal S6 kinase,[10] and DNA–protein kinase[11] (Figure 2). In addition the major glycosylation site of SRF, serine^{383} (the protein is glycosylated at four different sites), is also localized in this part of the protein (Figure 2).[12] This accumulation of regulatory modifications within a short stretch of amino acids in the transactivation domain of the transcription factor suggests that the correct orchestration of

---

[a] Prof. Dr. H. Waldmann
Max-Planck-Institut für Molekulare Physiologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
and
Universität Dortmund, Fachbereich 3, Organische Chemie
Fax: (+49) 231-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de

[b] Dr. J. Sander
Universität Karlsruhe, Institut für Organische Chemie

0947-6539/00/0609-1564 $ 17.50+.50/0

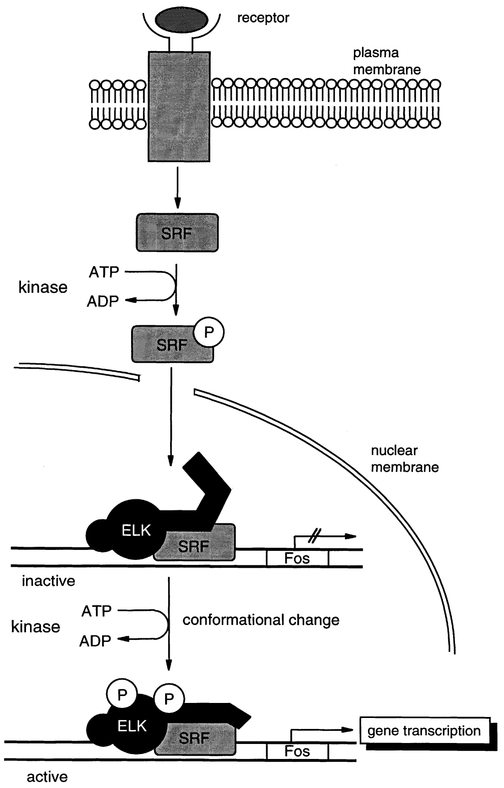


Figure 1. Activation of gene transcription after phosphorylation of the serum response factor.

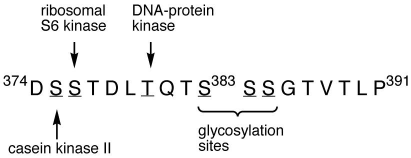
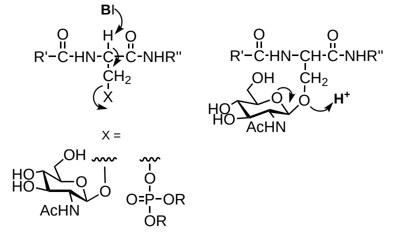


Figure 2. Phosphorylation and glycosylation sites in the transactivation domain of serum response factor.

SRF phosphorylation and glycosylation as well as the controlled removal of these covalent protein modifications may be paramount to regulation of gene transcription under the control of the serum response factor. To study the biological phenomena associated with such covalently modified protein conjugates characteristic partial structures which embody both types of modifications and also underivatized hydroxyamino acids may be efficient tools.[13, 14] Furthermore the precise location of the glycosylation sites may be determined by comparison of glycosylated peptides obtained from natural *O*-GlcNAc-containing glycopeptides with synthetic analogues. However, the synthesis of phospho- or glycopeptides itself is severely complicated by their pronounced acid and base sensitivity as well as their multifunctionality.[15] Thus, under weakly basic conditions (pH > 8) the phosphates and the glycosides may be lost by β-elimination reactions, and at low pH an anomerization or a rupture of the *O*-glycosidic bond may occur (Scheme 1).

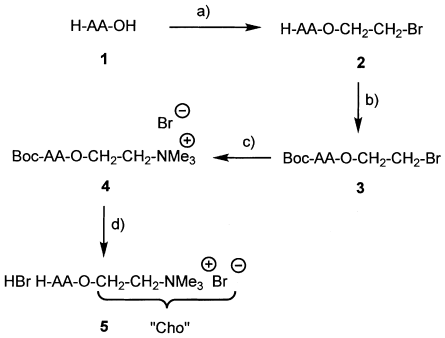
These properties make it necessary to apply a variety of protecting groups that can be removed selectively under the mildest and preferably neutral conditions. In the construction of glycophosphopeptides that embody both modifications, these difficulties are potentiated and consequently, many of the classical chemical protecting groups cannot be applied. Enzymatic protecting group techniques[13, 15, 16, 17] offer viable alternatives to the established classical chemical methods. Thus, on the one hand enzymatic transformations can be carried out under characteristically mild reaction conditions (pH 6–8, room temperature). On the other hand, enzymes often combine a high specificity for the structures they recognize and the reactions they catalyze with a broad substrate tolerance. In this paper, we report that by means of a combination of enzyme labile protecting groups glycophosphopeptides can be built up efficiently.[18]



Scheme 1. Acid and base sensitivity of phospho- and glycopeptides.

Results and Discussion

Enzymatic deprotection of glycopeptides: As enzyme-labile protecting group for the C-terminus of the peptide chain the choline ester was chosen. The choline ester had previously been investigated as possible blocking group for glycopeptide synthesis, but under the conditions required for its cleavage in a non-enzymatic reaction (pH 10–11) the sensitive O-glycosylated peptides were destroyed.[19] However, the results gained in subsequent successful applications of this blocking function in lipopeptide[20] and nucleopeptide chemistry[21] suggested that it might also be an efficient enzyme-labile protecting group for phospho-, glyco-, and glycophosphopeptide chemistry. Amino acid choline esters were synthesized by analogy to published procedures,[19–21] as shown in Scheme 2. Thus, amino acids **1** were converted into their 2-bromoethyl esters **2** and the N-terminus was masked with a Boc (*tert*-butyloxycarbonyl) urethane group. Intermediates **3** were then treated with trimethylamine to give choline esters **4** from

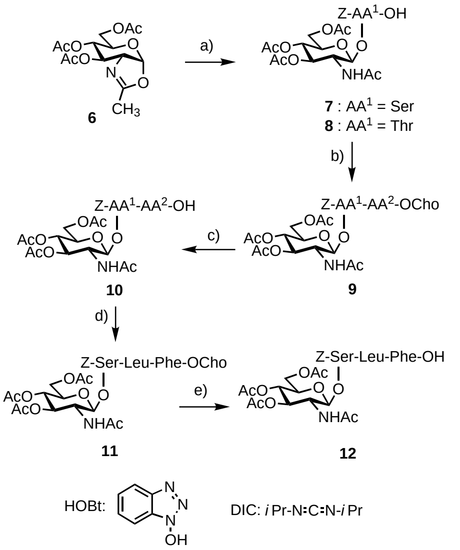


Scheme 2. Synthesis of amino acid choline esters: a) $HO(CH_2)_2Br$ (87–95%), b) Boc_2O (80–97%), c) NMe_3 (97–98%), d) HBr/HOAc (83–95%).

0947-6539/00/0609-1565 $ 17.50+.50/0

which the Boc group was cleaved by treatment with HBr in acetic acid to give the desired esters **5** in high yields.

In order to investigate the suitability of the enzymatic choline ester cleavage for glycopeptide synthesis, *O*-GlcNAc modified serine and threonine building blocks **7** and **8** were built-up by glycosylation of the corresponding Z (benzyloxycarbonyl) N-protected amino acids with oxazoline **6**[22] (Scheme 3). The C-terminally unmasked serine and threonine derivatives were then condensed with amino acid choline esters **5** in the presence of diisopropylcarbodiimide (DIC) and *N*-hydroxybenzotriazole (HOBt) to give glycodipeptides **9** in high yields (Table 1). For removal of the ester protecting group the enzyme butyrylcholine esterase was utilized. This biocatalyst recognizes positively charged choline esters and cleaves the ester group under very mild conditions (pH approx. 7, 27–37 °C). Butyrylcholine esterase is commercially available (Sigma). Alternatively, this enzyme can be isolated in a straightforward procedure from horse serum by a combination of anion-exchange chromatography and affinity chromatography on a procainamide column.[23]



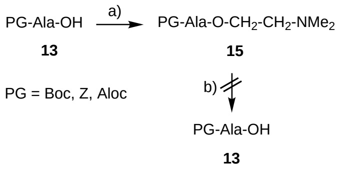
Scheme 3. Synthesis and selective enzymatic deprotection of glycopeptide choline esters by butyrylcholine esterase from horse serum: a) Z-Ser-OH or Z-Thr-OH (55 %), b) HBr × H-AA^{2}OCho (**5**), DIC, HOBt, NEt$_3$, CH$_2$Cl$_2$ (62–91 %), c) butyrylcholine esterase, 0.1M phosphate buffer, pH 6.5, 37 °C (37–80 %), d) AA^1 = Ser, AA^2 = Leu, HBr × H-PheOCho (**5d**), DIC, HOBt, NEt$_3$, CH$_2$Cl$_2$ (85 %), e) butyrylcholine esterase, 0.1M phosphate buffer, pH 6.5, 37 °C, 75 %.

Table 1. Results of the synthesis and enzymatic deprotection of glycopeptide choline esters **9**.

Entry	Compound	AA¹	AA²	coupling yield [%]	yield for choline ester cleavage [%]
1	**9a**	Ser	Ala	91	68
2	**9b**	Ser	Ile	78	37
3	**9c**	Ser	Leu	81	78
4	**9d**	Ser	Phe	88	76
5	**9e**	Ser	Val	89	61
6	**9f**	Thr	Ala	73	70
7	**9g**	Thr	Leu	74	70
8	**9h**	Thr	Phe	74	80
9	**9i**	Thr	Val	62	64

Upon treatment of an aqueous solution of the choline esters **9** with this enzyme at pH 6.5 and 37 °C, the C-terminal ester was cleaved smoothly and without any undesired side reaction. The acetates and the N-terminal urethane were not attacked and anomerization or β-elimination of the carbohydrate did not occur. The enzyme accepts different amino acid combinations and also tolerates sterically demanding residues such as phenylalanine at the C-terminus. In general, the desired selectively unmasked glycodipeptides **10** were isolated in yields of 60–80 %, only in the case of **10b** a lower yield was recorded. In order to investigate if the butyrylcholine esterase-catalyzed C-terminal deprotection can also be used successfully after further elongation of the peptide chain, glycodipeptide **10c** was condensed with phenylalanine choline ester. The resulting glycotripeptide choline ester **11** was also accepted as substrate by butyrylcholine esterase and the C-terminal protecting group was cleaved once more in high yield (Scheme 3). These results clearly indicate that the enzymatic removal of the choline ester can advantageously be applied in glycopeptide chemistry. Particularly rewarding is the high solubility of the charged substrates in the purely aqueous buffer. A solubility enhancing co-solvent that might denature the enzyme is not required. These properties of the choline ester group should be particularly advantageous for enzymatic transformation of hydrophobic substrates, which otherwise would be only sparingly soluble in the aqueous media needed for optimal performance of the enzyme. On the other hand, the fact that the choline esters are charged may complicate their isolation and purification by well-established chromatographic procedures. Therefore, to obtain optimal yields of selectively deprotected glycopeptides **10**, after completion of the peptide coupling reaction choline esters **9** were precipitated from the crude reaction mixture by addition of ether. The precipitates were used for the subsequent deprotection step without further purification. Under these conditions only triethylammonium bromide co-precipitates. This salt does not influence the efficiency of the enzymatic choline ester hydrolysis and is readily separated from the carboxylic acids **10**.

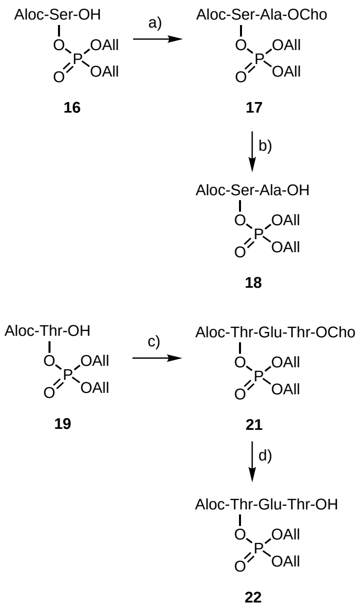
In an attempt to circumvent the sometimes unconvenient handling of permanently charged choline esters, we investigated peptide 2-(dimethylamino)ethyl esters as possible alternative substrates for butyrylcholine esterase. These compounds would be protonated at the tertiary amine group under weakly acidic conditions giving a charged and also highly soluble ammonium intermediate that might be accepted as substrate by the enzyme. Z-, Boc-, and Aloc-protected alanine (dimethylamino)ethyl esters **15** were synthesized by esterifying masked alanine derivatives **13** with 2-dimethylaminoethanol (**14**) (Scheme 4). Subsequently, these amino acid esters were treated with butyrylcholine esterase at pH 6.0, 6.5, 7.0, and 8.0 without additional co-solvent or in the presence of 10 vol. % or 20 vol. % methanol. However, in neither case was the C-terminal ester attacked. In a second series of experiments lipase AP from *Aspergillus niger* (Fluka), lipase F-AP14 from *Rhizopus oryzae*, lipase M from *Mucor javanicus*, lipase P from *Pseudomonas sp.*, lipase N from *Rhizopus*



Scheme 4. Synthesis and attempted enzymatic deprotection of amino acid 2-(dimethylamino)ethyl esters: a) $HO(CH_2)_2NMe_2$ (**14**), DCC, DMAP, b) lipase or butyrylcholine esterase.

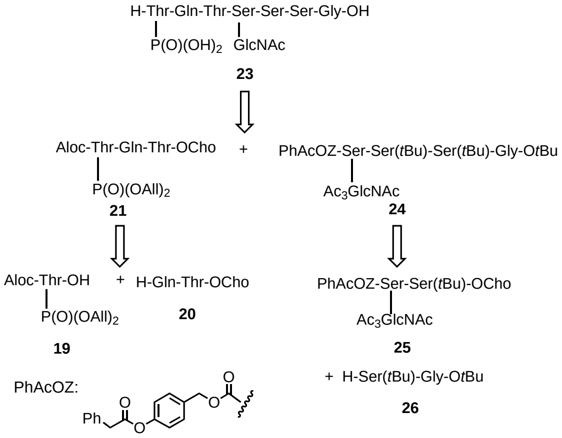
niveus, lipase D from *Rhizopus delemar*, and lipase R from *Penicillium roquefortii* (all obtained from Amano) were used at the different pH values with or without additional cosolvent as described above instead of the choline esterase. However, these lipases also did not attack the 2-(dimethylamino)ethyl esters at all. These findings correspond well with the observation that peptide 2-(diethylamino)ethyl esters are not saponified by lipases.[24]

Enzymatic deprotection of phosphopeptides: To investigate the suitability of the choline ester group for phosphopeptide synthesis *O*-phosphorylated serine[25] and threonine derivatives **16** and **19** were condensed with alanine choline ester **5a** and selectively deprotected dipeptide choline ester **20**, respectively (Scheme 5). Phosphorylated threonine carboxylic acid **19** was synthesized by *O*-phosphitylation of Aloc-threonine methyl ester and papain-catalyzed selective cleavage of the methyl ester (Scheme 8). Glutaminyl threonine choline ester **20** was obtained by condensation of Boc-glutamine with threonine 2-bromoethyl ester, conversion of the resulting dipeptide bromoethyl ester to the corresponding choline ester by treatment with NMe_3 and subsequent cleavage of the Boc group with HBr/AcOH (see Scheme 8). *O*-Phosphorylated di- and tripeptide choline esters **17** and **21** were then subjected to enzymatic hydrolysis with butyrylcholine esterase following the procedure described above. In the ensuing biocatalyzed transformations both phosphopeptides were converted into the desired selectively unmasked phosphopeptide carboxylic acids **18** and **22**. In these cases, too, no β-elimination of the phosphate was observed, and the other functional groups present were also not attacked. Dipeptide **18** was obtained in high yield, whereas tripeptide **22** could be isolated from the product mixture only in 35% yield. We note, however, that the enzymatic cleavage of the choline ester present in **21** was essentially quantitative. The low yield is caused by isolation problems during work-up procedure.



Scheme 5. Synthesis and selective enzymatic deprotection of phosphopeptide choline esters by butyrylcholine esterase from horse serum: a) H-Ala-OCho (**5a**), DIC, HOBt, NEt_3, CH_2Cl_2 (85%), b) butyrylcholine esterase, 0.1M phosphate buffer, pH 6.5 (73%), c) H-Gln-Thr-OCho (**20**), DIC, HOBt, NEt_3, CH_2Cl_2 (82%), d) butyrylcholine esterase, 0.1M phosphate buffer, pH 6.5 (35%).

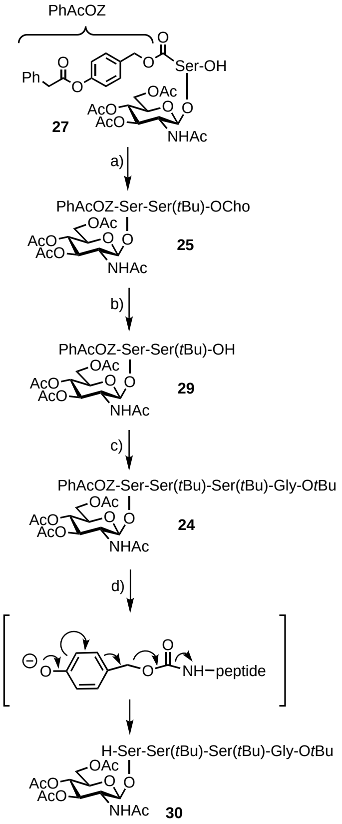
Enzymatic synthesis of a glycophosphopeptide from human serum response factor: Since the choline ester had proven to be an efficient enzyme-labile blocking group for glyco- and phosphopeptide chemistry its application for the construction of peptide conjugates, namely glycophosphopeptides, embodying both modifications appeared to be very promising as well. As a biologically relevant target compound, the heptapeptide **23** was chosen (Scheme 6). Compound **23** represents a characteristic partial structure of a glycosylated and phosphorylated sequence from the transactivation domain of serum response factor, incorporating the major glycosylation site of this transcription factor (see Figure 2).



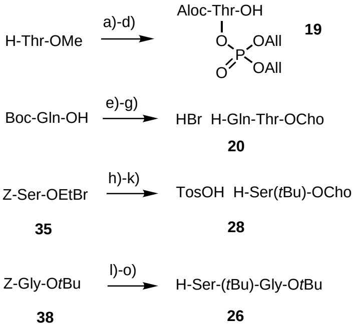
Scheme 6. Retrosynthetic analysis of the target glycophosphoheptapeptide **23** from serum response factor.

