Selective Enzymatic Removal of Protecting Groups from Phosphopeptides: Chemoenzymatic Synthesis of a Characteristic Phosphopeptide Fragment of the Raf-1 Kinase

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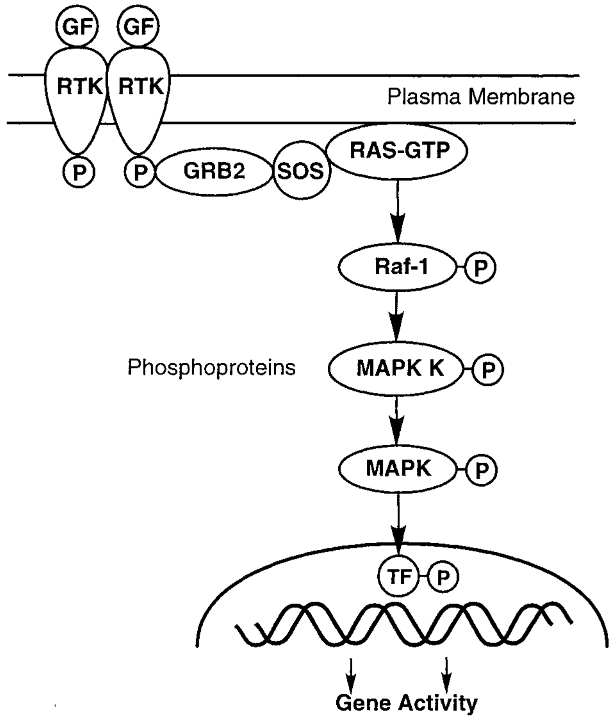
Base-labile biologically relevant phosphopeptides can advantageously be synthesized under very mild conditions and without undesired side reactions by employing the heptyl (Hep) ester as *C*-terminal or the phenylacetamide (PhAc) as *N*-terminal enzyme-labile protecting group. The lipase-mediated saponification of the heptyl ester blocking function was successfully applied in the construction of a characteristic selectively phosphorylated hydroxyamino acid fragment of Raf-1 kinase, an important member of the so-called Ras signal transduction pathway.



GF = Growth Factor
RTK = Receptor Tyrosine Kinase
TF = Transcription Factor

Figure. Phosphorylated proteins in the Ras pathway of signal transduction.

Introduction

The transmission of extracellular signals to their intracellular targets is mediated by a complex network of interacting proteins that regulate a large number of different cellular processes. In particular, the phosphorylation and dephosphorylation of proteins on serine, threonine and tyrosine residues play key roles in the events determining the intracellular response to different extracellular stimuli. Such covalent protein modifications are employed by a variety of organisms for numerous purposes, e.g. the regulation of the transcription of the genetic code by transcription factors, the regulation of cell growth and proliferation and also the control of the cell cycle.[1]

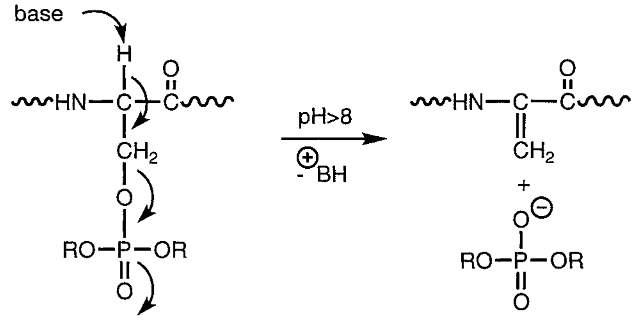
An illustrative and particularly relevant example for a signal-transducing protein which is regulated by phosphorylation is the Raf-1 serine/threonine kinase – a product of the c-raf gene, the normal cellular counterpart of v-raf, the transforming gene of murine sarcoma virus.[2] Raf is expressed in numerous different cell types and represents an important member of the so-called Ras signal transduction pathway by which extracellular signals are transduced across the plasma membrane to the cell nucleus.[3,4] In this pathway growth factors or mitogens induce an autophosphorylation of their respective transmembrane receptors in their cytosolic domain. The phosphotyrosine residues are then recognized by so-called adapter proteins such as Grb2 with no intrinsic enzymatic activity (Figure) which then recruit guanine nucleotide exchange factors like Sos to the membrane thereby bringing them in close proximity to G-proteins like Ras.

Sos activates Ras by exchange of GDP for GTP. Ras then interacts with Raf-1, a 70–75 kDa protein serine/threonine kinase and recruits it to the plasma membrane. At the membrane, Raf becomes hyperphosphorylated and thereby activated. Raf-1 represents the first member of a kinase cascade which by subsequent phosphorylations transduces the signal further on towards the nucleus where, in response to the arriving signal, gene expression is modulated. The kinase activity and thereby also the signal transducing ability of Raf and other kinases is decreased by dephosphorylation by different phosphatases.

If these sensitive interactions of protein kinases and phosphatases are disturbed or interrupted, disease may result. This is particularly the case in the manifestation of cancer (the Raf gene is an oncogene)[2] but also various other illnesses are associated with an aberrant phosphorylation state of different proteins.[2–5]

For the understanding of the underlying biological phenomena on a molecular level and for the development of therapeutics which target intracellular phosphorylation and dephosphorylation processes, peptides which embody the characteristic phosphorylation sites of their parent naturally occurring phosphoproteins are interesting tools and reagents.[6] As a consequence in the last few years a significant amount of effort has been directed to the development of methodologies for the efficient synthesis of phosphopeptides in solution and on a solid support.[7-10]

One of the most flexible strategies for phosphopeptide synthesis which is particularly important for the construction of peptides embodying several different phosphorylated and unphosphorylated serine/threonine residues, is the stepwise assembly of the target compound from prephosphorylated building blocks. However the synthesis of such phosphopeptides is severely complicated by their multifunctionality as well as their pronounced base lability which make the application of a variety of protecting groups neccessary that can be split off under the mildest conditions. In particular even under weakly basic conditions, i.e. $pH > 8$, a rapidly occurring β-elimination[7,8] of the phosphate has to be feared (Scheme 1). Consequently classical base-labile protecting groups cannot be applied for the synthesis of fully masked phosphopeptides.[10]



Scheme 1

Viable alternatives to classical chemical methods are offered by enzymatic protecting group techniques.[11] In particular, the characteristically mild reaction conditions (pH 6–8, room temperature) under which enzymatic transformations can be carried out has allowed for the chemoenzymatic synthesis of sensitive lipo- and glycopeptides.[12] In the light of these findings we have investigated the application of enzymatically removable protecting groups in phosphopeptide chemistry.[13]

Results and Discussion

In this paper we describe in full detail two alternative enzymatic protecting group techniques for the selective *C*- and *N*-terminal deprotection of fully masked phosphopeptides. The phenylacetamide (PhAc) group was chosen as *N*-terminal protecting group and had been used prior to this for the unmasking of simple peptides by means of the biocatalyst penicillin G acylase.[14] In addition, we report that the enzymatic removal of the *C*-terminal heptyl (Hep) ester protecting group which was also previously used in the construction of simple peptides,[15] under neutral conditions (pH 7) and at 37 °C is an efficient method for the construction of sensitive base-labile serine/threonine phosphopeptides.

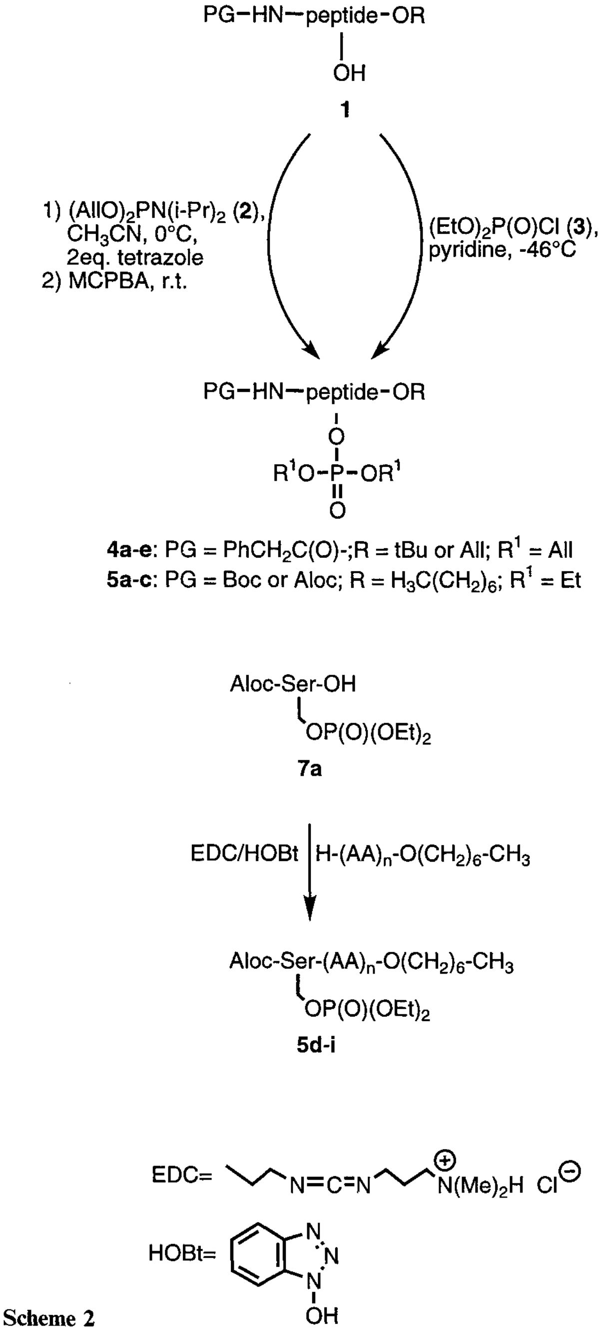
In order to investigate if the removal of the PhAc group by penicillin G acylase[14] and the lipase-mediated saponification of heptyl esters[15] can be employed successfully in phosphopeptide chemistry the model compounds **4a–e** and **5a–i** were built up from the unphosphorylated peptides **1**.[16] Thus, the diallyl phosphates **4a–e** were obtain-

Biographical Sketches

Herbert Waldmann, born in 1957, studied chemistry at the Johannes-Gutenberg-Universität Mainz and received his Dr. rer. nat. in 1985 under the guidance of Horst Kunz. After postdoctoral studies from 1985 to 1986 with George Whitesides at Harvard University he returned to the Universität Mainz and received his habilitation in 1991. After holding a professorship at the Rheinische Friedrich-Wilhelms-Universität Bonn from 1991 to 1993 he moved to the Universität Karlsruhe where he is now Full Professor of Organic Chemistry. Herbert Waldmann has been the recipient of the Friedrich Weygand Award for the advancement of peptide chemistry and of the Carl Duisberg Award of the Gesellschaft Deutscher Chemiker.
His current research interests include bioorganic chemistry, in particular the synthesis and biological evaluation of peptide conjugates and natural products which are involved in biological signal transduction processes, biocatalysis, stereoselective synthesis, peptide, carbohydrate and alkaloid chemistry as well as combinatorial chemistry.