For the synthesis of glycophosphopeptide **23**, the target compound was retrosynthetically divided into the N-terminal phosphotripeptide **21** and the C-terminal glycotetrapeptide **24** (see Scheme 6). Glycotetrapeptide **24** was traced back to glycodipeptide **25** and dipeptide **26**. The protecting group strategy employed the use of *O*-acetates for the GlcNAc residues of the glycopeptide fragments, since *O*-acetates can be cleaved from glycosylated peptides under weakly basic conditions without β-elimination or racemization of the amino acids.[26] In addition, this allowed for the use of the *tert*-butyl ester as permanent C-terminal blocking function and *tert*-butyl ethers as protecting groups for the serine OH-groups. Under the conditions necessary to split these acid-labile groups acetyl-masked glycosides remain unattacked and phosphopeptides are not destroyed under these conditions either.[27, 28] For the masking of the phosphate and the permanent protection of the N-terminus allyl-blocking groups were applied which can be removed from phosphoglycopeptides without undesired side reactions.[27, 28] As temporary protecting groups for the termini of the intermediary synthesized peptides two enzyme-labile blocking functions were chosen. The C-terminus of phosphopeptide **21** and of glycopeptide **25** were marked as choline esters. The N-terminus of glycopeptides **24** and **25** were protected as *p*-(phenylacetoxy)benzyloxycarbonyl (PhAcOZ) urethane. This enzyme-labile urethane protecting group was developed by us earlier for glycophosphopeptide synthesis;[27] it has, however, not been combined with a second enzyme-labile blocking group. The cleavage of the PhAcOZ urethane is initiated by penicillin G acylase mediated hydrolysis of the phenylacetate (at pH 7.5) incorporated into this protecting group. The liberated phenolate then undergoes a spontaneous non-enzymatic fragmentation resulting in the formation of the desired peptide or peptide conjugate (Scheme 7; **24**→**30**).[27]

The synthesis of the desired glycophosphopeptide commenced with the coupling of PhAcOZ-protected serine building block **27**[27] with *tert*-butyl protected serine choline ester **28** (Scheme 8). Glycodipeptide choline ester **25** obtained thereby was then selectively deprotected at the C-terminus by butyrylcholine esterase mediated saponification of the choline ester. PhAcOZ-protected glycodipeptide **29** was obtained in high yield. In this conversion once more no undesired side reactions were observed. The substrate specificity of the choline esterase and the very mild reaction conditions guarantee that the glycoside and the phenol ester of phenylacetic acid incorporated into the N-terminal protecting group are not attacked.



Scheme 7. Enzymatic synthesis of the N-terminally deprotected glycotetrapeptide **30**: a) HBr × H-Ser(*t*Bu)Ocho (**28**), DIC, HOBt, NEt_3, CH_2Cl_2 (64%), b) butyrylcholine esterase, 0.1M phosphate buffer, pH 6.5 (65%), c) H-Ser(*t*Bu)-Gly-O*t*Bu (**26**), DIC, HOBt, NEt_3, CH_2Cl_2 (78%), d) penicillin G acylase, pH 7 (68%).

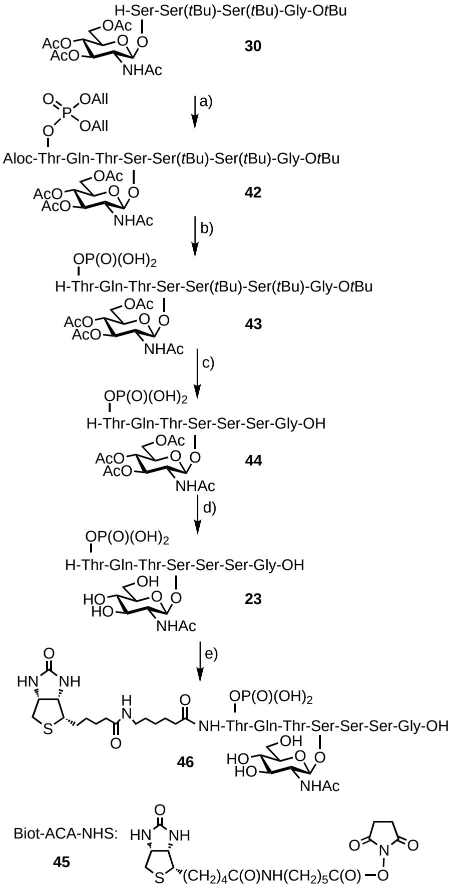


Scheme 8. Synthesis of amino acid and peptide esters **20**, **26** and **28**: a) Aloc-Cl (→ **31**), b) $P(OAll)_2NiPr$, c) *m*CPBA (→ **32**), d) papain, e) H-ThrOEtBr (→ **33**), f) NMe_3 (→ **34**), g) HBr/HOAc, h) $Cl_3CC(NH)OtBu$ (→ **36**), i) NMe_3 (→ **37**), k) H_2, Pd/C, TosOH, l) H_2, Pd/C (→ **39**), m) Z-Ser-OH, DIC, HOBt (→ **40**), n) $Cl_3CC(NH)OtBu$ (→ **41**), o) H_2, Pd/C.

The peptide chain was then elongated with dipeptide **26**, and the resulting glycotetrapeptide **24** was subsequently selectively unmasked at the N-terminus. Upon treatment of **24** with penicillin G acylase at pH 7.5 the phenylacetic acid ester was selectively saponified. The acetate protecting groups of the carbohydrate were not affected. The intermediary formed phenolate underwent the expected non-enzymatic fragmentation to give after decarboxylation of the resulting carbonic acid derivative the desired deprotected glycotetrapeptide **30** in high yield. In the course of this fragmentation the phenolate is converted to a quinone methide that is trapped by water (Scheme 7).

Peptide esters **28** and **26** employed in the synthesis detailed above were synthesized as shown in Scheme 8. In order to synthesize **28**, Z-serine bromoethyl ester (**35**)[19] was converted into the *O-tert*-butyl ether by treatment with *tert*-butyl trichloroacetimidate.[29] The 2-bromoethyl ester **36** was then converted to the choline ester and finally the Z group was removed by hydrogenation in the presence of toluenesulfonic acid. For the construction of selectively deprotected dipeptide **26**, glycine derivative **38**[30] was unmasked at the N-terminus and the resulting amino acid ester was condensed with Z-serine. After introduction of the side chain *O-tert*-butyl ether, the N-terminal Z group was removed by hydrogenolysis.

The peptide chain of glycotetrapeptide **30** was further elongated by coupling with selectively unmasked phosphotripeptide **22** obtained by enzymatic choline ester cleavage as shown in Scheme 5 (see above). Glycophosphoheptapeptide **42** was obtained in high yield (Scheme 9). From this complex fully masked peptide conjugate all blocking functions were removed. To this end, first the N-terminal Aloc group and the allyl phosphate protecting functions were cleaved by treatment with a Pd^0 catalyst and formic acid/*n*-butylamine.[31]



Scheme 9. Synthesis of a biotinylated glycophosphoheptapeptide from the transactivation domain of serum response factor: a) **22**, EDC, HOBt, CH_2Cl_2 (74%), b) $[Pd(PPh_3)_4]$, HCOOH, nBuNH_2 (86%), c) CF_3COOH (84%), d) hydrazine hydrate, methanol (56%), e) Biot-ACA-NHS (**45**), DMF (68%).

Under these weakly acidic conditions the phosphate and the carbohydrate are not lost through β-elimination.[27] Thus, the partially deprotected heptapeptide **43** was obtained in high yield without undesired side reactions. Next, the C-terminal *tert*-butyl ester and the serine *tert*-butyl ethers were cleaved selectively by treatment of **43** with trifluoroacetic acid. Under these conditions the glycoside was not anomerized or cleaved. Then the hydroxy groups of the carbohydrate were unmasked by means of hydrazine-mediated transesterification of the acetic acid esters with methanol.

In this final deprotection sequence, the order in which the different blocking functions were removed had to be chosen very carefully. Under the basic conditions necessary to split the carbohydrate ester protecting groups, the diallyl phosphate would most probably have been lost by β-elimination. Therefore, the N-terminal Aloc group and the allyl phosphates were cleaved first. The resulting phosphoric acid monoester is deprotonated under basic conditions and, is therefore, no longer prone to base-induced elimination. The second step had to be the cleavage of the *tert*-butyl ester and ethers under acidic conditions in the presence of peracetylated GlcNAc residues. Glycosidic bonds to acetylated carbohydrates are significantly more acid stable than, for example bonds to benzylated or deprotected sugars. Thus, by performing the C-terminal deprotection as the second step a possible anomerization or cleavage of the *O*-glycosides was prevented.

Finally, the glycophosphoheptapeptide **23** was equipped with a biotin label by selective N-acylation with biotinylamidocaproic acid *N*-hydroxysuccinimide (**45**) (Biot-Aca-NHS; Scheme 9). Biotinylated glycophosphopeptide **46** thus formed may serve as efficient molecular probe. The biotin label forms a very stable complex with the protein streptavidin, so that a neo-glycophosphoprotein can readily be generated. Furthermore, streptavidin is available in fluorescently labeled form as well as modified with colloidal gold. Thus, the biological fate of such a glycosylated and phosphorylated model protein as for example after injection in eukaryotic cells[32] can be monitored by fluorescence microscopy and electron microscopy.[33]

Conclusion

We have developed a new and efficient strategy for the synthesis of glycosylated and phosphorylated peptides as well as complex peptide conjugates carrying both types of covalent modification. This strategy is based on the use of suitable enzyme-labile protecting groups, and their combination with classical chemical protecting functions. Its usefulness was demonstrated by the construction of a glycophosphoheptapeptide which represents a characteristic partial structure of a glycosylated and phosphorylated sequence from the transactivation domain of human serum response factor. In all enzymatic and non-enzymatic transformations described above, no undesired side reactions were observed. Thus, the peptide backbone and the glycosidic bonds of the carbohydrates as well as the phosphate remained intact. In addition, an anomerization of the glycosidic bonds which may occur under acidic conditions was not observed. Also a base-mediated β-elimination of the phosphate or the carbohydrates could not be detected. When enzymatic and classical chemical protecting group techniques are combined as described above or in similar ways, further sensitive *O*-phosphorylated and *O*-glycosylated peptide conjugates will be accessible. The use of these compounds may open up new avenues of research in bioorganic chemistry and biology. In particular they should serve to unravel the chemical biology of the serum response factor and the importance of its posttranslational modification in molecular detail.

Experimental Section

General procedures: ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AC250, AM400, and DRX500 spectrometers. Mass spectra were measured on a Finnigan MAT MS 70 spectrometer and a Perseptive Biosystems VoyagerDE RP Biospectrometry MALDI-TOF spectrometer. Analytical thin-layer chromatography was performed on E. Merck silica gel F_{254} plates. Flash chromatography was performed on Baker silica gel (40–60 μm). Tetrahydrofuran was distilled from potassium metal, CH_2Cl_2 from lithium aluminium hydride, and toluene from sodium. Penicillin G acylase was obtained in immobilized form on Eupergit C from Boehringer Mannheim, butyrylcholine esterase from Sigma Chemie GmbH, and papain from Fluka. The lipases were purchased from Amano, Japan.

Synthesis of amino acid 2-bromoethyl esters (2): A mixture of L-amino acid (32.7 mmol), *p*-toluenesulfonic acid monohydrate (12.44 g, 65.4 mmol), and 2-bromoethanol (24 mL, 0.34 mmol) in benzene (100 mL) was refluxed at a Dean–Stark trap for 4–5 h. The majority of benzene was removed under reduced pressure and the product was precipitated by addition of with diethyl ether (500 mL). The product was purified by recrystallization from methanol/diethyl ether.

L-Serine 2-bromoethyl ester hydrotosylate (2a): colorless crystals (95 %); m.p.: 118–119 °C; ^{1}H NMR (500 MHz, CD_3OD): δ=7.71 (d, $J=8.3$ Hz, 2H), 7.24 (d, $J=8.3$ Hz, 2H), 4.53 (t, $J=5.8$ Hz, 2H), 4.20 (t, $J=3.7$ Hz, 1H), 4.04 (dd, $J=10.5$, 4.6 Hz, 1H), 3.95 (dd, $J=10.5$, 3.7 Hz, 1H), 3.63 (t, $J=5.9$ Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (125.8 MHz, CD_3OD): $\delta=168.63$, 143.27, 141.80, 129.84 (2 CH_2, tosyl), 126.90 (2 CH_2, tosyl), 66.84 (CH_2), 60.68 (CH_2), 56.02 (α-CH), 29.18 (CH_2), 21.31 (CH_3); $[\alpha]^{20}_{D}$: −2.7 ($c=1$, CH_3OH); anal. calcd for $C_{12}H_{18}BrNO_6S$ (370.23): C 37.81, H 4.72, N 3.67; found: C 38.03, H 4.72, N 3.43.

L-Threonine 2-bromoethyl ester hydrotosylate (2b): colorless crystals (88 %); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.02$ (br s, 3H), 7.71 (d, $J=8.0$ Hz, 2H), 7.15 (d, $J=8.0$ Hz, 2H), 4.27–4.32 (m, 3H), 3.92 (br s, 1H), 3.88 (m, 1H), 3.29 (t, $J=6.2$ Hz, 2H), 2.36 (s, 3H), 1.28 (d, $J=6.3$ Hz, 3H); ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta=167.53$, 141.13, 140.22, 129.06 (2 CH_2, tosyl), 126.05 (2 CH_2, tosyl), 65.77 (β-CH), 65.56 (CH_2), 59.11 (α-CH), 27.82 (CH_2), 21.42 (CH_3), 19.97 (CH_3); $[\alpha]^{20}_{D}$: −7.6 ($c=1$, CH_3OH); anal. calcd for $C_{13}H_{20}BrNO_6S$ (398.27): C 39.21, H 5.06, N 3.52; found: C 39.40, H 5.01, N 3.23.

L-Valine 2-bromoethyl ester hydrotosylate (2c): light yellow solid (87 %); m.p.: 85 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.72$ (d, $J=8.0$ Hz, 2H), 7.16 (d, $J=8.0$ Hz, 2H), 6.80 (br s, 3H), 4.30 (m, 2H), 3.90 (m, 1H), 3.34 (ddd, $J=22.3$, 10.9, 6.1 Hz, 1H), 2.26 (s, 3H), 2.20 (m, 1H), 0.97 (d, $J=6.9$ Hz, 3H), 0.94 (d, $J=6.9$ Hz, 3H); ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta=168.16$, 141.04, 140.36, 129.01 (2 CH_2, tosyl), 126.05 (2 CH_2, tosyl), 65.21 (CH_2), 58.56 (α-CH), 29.64 (β-CH), 27.75 (CH_2), 21.38 (CH_3), 18.25 (CH_3), 17.49 (CH_3); $[\alpha]^{20}_{D}$: +6.4 ($c=1$, CH_3OH); anal. calcd for $C_{14}H_{22}BrNO_5S$ (396.29): C 42.43, H 5.60, N 3.53; found: C 42.21, H 5.59, N 3.21.

Synthesis of *N-tert*-butyloxycarbonyl-L-amino acid 2-bromoethyl esters (3): Triethylamine (2.5 mL, 18.2 mmol) and a solution of di-*tert*-butyl dicarbonate (4.82 g, 25.9 mmol) in dioxane (50 mL) were added to a solution of L-amino acid 2-bromoethyl ester **2** (18.2 mmol) in a mixture of dioxane and water (150 mL, 2:1) at 0 °C. After the reaction mixture was stirred for 30 min at 0 °C and 24 h at room temperature the solvent was removed in vacuo. The remaining residue was dissolved in water (100 mL) and the solution was extracted three times with ethyl acetate (100 mL). The organic layer was washed with water (50 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure.

***N-tert*-Butyloxycarbonyl-L-isoleucine 2-bromoethyl ester (3a)**: white solid (95 %); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=5.09$ (d, $J=8.5$ Hz, 1H), 4.48 (m, 1H), 4.41 (m, 1H), 4.29 (dd, $J=8.8$, 4.7 Hz, 1H), 3.52 (t, $J=6.5$ Hz, 2H), 1.90 (m, 2H), 1.74 (m, 1H), 1.45 (s, 9H), 0.95 (d, $J=7.0$ Hz, 3H), 0.92 (t, $J=7.4$ Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta=171.82$, 155.42, 79.62, 64.22 (CH_2), 57.78 (α-CH), 37.74 (β-CH), 34.78 (CH_2), 28.17 (3 CH_3), 24.86 (CH_2), 15.44 (CH_3), 11.47 (CH_3); $[\alpha]^{20}_{D}$: +16.3 ($c=1$, $CHCl_3$); anal. calcd for $C_{13}H_{24}BrNO_4$ (338.24): C 46.16, H 7.15, N 4.14; found: C 46.20, H 7.12, N 4.02.

***N-tert*-Butyloxycarbonyl-L-serine 2-bromoethyl ester (3b)**: colorless crystals (97 %); m.p.: 64 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=5.57$ (d, $J=7.9$ Hz, 1H), 4.56–4.43 (m, 3H), 4.02 (dd, $J=11.3$, 3.4 Hz, 1H), 3.91 (dd, $J=11.2$, 3.4 Hz, 1H), 3.55 (t, $J=6.0$ Hz, 2H), 2.81 (br s, 1H), 1.46 (s, 9H); ^{13}C NMR (100.7 MHz, $CDCl_3$): $\delta=170.49$, 155.74, 80.37, 64.65 (CH_2), 63.35 (CH_2), 55.69 (α-CH), 28.48 (CH_2), 28.29 (3 CH_3); $[\alpha]^{20}_{D}$: −20.9 ($c=1$, CH_3OH); anal. calcd for $C_{10}H_{18}BrNO_5$ (312.16): C 38.48, H 5.81, N 4.49; found: C 38.47, H 5.86, N 4.59.

***N-tert*-Butyloxycarbonyl-L-threonine 2-bromoethyl ester (3c)**: light yellow oil (96 %); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=5.49$ (d, $J=9.1$ Hz, 1H), 4.46–4.17 (m, 4H), 3.47 (t, $J=6.0$ Hz, 2H), 1.38 (s, 9H), 1.18 (d, $J=6.0$ Hz, 3H); ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta=171.05$, 156.22, 80.04, 67.79 (β-CH), 64.52 (CH_2), 58.87 (α-CH), 28.51 (CH_2), 28.25 (3 CH_3), 19.92 (CH_3); $[\alpha]^{20}_{D}$: −16.9 ($c=1$, CH_3OH); anal. calcd for $C_{11}H_{20}BrNO_5$ (326.19): C 40.50, H 6.18, N 4.29; found: C 40.47, H 6.09, N 4.50.

***N-tert*-Butyloxycarbonyl-L-valine 2-bromoethyl ester (3d)**: colorless oil (80 %); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=5.07$ (d, $J=9.0$ Hz, 1H), 4.31 (ddt, $J=6.2$, 5.8 Hz, 2H), 4.11 (q, $J=4.7$ Hz, 1H), 3.41 (t, $J=6.1$ Hz, 2H), 2.05 (q, $J=6.4$ Hz, 1H), 1.31 (s, 9H), 0.86 (d, $J=7.0$ Hz, 3H), 0.79 (d, $J=7.0$ Hz, 3H); ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta=171.65$, 155.34, 79.32, 64.06 (CH_2), 58.31 (CH), 30.84 (CH), 28.12 (CH_2), 28.01 (3 CH_3), 18.75 (CH_3), 17.34 (CH_3); $[\alpha]^{20}_{D}$: −24.7 ($c=1$, CH_3OH); anal. calcd for $C_{12}H_{22}BrNO_4$ (324.21): C 44.46, H 6.84, N 4.32; found: C 44.50, H 6.68, N 4.23.

Synthesis of *N-tert*-butyloxycarbonyl-L-amino acid choline esters (4): Trimethylamine (ca. 5 mL) at −78 °C was added to a solution of *N-tert*-butyloxycarbonyl-L-amino acid 2-bromoethyl ester **3a–d** (17.5 mmol) in acetone (50 mL). The solution was stirred for 3 d at room temperature and the solvent was removed in vacuo. The product was purified by recrystallization from methanol/diethyl ether. In the case of L-serine and L-threonine derivatives the solvent was removed in vacuo after 24 h. The crude oil was dissolved in water (50 mL) and extracted three times with CH_2Cl_2 (20 mL). After lyophilisation of the aqueous layer, a pure product was obtained while the unreacted starting material was recovered from the organic layer.

***N-tert*-Butyloxycarbonyl-L-isoleucine choline ester bromide (4a)**: white, highly hygroscopic solid (97 %); ^{1}H NMR (500 MHz, CD_3OD): $\delta=4.70$ (d, $J=14.3$ Hz, 1H), 4.50 (ddt, $J=14.4$, 6.2 Hz, 1H), 4.07 (d, $J=6.2$ Hz, 1H), 4.03 (m, 2H), 3.27 (s, 9H), 1.92–1.74 (m, 3H), 1.45 (s, 9H), 0.93 (d, $J=4.4$ Hz, 3H), 0.91 (t, $J=3.7$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta=174.34$, 158.06, 80.49, 68.97 (CH_2), 59.64 (α-CH), 57.08 (CH_2), 54.77 (3 CH_3), 38.37 (β-CH), 35.97 (CH_2), 28.71 (3 CH_3), 11.70 (CH_3), 11.61 (CH_3); $[\alpha]^{20}_{D}$: −21.1 ($c=2$, CH_3OH); anal. calcd for $C_{16}H_{33}BrN_2O_4 \times H_2O$ (415.37): C 46.27, H 8.49, N 6.74; found: C 46.36, H 8.20, N 6.69.

***N-tert*-Butyloxycarbonyl-L-serine choline ester bromide (4b)**: white solid (57 %); m.p.: 50–52 °C; ^{1}H NMR (500 MHz, CD_3OD): $\delta=4.66$ (m, 2H), 4.28 (t, $J=4.6$ Hz, 1H), 3.92 (dd, $J=11.3$, 4.7 Hz, 1H), 3.87 (t, $J=4.5$ Hz, 2H), 3.80 (dd, $J=11.3$, 4.4 Hz, 1H), 3.31 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=171.80$, 157.68, 80.80, 65.85 (CH_2), 62.66 (CH_2), 59.85 (CH_2), 57.30 (CH), 54.64 (3 CH_3), 28.69 (3 CH_3); $[\alpha]^{20}_{D}$: −13.0 ($c=1$, CH_3OH); anal. calcd for $C_{13}H_{27}BrN_2O_4 \times 1.5\,H_2O$ (382.29): C 39.20, H 7.59, N 7.03; found: C 39.29, H 7.58, N 6.96.

***N-tert*-Butyloxycarbonyl-L-threonine choline ester bromide (4c)**: white solid (41 %); m.p.: 60–62 °C; ^{1}H NMR (500 MHz, CD_3OD): $\delta=4.63$ (t, $J=2.5$ Hz, 2H), 4.29 (dq, $J=6.5$, 3.0 Hz, 1H), 4.20 (d, $J=3.0$ Hz, 1H), 3.80 (t, $J=4.5$ Hz, 2H), 3.26 (s, 9H), 1.46 (s, 9H), 1.22 (d, $J=6.5$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=171.96$, 158.33, 80.99, 68.27 (β-CH), 66.02 (CH_2), 60.74 (α-CH), 59.57 (CH_2), 54.57 (3 CH_3), 28.66 (3 CH_3), 20.13 (CH_3); $[\alpha]^{20}_{D}$: −16.5 ($c=1$, CH_3OH); anal. calcd for $C_{14}H_{29}BrN_2O_5 \times 0.5\,H_2O$ (394.30): C 42.65, H 7.67, N 7.10; found: C 42.91, H 7.65, N 6.99.

***N-tert*-Butyloxycarbonyl-L-valine choline ester bromide (4d)**: white, highly hygroscopic solid (98 %); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=5.07$ (d, $J=7.9$ Hz, 1H), 4.67 (m, 1H), 4.18 (m, 2H), 4.02 (m, 1H), 3.57 (s, 9H), 2.09 (m, 1H), 1.43 (s, 9H), 0.99 (d, $J=6.8$ Hz, 3H), 0.95 (d, $J=6.9$ Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=172.06$, 155.91, 80.33, 64.86 (CH_2), 59.30 (α-CH), 58.50 (CH_2), 54.45 (3 CH_3), 30.33 (β-CH), 28.30 (3 CH_3), 19.27 (CH_3), 17.11 (CH_3); $[\alpha]^{20}_{D}$: −13.7 ($c=1$, CH_3OH); anal. calcd for $C_{15}H_{31}BrN_2O_4 \times 0.25\,H_2O$ (387.81): C 46.45, H 8.19, N 7.22; found: C 46.44, H 8.06, N 6.99.

Synthesis of amino acid choline ester hydrobromides (5): A solution of *N-tert*-butyloxycarbonyl-L-amino acid choline ester bromide **4a–d** (3.25 g, 8.2 mmol) in a solution of HBr in glacial acetic acid (45 %; 25 mL) was stirred for 30 min at room temperature. The product was precipitated by addition of diethyl ether (150 mL). The crude product was recrystallized from methanol/diethyl ether.

L-Isoleucine choline ester bromide hydrobromide (5a): white, hygroscopic solid (83 %); m.p.: 235 °C; ^{1}H NMR (500 MHz, CD_3OD): $\delta=4.86$ (dt, $J=13.3$, 8.1, 5.2 Hz, 1H), 4.70 (dt, $J=13.2$, 8.0, 5.2 Hz, 1H), 4.22 (d, $J=4.1$ Hz, 1H), 3.94 (dt, $J=4.2$ Hz, 2H), 3.33 (s, 9H), 2.08 (m, 1H), 1.60 (m, 1H), 1.40 (m, 1H), 1.08 (d, $J=7.0$ Hz, 3H), 1.02 (t, $J=7.4$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=169.45$, 65.68 (CH_2), 60.70 (CH_2), 58.54 (α-CH), 54.59 (3 CH_3), 37.56 (β-CH), 26.54 (CH_2), 15.06 (CH_3), 12.00 (CH_3); $[\alpha]^{20}_{D}$: +24.3 ($c=1$, CH_3OH); HR-MS (FAB): m/z: 217.1896 $[M-HBr_2]^+$ (calcd for $C_{11}H_{25}N_2O_2$ 217.1916); anal. calcd for $C_{11}H_{26}Br_2N_2O_2$ (378.15): C 34.94, H 6.93, N 7.41; found: C 34.94, H 6.77, N 7.57.

L-Valine choline ester bromide hydrobromide (5b): light yellow, hygroscopic solid (95 %); m.p.: 220 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta=4.87$ (d, $J=14.4$ Hz, 1H), 4.75 (d, $J=14.2$ Hz, 1H), 4.15 (d, $J=4.8$ Hz, 1H), 4.02

0947-6539/00/0609-1570 $ 17.50+.50/0

(t, $J=4.6$ Hz, 2H), 3.38 (s, 9H), 2.41 (m, 1H), 1.12 (d, $J=7.0$ Hz, 6H); ^{13}C NMR (100.5 MHz, CD$_3$OD): $\delta=169.35$, 65.60 (CH$_2$), 60.82 (CH$_2$), 59.52 (α-CH), 54.65 (3CH$_3$), 30.67 (β-CH), 18.61 (CH$_3$); $[\alpha]_D^{20}$: +10.2 ($c=1$, CH$_3$OH); HR-MS (FAB): m/z: 202.1785 $[M\cdot HBr_2]^+$ (calcd for $C_{10}H_{23}N_2O_2$ 203.1760); anal. calcd for $C_{10}H_{24}Br_2N_2O_2 \times 0.5H_2O$ (373.13): C 32.99, H 6.64, N 7.69; found: C 33.00, H 6.34, N 7.60.

Variaton of the method for the synthesis of the serine and threonine derivatives: A solution of *N*-*tert*-butyloxycarbonyl-L-amino acid choline ester bromide **4b,c** (4.89 mmol) in CH$_2$Cl$_2$ (40 mL) was cooled to −50 °C. A solution of HBr in glacial acetic acid (45 %, 13 mL) was then added and the mixture was stirred for 10 min at this temperature. The crude product was precipitated by addition diethyl ether (100 mL). The product was purified by recrystalization from methanol/diethyl ether.

L-Serine choline ester bromide hydrobromide (5c): colorless solid (91 %); m.p.: 210 °C; ^{1}H NMR (400 MHz, CD$_3$OD): $\delta=4.76$ (dd, $J=2.0$ Hz, 2H), 4.34 (t, $J=3.5$ Hz, 1H), 4.11 (dd, $J=11.9$, 4.0 Hz, 1H), 3.96 (dd, $J=11.9$, 3.9 Hz, 1H), 3.89 (m, 2H), 3.29 (s, 9H); ^{13}C NMR (100.5 MHz, CD$_3$OD): $\delta=168.58$, 65.74 (CH$_2$), 60.87 (CH$_2$), 60.52 (CH$_2$), 56.25 (α-CH), 54.55 (3CH$_3$); $[\alpha]_D^{20}$: −3.7 ($c=1$, CH$_3$OH); anal. calcd for $C_8H_{19}Br_2N_2O_3 \times 0.5H_2O$ (283.99): C 26.61, H 5.86, N 7.76; found: C 26.23, H 5.72, N 7.44.

L-Threonine choline ester bromide hydrobromide (5d): colorless solid (94 %); m.p.: 50–54 °C; ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=4.76$ (t, $J=2.2$ Hz, 2H), 4.42 (q, $J=3.3$ Hz, 1H), 4.17 (d, $J=3.3$ Hz, 1H), 3.89 (t, $J=4.8$ Hz, 2H), 3.29 (s, 9H), 1.35 (d, $J=6.5$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta=168.85$, 66.07 (CH$_2$), 65.74 (β-CH), 60.86 (CH$_2$), 59.73 (α-CH), 54.57 (3CH$_3$), 20.28 (CH$_3$); $[\alpha]_D^{20}$: −9.9 ($c=1$, CH$_3$OH); anal. calcd for $C_9H_{22}Br_2N_2O_3 \times 1.5H_2O$ (393.11): C 27.50, H 6.41, N 7.13; found: C 27.69, H 6.34, N 7.18.

Synthesis of glycosylated dipeptides (9): A solution of L-amino acid choline ester bromide hydrobromide **5** (0.37 mmol) and triethylamine (52 µL, 0.37 mmol) in DMF (10 mL) were added to a solution of *N*-terminally protected, glycosylated amino acid **7** or **8** (0.37 mmol), HOBt (100 mg, 0.74 mmol) and DIC (141 µL, 0.74 mmol) in CH$_2$Cl$_2$ (30 mL). The mixture was stirred for 24 h at room temperature. After removal of the solvent under reduced pressure the residue was dissolved in methanol (5 mL) and the product was precipitated by addition of diethyl ether (100 mL). This crude precipitate was characterized by NMR and mass spectroscopy and used without further purification in the subsequent enzymatic deprotection step.

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-alanine choline ester bromide (9a)**: light yellow, highly hygroscopic solid [91 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.05–1.90 (4s, 4CH$_3$CO, 12H) and 1.36 (t, NEt$_3$ × HBr, 9H)]; ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=7.37$ (m, 5H), 5.22 (dd, $J=9.9$ Hz, 1H), 5.13 (m, 2H), 4.99 (dd, $J=9.7$ Hz, 1H), 4.77 (d, $J=8.5$ Hz, 1H), 4.59 (brs, 2H), 4.56 (q, $J=7.2$ Hz, 1H), 4.37 (t, $J=5.4$ Hz, 1H), 4.31 (dd, $J=12.2$ Hz, 1H), 4.14 (d, $J=12.2$ Hz, 1H), 4.01 (m, 1H), 3.92 (m, 3H), 3.76 (m, 1H), 3.31 (s, 9H), 2.05 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.90 (s, 3H), 1.62 (d, $J=7.3$ Hz, 1H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta=173.67$, 172.93, 172.41, 171.77, 171.34 (6C), 158.12, 138.03, 129.57 (2CH), 129.14 (CH), 128.86 (2CH), 102.06 (CH), 74.03 (CH), 73.96 (CH), 70.20 (CH$_2$), 69.99 (CH), 67.88 (CH$_2$), 65.91 (CH$_2$), 63.17 (CH$_2$), 59.77 (CH$_2$), 56.40 (CH), 55.08 (CH), 54.60 (3CH$_3$), 49.71 (CH), 23.53 (CH$_3$), 20.76 (CH$_3$), 20.64 (CH$_3$), 20.60 (CH$_3$), 16.07 (CH$_3$); HR-MS (FAB): m/z: 725.3353 $[M]^+$ (calcd for $C_{33}H_{49}N_4O_{14}$ 725.3245).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-isoleucine choline ester bromide (9b)**: light yellow, highly hygroscopic solid [78 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.04–1.89 (4s, 4CH$_3$CO, 12H) and 1.34 (t, NEt$_3$ × HBr, 9H)]; ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=7.36$ (m, 5H), 5.17 (dd, $J=9.4$ Hz, 1H), 5.13 (d, $J=12.5$ Hz, 1H), 5.09 (d, $J=12.6$ Hz, 1H), 4.98 (dd, $J=9.7$ Hz, 1H), 4.69 (d, $J=8.5$ Hz, 1H), 4.65 (d, $J=15.1$ Hz, 1H), 4.50 (d, $J=15.5$ Hz, 1H), 4.39 (t, $J=7.0$ Hz, 1H), 4.32 (dd, $J=12.3$, 5.3 Hz, 1H), 4.27 (d, $J=6.5$ Hz, 1H), 4.12 (dd, $J=12.2$ Hz, 1H), 4.00 (m, 1H), 3.96–3.84 (m, 3H), 3.72 (dd, $J=6.6$ Hz, 1H), 3.22 (s, 9H), 2.04 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.89 (s, 3H), 1.54 (m, 2H), 1.38 (m, 1H), 1.28 (m, 1H), 0.95 (m, 6H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta=173.63$, 172.38, 172.01, 171.73, 171.31 (6C), 158.10, 138.12, 129.58 (2CH), 129.15 (CH), 128.87 (2CH), 102.57 (CH), 74.02 (CH), 73.10 (CH), 70.43 (CH$_2$), 70.04 (CH), 67.89 (CH$_2$), 65.90 (CH$_2$), 63.32 (CH$_2$), 59.52 (CH$_2$), 58.54 (CH), 58.54 (CH), 55.04 (CH), 49.51 (3CH$_3$), 37.88 (CH), 26.23 (CH$_2$), 22.91 (CH$_3$), 20.74 (CH$_3$), 20.58 (CH$_3$), 20.55 (CH$_3$), 15.97 (CH$_3$), 11.57 (CH$_3$); HR-MS (FAB): m/z: 767.3643 $[M]^+$ (calcd for $C_{36}H_{55}N_4O_{14}$ 767.3715).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-leucine choline ester bromide (9c)**: light yellow, highly hygroscopic solid [81 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.04–1.89 (4s, 4CH$_3$CO, 12H) and 1.35 (t, NEt$_3$ × HBr, 9H)]; $R_f=0.40$ (CHCl$_3$/methanol/acetic acid 80:1); ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=7.36$ (m, 5H), 5.20 (dd, $J=9.5$ Hz, 1H), 5.12 (s, 2H), 4.97 (dd, $J=9.7$ Hz, 1H), 4.74 (d, $J=8.4$ Hz, 1H), 4.56 (brs, 2H), 4.39 (t, $J=7.0$ Hz, 1H), 4.36 (t, $J=5.1$ Hz, 1H), 4.30 (m, 1H), 4.12 (dd, $J=12.2$, 2.1 Hz, 1H), 4.00 (m, 1H), 3.92–3.84 (m, 3H), 3.72 (d, $J=5.1$ Hz, 1H), 3.23 (s, 9H), 2.04 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.89 (s, 3H), 1.74 (dd, $J=4.7$ Hz, $J=12.0$ Hz, 2H), 1.66 (m, 1H), 0.98 (d, $J=6.0$ Hz, 3H), 0.92 (d, $J=5.9$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta=173.69$, 172.77, 172.35, 171.72, 171.29 (6C), 158.09, 138.08, 129.57 (2CH), 129.14 (CH), 128.84 (2CH), 102.10 (CH), 74.01 (CH), 72.96 (CH), 70.14 (CH$_2$), 70.04 (CH), 67.89 (CH$_2$), 65.90 (CH$_2$), 63.23 (CH$_2$), 59.71 (CH$_2$), 56.57 (CH$_2$), 55.11 (CH), 54.59 (3CH$_3$), 52.21 (CH), 40.64 (CH$_2$–CH$_2$), 23.46 (CH$_3$), 22.96 (CH$_3$), 21.78 (CH$_3$), 20.76 (CH$_3$), 20.61 (CH$_3$), 20.57 (CH$_3$); HR-MS (FAB): m/z: 767.3804 $[M]^+$ (calcd for $C_{36}H_{55}N_4O_{14}$ 767.3715).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-phenylalanine choline ester bromide (9d)**: white, highly hygroscopic solid [88 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.02–1.94 (4s, 4CH$_3$CO, 12H) and 1.32 (t, NEt$_3$ × HBr, 9H)]; ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=7.37$–7.25 (m, 10H), 5.19 (dd, $J=9.9$ Hz, 1H), 5.11 (s, 2H), 4.98 (dd, $J=9.7$ Hz, 1H), 4.70 (d, $J=8.5$ Hz, 1H), 4.57 (dd, $J=11.2$ Hz, 2H), 4.46 (m, 1H), 4.33 (t, $J=5.4$ Hz, 1H), 4.28 (dd, $J=12.4$, 4.9 Hz, 1H), 4.12 (d, $J=12.2$ Hz, 1H), 3.90–3.77 (m, 4H), 3.70 (m, 2H), 3.62 (dd, $J=7.3$ Hz, $J=14.0$ Hz, 2H), 3.14 (s, 9H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta=173.63$, 172.38, 172.10, 171.73, 171.30 (6C), 158.09, 138.10, 138.06, 130.47, 130.27, 129.70, 129.59, 129.15, 128.85 (10CH), 102.22 (CH), 74.01 (CH), 73.01 (CH), 70.17 (CH$_2$), 70.00 (CH), 67.89 (CH$_2$), 65.79 (CH$_2$), 63.23 (CH$_2$), 59.71 (CH$_2$), 56.44 (CH), 55.85 (CH), 55.04 (CH), 54.51 (3CH$_3$), 38.03 (CH$_2$), 22.94 (CH$_3$), 20.76 (CH$_3$), 20.61 (CH$_3$), 20.58 (CH$_3$); HR-MS (FAB): m/z: 801.3675 $[M]^+$ (calcd for $C_{39}H_{53}N_4O_{14}$ 801.3558).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-valine choline ester bromide (9e)**: white, highly hygroscopic solid [89 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.06–1.97 (4s, 4CH$_3$CO, 12H) and 1.36 (t, NEt$_3$ × HBr, 9H)]; ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=7.36$ (m, 5H), 5.19 (dd, $J=9.4$ Hz, 1H), 5.12 (d, $J=12.3$ Hz, 1H), 5.09 (d, $J=12.5$ Hz, 1H), 4.97 (dd, $J=9.6$ Hz, 1H), 4.73 (d, $J=8.4$ Hz, 1H), 4.46 (brs, 2H), 4.44 (t, $J=6.8$ Hz, 1H), 4.37–4.28 (m, 2H), 4.13 (d, $J=12.0$ Hz, 1H), 4.01 (m, 1H), 3.97–3.82 (m, 3H), 3.74 (m, 2H), 3.23 (s, 9H), 2.06 (m, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 0.99 (d, $J=6.7$ Hz, 3H), 0.94 (d, $J=6.8$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta=173.64$, 172.44 (2C), 171.98, 171.75, 171.34, 158.13, 138.10, 129.57 (2CH), 129.14 (CH), 128.88 (2CH), 102.48 (CH), 74.08 (CH), 73.07 (CH), 70.41 (CH$_2$), 70.06 (CH), 67.89 (CH$_2$), 65.90 (CH$_2$), 63.31 (CH$_2$), 59.59 (CH$_2$), 59.50 (CH), 56.39 (CH), 55.06 (CH), 54.55 (3CH$_3$), 31.53 (CH), 22.92 (CH$_3$), 20.75 (CH$_3$), 20.59 (CH$_3$), 20.56 (CH$_3$), 18.67 (CH$_3$), 18.50 (CH$_3$); HR-MS (FAB): m/z: 753.3633 $[M]^+$ (calcd for $C_{35}H_{53}N_4O_{14}$ 753.3558).