Dagmar Sebastian was born in Bad Neuenahr, Germany, in 1966. She studied chemistry at the Rheinische Friedrich-Wilhelms Universität in Bonn and received her diploma degree in 1992. She then joined the research group of Professor Herbert Waldmann at the University of Karlsruhe and obtained her Ph. D. in 1997. In her thesis she concentrated on the development of chemoenzymatic techniques for the synthesis of biologically relevant phosphopeptides.

ed by application of the phosphoramidate method[7–10,17] employing the phosphoramidate **2**[17] followed by oxidation of the primarily formed phosphites to the phosphates by means of *m*-chloroperbenzoic acid (MCPBA) (Scheme 2).

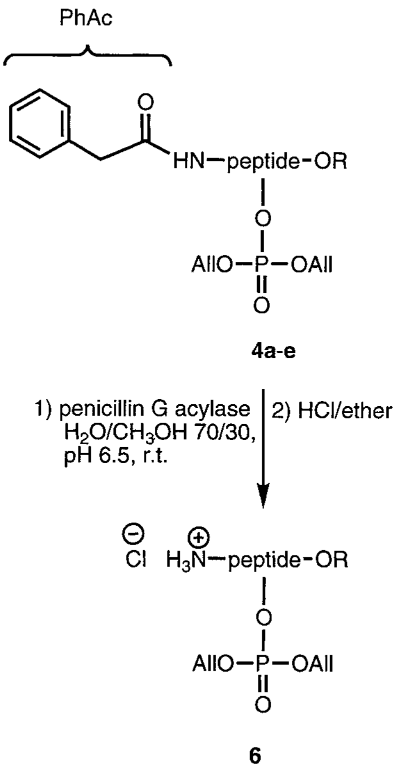


4a-e: PG = $PhCH_2C(O)$-;R = tBu or All; R^1 = All
5a-c: PG = Boc or Aloc; R = $H_3C(CH_2)_6$; R^1 = Et

Scheme 2

The diethyl phosphates **5a–c** were obtained from the peptides **1** by applying the phosphochloridate method[7,18] and using diethyl phosphochloridate (**3**) as the phosphorylating reagent (Scheme 2). In addition, the peptides **5d–i** were obtained from the serine building block **7a** (vide infra) by elongation of the peptide chain (Scheme 2). The synthesis of the substrates **4** and **5** follows well-established routes and, therefore, is not further commented on. Details are given in the Experimental Section.

The phenylacetamido protected peptides **4a–e** were then subjected to the enzymatic deprotection by penicillin G acylase at pH 6.5 and room temperature (Scheme 3). Gratifyingly, in the ensuing biocatalyzed hydrolysis of the *N*-terminal phenylacetyl protecting group, the phosphorylated di- and tripeptides **4** were all accepted as substrates. Under the mild reaction conditions (pH 6, room temperature, water/methanol mixture) only the PhAc blocking group was attacked, the *C*-terminal esters and the peptide bonds remained intact. In particular, the reaction conditions are so mild that no traces of *β*-elimination of the phosphate group could be detected. Reaction control by t.l.c. showed completion of the enzymatic deprotection within a few hours. The presence of 30 vol% of methanol as cosolvent guarantees that the protected phosphopeptides are soluble in the aqueous reaction mixture to a satisfying extent. The isolated yields of 40–72 % of the *N*-terminally deprotected peptides **6a–e** are due to losses upon isolation of the hydrophilic products from the aqueous solutions.

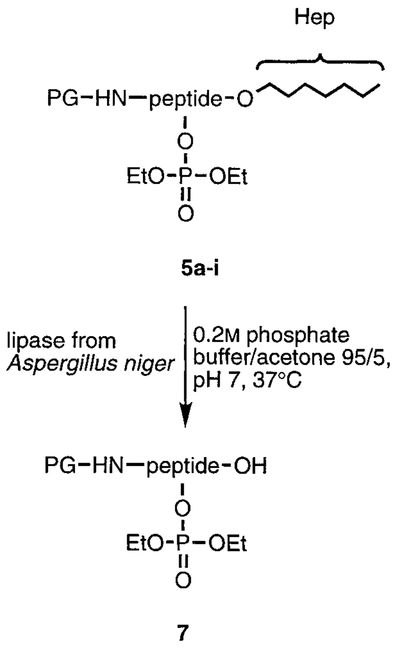


No.	peptide-OR	yield [%]
6a	Ala-Ser(P)-OtBu	55
6b	Ala-Thr(P)-OtBu	60
6c	Leu-Ala-Thr(P)-OAll	40
6d	Leu-Ala-Ser(P)-OAll	72
6e	Ala-Ala-Ser(P)-OtBu	62

Scheme 3

For the lipase-mediated saponification of the phosphopeptide heptyl esters **5a–i** several biocatalysts stemming from different biological sources (*Aspergillus niger*, *Rhizopus niveus*, *Rhizopus javanicus*, *Pseudomonas fluores-*

cens, *Mucor javanicus*, *Mucor miehei*, *Rhizopus oryzae*, *Candida antarctica*, *Bacillus thermocatenulatus*, *Penicillium roquefortii*, *Candida rugosa*, wheat germ) were investigated. The different peptides were accepted as substrates by most of the lipases. The best results were obtained, however, in the presence of a lipase from *Aspergillus niger*. This enzyme accepts variations of the side chains of the amino acids, in the position of the phosphate moiety in the peptide, the length of peptide chain and in the structure of the *N*-terminal protecting group (Scheme 4). In all cases the selectively unmasked phosphopeptides **7a–i** were isolated in high yields. The enzymatic transformations proceeded without undesired side reactions, i.e. neither the *N*-terminal blocking groups or the peptide bonds nor the phosphates were attacked. Again the reaction conditions were so mild (pH 7, 37 °C) that β-elimination of the phosphate resulting in the formation of dehydroalanyl peptides was not observed at all. We note, however, that the lipase only tolerates a phosphorylated serine residue at the *C*-terminus of the phosphopeptides. If instead of the Aloc-protected serine heptyl ester **5a** the respective threonine or also the analogous *allo* threonine analogue was subjected to the enzymatic reaction conditions, no hydrolysis of the ester group could be detected. This behaviour was also observed for several other lipases stemming from different biological sources.



No.	PG-peptide-OH	yield [%]
7a	Aloc-Ser(P)-OH*	89
7b	Boc-Ser(P)-Ala-OH	96
7c	Boc-Ala-Ser(P)-OH	78
7d	Aloc-Ser(P)-Gly-OH	90
7e	Aloc-Ser(P)-Leu-OH	90
7f	Aloc-Ser(P)-Val-OH	89
7g	Aloc-Ser(P)-Tyr-OH	56
7h	Boc-Ser(P)-Phe-OH	78
7i	Boc-Ser(P)-Ala-Ser-Ala-OH	57