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-alanine choline ester bromide (9f)**: white, highly hygroscopic solid [73 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.00–1.93 (4s, 4CH$_3$CO, 12H) and 1.36 (t, NEt$_3$ × HBr, 9H)]; ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=7.37$ (m, 5H), 5.23 (dd, $J=10.0$ Hz, 1H), 5.13 (s, 2H), 4.95 (dd, $J=9.7$ Hz, 1H), 4.74 (d, $J=8.5$ Hz, 1H), 4.56 (brs, 2H), 4.43 (m, 1H), 4.31 (m, 1H), 4.14 (d, $J=12.4$ Hz, 1H), 4.07 (dd, 1H), 3.92 (m, 1H), 3.84 (m, 2H), 3.73 (m, 2H), 3.31 (s, 9H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.62 (d, $J=7.3$ Hz, 3H), 1.93 (d, $J=6.2$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta=173.77$, 172.95, 172.30, 171.65, 171.27, 170.34, 158.20, 138.20, 129.54 (2CH), 129.11 (CH), 128.67 (2CH), 100.95 (CH), 73.78 (CH), 72.71 (CH), 70.00 (CH), 67.80 (CH$_2$), 65.85 (CH$_2$), 63.16 (CH$_2$), 60.90 (CH), 59.72 (CH$_2$), 55.24 (CH), 54.58 (3CH$_3$), 49.93 (CH), 49.51

(CH), 22.88 (CH_3), 20.80 (CH_3), 20.65 (CH_3), 20.60 (CH_3), 16.08 (CH_3); HR-MS (FAB): m/z: 739.3472 $[M]^+$ (calcd for $C_{34}H_{51}N_4O_{14}$ 739.3402).

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-leucine choline ester bromide (9g)**: light yellow, highly hygroscopic solid [74%, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.00–1.93 (4s, 4 CH_3CO, 12H) and 1.38 (t, $NEt_3 \times HBr$, 9H)]; ^1H NMR (500 MHz, CD_3OD): $\delta=$ 7.36 (m, 5H), 5.24 (dd, $J=9.8$ Hz, 1H), 5.13 (s, 2H), 4.95 (dd, $J=9.7$ Hz, 1H), 4.76 (d, $J=8.4$ Hz, 1H), 4.56 (m, 2H), 4.29 (dd, $J=12.2$, 5.1 Hz, 1H), 4.19 (m, 2H), 4.07 (dd, $J=12.3$ Hz, 1H), 3.85 (m, 1H), 3.78 (m, 1H), 3.72 (dd, $J=5.4$ Hz, 1H), 3.23 (s, 9H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.79 (m, 1H), 1.75 (m, 1H), 1.67 (m, 1H), 1.00 (d, $J=5.2$ Hz, 3H), 0.96 (d, $J=6.2$ Hz, 3H), 0.90 (d, $J=5.2$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=173.37$, 172.78, 172.24, 171.61, 171.24 (6C), 158.14, 138.17, 129.57 (2CH), 129.10 (CH), 128.68 (2CH), 100.40 (CH), 73.76 (CH), 72.73 (CH), 68.43 (CH), 67.80 (CH_2), 65.82 (CH_2), 63.36 (CH_2), 61.04 (CH), 59.63 (CH_2), 55.27 (CH), 54.53 (3 CH_3), 52.48 (CH), 52.38 (CH), 40.94 (CH_2) 29.24 (CH), 25.64 (CH_3), 22.90 (CH_3), 22.02 (CH_3), 20.80 (CH_3), 20.63 (CH_3), 20.58 (CH_3), 16.76 (CH_3); HR-MS (FAB): m/z: 781.3718 $[M]^+$ (calcd for $C_{37}H_{57}N_4O_{14}$ 781.3871).

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-phenylalanine choline ester bromide (9h)**: light yellow oil [74%, determined from the NMR spectrum of the crude precipitate by integration of the signals at 1.99–1.89 (4s, 4 CH_3CO, 12H) and 1.34 (t, $NEt_3 \times HBr$, 9H)]; ^1H NMR (500 MHz, CD_3OD): $\delta=7.37$–7.28 (m, 10H), 5.28 (dd, $J=9.9$ Hz, 1H), 5.13 (d, $J=12.8$ Hz, 1H), 5.10 (d, $J=12.8$ Hz, 1H), 4.95 (dd, $J=9.7$ Hz, 1H), 4.83 (d, $J=8.5$ Hz, 1H), 4.69 (q, $J=6.8$ Hz, 1H), 4.49 (brs, 2H), 4.26 (dd, $J=12.2$, 4.7, 1H), 4.13 (t, $J=5.5$ Hz, 1H), 4.06 (d, $J=11.0$ Hz, 1H), 3.89 (d, $J=8.6$ Hz, 1H), 3.86 (d, $J=8.8$ Hz, 1H), 3.64 (brs, 2H), 3.21 (d, $J=7.3$ Hz, 2H), 3.19 (s, 9H), 1.99 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.89 (s, 3H), 1.15 (d, $J=7.1$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=173.52$, 172.17, 171.87, 171.70, 171.53, 171.15, 157.97, 138.06, 138.76, 129.78, 129.59, 129.54, 129.51, 129.03, 128.52, 128.20 (10CH), 100.92 (CH), 73.69 (CH), 72.55 (CH), 69.94 (CH), 67.63 (CH_2), 65.54 (CH_2), 63.14 (CH_2), 60.80 (CH), 59.63 (CH_2), 55.63 (CH), 55.30 (CH), 55.10 (CH), 54.46 (3 CH_3), 37.87 (CH_2), 23.32 (CH_3), 20.77 (CH_3), 20.73 (CH_3), 20.68 (CH_3), 17.10 (CH_3); HR-MS (FAB): m/z: 815.3795 $[M]^+$ (calcd for $C_{40}H_{55}N_4O_{14}$ 815.3715).

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-valine choline ester bromide (9i)**: white, highly hygroscopic solid [62%, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.01–1.95 (4s, 4 CH_3CO, 12H) and 1.36 (t, $NEt_3 \times HBr$, 9H)]; ^1H NMR (500 MHz, CD_3OD): $\delta=7.37$ (m, 5H), 5.27 (dd, $J=9.9$ Hz, 1H), 5.15 (d, $J=12.8$ Hz, 1H), 5.11 (d, $J=12.6$ Hz, 1H), 4.93 (dd, $J=9.7$ Hz, 1H), 4.86 (d, $J=8.4$ Hz, 1H), 4.61 (dd, $J=8.8$ Hz, 2H), 4.21 (d, $J=4.1$ Hz, 1H), 4.17 (q, $J=5.6$ Hz, 1H), 4.12 (d, $J=4.8$ Hz, 1H), 4.06 (dd, $J=11.6$ Hz, 1H), 3.92 (dd, 1H), 3.84 (m, 2H), 3.77 (dd, $J=6.7$ Hz, 2H), 3.35 (s, 9H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.24 (m, 1H), 1.22 (d, $J=6.3$ Hz, 3H), 1.03 (d, $J=5.3$ Hz, 3H), 0.98 (d, $J=6.8$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=173.60$, 172.19, 171.98, 171.83, 171.57, 171.24, 157.99, 138.09, 129.54 (2CH), 129.51 (CH), 128.55 (2CH), 100.84 (CH), 76.74 (CH), 73.71 (CH), 70.00 (CH), 67.63 (CH_2), 65.68 (CH_2), 63.21 (CH_2), 61.62 (CH), 60.69 (CH), 59.56 (CH_2), 59.49 (CH), 54.75 (CH), 54.59 (3 CH_3), 31.14 (CH), 22.98 (CH_3), 20.88 (CH_3), 20.77 (CH_3), 20.70 (CH_3), 18.69 (CH_3), 18.57 (CH_3), 17.03 (CH_3); HR-MS (FAB): m/z: 767.3880 $[M]^+$ (calcd for $C_{36}H_{55}N_4O_{14}$ 767.3715).

Enzymatic cleavage of the choline ester groups: Butyrylcholine esterase (100 U) was added to a solution of 100 μmol of dipeptide choline ester **7** in phosphate buffer (30 mL, pH 6.5, 0.01 M). The mixture was shaken at 37 °C for 3 d and then lyophilized. The product was extracted three times with $CHCl_3$ (20 mL), the organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by flash chromatography (hexane/ethyl acetate 2:1).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-alanine (10a)**: colorless, amorphic solid (68%); m.p.: 111–115 °C; ^1H NMR (500 MHz, CD_3OD): $\delta=7.35$ (m, 5H), 5.21 (dd, $J=9.9$ Hz, 1H), 5.12 (d, $J=12.2$ Hz, 1H), 5.09 (d, $J=12.4$ Hz, 1H), 5.01 (dd, $J=9.7$ Hz, 1H), 4.77 (m, 1H), 4.44 (q, $J=6.8$ Hz, 2H), 4.28–4.23 (m, 2H), 4.14 (d, $J=12.3$ Hz, 1H), 3.95–3.76 (m, 4H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.12 (d, $J=6.7$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=173.00$, 171.97, 171.45, 170.91, 170.69 (6C), 157.34, 138.03, 129.04 (2CH), 128.72 (CH), 128.50 (2CH), 101.56 (CH), 73.40 (CH), 72.39 (CH), 69.92 (CH_2), 67.55 (CH_2), 62.80 (CH_2), 57.57 (CH), 55.24 (CH), 54.59 (CH), 23.44 (CH_3), 20.79 (CH_3), 20.73 (CH_3), 20.68 (CH_3), 15.71 (CH_3); $[\alpha]_D^{20}$: −4.8 ($c=0.15$, CH_3OH); HR-MS (FAB): m/z: 640.2419 $[M+H]^+$ (calcd for $C_{28}H_{37}N_3O_{14}$ 640.2354).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-isoleucine (10b)**: light yellow oil (37%); ^1H NMR (400 MHz, $CDCl_3$): $\delta=7.32$ (m, 5H), 6.90 (d, $J=8.6$ Hz, 1H), 6.10 (m, 1H), 6.07 (d, $J=7.7$ Hz, 1H), 5.25 (dd, $J=10.2$ Hz, 1H), 5.05 (m, 3H), 4.72 (d, $J=8.2$ Hz, 1H), 4.49 (m, 2H), 4.24 (dd, $J=11.8$, 4.2 Hz, 1H), 4.11 (d, $J=11.8$ Hz, 1H), 3.98 (m, 2H), 3.76 (d, $J=6.4$ Hz, 2H), 2.04 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.87 (s, 3H), 1.50 (m, 1H), 1.22 (m, 2H), 0.91 (m, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=173.54$, 172.20, 171.01, 170.05, 169.53 (6C), 156.07, 136.05, 128.51 (2CH), 128.21 (CH), 128.05 (2CH), 101.26 (CH), 72.46 (CH), 71.83 (CH), 69.82 (CH_2), 68.53 (CH), 67.15 (CH_2), 62.08 (CH_2), 57.10 (CH), 57.09 (CH), 54.22 (CH), 37.44 (CH), 25.01 (CH_2), 22.88 (CH_3), 20.65 (3 CH_3), 15.35 (CH_3), 11.47 (CH_3); $[\alpha]_D^{20}$: −13.1 ($c=1$, CH_3OH); HR-MS (FAB): m/z: 682.2751 $[M+H]^+$ (calcd for $C_{31}H_{43}N_3O_{14}$ 682.2823).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-leucine (10c)**: colorless solid (78%); m.p.: 80 °C; $R_f=0.21$ ($CHCl_3$/methanol/acetic acid 80:10:1); ^1H NMR (500 MHz, $CDCl_3$): $\delta=7.32$ (m, 5H), 6.66 (d, $J=8.7$ Hz, 1H), 6.01 (d, $J=7.9$ Hz, 1H), 5.20 (dd, $J=10.0$ Hz, 1H), 5.11 (m, 1H), 5.09 (s, 2H), 5.06 (dd, $J=9.8$ Hz, 1H), 4.63 (d, $J=8.6$ Hz, 1H), 4.61 (dd, $J=4.3$ Hz, 2H), 4.50 (m, 1H), 4.25 (dd, $J=12.1$, 4.5 Hz, 1H), 4.11 (m, 2H), 4.00 (dd, $J=9.6$ Hz, 1H), 3.74 (m, 2H), 2.06 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.86 (s, 3H), 1.70 (m, 2H), 1.60 (m, 1H), 0.96 (d, $J=6.3$ Hz, 3H), 0.94 (d, $J=6.0$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta=174.68$, 172.26, 171.17, 170.90, 170.05, 169.48, 156.06, 136.10, 128.55 (2CH), 128.25 (CH), 128.11 (2CH), 101.27 (CH), 72.50 (CH), 71.87 (CH), 69.76 (CH_2), 68.44 (CH), 67.24 (CH_2), 62.01 (CH_2), 54.24 (2CH), 51.12 (CH), 41.06 (CH_2), 29.21 (CH), 24.72 (CH_3), 22.98 (CH_3), 21.80 (CH_3), 20.72 (CH_3), 20.69 (CH_3), 20.63 (CH_3); $[\alpha]_D^{20}$: −15.0 ($c=0.65$, $CHCl_3$); HR-MS (FAB): m/z: 682.2893 $[M+H]^+$ (calcd for $C_{31}H_{43}N_3O_{14}$ 682.2823); anal. calcd for $C_{31}H_{43}N_3O_{14} \times 2H_2O$ (717.12): C 51.88, H 6.60, N 6.16; found: C 51.82, H 6.61, N 6.42.

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-phenylalanine (10d)**: colorless solid (76%); m.p.: 212–215 °C; ^1H NMR (500 MHz, CD_3OD): $\delta=7.34$ (m, 5H), 7.22 (m, 5H), 5.17 (dd, $J=9.9$ Hz, 1H), 5.09 (s, 2H), 4.99 (dd, $J=9.7$ Hz, 1H), 4.74 (t, $J=5.9$ Hz, 1H), 4.62 (d, $J=8.4$ Hz, 1H), 4.40 (t, $J=6.0$ Hz, 2H), 4.15 (m, 2H), 3.87 (m, 2H), 3.76 (m, 2H), 3.20 (dd, $J=14.0$, 6.4 Hz, 1H), 3.07 (dd, $J=6.4$ Hz, $J=13.8$ Hz, 1H), 2.03 (s, 3H), 2.01 (s, 6H), 1.84 (s, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta=172.55$, 171.79, 171.00, 170.69, 169.63, 169.57, 156.11, 135.90, 135.79, 129.08, 128.23, 128.13, 127.94, 127.67, 126.65 (10CH), 100.67 (CH), 72.45 (CH), 71.47 (CH), 68.96 (CH_2), 68.36 (CH), 66.83 (CH_2), 61.81 (CH_2), 53.92 (CH), 53.55 (CH), 37.09 (CH_2), 22.17 (CH_3), 20.16 (2 CH_3), 20.12 (CH_3); $[\alpha]_D^{20}$: +9.6 ($c=0.4$, $CHCl_3$); HR-MS (FAB): m/z: 716.2604 $[M+H]^+$ (calcd for $C_{34}H_{41}N_3O_{14}$ 716.2667); anal. calcd for $C_{34}H_{41}N_3O_{14}$ (716.27): C 57.06, H 5.77, N 5.87; found: C 57.74, H 5.99, N 5.94.

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-valine (10e)**: yellow oil (61%); $R_f=0.21$ ($CHCl_3$/methanol/acetic acid 80:10:1); ^1H NMR (500 MHz, $CDCl_3$): $\delta=7.31$ (m, 5H), 6.46 (d, $J=8.3$ Hz, 1H), 5.98 (d, $J=7.9$ Hz, 1H), 5.24 (d, $J=10.6$ Hz, 1H), 5.20 (dd, $J=9.8$ Hz, 1H), 5.11 (d, 1H), 5.09 (d, 1H), 5.03 (dd, $J=9.5$ Hz, 1H), 4.66 (d, $J=8.4$ Hz, 1H), 4.49 (m, 2H), 4.24 (dd, $J=12.0$, 4.9 Hz, 1H), 4.11 (dd, $J=12.1$ Hz, 1H), 4.01 (m, 2H), 3.74 (m, 2H), 2.18 (q, $J=6.3$ Hz, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.86 (s, 3H), 0.96 (dd, $J=6.5$ Hz, 6H), 0.94 (d, $J=6.5$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta=173.38$, 172.08, 171.12, 170.85, 170.04, 169.47, 156.00, 136.10, 128.54 (2CH), 128.23 (CH), 128.13 (2CH), 101.44 (CH), 72.41 (CH), 71.96 (CH), 70.01 (CH_2), 68.41 (CH), 67.20 (CH_2), 62.06 (CH_2), 57.78 (CH), 54.32 (CH), 52.34 (CH), 31.08 (CH), 22.95 (CH_3), 21.67 (3 CH_3), 19.05 (CH_3), 17.90 (CH_3); $[\alpha]_D^{20}$: −9.4 ($c=0.26$, $CHCl_3$); HR-MS (FAB): m/z: 668.2762 $[M+H]^+$ (calcd for $C_{30}H_{41}N_3O_{14}$ 668.2667).

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-alanine (10f)**: light yellow solid (70%); m.p.: 210 °C; $R_f=0.35$ ($CHCl_3$/methanol/acetic acid 80:10:1); ^1H NMR

(500 MHz, CD_3OD): $\delta = 7.35$ (m, 5H), 5.19 (dd, $J = 9.9$ Hz, 1H), 5.11 (d, 1H), 5.10 (d, 1H), 5.02 (dd, $J = 9.7$ Hz, 1H), 4.69 (d, $J = 8.5$ Hz, 1H), 4.45 (m, 2H), 4.29 (dd, $J = 12.2$, 4.9 Hz, 1H), 4.11 (m, 2H), 3.91 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.91 (s, 3H), 1.44 (d, $J = 7.2$ Hz, 3H), 1.12 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 174.29$, 171.95, 171.29, 171.13, 170.00, 168.85, 156.52, 136.26, 128.65 (2CH), 128.33 (CH), 128.00 (2CH), 100.60 (CH), 76.48 (CH), 72.68 (CH), 71.81 (CH), 68.65 (CH), 67.19 (CH_2), 62.12 (CH_2), 54.20 (CH), 49.79 (CH), 22.71 (CH_3), 20.64 ($3CH_3$), 17.85 (CH_3), 15.39 (CH_3); $[\alpha]^{20}_{D}$: -9.4 ($c = 1$, CH_3OH); HR-MS (FAB): m/z: 654.2369 $[M{+}H]^+$ (calcd for $C_{29}H_{39}N_3O_{14}$ 654.2510); anal. calcd for $C_{29}H_{39}N_3O_{14}$ (654.24): C 53.29, H 6.01, N 6.43; found: C 53.43, H 6.21, N 6.65.

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-leucine (10g)**: light brown solid (70%); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.29$ (m, 5H), 7.10 (d, $J = 8.1$ Hz, 1H), 6.53 (d, $J = 9.0$ Hz, 1H), 6.08 (d, $J = 6.5$ Hz, 1H), 5.22 (dd, $J = 10.0$ Hz, 1H), 5.10 (d, 1H), 5.08 (d, 1H), 5.04 (dd, $J = 9.5$ Hz, 1H), 4.73 (d, $J = 8.3$ Hz, 1H), 4.55 (m, 1H), 4.22 (m, 4H), 3.99 (dd, $J = 10.0$ Hz, 2H), 3.78 (m, 1H), 2.02, 2.01 (s, 9H), 1.89 (s, 3H), 1.69 (m, 3H), 1.09 (d, $J = 6.3$ Hz, 3H), 0.93 (d, $J = 7.4$ Hz, 3H), 0.90 (d, $J = 7.5$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 175.18$, 171.73, 171.25, 170.83, 169.54 (5C), 156.09, 136.15, 130.94 (CH), 128.84 (CH), 128.58 (CH), 128.25 (CH), 127.98 (CH), 100.49 (CH), 75.98 (CH), 72.35 (CH), 68.51 (CH), 67.08 (CH_2), 62.10 (CH_2), 58.64 (CH), 57.19 (CH), 54.52 (CH), 51.40 (CH), 40.97 (CH_2) 24.85 (CH_3), 22.96 (CH_3), 21.80 (CH_3), 20.71, 20.66 ($3CH_3$), 14.10 (CH_3); $[\alpha]^{20}_{D}$: -20.2 ($c = 1.1$, CH_3OH); HR-MS (FAB): m/z: 696.3060 $[M{+}H]^+$ (calcd for $C_{32}H_{45}N_3O_{14}$ 696.2980).

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-phenylalanine (10h)**: colorless solid (80%); m.p.: 165°C; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.35$–7.23 (m, 10H), 7.13 (d, $J = 7.7$ Hz, 1H), 6.12 (d, $J = 8.8$ Hz, 1H), 5.98 (d, $J = 6.8$ Hz, 1H), 5.21 (dd, $J = 10.0$ Hz, 1H), 5.06 (s, 2H), 4.99 (dd, $J = 9.6$ Hz, 1H), 4.69 (d, $J = 8.3$ Hz, 1H), 4.50 (m, 1H), 4.20 (m, 2H), 4.13 (dd, $J = 5.3$ Hz, $J = 12.1$ Hz, 1H), 4.07 (dd, $J = 12.1$ Hz, 1H), 3.79 (dd, $J = 9.8$ Hz, 1H), 3.72 (brs, 1H), 3.18 (m, 2H), 2.03 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.87 (s, 3H), 1.07 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 173.08$, 172.64, 171.17, 170.81, 169.50, 168.91, 155.94, 136.30, 136.18, 129.36, 128.65, 128.55, 128.20, 127.92, 127.13 (10CH), 100.11 (CH), 75.60 (CH), 72.17 (CH), 71.88 (CH), 68.47 (CH), 67.01 (CH_2), 62.03 (CH_2), 57.23 (CH), 54.71 (CH), 53.69 (CH), 37.13 (CH_2), 23.01 (CH_3), 20.69 (CH_3), 20.62 (CH_3), 20.56 (CH_3), 14.76 (CH_3); $[\alpha]^{20}_{D}$: -3.0 ($c = 0.4$, CH_3OH); HR-MS (FAB): m/z: 730.2720 $[M{+}H]^+$ (calcd for $C_{35}H_{43}N_3O_{14}$ 730.2823); anal. calcd for $C_{35}H_{43}N_3O_{14}$ (730.28): C 57.61, H 5.94, N 5.76; found: C 57.12, H 6.00, N 5.80.

***N*-Benzyloxycarbonyl-L-threonyl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-valine (10i)**: light yellow solid (64%); m.p.: 63°C; $R_f = 0.21$ ($CHCl_3$/methanol/acetic acid 80:10:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.32$ (m, 5H), 6.69 (d, $J = 9.1$ Hz, 1H), 6.25 (d, $J = 7.0$ Hz, 1H), 6.05 (d, $J = 7.9$ Hz, 1H), 5.27 (dd, $J = 10.4$ Hz, 1H), 5.13 (d, $J = 12.3$ Hz, 1H), 5.09 (d, $J = 12.4$ Hz, 1H), 5.05 (dd, $J = 9.7$ Hz, 1H), 4.86 (d, $J = 8.3$ Hz, 1H), 4.33–4.21 (m, 3H), 4.13 (m, 2H), 3.98 (dd, $J = 8.1$ Hz, 1H), 3.82 (m, 1H), 2.23 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.91 (s, 3H), 1.16 (d, $J = 6.3$ Hz, 3H), 0.93 (d, $J = 6.9$ Hz, 3H), 0.90 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 174.37$, 171.94, 171.19, 169.60 (5C), 156.89, 136.02, 128.56 (2CH), 128.26 (CH), 128.00 (2CH), 101.04 (CH), 77.04 (CH), 72.34 (CH), 71.98 (CH), 67.30 (CH_2), 62.17 (CH_2), 58.40 (CH), 57.99 (CH), 57.40 (CH), 54.67 (CH), 30.65 (CH), 22.91 (CH_3), 21.68 (CH_3), 20.61 (CH_3), 20.58 (CH_3), 19.07 (CH_3), 17.94 (CH_3), 17.51 (CH_3); $[\alpha]^{20}_{D}$: -14.3 ($c = 0.12$, CH_3OH); HR-MS (FAB): m/z: 682.2699 $[M{+}H]^+$ (calcd for $C_{31}H_{43}N_3O_{14}$ 682.2823).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-leucyl-L-phenylalanine choline ester bromide (11)**: The tripeptide **11** was synthesized as described for the dipeptides **9**. Colorless, hygroscopic solid [85%, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.02–1.90 (4s, $4CH_3CO$, 12H) and 1.35 (t, $NEt_3 \times HBr$, 9H)]; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.36$–7.24 (m, 10H), 5.20 (dd, $J = 9.4$ Hz, 1H), 5.11 (d, $J = 12.5$ Hz, 1H), 5.08 (d, $J = 12.6$ Hz, 1H), 4.98 (dd, $J = 9.7$ Hz, 1H), 4.71 (d, $J = 8.5$ Hz, 1H), 4.60 (m, 2H), 4.40 (m, 2H), 4.32 (m, 2H), 4.11 (dd, $J = 12.3$, 2.1 Hz, 1H), 3.97 (m, 1H), 3.89 (m, 1H), 3.61 (dd, $J = 6.8$ Hz, $J = 14.4$ Hz, 2H), 3.23 (m, 2H), 3.14 (s, 9H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.90 (s, 3H), 1.61 (m, 1H), 1.55 (m, 1H), 1.44 (m, 1H), 0.93 (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 174.66$, 173.69, 173.37, 172.38, 171.89, 171.77, 171.33, 158.46, 138.21, 138.08, 130.38, 130.26, 129.68, 129.56, 129.16, 129.08, 128.91, 128.02 (10CH), 102.23 (CH), 74.04 (CH), 72.97 (CH), 70.02 (CH_2), 70.01 (CH), 67.89 (CH_2), 65.84 (CH_2), 63.26 (CH_2), 59.67 (CH_2), 55.83 (CH), 55.37 (CH), 55.08 (CH), 54.51 ($3CH_3$), 53.38 (CH), 41.65 (CH_2), 38.02 (CH_2), 23.51 (CH), 23.00 (CH_3), 22.20 (CH_3), 21.78 (CH_3), 20.77, 20.61 ($3CH_3$); HR-MS (FAB): m/z: 914.4294 $[M]^+$ (calcd for $C_{45}H_{64}N_5O_{15}$ 914.4399).

***N*-Benzyloxycarbonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-leucyl-L-phenylalanine (12)**: The choline ester was removed as described for **10a–i**. Colorless solid (75%); m.p.: 150°C; ^1H NMR (500 MHz, CD_3OD): $\delta = 7.36$–7.30 (m, 10H), 5.20 (dd, $J = 9.9$ Hz, 1H), 5.09 (s, 2H), 4.96 (dd, $J = 9.7$ Hz, 1H), 4.65 (d, $J = 8.5$ Hz, 1H), 4.43 (d, $J = 7.4$ Hz, 2H), 4.33 (t, $J = 5.9$ Hz, 1H), 4.27 (dd, $J = 12.0$ Hz, 1H), 4.11 (dd, $J = 11.8$ Hz, 1H), 3.92–3.74 (m, 2H), 3.04 (m, 2H), 2.01 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.86 (s, 3H), 1.68 (m, 1H), 1.54 (m, 2H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.89 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 174.35$, 174.21, 173.77, 172.39, 171.88, 171.81, 171.27, 158.99, 138.35, 138.08, 130.43 (2CH), 129.52 (2CH), 129.48 (2CH), 129.13 (CH), 129.04 (2CH), 127.79 (CH), 101.96 (CH), 74.00 (CH), 73.08 (CH), 70.15 (CH_2), 70.07 (CH), 67.95 (CH_2), 63.26 (CH_2), 56.34 (CH), 55.31 (CH), 55.04 (CH), 53.19 (CH), 48.04 (CH), 41.99 (CH_2), 38.28 (CH_2), 23.50 (CH_3), 22.89 (CH_3), 22.02 (CH_3), 20.69 (CH_3), 20.57 (CH_3), 20.54 (CH_3); $[\alpha]^{20}_{D}$: -13.2 ($c = 0.25$, CH_3OH); HR-MS (FAB): m/z: 829.3252 $[M]^+$ (calcd for $C_{40}H_{52}N_4O_{15}$ 829.3507).

Synthesis of *N*-terminally protected L-amino acid 2-(dimethylamino)ethyl esters (15): A solution of *N*-terminally protected L-alanine **13** (50 mmol), DMAP (5 mmol) and 2-(dimethylamino)ethanol (**14**, 60 mmol) in CH_2Cl_2 (250 mL) was cooled to 0°C and a solution of dicyclohexyl carbodiimde (DCC, 60 mmol) in CH_2Cl_2 (50 mL) was added. The mixture was stirred overnight at room temperature. After removal of the precipitated urea by filtration, the solution was washed three times with a Na_2CO_3 solution, dried over $MgSO_4$ and the solvent was removed in vacuo. The crude product was purified by flash chromatography (ethyl acetate/methanol 5:1).

***N*-*tert*-Butyloxycarbonyl-L-alanine 2-(dimethylamino)ethyl ester (15a)**: colorless oil (52%); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 5.20$ (d, $J = 6.6$ Hz, 1H), 4.32 (m, 1H), 4.27 (t, $J = 6.9$ Hz, 2H), 2.59 (t, $J = 6.8$ Hz, 2H), 2.29 (s, 6H), 1.44 (s, 9H), 1.39 (d, $J = 7.5$ Hz, 3H); $[\alpha]^{20}_{D}$: -1.0 ($c = 1$, CH_3OH).

***N*-Benzyloxycarbonyl-L-alanine 2-(dimethylamino)ethyl ester (15b)**: colorless oil (51%); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.38$ (m, 5H), 5.52 (d, $J = 7.9$ Hz, 1H), 5.10 (s, 2H), 4.41 (m, 1H), 4.24 (t, $J = 5.0$ Hz, 2H), 2.55 (t, $J = 4.6$ Hz, 2H), 2.26 (s, 6H), 1.40 (d, $J = 6.8$ Hz, 3H); $[\alpha]^{20}_{D}$: -4.0 ($c = 1$, CH_3OH); HR-MS (EI): m/z: 294.1572 $[M]^+$ (calcd for $C_{15}H_{22}N_2O_4$ 294.1579).

***N*-Allyloxycarbonyl-L-alanine 2-(dimethylamino)ethyl ester (15c)**: colorless oil (39%); $R_f = 0.15$ (ethyl acetate/methanol 10:1); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 6.02$–5.78 (brs, 2H), 5.41–5.15 (m, 2H), 4.35 (m, 1H), 4.21 (m, 2H), 2.55 (m, 2H), 2.25 (s, 6H), 1.40 (d, $J = 6.8$ Hz, 3H); $[\alpha]^{20}_{D}$: -14.4 ($c = 2.5$, CH_3OH); HR-MS (EI): m/z: 244.1437 $[M]^+$ (calcd for $C_{11}H_{20}N_2O_4$ 244.1423).

***N*-Allyloxycarbonyl-L-seryl(*O*-diallylphosphato)-L-alanine choline ester bromide (17)**: A solution of *N*-allyloxycarbonyl-L-serine(*O*-diallylphosphate) (**16**, 12.6 mg, 0.036 mmol), HOBt (5.3 mg, 0.039 mmol) and DIC (6.03 µL, 0.039 mmol) in CH_2Cl_2 (3 mL) were added to a solution of L-alanine choline ester bromide hydrobromide (**5a**, 14.2 mg, 0.036 mmol) and tributylamine (8.47 µL, 0.036 mmol) in dry DMF (3 mL). The solution was stirred for 14 h at room temperature and then concentrated in vacuo. The product was precipitated from diethyl ether to yield **17** (16 mg, 85%) as a light yellow oil; ^1H NMR (500 MHz, CD_3OD): $\delta = 6.01$–5.90 (m, 3H), 5.41–5.37 (m, 2H), 5.37–5.34 (m, 2H), 5.28–5.25 (m, 2H), 5.19 (d, $J = 10.5$ Hz, 1H), 4.75 (t, $J = 2.5$ Hz, 2H), 4.58–4.48 (m, 6H), 4.42–4.30 (m, 4H), 3.93 (t, $J = 4.7$ Hz, 2H), 3.32 (s, 9H), 1.45 (d, $J = 7.3$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 171.90$, 170.38, 158.11, 134.12 (CH), 133.72 (2CH), 118.95 ($2CH_2$), 117.70 (CH_2), 69.80 ($2CH_2$), 68.53 (CH_2), 66.70 (CH_2), 65.73 (CH_2), 60.86 (CH_2), 55.67 (CH), 54.60 ($3CH_3$), 49.91 (CH), 16.11 (CH_3); $[\alpha]^{20}_{D}$: -10.9 ($c = 1$, CH_3OH); HR-MS (FAB): m/z: 506.2206 $[M]^+$ (calcd for $C_{21}H_{37}N_3O_9P$ 506.2267).