* 0.1 equiv. of deoxytaurocholic acid sodium salt was added

Scheme 4

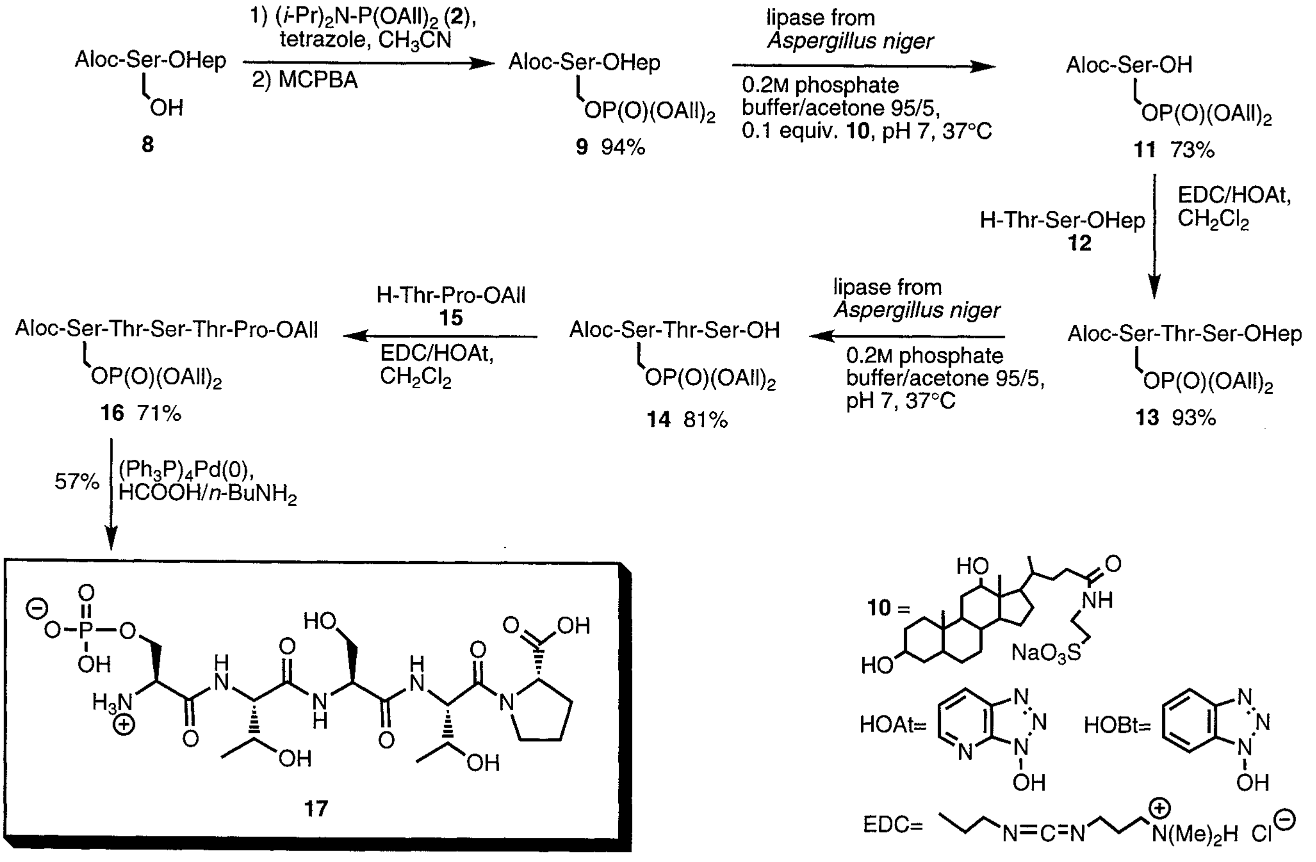
After having established the enzymatic protecting group techniques in principle we furthermore demonstrated the capacity of these biocatalyzed transformations by the construction of a more complex biologically relevant phosphopeptide. To this end, the synthesis of the peptide conjugate **17** was carried out by employing the lipase-catalyzed hydrolysis of Hep esters as the key steps (Scheme 5). Compound **17** represents a consensus sequence of the Raf-1 kinase whose phosphorylation influences the activity of Raf-1 and therefore plays an important role in the regulation of this signal transducing protein.[19]

For the synthesis of the phosphopentapeptide **17** a stepwise approach using phosphorylated and unphosphorylated amino acid building blocks together with selective deprotection in the presence of a sensitive serine phosphate was chosen, because **17** embodies several hydroxyamino acids of which only one should be phosphorylated.

First the Aloc-protected serine heptyl ester **8** was converted to the diallyl phosphate **9** in high yield by phosphitylation with the phosphoramidite **2** (vide supra) followed by oxidation of the resulting phosphite to the phosphate by means of MCPBA (Scheme 5). Next the *C*-terminal heptyl ester of **9** was split off with complete selectivity and without undesired side reactions under the agency of lipase from *Aspergillus niger*. In order to obtain the selectively deprotected phosphorylated amino acid **11**[9] in consistently high yield, the addition of 0.1 equivalent of deoxytaurocholic acid sodium salt **10** as emulsifying agent proved to be particularly useful. The emulsifier guarantees that an emulsion of **9** in the aqueous mixture is formed in which the lipase works well. A similar observation was also made in the deprotection of the serine derivative **5a** (see Scheme 4). Coupling of **11** with the dipeptide **12** then delivered the selectively phosphorylated tripeptide heptyl ester **13** in high yield. From **13** once more the lipase from *Aspergillus niger* removed the *C*-terminal heptyl ester without detectable side reactions. For the enzymatic transformation of **13** the addition of deoxytaurocholic acid sodium salt **10** was not necessary to obtain a high yield. The condensation of the selectively unmasked phosphotripeptide **14** and L-threonyl-L-proline allyl ester **15** resulted in the desired phosphopentapeptide **16**. Finally the removal of all remaining protecting groups from **16** had to be accomplished. All allyl-based blocking functions were removed simultaneously under weakly acidic conditions by treatment with formic acid and butylamine at room temperature in the presence of a catalytic amount of $Pd(PPh_3)_4$[20] affording the fully deprotected phosphopentapeptide **17**.

Conclusion

We have developed two different enzymatic techniques for the synthesis of phosphopeptide conjugates under particularly mild reaction conditions. By means of these two methods the phosphopeptide chain can be elongated selectively into the *C*- or the *N*-terminal direction. In the case of the lipase-mediated saponification of the heptyl ester we have demonstrated the capacity of the technique



Scheme 5

in the construction of the sensitive phosphopentapeptide **17** which represents a characteristic partial structure of the Raf-1 serine/threonine kinase. In all enzymatic and chemical transformations described above, no undesired side reactions were observed. Thus the peptide backbone remained intact because neither the lipase nor the penicillin G acylase has an intrinsic protease activity. The enzymatic steps proceeded with complete selectivity and no β-elimination of the phosphate to give the respective dehydroalanine peptides could be detected.

Together with recently developed classical chemical methods[7–10] these enzymatic techniques make sensitive *O*-phosphorylated peptide conjugates accessible, which may open up new alternatives for the study of biological phenomena by means of a bioorganic approach.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 250, AM 400 and DRX 500. Mass spectra were measured on a Finnigan MAT MS 70 spectrometer and with a Perseptive Biosystem Voyger™ Biospectrometry™ spectrometer (MALDI-TOF, nitrogen-UV-laser $\lambda = 237$ nm, accelerating voltage = 28 kV). Analytical chromatography was performed on Merck silica gel 60 F_{254} plates. Flash chromatography was performed on Baker silica gel (40–64 μm). THF was distilled from K metal and CH_2Cl_2 from P_2O_5. Penicillin G acylase was immobilized on Eupergit C. Lipase from *Aspergillus niger* (crude preparation) was a generous gift of Amano Ltd. Japan.

Phosphorylation of Protected Amino Acids/Peptides Using Bis(allyloxy)-(*N*,*N*-diisopropyl)phosphoramidite (2);[17] General Procedure:
To a mixture of the protected amino acid/peptide (5 mmol) and 1*H*-tetrazole (10 mmol, 2 equiv) in anhyd MeCN (60 mL) at 0 °C was added slowly bis(allyloxy)-(*N*,*N*-diisopropyl)phosphoramidite (**2**) (1.51 g, 6 mmol, 1.2 equiv). After several minutes a white precepitate formed. The mixture was stirred at r.t. for 2–10 h, MCPBA (80 %, 9 mmol, 1.8 equiv) was added and stirring was continued for additional 2–15 h. The mixture was poured into Et_2O (250 mL), the organic phase was extracted with 5 % $NaHSO_3$ solution (3 × 50 mL), sat. $NaHCO_3$ (3 × 50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was purified by flash chromatography using EtOAc/hexane mixtures as eluent.

According to this procedure the following compounds were prepared:

*N-Phenylacetyl-*L*-alanyl-(O)-diallylphosphato-*L*-serine tert-Butyl Ester* (**4a**): colorless oil, yield 70 %, $[\alpha]_D^{20} = -27.0$ ($c = 1$, MeOH).

^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.30$ (m, 5 H, 5 × CH_{arom}), 7.17 (d, 1 H, $J = 7.5$ Hz, NH), 6.47 (d, 1 H, $J = 7.4$ Hz, NH), 5.91 (m, 2 H, 2 × C*H*=CH_2), 5.35 (dd, 2 H, $J_{trans} = 17$ Hz, $J_{gem} = 1.5$ Hz, 2 × CH=CH_{2trans}), 5.25 (dd, 2 H, $J_{cis} = 7.5$ Hz, $J_{gem} = 1.5$ Hz, 2 × CH=CH_{2cis}), 4.51 (m, 6 H, 2 × α-CH, 2 × CH_2 CH=CH_2), 4.38 (m, 1 H, β-CH_{2a} Ser), 4.31 (m, 1 H, β-CH_{2b} Ser), 3.58 (s, 2 H, CH_2 PhAc), 1.47 (s, 9 H, $C(CH_3)_3$), 1.34 (d, 3 H, $J = 7.5$ Hz, CH_3).

^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 172.0$, 170.8, 167.3 (3 × CO), 134.7 (ipso-C), 132.1, 132.0 (2 × *C*H=CH_2), 129.3, 128.9, 127.2 (5 × $CH_{aromat.}$), 118.8, 118.7 (2 × CH=*C*H_2), 82.6 (*C*$(CH_3)_3$), 68.6 (2 × *C*H_2 CH=CH_2), 67.3 (β-CH_2 Ser), 53.4 (α-CH Ser), 48.7 (α-CH Ala), 43.5 (CH_2 PhAc), 27.9 (3 × C(*C*H_3)$_3$), 18.2 (CH_3 Ala).

HRMS: calcd: 510.2131, found: 510.2104.