***N*-Allyloxycarbonyl-L-seryl(*O*-diallylphosphato)-L-alanine (18)**: Butyrylcholine esterase (30 U) was added to a solution of choline ester **17** (15.2 mg, 0.03 mmol) in phosphate buffer (20 mL, pH 6.5, 0.05 M). The reaction mixture was shaken for 64 h at 37 °C. After lyophilisation, the crude solid was extracted once with each $CHCl_3$ (10 mL) and methanol (10 mL). The solvent was removed in vacuo. The residue was dissolved in methanol (2 mL). The product was precipitated by addition of diethyl ether (100 mL) to yield **18** as a colorless oil (9.2 mg, 73 %); ^1H 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, $J = 7.3$ Hz, 3H); $[\alpha]_D^{20}$: -3.0 ($c = 0.5$, CH_3OH); HR-MS (FAB): *m*/*z*: 421.1439 $[M+H]^+$ (calcd for $C_{16}H_{25}N_2O_9P$ 421.1376).

***N*-Allyloxycarbonyl-L-threonine methyl ester (31)**: Triethylamine (1.64 mL, 11.8 mmol) was added to a solution of L-threonine methyl ester (1.0 g, 5.9 mmol) in CH_3CN (50 mL) and at 0 °C allyloxycarbonyl chloride (6.3 mL, 5.9 mmol) was added. After stirring at 0 °C for 30 min. and an additional 18 h at room temperature, the solution was washed three times with HCl (pH 3, 30 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield **31** as colorless crystals (1.2 g, 98 %): m.p.: 65 – 67 °C; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.92$ (ddd, $J = 17.2$, 10.8, 5.4 Hz, 1H), 5.76 (d, $J = 8.8$ hz, 1H), 5.32 (d, $J = 17.0$ Hz, 1H), 5.22 (dd, $J = 10.5$, 1.0 Hz, 1H), 4.59 (m, 2H), 4.33 (m, 2H), 3.77 (s, 3H), 2.81 (brs, 1H), 1.25 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 171.78$, 156.67, 132.51 (CH), 117.82 (CH_2), 67.87 (CH), 66.01 (CH_2), 59.16 (CH), 52.57 (CH_3), 19.83 (CH_3); $[\alpha]_D^{20}$: -17.8 ($c = 1$, $CHCl_3$); anal. calcd for $C_9H_{15}NO_5$ (205.21): C 49.76, H 6.96, N 6.45; found: C 49.86, H 6.85, N 6.35.

***N*-Allyloxycarbonyl-L-threonine(*O*-diallylphosphato) methyl ester (32)**: Bisallyloxy (*N*,*N*-diisopropylamine)phosphoramidite (0.79 mL) was added dropwise during 3 h at 0 °C to a solution of *N*-allyloxycarbonyl-L-threonine methyl ester (700 mg, 3.23 mmol) and tetrazole (270 mg, 3.74 mmol) in CH_2Cl_2 (10 mL). After stirring for 18 h at room temperature, the solution was cooled to 0 °C and *m*-chloroperoxybenzoic acid (2.5 g, 14.5 mmol) was added. After removal of the cooling bath and an additional 2 h of stirring at room temperature the solution was washed twice with a $NaHSO_3$ solution (10 %, 10 mL). The aqueous layer was extracted twice with $CHCl_3$ and the combined organic layers were washed with a saturated $NaHCO_3$ solution (10 mL) and water (10 mL). After drying over $MgSO_4$, the solvent was removed under reduced pressure. Purification by flash chromatography (hexane/ethyl acetate 3:1) yielded **32** as a colorless oil (986 mg, 81 %): $R_f = 0.35$ (hexane/ethyl acetate 1:1); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.92-5.88$ (m, 3H), 5.62 (d, $J = 9.5$ Hz, 1H), 5.39 – 5.20 (m, 6H), 5.05 – 5.00 (m, 1H), 4.61 – 4.44 (m, 7H), 3.78 (s, 3H), 1.43 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 170.03$, 156.43, 132.44 (CH), 132.26 (CH), 132.22 (CH), 118.47 (CH_2), 118.42 (CH_2), 117.97 (CH_2), 75.38 (2s, CH), 68.35 (2s, CH_2), 66.13 (CH_2), 58.36 (d, $J = 7.0$ Hz, CH), 52.82 (CH_3), 18.56 (CH_3); $[\alpha]_D^{20}$: $+16.8$ ($c = 1$, $CHCl_3$); HR-MS (EI): *m*/*z*: 377.1227 $[M]^+$ (calcd for $C_{15}H_{24}NO_8P$ 377.1239); anal. calcd for $C_{15}H_{24}NO_8P$ (377.12): C 47.75, H 6.41, N 3.71; found: C 48.02, H 6.51, N 3.59.

***N*-Allyloxycarbonyl-L-threonine(*O*-diallylphosphato) (19)**: A solution of methyl ester **32** (150 mg, 0.40 mmol) in acetone (3.6 mL) was added to a solution of papain (160 mg) and L-cysteine (130 mg, 1 mmol) in phosphate buffer (36 mL, pH 6.5, 0.01 M). The mixture was shaken at 37 °C for 3 – 4 h. After the biocatalyst was filtered off, the solution was extracted three times with $CHCl_3$ (15 mL) and the combined organic layers were dried over $MgSO_4$. After removal of the solvent under reduced pressure, the product was purified by flash chromatography ($CHCl_3$/ethanol 40:1 → 10:1) to yield **19** as a colorless oil (136 mg, 94 %): $R_f = 0.10$ (hexane/ethyl acetate 1:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.46$ (brs, 1H), 5.98 – 5.85 (m, 3H), 5.60 (d, $J = 9.4$ Hz, 1H), 5.39 – 5.22 (m, 6H), 5.11 (t, $J = 6.3$ Hz, 1H), 4.60 – 4.44 (m, 7H), 1.42 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 170.82$, 156.57, 132.44 (CH), 132.11 (d, $J_{C-P} = 7.1$ Hz, CH), 131.93 (d, $J_{C-P} = 7.1$ Hz, CH), 118.73 (CH_2), 118.67 (CH_2), 117.91 (CH_2), 76.12 (d, $J = 5.2$ Hz, CH), 68.76 (CH_2), 68.71 (CH_2), 66.09 (CH_2), 58.08 (CH), 18.50 (CH_3); $[\alpha]_D^{20}$: $+36.9$ ($c = 1$, CH_3OH); anal. calcd for $C_{14}H_{22}NO_8P$ (363.30): C 46.29, H 6.10, N 3.86; found: C 46.61, H 6.14, N 3.97.

***N*-Allyloxycarbonyl-L-threonyl(*O*-diallylphosphato)-L-glutaminyl-L-threonine choline ester bromide (21)**: Triethylamine (48 µL, 0.18 mmol) and DIC (57 µL) were added to a solution of acid **19** (65 mg, 0.18 mmol), choline ester **20** (103 mg, 0.18 mmol), and HOBt (48 mg, 0.36 mmol) in CH_2Cl_2 (20 mL). The solution was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the crude product was dissolved in methanol (5 mL) and precipitated by addition of diethyl ether (100 mL). The crude precipitate was characterized by NMR and mass spectroscopy and was used without further purification in the subsequent enzymatic deprotection step (82 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 6.01 – 5.94 (m, 3CH, 3H) and 1.35 (t, $NEt_3 \times HBr$, 9H)]; colorless, hygroscopic solid; ^1H NMR (500 MHz, CD_3OD): $\delta = 6.01-5.94$ (m, 3H), 5.42 – 5.20 (m, 6H), 4.89 (m, 1H), 4.75 (m, 1H), 4.63 – 4.52 (m, 8H), 4.48 (d, $J = 3.1$ Hz, 1H), 4.36 (m, 2H), 3.81 (dd, $J = 4.5$ Hz, 2H), 3.22 (s, 9H), 2.37 – 2.34 (m, 2H), 2.24 – 2.17 (m, 1H), 1.99 – 1.94 (m, 1H), 1.33 (d, $J = 6.4$ Hz, 3H), 1.14 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 177.68$, 173.91, 171.31, 171.21, 158.54, 134.12 (CH), 133.82 (d, $J = 7.6$ Hz, CH), 133.72 (d, $J = 7.6$ Hz, CH), 118.85 (CH_2), 118.80 (CH_2), 117.97 (CH_2), 72.80 (CH), 69.20 (CH_2), 69.04 (CH), 67.37 (CH_2), 67.33 (CH_2), 66.93 (CH_2), 60.99 (CH_2), 60.76 (CH), 58.67 (CH), 54.74 (CH_3), 54.71 (CH_3), 54.68 (CH_3), 32.49 (CH_2), 28.77 (CH_2), 20.36 (CH_3), 19.07 (CH_3); HR-MS (FAB): *m*/*z*: 678.2988 $[M]^+$ (calcd for $C_{28}H_{49}N_5O_{12}P$ 678.3115).

***N*-Allyloxycarbonyl-L-threonyl(*O*-diallylphosphato)-L-glutaminyl-L-threonine (22)**: Butyrylcholine esterase (350 U) was added to a solution of choline ester **21** (85 mg, 0.11 mmol) in phosphate buffer (30 mL, pH 6.5, 0.01 M). The mixture was shaken for 5 d at 37 °C. After lyophilisation, the crude product was extracted three times with a mixture of $CHCl_3$ and methanol (10:1) and purified by flash chromatography ($CHCl_3$/methanol 10:1) to yield a white solid (26 mg, 35 %): ^1H NMR (500 MHz, $CDCl_3$): $\delta = 6.01-5.93$ (m, 3H), 5.41 – 5.12 (m, 6H), 4.66 – 4.48 (m, 2H), 4.43 – 4.22 (m, 8H), 4.21 (t, $J = 3.9$ Hz, 1H), 2.36 (t, $J = 5.2$ Hz, 2H), 2.27 – 2.12 (m, 1H), 2.06 – 1.92 (m, 1H), 1.33 (d, $J = 6.4$ Hz, 3H), 1.18 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 177.96$, 175.14, 173.46, 172.43, 158.44, 135.99 (CH), 133.92 (CH), 134.15 (CH), 118.02 (CH_2), 117.86 (CH_2), 116.33 (CH_2), 72.82 (CH), 68.95 (CH_2), 68.72 (CH), 67.82 (CH_2), 66.87 (CH_2), 60.20 (CH), 60.11 (CH), 54.11 (CH), 32.41 (CH_2), 28.84 (CH_2), 20.46 (CH_3), 18.94 (CH_3); $[\alpha]_D^{20}$: -5.5 ($c = 1$, CH_3OH); HR-MS (FAB): *m*/*z*: 615.2084 $[M+Na]^+$ (calcd for $C_{23}H_{37}N_4NaO_{12}P$ 615.2043).

***N*-(4-Phenylacetoxybenzyloxycarbonyl)-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-serine(*O*-*tert*-butyl) choline ester bromide (25)**: DIC (56 µL, 0.36 mmol), HOBt (49 mg, 0.36 mmol), and triethylamine (41 µL, 0.30 mmol) were added to a solution of *N*-(4-phenylacetoxybenzyloxycarbonyl)-L-serine(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)[27] (**27**, 211 mg, 0.30 mmol) and choline ester **28** (150 mg, 0.30 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was stirred for 18 h at room temperature. The majoriy of the solvent was removed under reduced pressure and the crude product precipitated by addition of diethylether (100 mL). This crude precipitate was characterized and used without further purification in the subsequent enzymatic deprotection step [64 %, determined from the NMR spectrum of the crude precipitate by integration of the signals at 2.03 – 1.86 (4s, 4 CH_3CO, 12H) and 1.35 (t, $NEt_3 \times HBr$, 9H)]; light yellow, highly hygroscopic solid; ^1H NMR (500 MHz, CD_3OD): $\delta = 7.44-7.24$ (m, 7H), 7.06 (d, $J = 8.4$ Hz, 2H), 5.21 (t, $J = 9.8$ Hz, 1H), 5.10 (s, 2H), 4.99 (t, $J = 9.5$ Hz, 1H), 4.74 (d, $J = 7.9$ Hz, 1H) 4.68 (m, 2H), 4.52 (m, 1H), 4.46 (t, $J = 6.1$ Hz, 1H), 4.22 (dd, $J = 11.7$, 4.2 Hz, 1H), 4.01 – 3.73 (m, 9H), 3.48 (t, $J = 4.9$ Hz, 2H), 3.21 (s, 9H), 2.03 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.86 (s, 3H), 1.21 (2, 9H); ^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 173.65$, 172.40, 172.02, 171.95, 171.70, 171.29 (7C), 151.91, 133.20, 132.00, 129.66, 128.40, 128.27, 127.99, 127.27, 127.17, 126.39 (9CH), 101.94 (CH), 74.78, 73.96 (CH), 72.79 (CH), 70.00 (CH_2), 68.84 (CH), 65.84 (CH_2), 65.50 (CH_2), 60.83 (CH_2), 60.62 (CH_2), 59.73 (CH_2), 56.28 (CH), 56.17 (CH), 55.12 (CH), 47.81 (3 CH_3), 27.52 (3 CH_3), 22.95 (CH_3), 21.33 (2 CH_3), 20.67 (CH_3); HR-MS (FAB): *m*/*z*: 933.4186 $[M]^+$ (calcd for $C_{45}H_{63}N_4O_{17}$ 933.4345).

***N*-(4-Phenylacetoxybenzyloxycarbonyl)-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-serine(*O*-*tert*-butyl) (29)**: Butyrylcholine esterase (150 U) was added to a solution of choline ester **25** (120 mg, 0.12 mmol) in phosphate buffer (40 mL, pH 6.5, 0.01 M). The reaction mixture was shaken for 3 d at 37 °C. After lyophilization, the remaining residue was extracted three times with $CHCl_3$ (ca. 20 mL), the organic layer was dried over $MgSO_4$ and the product was purified by flash chromatography ($CHCl_3$/methanol 10:1) to yield the product (65 mg, 65 %) as a colorless, amorphous solid: ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.37-7.29$ (m, 7H), 7.02 (d, $J = 8.1$ Hz, 2H), 6.65 (d, $J = 8.5$ Hz, 1H), 5.94 (d, $J = 7.7$ Hz, 1H), 5.27 (t, $J = 9.7$ Hz, 1H), 5.06 (d, $J = 12.5$ Hz, 1H), 5.04 (d, 1H), 5.01 (t, $J = 9.5$ Hz, 1H), 4.70 (d, $J = 8.2$ Hz, 1H) 4.64 (t, $J = 3.7$ Hz, 1H), 4.49 (t, $J = 5.5$ Hz, 1H), 4.22 (dd, $J = 11.7$, 4.9 Hz, 1H), 4.12 (d, $J = 11.7$ Hz, 1H), 3.90 (m, 2H), 3.86 (s, 2H), 3.83 (d, $J = 3.2$ Hz, 1H), 3.73 (m, 2H), 3.59 (dd, $J = 5.0$ Hz, 1H), 2.05 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.82 (s, 3H), 1.15 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 171.91, 170.90, 170.13, 170.06, 169.53 (7C), 155.89, 133.92, 133.32, 129.34, 128.77, 128.70, 128.60, 127.43, 121.61 (9CH), 100.79 (CH), 74.15, 72.38 (CH), 71.82 (CH), 69.25 (CH_2), 68.86, 66.42 (CH_2), 62.14 (CH_2), 61.53 (CH_2), 54.45 (CH), 54.09 (CH), 53.11 (CH), 41.35 (CH_2), 27.34 (3CH_3), 23.16 (CH_3), 20.73 (CH_3), 20.68 (CH_3), 20.64 (CH_3); $[\alpha]_D^{20}$: +3.2 (c = 1, CH_3OH); HR-MS (FAB): *m/z*: 846.3370 $[M+H]^+$ (calcd for $C_{40}H_{51}N_3O_{17}$ 846.3297).

***N*-(4-Phenylacetoxybenzyloxycarbonyl)-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-seryl(*O*-*tert*-butyl)-L-seryl(*O*-*tert*-butyl)-glycine *tert*-butyl ester (24)**: Triethylamine (13.4 µL, 0.096 mmol) was added to a solution of serine derivative **29** (37 mg, 0.044 mmol), L-seryl(*O*-*tert*-butyl)-glycine *tert*-butyl ester hydrotosylate (**26**, 20 mg, 0.044 mmol), and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, 12.9 mg, 0.052 mmol) in CH_2Cl_2 (15 mL)and the solution was stirred for 18 h at room temperature. After washing with HCl (pH 2, 10 mL) and $NaHCO_3$ solution (10 mL, 0.5 M), the organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product was purified by flash chromatography (hexane/ethyl acetate 2:1) to yield a light yellow, amorphous solid (38 mg, 78%): ^1H NMR (500 MHz, CD_3OD): δ = 7.38–7.18 (m, 7H), 7.05 (d, J = 8.2 Hz, 2H), 5.21 (t, J = 9.8 Hz, 1H), 5.10 (s, 2H), 4.98 (t, J = 9.9 Hz, 1H), 4.73 (d, J = 8.0 Hz, 1H) 4.49 (m, 1H), 4.43 (d, J = 5.4 Hz, 1H), 4.29 (m, 1H), 4.12 (m, 1H), 4.03–3.92 (m, 3H), 3.89 (s, 2H), 3.87–3.56 (m, 8H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.45 (s, 9H), 1.19 (s, 9H), 1.18 (s, 9H); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 173.63, 172.38, 172.02, 171.81, 171.77, 171.25, 169.99 (9C), 152.06, 136.47, 131.34, 131.13, 129.78, 129.13, 128.27, 128.12, 127.35, 126.94, 126.63, 126.27, 122.71 (9CH), 102.23 (CH), 82.87, 79.51, 79.24, 74.79 (CH), 73.07 (CH), 70.26 (CH_2), 70.05 (CH), 67.28 (CH_2), 62.59 (CH_2), 62.40 (CH_2), 62.27 (CH_2), 57.63 (CH), 56.29 (CH), 55.40 (CH), 55.25 (CH), 42.80 (CH_2), 41.84 (CH_2), 28.31 (3CH_3), 27.79 (3CH_3), 27.75 (3CH_3), 22.94 (CH_3), 20.76 (CH_3), 20.59 (2CH_3); $[\alpha]_D^{20}$: −0.6 (c = 1, CH_3OH); HR-MS (FAB): *m/z*: 1124.4 $[M+Na]^+$ (calcd for $C_{53}H_{75}NaN_5O_{20}$ 1124.4).

L-Seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-seryl(*O*-*tert*-butyl)-L-seryl(*O*-*tert*-butyl)-glycine *tert*-butyl ester hydrotosylate (30): Penicillin G acylase (300 U) was added to a solution of glycopeptide **24** (51 mg, 0.05 mmol) in a mixture of methanol (33 mL) and phosphate buffer (68 mL, pH 7.5, 0.07 M). The mixture was stirred for 24 h at room temperature. After filtering off the biocatalyst, the methanol was removed under reduced pressure. The aqueous layer was extracted three times with $CHCl_3$ (20 mL). After drying over $MgSO_4$ and removal of the solvent in vacuo the product was purified by flash chromatography ($CHCl_3$/methanol 10:1) to yield **30** as a colorless, amorphous solid (34 mg, 68%); ^1H NMR (500 MHz, CD_3OD): δ = 7.72 (d, J = 8.2 Hz, 2H), 7.24 (d, J = 8.1 Hz, 2H), 5.20 (t, J = 9.8 Hz, 1H), 5.01 (t, J = 9.7 Hz, 1H), 4.75 (d, J = 8.5 Hz, 1H) 4.28 (dd, J = 12.5, 4.5 Hz, 1H), 4.24–4.15 (m, 2H), 4.13 (d, J = 4.5 Hz, 1H), 4.05 (dd, J = 11.0, 8.6 Hz, 1H), 3.99–3.89 (m, 3H), 3.87 (d, J = 2.0 Hz, 1H), 3.86–3.79 (m, 3H), 3.71 (dd, J = 9.5, 5.1 Hz, 1H), 3.65–3.55 (m, 2H), 2.37 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.47 (s, 9H), 1.22 (s, 9H), 1.20 (s, 9H); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 174.03, 172.38, 172.27, 171.71, 171.52, 171.22, 169.98, 167.33, 143.46, 141.74, 129.86 (CH), 126.86 (CH), 101.81 (CH), 82.87, 75.41, 74.89, 73.89 (CH), 73.17 (CH), 69.44 (CH_2), 68.71 (CH), 63.01 (CH_2), 62.72 (CH_2), 62.62 (CH_2), 55.43 (CH), 55.24 (CH), 54.96 (CH), 54.48 (CH), 42.77 (CH_2), 28.29 (3CH_3), 27.70 (6CH_3), 22.91 (CH_3), 21.32 (CH_3), 20.73 (CH_3), 20.58 (CH_3), 20.54 (CH_3); $[\alpha]_D^{20}$: −4.1 (c = 1.5, CH_3OH); HR-MS (FAB): *m/z*: 834.4411 $[M+H]^+$ (calcd for $C_{44}H_{71}N_5O_{19}S$ 834.4348).

***N*-*tert*-Butyloxycarbonyl-L-glutaminyl-L-threonine 2-bromoethyl ester (33)**: Triethylamine (138 µL, 1 mmol) was added to a solution of L-threonine 2-bromoethyl ester hydrotosylate (**2b**, 398 mg, 1 mmol), *N*-*tert*-butyloxycarbonyl-L-glutamine (295 mg, 1.2 mmol) and EEDQ (270 mg, 1.2 mmol) in CH_2Cl_2 (40 mL). The solution was stirred overnight at room temperature. After removal of the solvent in vacuo, the crude product was purified by flash chromatography (ethyl acetate/methanol 10:1) to yield **33** as a light yellow solid (340 mg, 91%): m.p.: 120 °C; R_f = 0.5 (ethyl acetate/methanol 10:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 8.14 (brs, 1H), 6.87 (brs, 1H), 6.39 (brs, 1H), 5.50 (d, J = 7.7 Hz, 1H), 4.64 (dd, J = 8.8 Hz, 1H), 4.53 (t, J = 6.0 Hz, 1H), 4.45 (dt, J = 6.0 Hz, 2H), 4.28 (t, J = 7.6 Hz, 1H), 3.55 (t, J = 6.1 Hz, 2H), 2.39 (t, J = 6.0 Hz, 2H), 2.05 (m, 2H), 1.43 (s, 9H), 1.27 (d, J = 6.4 Hz, 3H); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 175.99, 172.29, 171.17, 156.00, 80.35, 67.81 (CH), 64.86 (CH_2), 58.05 (CH), 53.12 (CH), 31.83 (CH_2), 29.65 (CH_2), 28.35 (CH_2), 28.36 (3CH_3), 20.04 (CH_3); $[\alpha]_D^{20}$: −14.5 (c = 1, CH_3OH); anal. calcd for $C_{16}H_{28}BrN_3O_7 \times 0.5\,H_2O$ (454.32): C 41.48, H 6.31, N 9.07; found: C 41.48, H 6.60, N 9.07.

***N*-*tert*-Butyloxycarbonyl-L-glutaminyl-L-threonine choline ester bromide (34)**: Trimethylamine (ca. 3 mL) was added at −78 °C to a solution of *N*-*tert*-butyloxycarbonyl-L-glutaminyl-L-threonine 2-bromoethyl ester (**33**, 1.0 g, 2.2 mmol) in acetone (70 mL) and the solution was then stirred at room temperature for 3 d. After removal of the solvent under reduced pressure, the crude product was dissolved in methanol (10 mL) and purified by precipitation with diethly ether (100 mL) to give **34** (1.15 g) in a quantitative yield as a white, highly hygroscopic solid; ^1H NMR (400 MHz, CD_3OD): δ = 4.62 (brs, 2H), 4.47 (d, J = 2.9 Hz, 1H), 4.35 (m, J = 2.9 Hz, J = 6.2 Hz, 1H), 4.16 (dd, J = 5.1 Hz, 1H), 3.78 (t, J = 4.6 Hz, 2H), 3.24 (s, 9H), 2.36 (t, J = 7.4 Hz, 2H), 2.10 (ddt, J = 12.5, 7.3 Hz, 1H), 1.88 (ddt, J = 12.5, 7.3 Hz, 1H), 1.45 (s, 9H), 1.22 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100.6 MHz, CD_3OD): δ = 177.79, 175.41, 171.25, 157.92, 80.79, 68.11 (CH), 65.92 (CH_2), 59.88 (CH_2), 59.34 (CH), 55.54 (CH), 54.50 (3CH_3), 32.52 (CH_2), 28.92 (CH_2), 28.71 (3CH_3), 20.19 (CH_3); $[\alpha]_D^{20}$: −18.7 (c = 1, CH_3OH); anal. calcd for $C_{19}H_{37}BrN_4O_7 \times 0.5\,H_2O$ (522.43): C 43.68, H 7.33, N 10.72; found: C 43.35, H 7.38, N 11.01.

L-Glutaminyl-L-threonine choline ester bromide hydrobromide (20): The Boc protecting group was removed as described for **5a–d**. White, hygroscopic solid (91%); ^1H NMR (400 MHz, CD_3OD): δ = 4.67 (brs, 2H), 4.59 (d, J = 3.0 Hz, 1H), 4.42 (m, 1H), 4.25 (t, J = 5.7 Hz, 1H), 3.88 (t, J = 1.8 Hz, 2H), 3.27 (s, 9H), 2.63 (t, J = 7.4 Hz, 2H), 2.21 (m, 2H), 1.27 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100.6 MHz, CD_3OD): δ = 177.58, 171.10, 170.45, 67.84 (CH), 65.87 (CH_2), 60.12 (CH_2), 59.73 (CH), 54.64 (3CH_3), 53.57 (CH), 31.50 (CH_2), 28.40 (CH_2), 20.31 (CH_3); $[\alpha]_D^{20}$: +2.4 (c = 0.45, CH_3OH); anal. calcd for $C_{14}H_{30}Br_2N_4O_5 \times 0.5\,H_2O$ (503.23): C 28.79, H 5.52, N 9.59; found: C 28.72, H 5.81, N 9.69.

***N*-Benzyloxycarbonyl-L-serine(*O*-*tert*-butyl) 2-bromoethyl ester (36)**: A solution of *tert*-butyl-trichloroacetimidate (765 mg, 3.5 mmol) in cyclohexane (10 mL) was added to a solution of *N*-benzyloxycarbonyl-L-serine 2-bromoethyl ester (**35**, 1.1 g, 3.2 mmol) in CH_2Cl_2/cyclohexane (10 mL, 1:1). After stirring for 10 min, $BF_3 \times Et_2O$ (64 µL, 0.5 mmol) was added. After an additional 30 min the precipitate of trichloroacetic acid amide was filtered off and the solvent was removed in vacuo. Flash chromatography (hexane/ethyl acetate 2:1) yielded the product as a colorless oil (821 mg, 64%): R_f = 0.66 (hexane/ethyl acetate 2:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.35 (m, 5H), 5.62 (d, J = 8.8 Hz, 1H), 5.13 (s, 2H), 4.51–4.41 (m, 3H), 4.01 (dd, J = 8.9, 2.8 Hz, 1H), 3.59 (dd, J = 8.9, 3.1 Hz, 1H), 3.49 (ddd, J = 6.2, 4.8, 1.4 Hz, 2H), 1.13 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 170.28, 156.15, 136.24, 128.54 (2CH), 128.21 (CH), 128.17 (2CH), 73.50, 67.06 (CH_2), 64.56 (CH_2), 61.95 (CH_2), 54.58 (CH), 28.25 (CH_2), 17.29 (3CH_3); $[\alpha]_D^{20}$: −4.2 (c = 1.75, $CHCl_3$); HR-MS (EI): *m/z*: 401.0849 $[M]^+$ (calcd for $C_{17}H_{24}BrNO_5$ 401.0838); anal. calcd for $C_{17}H_{24}BrNO_5$ (401.08): C 50.76, H 6.01, N 3.48; found: C 50.97, H 6.00, N 3.44.

***N*-Benzyloxycarbonyl-L-serine(*O*-*tert*-butyl) choline ester bromide (37)**: Trimethylamine (ca. 5 mL) was added at −78 °C to a solution of *N*-benzyloxycarbonyl-L-serine(*O*-*tert*-butyl) 2-bromoethyl ester (**36**, 85 mg, 0.21 mmol) in acetone (20 mL). The solution was allowed to warm to room temperature and was stirred for 3 d. Removal of the solvent in vacuo yielded the product (97 mg, 99%) as a colorless, hygroscopic solid: ^1H NMR (500 MHz, CD_3OD): δ = 7.38–7.32 (m, 5H), 5.11 (s, 2H), 4.60 (dd, J = 13.6 Hz, 2H), 4.41 (t, J = 4.4 Hz, 1H), 3.81–3.76 (m, 3H), 3.67 (dd, J = 9.4, 4.3 Hz, 1H), 3.21 (s, 9H), 1.17 (s, 9H); ^{13}C NMR (125.7 MHz, CD_3OD): δ = 171.50, 158.37, 138.10, 129.53 (2CH), 129.13 (CH), 128.94 (2CH), 74.84, 67.74 (CH_2), 62.73 (CH_2), 59.84 (CH_2), 56.35 (CH), 54.59 (3CH_3), 28.25 (CH_2), 27.65 (3CH_3); $[\alpha]_D^{20}$: −3.5 (c = 0.55, CH_3OH); HR-MS (FAB): *m/z*: 381.2429 $[M]^+$ (calcd for $C_{20}H_{33}N_2O_5$ 381.2398); anal. calcd for $C_{20}H_{33}BrN_2O_5 \times 2.5\,H_2O$ (506.43): C 47.43, H 7.56, N 5.53; found: C 47.86, H 7.83, N 5.76.

L-Serine(*O*-*tert*-butyl) choline ester bromide hydrotosylate (28): A catalytic amount of Pd/C (ca. 10 mg) was added to a solution of *N*-benzyloxycarbonyl-L-serine(*O*-*tert*-butyl) choline ester bromide (**37**, 97 mg, 0.21 mmol) and *p*-toluenesulfonic acid (40 mg, 0.21 mmol) in methanol (25 mL). The solution was stirred for 6 h under a H_2 atmosphere. After filtering the solvent was removed under reduced pressure to yield the product (35 mg, 99%) as a colorless, hygroscopic solid: ^1H NMR (500 MHz, CD_3OD): δ = 7.71 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H) 4.63 (dd, J = 13.6, 2.7 Hz, 2H), 4.41 (t, J = 3.7 Hz, 1H), 3.92 (dd, J = 10.5, 4.3 Hz, 1H), 3.85 (t, $J=3.0$ Hz, 2H), 3.83 (dd, $J=10.4$, 5.0 Hz, 1H), 3.25 (s, 9H), 2.37 (s, 3H), 1.21 (s, 9H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta = 168.30$, 143.49, 141.82, 129.92 (2CH), 126.89 (2CH), 75.58, 65.56 (CH$_2$), 60.68 (CH$_2$), 60.65 (CH$_2$), 54.84 (CH), 54.51 (3CH$_3$), 27.53 (3CH$_3$), 21.33 (CH$_3$); $[\alpha]_D^{20}$: −0.7 ($c=0.6$, CH$_3$OH); HR-MS (FAB): *m/z*: 247.2066 $[M-\mathrm{HBr}_2]^+$ (calcd for $C_{12}H_{28}N_2O_3$ 247.2022); anal. calcd for $C_{19}H_{35}BrN_2O_6S$ (499.46): C 45.59, H 7.06, N 5.61; found: C 45.16, H 6.79, N 5.57.

Glycine *tert*-butyl ester hydrotosylate (39): A catalytic amount of Pd/C was added to a solution of *N*-benzyloxycarbonyl-glycine *tert*-butyl ester (**38**, 1.6 g, 5.9 mmol) and *p*-toluenesulfonic acid (1.08 g, 5.8 mmol) in methanol (35 mL). The mixture is stirred for 12 h under a H$_2$ atmosphere. After removal of the catalyst the solvent was removed under reduced pressure to yield **39** as a white solid (1.7 g, 96%): ^{1}H NMR (250 MHz, CD$_3$OD): $\delta =$ 7.70 (d, $J=8.2$ Hz, 2H), 7.24 (d, $J=8.0$ Hz, 2H), 3.71 (2, 2H), 2.37 (s, 3H), 1.51 (s, 9H); anal. calcd ($C_{13}H_{21}NO_5S \times 0.5\,H_2O$): C 49.98, H 7.10, N 4.48; found: C 50.09, H 7.00, N 4.45.

***N*-Benzyloxycarbonyl-L-seryl-glycine *tert*-butyl ester (40)**: Triethylamine (320 µmol) was added to a solution of glycine *tert*-butyl ester hydrotosylate (**39**, 690 mg, 2.3 mmol) in CH$_2$Cl$_2$ (20 mL). Then a solution of *N*-benzyloxycarbonyl-L-serine (550 mg, 2.3 mmol) and EEDQ (720 mg, 2.9 mmol) in DMF (5 mL) was added. The mixture was stirred for 18 h at room temperature before washing with HCl (pH 3) and a 0.5 M NaHCO$_3$ solution. After drying over MgSO$_4$ and removal of the solvent under reduced pressure the product is purified by flash chromatography (hexane/ethyl acetate 4:1) to yield a colorless oil (665 mg, 62%): $R_f = 0.3$ (hexane/ethyl acetate 2:1); ^{1}H NMR (250 MHz, CDCl$_3$): $\delta = \delta = 7.35$–7.29 (m, 5H), 7.02 (brs, 1H), 5.92 (d, $J=6.1$ Hz, 1H), 5.12 (s, 1H), 4.29 (brs, 1H), 4.11 (m, 1H), 3.93 (d, $J=4.6$ Hz, 2H), 3.71 (dd, $J=7.7$ Hz, $J=8.6$ Hz, 1H), 1.44 (s, 9H).

***N*-Benzyloxycarbonyl-L-seryl(*O-tert*-butyl)-glycine *tert*-butyl ester (41)**: A solution of *tert*-butyl-trichloroacetimidate (312 mg, 1.4 mmol) in cyclohexane (5 mL) was added to a solution of *N*-benzyloxycarbonyl-L-seryl-glycine *tert*-butyl ester (**40**, 450 mg, 1.3 mmol) in CH$_2$Cl$_2$ (15 mL). After 10 min stirring at room temperature BF$_3$ × Et$_2$O (27 µL, 0.2 mmol) was added and the mixture was stirred for an additional 5 h. After removal of the solvent under reduced pressure the product was purified by flash chromatography (hexane/ethyl acetate 4:1) to yield **41** as a colorless oil (236 mg, 61%): $R_f = 0.7$ (hexane/ethyl acetate 1:1); ^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 7.37$–7.29 (m, 5H), 7.19 (d, 1H), 6.02 (d, $J=6.2$ Hz, 1H), 5.12 (s, 2H), 4.27 (brs, 1H), 3.93 (d, $J=4.6$ Hz, 2H) 3.83 (brs, 1H), 3.41 (dd, $J=7.7$ Hz, $J=8.6$ Hz, 1H), 1.20 (s, 9H); ^{13}C NMR (125.7 MHz, CDCl$_3$): $\delta =$ 170.32, 168.49, 156.12, 136.18, 128.51 (2CH), 128.16 (CH), 128.10 (2CH), 82.17, 74.18, 67.03 (CH$_2$), 61.60 (CH$_2$), 54.58 (CH), 42.18 (CH$_2$), 28.00 (3CH$_3$), 27.32 (3CH$_3$); $[\alpha]_D^{20}$: +14.3 ($c=0.45$, CHCl$_3$); HR-MS (EI): *m/z*: 408.2248 $[M]^+$ (calcd. for $C_{21}H_{23}N_2O_6$ 408.2260).