$C_{24}H_{35}N_2O_8P$	(510.52)	calcd	C 56.45	H 6.91	N 5.49
		found	56.13	6.82	6.42

*N-Phenylacetyl-*L*-alanyl-(O)-diallylphosphato-*L*-threonine tert-Butyl Ester* (**4b**): colorless solid, yield: 73 %, $[\alpha]_D^{20} = -7.1$ ($c = 1$, MeOH).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.23$–7.18 (m, 5 H, C_6H_5), 7.13 (d, 1 H, $J = 9$ Hz, NH), 7.07 (d, 1 H, $J = 7.5$ Hz, NH), 5.87–5.80

(m, 2 H, 2 × C*H*=CH$_2$), 5.29–5.25 (m, 4 H, 2 × CH=C*H*$_2$), 4.92–4.89 (m, 1 H, β-CH Thr), 4.6–4.4 (m, 6 H, 2 × α-CH, 2 × C*H*$_2$ CH=CH$_2$), 3.48 (s, 2 H, CH$_2$ PhAc), 1.38 (s, 9 H, C(CH$_3$)$_3$), 1.28 (d, 3 H, $J = 7$ Hz, CH$_3$ Thr), 1.24 (d, 3 H, $J = 6.5$ Hz, CH$_3$ Ala).

^{13}C NMR (125.7 MHz, CDCl$_3$): $\delta = 172.6$, 170.6, 167.6 (3 × CO), 134.7 (ipso-C), 132.1, 132.0 (2 × *C*H=CH$_2$), 129.0, 128.5, 126.9 (5 × CH$_{\text{arom}}$), 118.1, 118.0 (2 × CH=*C*H$_2$ allyl), 82.6 (*C*(CH$_3$)$_3$), 75.2 (d, $J_{\text{C,P}} = 5.5$ Hz, β-CH Thr), 68.0, 67.9 (2 × *C*H$_2$ CH=CH$_2$), 56.8 (d, $J_{\text{C,P}} = 7.5$ Hz, α-CH Thr), 48.5 (α-CH Ala), 43.1 (CH$_2$ PhAc), 27.7 (3 × C(*C*H$_3$)$_3$), 18.2 (CH$_3$ Thr), 18.0 (CH$_3$ Ala).

HRMS: calcd: 525.2189, found: 535.2365.

$C_{25}H_{37}N_2O_8P$ (525.5)	calcd	C 57.24	H 7.11	N 5.34
	found	56.12	6.86	5.85

*N-Phenylacetyl-*L*-leucyl-*L*-alanyl-(O)-diallylphosphato-*L*-threonine Allyl Ester* (**4c**): colorless solid, yield 89%, mp 65 °C, $[\alpha]_D^{20} = -14.5$ ($c = 1.5$, MeOH).

^{1}H NMR (500 MHz, CD$_3$OD): $\delta = 7.28$–7.2 (m, 5 H, C$_6$H$_5$), 6.01–5.93 (m, 3 H, 3 × C*H*=CH$_2$), 5.40–5.20 (m, 6 H, 3 × CH=C*H*$_2$), 5.10 (m, 1 H, β-CH Thr), 4.81–4.42 (m, 9 H, 3 × α-CH, 3 × C*H*$_2$–CH=CH$_2$), 3.54 (s, 2 H, CH$_2$ PhAc), 1.63–1.59 (m, 3 H, C*H*(CH$_3$)$_2$, β-CH$_2$ Leu), 1.40 (d, 3 H, $J = 7$ Hz, CH$_3$), 1.38 (d, 3 H, $J = 7$ Hz, CH$_3$), 0.92 (d, 3 H, $J = 6.5$ Hz, CH$_3$ Leu), 0.86 (d, 3 H, $J = 6.5$ Hz, CH$_3$ Leu).

^{13}C NMR (125.7 MHz, CD$_3$OD): $\delta = 175.5$, 174.5, 173.9, 170.1 (4 × CO), 136.8 (ipso-C), 133.7, 133.6, 133.0 (3 × *C*H=CH$_2$), 130.1, 129.5, 127.8 (5 × CH$_{\text{arom}}$), 119.1, 118.8, 118.7 (3 × CH=*C*H$_2$), 77.0 (d, $J_{\text{C,P}} = 5$ Hz, β-CH Thr), 69.7, 69.6, 67.4 (3 × *C*H$_2$ CH=CH$_2$), 57.6 (d, $J_{\text{C,P}} = 8$ Hz, α-CH Thr), 52.9 (α-CH), 49.5 (α-CH), 43.5 (CH$_2$ PhAc), 41.9 (β-CH$_2$ Leu), 25.8 (*C*H(CH$_3$)$_2$), 23.5, 21.9, 18.7, 18.0 (4 × CH$_3$).

$C_{30}H_{44}N_3O_9P$ (621.7)	calcd	C 57.96	H 7.13	N 6.76
	found	57.55	7.10	6.92

*N-Phenylacetyl-*L*-leucyl-*L*-alanyl-(O)-diallylphosphato-*L*-serine Allyl Ester* (**4d**): yellow solid, yield 72%, mp 136 °C, $[\alpha]_D^{20} = -40.0$ ($c = 1$, MeOH).

^{1}H NMR (500 MHz, DMSO-d$_6$): $\delta = 8.48$ (d, 1 H, $J = 8$ Hz, NH), 8.17 (d, 1 H, $J = 8$ Hz, NH), 8.03 (d, 1 H, $J = 8$ Hz, NH), 7.28–7.18 (m, 5 H, C$_6$H$_5$), 5.96–5.86 (m, 3 H, 3 × C*H*=CH$_2$), 5.36–5.30 (m, 3 H, 3 × CH=C*H*$_{2trans}$), 5.25–5.20 (m, 3 H, 3 × CH=C*H*$_{2cis}$), 4.68 (m, 1 H, α-CH), 4.60 (dd, $J = 6$, 1 Hz, 2 H, C*H*$_2$ CH=CH$_2$), 4.48 (m, 4 H, 2 × CH$_2$ CH=CH$_2$), 4.40–4.13 (m, 4 H, 2 × α-CH, 2 × β-CH$_2$ Ser), 3.45 (d, $J = 9$ Hz, 2 H, CH$_2$ PhAc), 1.54 (m, 1 H, C*H*(CH$_3$)$_2$), 1.43 (dd, 2 H, $J_2 = J_2 = 7$ Hz, β-CH$_2$ Leu), 1.20 (d, 3 H, $J = 7$ Hz, CH$_3$ Ala), 0.85 (d, 3 H, $J = 6.5$ Hz, CH$_3$ Leu), 0.80 (d, 3 H, $J = 6.5$ Hz, CH$_3$ Leu).

^{13}C NMR (125.7 MHz, DMSO-d$_6$): $\delta = 172.6$, 171.7, 170.0, 168.5 (4 × CO), 136.4 (ipso-C), 132.9, 132.0 (3 × *C*H=CH$_2$), 128.9, 128.1, 126.2 (5 × CH$_{\text{arom}}$), 117.9 (3 × CH=*C*H$_2$), 67.7, 65.3 (3 × *C*H$_2$ CH=CH$_2$), 66.1 (β-CH$_2$ Ser), 52.2 (d, $J_{c,p} = 8$ Hz, α-CH Ser), 50.8 (α-CH), 47.7 (α-CH), 42.1 (CH$_2$ PhAc), 40.8 (β-CH$_2$ Leu), 24.1 ((CH$_3$)$_2$*C*H), 23.1, 21.5, 18.1 (CH$_3$).

$C_{27}H_{40}N_3O_9P$ (607.32)	calcd	C 57.32	H 6.97	N 6.92
	found	56.93	6.82	7.18

*N-Phenylacetyl-*L*-alanyl-*L*-alanyl-(O)-diallylphosphato-*L*-serine tert-Butyl Ester* (**4e**): colorless oil, yield 35%, $[\alpha]_D^{20} = -20.9$ ($c = 1$, MeOH).

^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 7.28$ (m, 5 H, C$_6$H$_5$), 7.12 (d, 1 H, $J = 7.5$ Hz, NH), 6.98 (d, 1 H, $J = 7.5$ Hz, NH), 6.25 (d, 1 H, $J = 7$ Hz, NH), 5.90 (m, 2 H, 2 × C*H*=CH$_2$), 5.36 (m, 2 H, 2 × CH=C*H*$_{2trans}$), 5.26 (dd, 2 H, $J_{cis} = 8$ Hz, $J_{gem} = 7.5$ Hz, 2 × CH=C*H*$_{2cis}$), 4.61 (m, 1 H, α-CH), 4.52–4.28 (m, 8 H, 2 × α-CH, β-CH$_2$ Ser, 2 × C*H*$_2$ CH=CH$_2$), 3.58 (s, 2 H, CH$_2$ PhAc), 1.47 (s, 9 H, C(CH$_3$)$_3$), 1.37 (d, 3 H, $J = 7$ Hz, CH$_3$), 1.34 (d, 3 H, $J = 7$ Hz, CH$_3$).

^{13}C NMR (125.7 MHz, CDCl$_3$): $\delta = 171.8$, 171.6, 170.8, 167.3 (4 × CO), 134.6 (*ipso*-C), 132.1, 132.0 (2 × *C*H=CH$_2$), 129.3, 128.9, 127.3 (5 × CH$_{\text{arom}}$), 118.9, 118.7 (2 × CH=*C*H$_2$), 82.8 (*C*(CH$_3$)$_3$), 68.7, 68.6 (2 × *C*H$_2$ CH=CH$_2$), 67.4 (β-CH$_2$ Ser), 53.4 (d, $J_{c,p} = 5$ Hz, α-CH, Ser), 48.9, 48.8 (2 × α-CH Ala), 43.5 (CH$_2$ PhAc), 27.9 (3 × C(*C*H$_3$)$_3$), 18.35, 18.0 (CH$_3$ Ala).

HRMS: calcd: 582.2580; found: 582.2544

$C_{27}H_{40}N_3O_9P$ (581.46).

Phosphorylation of Protected Amino Acid/Peptide Derivatives Using Diethyl Phosphochloridate (3);[18] General Procedure:

A solution of the respective protected amino acid/peptide (2 mmol) in anhyd pyridine (10 mL) was added to a solution of diethyl phosphochloridate (1.04 g, 6 mmol) in anhyd pyridine (15 mL) at −46 °C. After stirring at this temperature for 30 min, the mixture was allowed to warm to −15 °C and stirred for further 18 h. After stirring at 0 °C for additional 6 h, EtOAc (100 mL) was added and the organic phase was extracted with 0.5 M HCl (3 × 30 mL), 0.5 M NaHCO$_3$ (1 × 50 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The resulting residue was purified by flash chromatography using EtOAc/hexane mixtures as eluents.

According to this procedure the following compounds were prepared:

*N-Allyloxycarbonyl-(O)-diethylphosphato-*L*-serine Heptyl Ester* (**5a**):

colorless oil, yield 86%, $[\alpha]_D^{20} = +8.0$ ($c = 0.83$, CHCl$_3$).

^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 5.96$–5.84 (m, 2 H, C*H*=CH$_2$, NH urethane), 5.32 (d, 1 H, $J_{trans} = 17.2$ Hz, $J_{gem} = 1.4$ Hz, CH=CH$_{2trans}$), 5.22 (d, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.4$ Hz, CH=C*H*$_{2cis}$), 4.60–4.52 (m, 3 H, C*H*$_2$ CH=CH$_2$, α-CH Ser), 4.46–4.41 (m, 1 H, β-CH$_{2a}$ Ser), 4.34–4.29 (m, 1 H, β-CH$_{2b}$ Ser), 4.17 (t, 2 H, $J = 6.7$ Hz, CO$_2$CH$_2$), 4.14–4.06 (m, 4 H, 2 × POCH$_2$), 1.67–1.62 (m, 2 H, CO$_2$CH$_2$C*H*$_2$), 1.35–1.28 (m, 14 H, 4 × CH$_2$ Hep, 2 × POCH$_2$C*H*$_3$), 0.88 (t, 3 H, $J = 6.8$ Hz, CH$_3$ Hep).

^{13}C NMR (125.7 MHz, CDCl$_3$): $\delta = 168.9$ (CO ester), 155.6 (CO urethane), 132.4 (*C*H=CH$_2$), 117.8 (CH=*C*H$_2$), 67.1 (d, $^2J_{\text{C,P}} = 5.1$ Hz, β-CH$_2$ Ser), 66.2 (2C, POCH$_2$), 65.9 (CO$_2$*C*H$_2$), 64.1 (*C*H$_2$CH=CH$_2$), 54.4 (d, $^3J_{\text{C,P}} = 6.9$ Hz, α-CH Ser), 31.6, 28.8, 28.4, 25.6, 22.5 (5 × CH$_2$ Hep), 16.0 (d, $^3J_{\text{C,P}} = 6.5$ Hz, 2C, POCH$_2$*C*H$_3$), 14.0 (CH$_3$ Hep).

HRMS: calcd: 423.2022 found: 423.2010

$C_{18}H_{34}N_1O_8P$ (423.44)	calcd	C 51.06	H 8.09	N 3.31
	found	51.38	8.23	3.33

*N-tert-Butyloxycarbonyl-(O)-diethylphosphato-*L*-seryl-*L*-alanine Heptyl Ester* (**5b**): colorless oil, 86%, $[\alpha]_D^{20} = -5.5$ ($c = 0.52$, CH$_2$Cl$_2$).

^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 7.30$ (br, 1 H, NH amide), 5.79 (br, 1 H, NH urethane), 4.57–4.51 (m, 1 H, α-CH Ala), 4.43–4.42 (br, 2 H, α-CH Ser, β-CH$_{2a}$ Ser), 4.29–4.07 (m, 7 H, CO$_2$CH$_2$, β-CH$_{2b}$ Ser, 2 × POCH$_2$), 1.66–1.59 (m, 2 H, CO$_2$CH$_2$C*H*$_2$), 1.46 (s, 9 H, C(CH$_3$)$_3$), 1.40 (d, 3 H, $J = 7.1$ Hz, β-CH$_3$ Ala), 1.38–1.28 (m, 14 H, 4 × CH$_2$ Hep, 2 × POCH$_2$C*H*$_3$), 0.88 (t, 3 H, $J = 6.9$ Hz, CH$_3$ Hep).

^{13}C NMR (125.6 MHz, CDCl$_3$): $\delta = 172.4$, 168.3 (CO ester, CO amide), 155.4 (CO urethane), 80.5 (*C*(CH$_3$)$_3$), 66.7 (d, $^2J_{\text{C,P}} = 5.4$ Hz, β-CH$_2$ Ser), 65.5 (CO$_2$*C*H$_2$), 64.2 (d, $^2J_{\text{C,P}} = 4.9$ Hz, 2C, POCH$_2$), 54.5 (α-CH Ser), 48.3 (α-CH Ala), 31.6, 28.7, 28.4 (3 × CH$_2$ Hep), 28.2 (3C, C(*C*H$_3$)$_3$), 25.6, 22.5 (2 × CH$_2$ Hep), 18.4 (β-CH$_3$ Ala), 16.0 (d, $^3J_{\text{C,P}} = 6.6$ Hz, 2C, POCH$_2$*C*H$_3$), 13.9 (CH$_3$ Hep).

HRMS: calcd: 511.2784, found: 511.2710

$C_{22}H_{43}N_2O_9P$ (510.57)	calcd	C 51.75	H 8.49	N 5.49
	found	51.79	8.51	5.69

*N-tert-Butyloxycarbonyl-*L*-alanyl-(O)-diethylphosphato-*L*-serine Heptyl Ester* (**5c**): colorless oil, yield 95%, $[\alpha]_D^{20} = -2.7$ ($c = 0.9$, CH$_2$Cl$_2$).

^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 7.28$ (d, 1 H, $J = 7.7$ Hz, NH amide), 5.33 (br, 1 H, NH urethane), 4.75–4.74 (m, 1 H, α-CH, Ser), 4.43–4.28 (m, 3 H, α-CH Ala, β-CH$_2$ Ser), 4.17 (t, 2 H, $J = 6.8$ Hz, CO$_2$CH$_2$), 4.14–4.06 (m, 4 H, 2 × POCH$_2$), 1.68–1.62 (m, 2 H, CO$_2$CH$_2$C*H*$_2$), 1.45 (s, 9 H, C(CH$_3$)$_3$), 1.40 (d, 3 H, $J = 7.1$ Hz, β-CH$_3$ Ala), 1.35–1.24 (m, 14 H, 4 × CH$_2$ Hep, 2 × POCH$_2$C*H*$_3$), 0.88 (t, 3 H, $J = 6.6$ Hz, CH$_3$ Hep).

^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 172.7$, 168.6 (CO ester, CO amide), 155.3 (CO urethane), 79.8 ($C(CH_3)_3$), 66.7 (d, $^2J_{C,P} = 5.0$ Hz, β-CH_2 Ser), 66.1 (CO_2CH_2), 64.2 (d, $^2J_{C,P} = 6.0$ Hz, 2C, $POCH_2$), 52.8 (d, $^3J_{C,P} = 5.5$ Hz, α-CH Ser), 50.0 (α-CH Ala), 31.5, 28.7, 28.4 (3 × CH_2 Hep), 28.2 (3C, $C(CH_3)_3$), 25.6, 22.4 (2 × CH_2 Hep), 18.5 (β-CH_3 Ala), 16.0 (d, $^3J_{C,P} = 6.5$ Hz, 2C, $POCH_2CH_3$), 13.9 (CH_3 Hep).

HRMS: calcd: 510.2706, found: 510.2721

$C_{22}H_{43}N_2O_9P$ (510.57)	calcd	C 51.75	H 8.49	N 5.49
	found	51.59	8.62	5.51

Synthesis of *N*-Allyloxycarbonyl-(*O*)-diethylphosphato-L-serylamino Acid Heptyl Esters; General Procedure:

To a solution of *N*-(allyloxycarbonyl)diethylphosphato-L-serine **(7a)** (100 mg, 3.1 mmol), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC) (118 mg, 6.1 mmol, 2 equiv) and *N*-hydroxybenzotriazole (HOBt) (84 mg, 6.1 mmol, 2 equiv) in anhyd CH_2Cl_2 (15 mL) was added at 0 °C a solution of the respective amino acid or peptide heptyl ester salt (307 μmol, 1 equiv) and Et_3N (31 mg, 3.1 mmol, 1 equiv) in anhyd CH_2Cl_2 (10 mL). After stirring for 2 h at this temperature, the mixture was left overnight at r.t., filtered and the filtrate was washed with 0.5 M HCl (3 × 10 mL), dried and concentrated under reduced pressure. If necessary the crude product was purified by flash chromatography (EtOAc/hexane).

According to this procedure the following compounds were synthesized:

*N-Allyloxycarbonyl-(O)-diethylphosphato-*L*-serylglycine Heptyl Ester* **(5d)**; colorless oil, yield 92%, $[\alpha]_D^{20} = -0.73$ ($c = 0.38$, CH_2Cl_2).