L-Seryl(*O-tert*-butyl)-L-glycine *tert*-butyl ester hydrotosylate (26): A catalytic amount of Pd/C was added to a solution of *N*-benzyloxycarbonyl-L-seryl(*O-tert*-butyl)-glycine *tert*-butyl ester (**41**, 80 mg, 0.20 mmol) and *p*-toluenesulfonic acid (37 mg, 0.20 mmol) in methanol (15 mL). The mixture was stirred in an H$_2$ atmosphere for 12 h, the catalyst was filtered off and the solvent was removed in vacuo to yield the product as a colorless wax (83 mg, 95%); ^{1}H NMR (500 MHz, CD$_3$OD): $\delta = 4.29$ (brs, 3H), 4.08 (m, 1H), 3.97 (dd, $J=8.3$ Hz, 1H) 3.74 (m, 2H), 3.42 (s, 2H), 1.48 (s, 9H), 1.23 (2, 9H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta = 171.92$, 170.20, 83.02, 75.05, 63.22 (CH$_2$), 55.66 (CH), 42.72 (CH$_2$), 28.26 (3CH$_3$), 27.63 (3CH$_3$); $[\alpha]_D^{20}$: −5.1 ($c=1$, CH$_3$OH); HR-MS (FAB): *m/z*: 275.2028 $[M+H]^+$ (calcd for $C_{20}H_{34}N_2O_7S$ 275.1971).

***N*-Allyloxycarbonyl-L-threonyl(*O*-diallylphosphato)-L-glutaminyl-L-threonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-seryl(*O-tert*-butyl)-L-seryl(*O-tert*-butyl)-glycine *tert*-butyl ester (42)**: EDC (2.7 mg, 13.9 µmol), HOBt (1.9 mg, 1.9 µmol), and triethylamine (3.5 µL, 25.2 µmol) was added to a solution of tripeptide **22** (6.9 mg, 11.6 µmol) and tetrapeptide **30** (11.7 mg, 11.6 µmol) in CH$_2$Cl$_2$ (15 mL). The mixture was stirred overnight at room temperature. After washing with HCl (pH 2, 10 mL) and a 0.5 M NaHCO$_3$ solution (10 mL), the organic layer was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The product was purified by flash chromatography (CHCl$_3$/methanol 10:1 → methanol) to yield a colorless, amorphous solid (12.1 mg, 74%): ^{1}H NMR (500 MHz, CD$_3$OD): $\delta = 5.95$–5.90 (m, 3H), 5.40–5.23 (m, 6H), 5.11 (m, 1H), 4.93 (m, 1H), 4.70–4.31 (m, 16H), 4.13–3.86 (m, 6H), 3.78–3.73 (m, 3H), 3.65–3.50 (m, 2H), 2.36 (m, 2H), 2.11–2.05 (m, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.92 (m, 1H), 1.46 (s, 9H), 1.34 (d, $J=7.1$ Hz, 3H), 1.22 (s, 9H), 1.20 (s, 9H), 1.18 (m, 3H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta = 177.77$, 174.65, 172.35, 171.96, 171.91, 170.33, 169.92 (12C), 158.46, 134.06 (CH), 133.71 (2CH), 118.32 (CH$_2$), 118.01 (CH$_2$), 117.96 (CH$_2$), 101.58 (CH), 82.83, 76.95 (CH), 75.30, 74.87, 69.75 (CH), 69.70 (CH), 68.75 (CH$_2$), 68.71 (CH), 68.33 (CH$_2$), 68.07 (CH), 67.83 (CH$_2$), 67.06 (CH$_2$), 62.61 (CH$_2$), 62.59 (2CH$_2$), 60.12 (CH), 59.33 (CH), 56.19 (3CH), 55.37 (CH), 54.34 (CH), 42.80 (CH$_2$), 32.32 (CH$_2$), 30.68 (CH$_2$), 28.31 (3CH$_3$), 27.74 (6CH$_3$), 22.93 (CH$_3$), 20.87 (CH$_3$), 20.63 (2CH$_3$), 18.84 (CH$_3$), 18.77 (CH$_3$); $[\alpha]_D^{20}$: −7.0 ($c=0.5$, CH$_3$OH); HR-MS (FAB): *m/z*: 1430.0 $[M+\mathrm{Na}]^+$ (calcd for $C_{60}H_{98}NaN_9O_{27}P$ 1430.1).

L-Threonyl(*O*-phosphato)-L-glutaminyl-L-threonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-seryl(*O-tert*-butyl)-L-seryl(*O-tert*-butyl)-glycine *tert*-butyl ester (43): A catalytic amount of [Pd(PPh$_3$)$_4$] (ca. 5–10 mg) was added to a solution of fully protected heptapeptide **42** (12.1 mg, 8.5 µmol) in THF (5 mL). After 5 min stirring under argon, a solution of formic acid (2.0 mg, 43 µmol) in THF (1 mL) and a solution of *n*-butylamine (1.5 mL, 21 µmol) in THF (0.5 mL) were added. The solution was stirred for 1 h at room temperature. After removal of the solvent under reduced pressure, the crude product was purificatied by flash chromatography (CHCl$_3$/methanol 5:1 → methanol) to yield a light yellow, amorphous solid (10.2 mg, 86%): ^{1}H NMR (500 MHz, CD$_3$OD): $\delta = 5.03$ (m, 1H), 4.94 (m, 1H), 4.83–4.49 (m, 10H), 3.96–3.72 (m, 11H), 2.37 (m, 2H), 2.10–2.05 (m, 1H), 2.07 (s, 3H), 2.01 (s, 6H), 1.98 (s, 3H), 1.93 (m, 1H), 1.47 (s, 9H), 1.28–1.22 (m, 6H), 1.22 (s, 9H), 1.20 (s, 9H); HR-MS (MALDI-TOF): *m/z*: 1243.11 $[M+H]^+$ (calcd for $C_{50}H_{86}N_9O_{25}P$ 1244.24).

L-Threonyl(*O*-phosphato)-L-glutaminyl-L-threonyl-L-seryl(*O*-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-seryl-L-seryl-glycine (44): A solution of heptapeptide **43** (10 mg, 8.0 µmol) in trifluoroacetic acid (1.6 mL) was stirred for 2 h at 0 °C. After codistillation with toluene (5 × s under reduced pressure) the product was precipitated by addition of diethylether to yield a light yellow, amorphous solid (7.8 mg, 84%): ^{1}H NMR (500 MHz, CD$_3$OD): $\delta = 5.03$ (m, 1H), 4.94 (m, 1H), 4.70–4.31 (m, 10H), 4.13–3.86 (m, 6H), 3.78–3.73 (m, 3H), 3.65–3.50 (m, 2H), 2.34 (m, 2H), 2.11–2.05 (m, 1H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.92 (m, 1H), 1.34 (d, $J=7.1$ Hz, 3H), 1.18 (m, 3H); HR-MS (MALDI-TOF): *m/z*: 1077.01 $[M-H]^+$ (calcd for $C_{38}H_{62}N_9O_{25}P$ 1075.92).

L-Threonyl(*O*-phosphato)-L-glutaminyl-L-threonyl-L-seryl(*O*-2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-L-seryl-L-seryl-glycine (23): Hydrazine monohydrate (0.5 mL, 10.3 µmol) was added to a solution of heptapeptide **44** (8 mg, 7.4 µmol) in methanol (3 mL). The solution was stirred for 3 h at room temperature, cooled to 0 °C, and acetone (3 mL) was added. After 1 h the solvent was removed in vacuo and the crude residue was codistilled with acetone (5 × under reduced pressure) to yield a light yellow, amorphous solid (3.9 mg, 56%): ^{1}H NMR (500 MHz, CD$_3$OD): $\delta = 5.11$ (m, 1H), 4.93 (m, 1H), 4.70–4.31 (m, 10H), 4.13–3.86 (m, 6H), 3.78–3.73 (m, 3H), 3.65–3.50 (m, 2H), 2.34 (m, 2H), 2.11–2.05 (m, 1H), 2.04 (s, 3H), 1.92 (m, 1H), 1.34 (d, $J=7.0$ Hz, 3H), 1.17 (m, 3H); ^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta = 175$–169 (9C), 101.04 (CH), 77.68 (CH), 75.23 (CH), 74.75 (CH), 73.59 (CH$_2$), 71.84 (CH$_2$), 68.45 (CH), 68.08 (CH), 64.90 (2CH$_2$), 62.52 (CH), 58.99 (CH), 56.81 (CH), 55.52 (CH), 52.48 (CH), 44.64 (CH$_2$), 31.65 (CH$_2$), 30.68 (CH$_2$), 24.20 (CH$_3$), 16.79 (CH$_3$), 16.36 (CH$_3$); $[\alpha]_D^{20}$: −0.42 ($c=0.5$, CH$_3$OH); HR-MS (MALDI-TOF): *m/z*: 950.36 $[M-H]^+$ (calcd for $C_{32}H_{56}N_9O_{22}P$ 949.811).

***N*-(Biotinyl-amidocaproyl)-L-threonyl(*O*-phosphato)-L-glutaminyl-L-threonyl-L-seryl(*O*-2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-L-seryl-L-seryl-glycine (46)**: A solution of (+)-biotinyl amidocaproic acid *N*-hydroxysuccinimide ester (**45**, 6.4 mg, 13.5 µmol) in DMF (1 mL) was added to a solution of fully deprotected heptapeptide **23** (8.5 mg, 9.0 µmol) in DMF (1 mL) at 0 °C. After stirring for 1 h at 0 °C, the solution was allowed to warm to room temperature and stirred overnight. After removal of the solvent in vacuo, the crude product was dissolved in water (2 mL), filtered and extracted twice with CHCl$_3$ (10 mL). Lyophilisation of the aqueous layer yielded a yellow, amorphous solid (7.9 mg, 68%): ^{1}H NMR (500 MHz, CD$_3$OD): $\delta = 5.10$ (m, 1H), 4.90 (m, 1H), 4.80–4.67 (m, 7H), 4.59 (dd, $J=8.9$, 5.2 Hz, 1H), 4.50–4.45 (m, 3H), 4.30 (dd, 1H, $J=8.0$, 4.3 Hz, 1H), 3.91–3.87 (m, 6H), 3.67–3.62 (m, 3H), 3.51 (m, 2H), 3.24–3.10 (m, 3H), 2.91 (dd, $J=12.7$, 5.0 Hz, 2H), 2.67–2.63 (m, 2H), 2.41–2.07 (m, 6H), 2.02 (s, 3H), 1.95–1.86 (m, 2H), 1.79–1.30 (m, 13H), 1.27–1.12

(m, 3H); $[\alpha]_D^{20}$: +17.7 ($c = 0.35$, CH_3OH); MS (FAB): m/z: 1327.8 $[M+Na]^+$ (calcd for $C_{48}H_{81}N_{12}NaO_{26}PS$ 1327.4).

Acknowledgement

This research was supported by the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft.

---

[1] a) T. Hunter, M. Karin, *Cell* **1992**, *70*, 375–387; b) A. Levitzki, *Eur. J. Biochem.* **1994**, *226*, 1–13; c) P. J. Casey, *Science* **1995**, *268*, 221–225; d) S. E. Egan, R. A. Weinberg, *Nature* **1993**, *365*, 781–783; e) M. S. Boguski, F. McCormick, *Nature* **1993**, *366*, 643–654.

[2] *Protein Phosphorylation* (Ed.: F. Marks), Wiley-VCH, Weinheim, **1996**.

[3] Review: G. W. Hart, L. K. Kreppel, F. I. Comer, C. S. Arnold, D. M. Snow, Z. Ye, X. Cheng, D. DellaManna, D. S. Caine, B. J. Earles, Y. Akimoto, R. N. Cole, B. K. Hayes, *Glycobiology* **1996**, *6*, 711–716.

[4] a) S. P. Jackson, R. Tjian, *Cell* **1988**, *55*, 125–133; b) S. Lichtsteiner, U. Schibler, *Cell* **1989**, *57*, 1179–1187.

[5] a) M. L. Privalsky, *J. Virol.* **1990**, *64*, 463–466; b) T.-Y. Chou, G. W. Hart, C. V. Dang, *J. Biol. Chem.* **1995**, *270*, 18961–18965.

[6] a) B. Datta, M. K. Ray, D. Chakrabarti, D. E. Wylie, N. K. Gupta, *J. Biol. Chem.* **1989**, *264*, 20620–20624; b) W. Meikrantz, D. M. Smith, M. M. Sladicka, R. A. Schlegel, *J. Cell. Sci.* **1991**, *98*, 303–307.

[7] a) K. G. Mullis, R. S. Haltiwanger, G. W. Hart, R. B. Marchase, J. A. Engler, *J. Virol.* **1990**, *64*, 5317–5323; b) M. Whitford, P. Faulkner, *J. Virol.* **1992**, *66*, 3324–3329; c) S. A. Gonzalez, O. R. Burrone, *Virology* **1992**, *182*, 8–16.

[8] a) C. Norman, M. Runswick, R. Pollock, R. Treisman, *Cell* **1988**, *55*, 989–1003; b) J. M. Almendral, D. Sommer, H. MacDonald-Bravo, J. Burckhardt, J. Perera, R. Bravo, *Mol. Cell Biol.* **1988**, *8*, 2140–2148.

[9] a) T. Carter, I. Vancurova, I. Sun, W. Lou, S. DeLeon, *Mol. Cell Biol.* **1990**, *10*, 6460–6471; b) S. P. Lees-Miller, Y. R. Chen, C. W. Anderson, *Mol. Cell Biol.* **1990**, *10*, 6472–6481.

[10] B. E. Kemp, R. B. Pearson, *Trends Biochem. Sci.* **1990**, *15*, 342–346.

[11] S.-H. Liu, J.-T. Ma, A. Y. Yueh, S. P. Lees-Miller, C. W. Anderson, S.-Y. Ng, *J. Biol. Chem.* **1993**, *268*, 21147–21154.

[12] A. J. Reason, M. R. Morris, M. Panico, R. Marais, R. H. Treisman, R. S. Haltiwanger, *J. Biol. Chem.* **1992**, *267*, 16911–16921.

[13] K. Hinterding, D. Alonso-Díaz, H. Waldmann, *Angew. Chem.* **1998**, *110*, 716–718; *Angew. Chem. Int. Ed.* **1998**, *37*, 688–749.

[14] For a very instructive example see: T. Tagawa, T. Kuroki, P. K. Vogt, K. Chida, *J. Cell Biol.* **1995**, *130*, 255–263; b) H. Waldmann, M. Schelhaas, E. Nägele, J. Kuhlmann, A. Wittinghofer, H. Schroeder, J. R. Silvius, *Angew. Chem.* **1997**, *109*, 2077–2080; *Angew. Chem. Int. Ed.* **1997**, *36*, 2238–2241; c) H. Schroeder, R. Leventis, S. Rex, M. Schelhaas, E. Nägele, H. Waldmann, J. R. Silvius, *Biochemistry* **1997**, *36*, 13102–13109.

[15] M. Schelhaas, H. Waldmann, *Angew. Chem.* **1997**, *108*, 2190–2219; *Angew. Chem. Int. Ed.* **1997**, *35*, 2056–2083.

[16] H. Waldmann, D. Sebastian, *Chem. Rev.* **1994**, *94*, 911–937.

[17] a) F. Eisele, D. Owen, H. Waldmann, *Bioorg. Med. Chem.* **1999**, *7*, 193–224; b) T. Kappes, H. Waldmann, *Liebigs Ann./Recueil* **1997**, 808–813; c) T. Schmittberger, H. Waldmann, *Synlett* **1998**, 574–584.

[18] Part of this work was published in preliminary form: J. Sander, H. Waldmann, *Angew. Chem.* **1999**, *111*, 1337–1339; *Angew. Chem. Int. Ed.* **1999**, *38*, 1250–1252.

[19] M. Buchholz, H. Kunz, *Liebigs Ann. Chem.* **1983**, 1859–1885.

[20] a) M. Schelhaas, S. Glomsda, M. Hänsler, H.-D. Jakubke, H. Waldmann, *Angew. Chem.* **1996**, *108*, 82–83; *Angew. Chem. Int. Ed.* **1996**, *35*, 106–109; b) M. Schelhaas, E. Nägele, N. Kuder, B. Bader, J. Kuhlmann, A. Wittinghofer, H. Waldmann, *Chem. Eur. J.* **1999**, *5*, 1239–1252; c) A. Cotté, B. Bader, J. Kuhlmann, H. Waldmann, *Chem. Eur. J.* **1999**, *5*, 922–936.

[21] S. Flohr, V. Jungmann, H. Waldmann, *Chem. Eur. J.* **1999**, *5*, 669–581.

[22] a) G. Arsequell, L. Krippner, R. A. Dwek, S. Y. C. Wong, *J. Chem. Soc. Chem. Commun.* **1994**, 2383–2384; b) G. Arsequell, J. S. Haurum, T. Elliott, R. A. Dwek, A. C. Lellouch, *J. Chem. Soc. Perkin Trans. 1* **1995**, 1739–1745.

[23] J. S. Ralston, A. R. Maier, B. F. Kilpatrick, A. L. Chasson, *Biochem. J.* **1983**, *211*, 243–250.

[24] M. Gewehr, H. Kunz, *Synthesis* **1997**, 1499–1511.

[25] D. Sebastian, A. Heuser, S. Schulze, H. Waldmann, *Synthesis* **1997**, 1098–1108.

[26] a) P. Schultheiss-Reimann, H. Kunz, *Angew. Chem.* **1983**, *95*, 64–65; *Angew. Chem. Int. Ed.* **1993**, *22*, 62–63; b) P. Sjölin, M. Elofsson, J. Kihlberg, *J. Org. Chem.* **1996**, *61*, 560–565.

[27] T. Pohl, H. Waldmann, *J. Am. Chem. Soc.* **1997**, *119*, 6702–6710 and references therein.

[28] T. Kappes, A. Gum. H. Waldmann, unpublished results.

[29] A. Armstrong, I. Brackenridge, R. F. W. Jackson, J. M. Kirk, *Tetrahedron Lett.* **1988**, *29*, 2483–2486.

[30] a) E. Vowinkel, *Chem. Ber.* **1967**, *100*, 16–20; b) J. F. Callahan, K. A. Newlander, J. L. Burgess, D. S. Eggleston, A. Nichols, *Tetrahedron* **1993**, *49*, 3479–3488.

[31] Y. Hayakawa, H. Kato, M. Uchiyama, H. Kajino, R. Noyori, *J. Org. Chem.* **1986**, *51*, 2402–2404.

[32] a) H. Kunz, H. Waldmann, J. März, *Liebigs Ann. Chem.* **1989**, 45–49; b) C. Unverzagt, H. Kunz, *Bioorg. Med. Chem.* **1994**, *2*, 1189–1201; c) S. Peters, T. L. Lowary, O. Hindsgaul, M. Meldal, K. Bock, *J. Chem. Soc. Perkin Trans. 1* **1995**, 3017–3022.

[33] G. T. Hermanson, *Bioconjugate Techniques*, *Academic Press*, San Diego, **1996**.

Received: August 10, 1999 [F1969]