^{13}C NMR (500 MHz, $CDCl_3$): $\delta = 7.06$ (t, 1 H, $J = 4.9$ Hz, NH amide), 6.17 (br, 1 H, NH urethane), 5.96–5.88 (m, 1 H, $CH{=}CH_2$), 5.32 (dd, 1 H, $J_{trans} = 17.2$ Hz, $J_{gem} = 1.2$ Hz, $CH{=}CH_{2trans}$), 5.23 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.2$ Hz, $CH{=}CH_{2cis}$), 4.60 (d, 2 H, $J = 5.6$ Hz, CH_2 $CH{=}CH_2$), 4.51–4.47 (m, 2 H, α-CH Ser, β-CH_{2a} Ser), 4.23–4.17 (m, 1 H, β-CH_{2b} Ser), 4.15–3.97 (m, 8 H, α-CH_2 Gly, 2 × $POCH_2$, CO_2CH_2), 1.66–1.61 (m, 2 H, $CO_2CH_2CH_2$), 1.35–1.25 (m, 14 H, 4 × CH_2 Hep, 2 × $POCH_2CH_3$), 0.88 (t, 3 H, $J = 6.8$ Hz, CH_3 Hep).

^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 169.3$, 168.7 (CO ester, CO amide), 156.1 (CO urethane), 132.3 ($CH{=}CH_2$), 118.1 ($CH{=}CH_2$), 66.6 (d, $^2J_{C,P} = 5.2$ Hz, β-CH_2 Ser), 66.2 (CO_2CH_2), 65.7 ($CH_2{-}CH{=}CH_2$), 64.5, 64.4 (d, $^2J_{C,P} = 6.7$ Hz, 2C, 2 × $POCH_2$), 54.9 (α-CH Ser), 41.4 (α-CH_2 Gly), 31.6, 28.8, 28.4, 25.7, 22.5 (5 × CH_2 Hep), 16.0 (d, $^3J_{C,P} = 6.5$ Hz, 2C, 2 × $POCH_2CH_3$), 14.0 (CH_3 Hep).

HRMS: calcd: 480.2236, found: 480.2256

$C_{20}H_{37}N_2O_9P$ (480.49)	calcd	C 49.99	H 7.76	N 5.83
	found	50.06	7.59	5.82

*N-Allyloxycarbonyl-(O)-diethylphosphato-*L*-seryl-*L*-leucine Heptyl Ester* **(5e)**: colorless oil, yield 90 %, $[\alpha]_D^{20} = -17$ ($c = 0.32$, CH_2Cl_2).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.04$ (d, 1 H, $J = 7.6$ Hz, NH amide), 6.13 (br, 1 H, NH urethane), 5.96–5.88 (m, 1 H, $CH{=}CH_2$), 5.32 (dd, 1 H, $J_{trans} = 17.1$ Hz, $J_{gem} = 1.3$ Hz, $CH{=}CH_{2trans}$), 5.22 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.3$ Hz, $CH{=}CH_{2cis}$), 4.61–4.56 (m, 3 H, CH_2 $CH{=}CH_2$, α-CH Leu), 4.50 (br, 1 H, α-CH Ser), 4.46–4.42 (m, 1 H, β-CH_{2a} Ser), 4.22–4.08 (m, 7 H, β-CH_{2b} Ser, 2 × $POCH_2$, CO_2CH_2), 1.67–1.59 (m, 4 H, β-CH_2 Leu, $CO_2CH_2CH_2$), 1.57–1.54 (m, 1 H, γ-CH Leu), 1.36–1.25 (m, 14 H, 4 × CH_2 Hep, 2 × $POCH_2CH_3$), 0.94–0.91 (m, 6 H, 2 × δ-CH_3 Leu), 0.88 (t, 3 H, $J = 6.8$ Hz, CH_3 Hep).

^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 172.4$, 168.3 (CO ester, CO amide), 156.1 (CO urethane), 132.3 ($CH{=}CH_2$), 118.1 ($CH{=}CH_2$), 66.8 (d, $^2J_{C,P} = 5.2$ Hz, β-CH_2 Ser), 66.2 (CO_2CH_2), 65.5 (CH_2 $CH{=}CH_2$), 64.4 (d, $^2J_{C,P} = 5.6$ Hz, 2C, 2 × $POCH_2$), 54.9 (α-CH Ser), 51.1 (α-CH Leu), 41.6 (β-CH_2 Leu), 31.6, 28.8, 28.4, 25.7 (4 × CH_2 Hep), 24.8 (δ-CH_3 Leu), 22.7 (γ-CH Leu), 22.5 (CH_2 Hep), 22.0 (δ-CH_3 Leu), 16.0 (d, $^3J_{C,P} = 6.5$ Hz, 2C, 2 × $POCH_2CH_3$), 14.0 (CH_3 Hep).

HRMS: calcd: 536.2862, found: 536.2882

$C_{24}H_{45}N_2O_9P$ (536.60)	calcd	C 53.72	H 8.45	N 5.22
	found	53.96	8.41	5.18

*N-Allyloxycarbonyl-(O)-diethylphosphato-*L*-seryl-*L*-valine Heptyl Ester* **(5f)**: colorless liquid, yield 94%, $[\alpha]_D^{20} = -4.9$ ($c = 0.5$, CH_2Cl_2).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.07$ (d, 1 H, $J = 7.9$ Hz, NH amide), 6.20 (br, 1 H, NH urethane), 5.95–5.89 (m, 1 H, $CH{=}CH_2$), 5.32 (d, 1 H, $J_{trans} = 17.1$ Hz, $CH{=}CH_{2trans}$), 5.22 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.1$ Hz, $CH{=}CH_{2cis}$), 4.61 (d, 2 H, $J = 5.6$ Hz, CH_2 $CH{=}CH_2$), 4.52–4.45 (m, 3 H, α-CH Ser, α-CH Val, β-CH_{2a} Ser), 4.22–4.09 (m, 7 H, β-CH_{2b} Ser, 2 × $POCH_2$, CO_2CH_2), 2.19–2.16 (m, 1 H, β-CH Val), 1.65–1.61 (m, 2 H, $CO_2CH_2CH_2$), 1.35–1.25 (m, 14 H, 4 × CH_2 Hep, 2 × $POCH_2CH_3$), 0.94–0.87 (m, 9 H, 2 × γ-CH_3 Val, CH_3 Hep).

^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 171.3$, 168.5 (CO ester, CO amide), 156.1 (CO urethane), 132.3 ($CH{=}CH_2$), 118.1 ($CH{=}CH_2$), 66.8 (d, $^2J_{C,P} = 5.4$ Hz, β-CH_2 Ser), 66.2 (CO_2CH_2), 65.5 (CH_2 $CH{=}CH_2$), 64.4 (d, $^2J_{C,P} = 5.3$ Hz, $POCH_2$), 64.3 (d, $^2J_{C,P} = 5.5$ Hz, $POCH_2$), 57.4, 55.1 (α-CH Ser, α-CH Val), 31.6 (CH_2 Hep), 31.3 (β-CH Val), 28.8, 28.5, 25.8, 22.5 (4 × CH_2 Hep), 18.9, 17.7 (2 × γ-CH_3 Val), 16.0 (d, $^3J_{C,P} = 6.7$ Hz, 2C, 2 × $POCH_2CH_3$), 14.0 (CH_3 Hep).

HRMS: calcd: 522.2706, found: 522.2692

$C_{23}H_{43}N_2O_9P$ (522.57)	calcd	C 52.87	H 8.29	N 5.36
	found	53.26	8.39	5.29

*N-Allyloxycarbonyl-(O)-diethylphosphato-*L*-seryl-*L*-tyrosine Heptyl Ester* **(5g)**: colorless wax, yield 75 %, $[\alpha]_D^{20} = +13.2$ ($c = 0.32$, CH_2Cl_2).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.02$ (d, 1 H, $J = 6.8$ Hz, NH amide), 6.92 (d, 2 H, $J = 8.4$ Hz, CH-2/6 Tyr), 6.75 (d, 2 H, $J = 8.4$ Hz, CH-3/5 Tyr), 6.02 (d, 1 H, $J = 6.7$ Hz, NH urethane), 5.92–5.86 (m, 1 H, $CH{=}CH_2$), 5.30 (d, 1 H, $J_{trans} = 17.1$ Hz, $CH{=}CH_{2trans}$), 5.21 (d, 1 H, $J_{cis} = 10.4$ Hz, $CH{=}CH_{2cis}$), 4.77 (ddd, 1 H, $J_1 = 6.8$ Hz, $J_2 = 5.9$ Hz, $J_3 = 5.5$ Hz, α-CH Tyr), 4.56–4.53 (m, 2 H, CH_2 $CH{=}CH_2$), 4.49 (br, 1 H, α-CH Ser), 4.38 (br, 1 H, β-CH_{2a} Ser), 4.19–4.13 (m, 1 H, β-CH_{2b} Ser), 4.12–4.05 (m, 6 H, 2 × $POCH_2$, CO_2CH_2), 3.11 (dd, 1 H, $J = 14.0$, 5.5 Hz, β-CH_{2a} Tyr), 2.97 (dd, 1 H, $J = 14.0$, 6.1 Hz, β-CH_{2b} Tyr), 1.65–1.59 (m, 2 H, $CO_2CH_2CH_2$), 1.33–1.25 (m, 14 H, 4 × CH_2 Hep, 2 × $POCH_2CH_3$), 0.89 (t, 3 H, $J = 6.8$ Hz, CH_3 Hep).

^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 171.1$, 168.1 (CO ester, CO amide), 156.1 (CO urethane), 155.6 (C-4 Tyr), 132.3 ($CH{=}CH_2$), 130.3 (2C, CH-2/6 Tyr), 126.8 (C-1 Tyr), 118.1 ($CH{=}CH_2$), 115.7 (2C, CH-3/5 Tyr), 66.7 (d, $^2J_{C,P} = 5.3$ Hz, β-CH_2 Ser), 66.3 (CO_2CH_2), 65.8 (CH_2 $CH{=}CH_2$), 64.5 (d, $^2J_{C,P} = 5.4$ Hz, $POCH_2$), 64.4 (d, $^2J_{C,P} = 5.1$ Hz, $POCH_2$), 54.8 (α-CH Ser), 53.7 (α-CH Tyr), 36.9 (β-CH_2 Tyr), 31.6, 28.8, 28.4, 25.7, 22.5 (5 × CH_2 Hep), 16.0 (d, $^3J_{C,P} = 6.6$ Hz, 2C, 2 × $POCH_2CH_3$), 14.0 (CH_3 Hep).

HRM: calcd: 586.6255, found: 586.2633

$C_{27}H_{43}N_2O_{10}P$ (586.62)	calcd	C 55.28	H 7.38	N 4.77
	found	55.24	7.16	4.59

*N-Allyloxycarbonyl-(O)-diethylphosphato-*L*-seryl-*L*-phenylalanine Heptyl Ester* **(5h)**: colorless oil, yield 94.8 %, $[\alpha]_D^{20} = +13.4$ ($c = 0.67$, CH_2Cl_2).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.29$–7.21 (m, 3 H, 3 × CH_{arom} Phe), 7.12–7.10 (m, 2 H, 2 × CH_{arom} Phe), 7.00 (brs, 1 H, NH amide), 5.97 (brs, 1 H, NH urethane), 5.92–5.87 (m, 1 H, $CH{=}CH_2$), 5.30 (d, 1 H, $J_{trans} = 17.1$ Hz, $CH{=}CH_{2trans}$), 5.22 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.0$ Hz, $CH{=}CH_{2cis}$), 4.81 (dd, 1 H, $J = 13.4$, 5.9 Hz, α-CH Phe), 4.59 (brs, 2 H, CH_2 $CH{=}CH_2$), 4.57–4.56 (m, 2 H, α-CH Ser, β-CH_{2a} Ser), 4.20–4.16 (m, 1 H, β-CH_{2b} Ser), 4.14–4.03 (m, 6 H, 2 × $POCH_2$, CO_2CH_2), 3.16–3.07 (m, 2 H, β-CH_2 Phe), 1.57 (t, 2 H, $J = 6.9$ Hz, $CO_2CH_2CH_2$), 1.34–1.27 (m, 14 H, 4 × CH_2 Hep, 2 × $POCH_2CH_3$), 0.89 (t, 3 H, $J = 6.8$ Hz, CH_3 Hep).

^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 170.9$, 168.1 (CO ester, CO amide), 156.1 (CO urethane), 132.3 ($CH{=}CH_2$), 135.7 (ipso-C Phe), 129.3, 128.5, 127.1 (5C, CH Phe), 118.1 ($CH{=}CH_2$), 66.6 (β-CH_2 Ser), 66.2 (CH_2 $CH{=}CH_2$), 65.7 (CO_2CH_2), 64.4 (2C, 2 × $POCH_2$), 54.8 (α-CH Ser), 53.6 (α-CH Phe), 37.9 (β-CH_2 Phe), 31.6, 28.8, 28.4, 25.7, 22.5 ($5 \times CH_2$ Hep), 16.0 (d, $^3J_{C,P} = 6.5$ Hz, 2C, $2 \times POCH_2CH_3$), 14.0 (CH_3 Hep).

HRMS: calcd: 570.2706, found: 570.2719

$C_{27}H_{43}N_2O_9P$ (570.62)	calcd	C 56.83	H 7.59	N 4.91
	found	56.69	7.43	5.29

N-tert-Butyloxycarbonyl-(O)-diethylphosphato-L-seryl-L-alanyl-L-seryl-L-alanine Heptyl Ester (5i):

To a solution of *N-tert*-butyloxycarbonyl-(*O*)-diethylphosphato-L-seryl-L-alanine (60 mg, 1.44 mmol), EDC (56 mg, 2.88 mmol, 2 equiv) and *N*-hydroxybenzotriazole (HOBt) (40 mg, 2.88 mmol, 2 equiv) in anhyd CH_2Cl_2 (15 mL) was added at 0 °C a solution of L-seryl-L-alanine heptyl ester hydrochloride (46 mg, 1.44 mmol, 1 equiv) and Et_3N (15 mg, 1.44 mmol, 1 equiv) in anhyd CH_2Cl_2. After stirring for 2 h at this temperature, the mixture was left overnight at r.t., filtered and the filtrate was washed with 0.5 M HCl (3×10 mL), dried and concentrated under reduced pressure.

Colorless wax, yield 88 mg (90 %), $[\alpha]_D^{20}$ −20 ($c = 0.07$, CH_2Cl_2).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.45$–4.39 (m, 4 H, $2 \times \alpha$-CH Ala, $2 \times \alpha$-CH Ser), 4.30–4.20 (m, 2 H, β-CH_2 Ser), 4.16–4.08 (m, 6 H, CO_2CH_2, $2 \times POCH_2$), 3.82–3.79 (m, 2 H, β-CH_2 Ser), 1.66–1.62 (m, 2 H, $CO_2CH_2CH_2$), 1.46 (s, 9 H, $C(CH_3)_3$), 1.40 (d, 3 H, $J = 2.6$ Hz, β-CH_3 Ala), 1.39 (d, 3 H, $J = 2.4$ Hz, β-CH_3 Ala), 1.37–1.29 (m, 14 H, $4 \times CH_2$ Hep, $2 \times POCH_2CH_3$), 0.90 (t, 3 H, $J = 6.8$ Hz, CH_3 Hep).

^{13}C NMR (125.6 MHz, CD_3OD): $\delta = 174.5$, 174.1, 171.8, 171.1 (CO ester, $3 \times CO$ amide), 157.7 (CO urethane), 81.1 ($C(CH_3)_3$), 68.0 (β-CH_2 Ser), 66.4 (CO_2CH_2), 65.7 (d, $^2J_{C,P} = 5.6$ Hz, 2C, $2 \times POCH_2$), 63.0 (β-CH_2 Ser), 56.7, 56.0 ($2 \times \alpha$-CH Ser), 50.8, 49.6 ($2 \times \alpha$-CH Ala), 32.8, 30.0, 29.6 ($3 \times CH_3$ Hep), 28.6 (3C, $C(CH_3)_3$), 26.8, 23.6 ($2 \times CH_2$ Hep), 18.0, 17.5 ($2 \times \beta$-CH_3 Ala), 16.4 (d, $^3J_{C,P} = 6.5$ Hz, 2C, $2 \times POCH_2CH_3$), 14.3 (CH_3 Hep).

$C_{28}H_{53}N_4O_{12}P \cdot 0.5\ H_2O$ (668.34)	calcd	C 49.62	H 8.03	N 8.26
	found	49.74	7.87	7.99

Enzymatic Deprotection of Phenylacetamido Protected Phosphopeptides; General Procedure:

To a solution of the fully protected phosphopeptide (0.1–0.3 mmol) in MeOH (30 mL), H_2O (70 mL) was added slowly. The pH value was adjusted to pH 6.5 by addition of 5 % HOAc or 0.02 M NaOH. The solution was stirred for 1 h, then penicillin G acylase [immobilized on Eupergit C (ca. 1200 units)] was added. The course of the reaction was monitored by t.l.c. (EtOAc/hexane/MeOH) or by HPLC (RP18, $MeOH/H_2O = 7/3$). After stirring the mixture for 6 to 30 h the reactions were completed. The enzyme was filtered off and washed successively with H_2O and MeOH. The MeOH was evaporated under reduced pressure, the pH value was adjusted to pH 1–2 with dil. HCl and the solution was extracted with Et_2O (3×50 mL). Then the pH was adjusted to pH 9 by addition of Na_2CO_3 and the aqueous solution was immediately extracted with CH_2Cl_2 (5×80 mL). The organic layer was dried ($MgSO_4$) and sat. HCl in Et_2O (5 mL) was added. The solvent was removed under reduced pressure and the *N*-terminally deprotected phosphopeptides were isolated as their colorless hydrochlorides.

L-*Alanyl-(O)-diallylphosphato*-L-*serine tert-Butyl Ester Hydrochloride* (**6a**): colorless oil, yield 55 %, $[\alpha]_D^{20} = -17.2$ ($c = 1$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 6.02$–5.86 (m, 2 H, $CH{=}CH_2$), 5.35 (m, 2 H, $2 \times CH{=}CH_{2trans}$), 5.25 (dd, 2 H, $2 \times CH{=}CH_{2cis}$), 4.70 (m, 1 H, α-CH), 4.59–4.56 (m, 4 H, $2 \times CH_2$ $CH{=}CH_2$), 4.45 (m, 1 H, α-CH), 4.40–4.28 (m, 2 H, β-CH_2 Ser), 4.02 (m, 1 H, α-CH), 1.52 (d, 3 H, $J = 7.5$ Hz, CH_3), 1.49 (s, 9 H, $C(CH_3)_3$).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 171.1$, 169.3, 168.6 ($3 \times CO$), 133.6, 133.5 ($2 \times CH{=}CH_2$), 119.1, 119.0 ($2 \times CH{=}CH_2$), 84.1 ($C(CH_3)_3$), 69.9 ($2 \times CH_2$ $CH{=}CH_2$), 69.0 (β-CH_2 Ser), 50.1 (α-CH Ser), 40.1 (α-CH Ala), 28.2 ($3 \times C(CH_3)_3$), 17.7 (CH_3 Ala).

HRMS: calcd: 393.1791; found: 393.1724

$C_{16}H_{30}N_2O_7$ (392.4)

L-*Alanyl-(O)-diallylphosphato*-L-*threonine tert-Butyl Ester Hydrochloride* (**6b**): yellow oil, yield 60 %, $[\alpha]_D^{20} = 4$ ($c = 0.95$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.49$ (s, 3 H, NH_3^+), 5.97–5.88 (m, 2 H, $2 \times CH{=}CH_2$), 5.39–5.21 (m, 4 H, $2 \times CH{=}CH_2$), 5.05–5.03 (m, 1 H, NH), 4.79 (m, 1 H, α-CH Thr), 4.66 (d, $J = 8$ Hz, 1 H, α-CH Ala), 4.60–4.53 (m, 4 H, $2 \times CH_2$ $CH{=}CH_2$), 3.23 (d, $J = 8$ Hz, 1 H, β-CH Thr), 1.70 (d, 3 H, $J = 8$ Hz, CH_3), 1.49 (s, 9 H, $C(CH_3)_3$), 1.45 (d, 3 H, $J = 8$ Hz, CH_3).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 170.5$, 167.6 ($2 \times CO$), 132.4, 132.3 ($2 \times CH{=}CH_2$), 118.5, 118.4 ($2 \times CH{=}CH_2$), 82.9 ($C(CH_3)_3$), 76.0 (β-CH Thr), 68.7, 68.6 ($2 \times CH_2$ $CH{=}CH_2$), 57.0 (d, $^3J_{C,P} = 8$ Hz, α-CH Thr), 49.3 (α-CH Ala), 28.0 ($3 \times C(CH_3)_3$), 18.6 (CH_3 Thr), 17.3 (CH_3 Ala).

HRMS: calcd: 407.1828; found: 407.1947

$C_{17}H_{32}N_2O_7PCl$ (442.9)	calcd	C 46.10	H 7.28	N 6.33
	found	45.80	7.29	6.47

L-*Leucyl*-L-*alanyl-(O)-diallylphosphato*-L-*threonine Allyl Ester Hydrochloride* (**6c**): yellow oil, yield 40 %, $[\alpha]_D^{20} = 5.9$ ($c = 2$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.47$ (d, $J = 9$ Hz, 1 H, NH), 6.0–5.92 (m, 3 H, $3 \times CH{=}CH_2$), 5.40–5.24 (m, 6 H, $3 \times CH{=}CH_2$), 4.58–4.52 (m, 8 H, α-CH Ala, α-CH Thr, $3 \times CH_2CH{=}CH_2$), 3.94–3.91 (m, 1 H, β-CH Thr), 1.78–1.65 (m, 2 H, β-CH_2 Leu), 1.58–1.52 (m, 1 H, γ-CH Leu), 1.44 (d, $J = 7$ Hz, 3 H, CH_3 Ala), 1.39 (d, $J = 6.5$ Hz, 3 H, CH_3 Thr), 1.00 (d, 3 H, $J = 7$ Hz, CH_3 Leu), 0.99 (d, 3 H, $J = 7$ Hz, CH_3 Leu).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 171.8$, 170.3, 169.9 ($3 \times CO$), 133.6, 132.9, 132.3 ($3 \times CH{=}CH_2$), 119.2, 118.8, 118.7 ($3 \times CH{=}CH_2$), 76.6 (d, $J_{C,P} = 5.6$ Hz, β-CH Thr), 67.9, 67.8 (d, $J_{C,P} = 5.5$ Hz, $CH_2CH{=}CH_2$), 66.8 ($CH_2CH{=}CH_2$), 57.8 (α-CH Thr), 50.3 (α-CH Leu), 49.5 (α-CH Ala), 41.6 (β-CH_2 Leu), 25.3 ($(CH_3)_2CH$), 22.2 (CH_3 Thr), 22.0 (CH_3 Ala), 17.8, 15.4 ($2 \times CH_3$ Leu).

HRMS: calcd: 504.2401; found: 504.2475

$C_{22}H_{38}N_3O_8PCl$ (538.9)	calcd	C 49.03	H 7.11	N 7.80
	found	47.74	7.25	7.87

L-*Leucyl*-L-*alanyl-(O)-diallylphosphato*-L-*serine Allyl Ester Hydrochloride* (**6d**): yellow oil, yield 72 %, $[\alpha]_D^{20} = -31.8$ ($c = 1$, MeOH).

^{1}H NMR (400 MHz, CD_3OD): $\delta = 6.03$–5.90 (m, 3 H, $3 \times CH{=}CH_2$), 5.42–5.31 (m, 3 H, $3 \times CH{=}CH_{2trans}$), 5.29–5.23 (m, 3 H, $3 \times CH{=}CH_{2cis}$), 4.68 (m, 1 H, α-CH), 4.62–4.53 (m, 6 H, $3 \times CH_2$ $CH{=}CH_2$), 4.51–4.18 (m, 4 H, $2 \times \alpha$-CH, β-CH_2 Ser), 1.71 (m, 3 H, β-CH_2 Leu, γ-CH Leu), 1.43 (d, 3 H, $J = 7$ Hz, CH_3 Ala), 1.01 (d, 3 H, $J = 6$ Hz, CH_3 Leu), 1.00 (d, 3 H, $J = 6$ Hz, CH_3 Leu).

^{13}C NMR (100.6 MHz, CD_3OD): $\delta = 174.5$, 170.4, 169.7 ($3 \times CO$), 133.7, 133.6, 132.4 ($3 \times CH{=}CH_2$), 119.1, 119.0 ($3 \times CH{=}CH_2$), 69.9, 69.8, 68.0 ($3 \times CH_2$ $CH{=}CH_2$), 67.4 (β-CH_2 Ser), 52.8 (α-CH Ser), 50.3 (α-CH), 50.1 (α-CH), 41.6 (β-CH_2 Leu), 25.3 ($(CH_3)_2CH$), 23.2, 22.0, 18.2 ($3 \times CH_3$).

HRMS: calcd: 490.2318; found: 490.2238

$C_{21}H_{37}N_3O_8P$ (490.2)

L-*Alanyl*-L-*alanyl-(O)-diallylphosphato*-L-*serine tert-Butyl Ester Hydrochloride* (**6e**): colorless oil, yield 62 %, $[\alpha]_D^{20} = -12.9$ ($c = 1$, MeOH).

^{1}H NMR (250 MHz, CD_3OD): $\delta = 6.00$–5.80 (m, 2 H, $2 \times CH{=}CH_2$), 5.40–5.10 (m, 4 H, $2 \times CH{=}CH_2$), 4.61 (m, 1 H, α-CH), 4.52–4.28 (m, 8 H, $2 \times \alpha$-CH, β-CH_2 Ser, $2 \times CH_2$ $CH{=}CH_2$), 1.48 (s, 9 H, $C(CH_3)_3$), 1.41 (d, 3 H, $J = 7$ Hz, CH_3), 1.33 (d, 3 H, $J = 7$ Hz, CH_3).

HRMS: calcd: 464.2163; found: 464.2178

$C_{19}H_{36}N_3O_8P$ (463.5)

Enzymatic Deprotection of Phosphopeptide Heptyl Esters Under the Agency of Lipases; General Procedure:

To a solution of lipase from *Aspergillus niger* (0.1–1.2 g, 0.4–4.8 units) in phosphate buffer (20–100 mL, 0.2 M, pH 7) was added a solution of the respective fully protected phosphopeptide (80–950 μmol) in acetone (2–10 mL) at 37 °C, and, if required, deoxytaurocholic acid sodium salt (0.1 equiv). The mixture was shaken at 37 °C for 12–48 h, saturated with NaCl, and the pH was adjusted to pH 4.5 with conc. HCl. The solution was extracted with EtOAc (5 × 100 mL), the combined organic layers were dried ($MgSO_4$) and evaporated to dryness. If necessary the crude product was purified by flash chromatography (EtOAc/hexane = 10/3 and 1–2 vol% CH_3CO_2H).

According to this procedure the following compounds were synthesized:

N-Allyloxycarbonyl-(O)-diethylphosphato-L-*serine* (**7a**): yellow oil, yield 89%, $[\alpha]_D^{20} = +16.3$ ($c = 0.2$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 5.98$–5.90 (m, 1 H, C*H*=CH$_2$), 5.32 (dd, 1 H, $J_{trans} = 17.1$ Hz, $J_{gem} = 1.3$ Hz, CH=C*H*$_{2trans}$), 5.19 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.3$ Hz, CH=C*H*$_{2cis}$), 4.57 (d, 2 H, $J = 5.3$ Hz, C*H*$_2$ CH=CH$_2$), 4.50–4.48 (m, 1 H, α-CH Ser), 4.39–4.30 (m, 2 H, β-CH$_2$ Ser), 4.15–4.08 (m, 4 H, 2 × POCH$_2$), 1.34–1.31 (m, 6 H, 2 × POCH$_2$C*H*$_3$).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 171.8$ (CO acid), 158.2 (CO urethane), 134.1 (*C*H=CH$_2$), 117.7 (CH=*C*H$_2$), 68.2 (d, $^2J_{C,P} = 5.3$ Hz, β-CH$_2$ Ser), 66.7 (*C*H$_2$CH=CH$_2$), 65.6 (d, $^2J_{C,P} = 5.4$ Hz, 2C, POCH$_2$), 55.6 (d, $^3J_{C,P} = 7.9$ Hz, α-CH Ser), 16.3 (d, $^3J_{C,P} = 6.4$ Hz, 2C, POCH$_2$*C*H$_3$).

HRMS: calcd: 325.0926, found: 325.0916

$C_{11}H_{20}NO_8P$ (325.26) calcd C 40.62 H 6.20 N 4.30
found 40.62 6.24 3.89

N-tert-Butyloxycarbonyl-(O)-diethylphosphato-L-*seryl*-L-*alanine* (**7b**): colorless oil, yield 96%, $[\alpha]_D^{20} = -5.3$ ($c = 0.13$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.42$–4.39 (m, 2 H, α-CH Ala, α-CH Ser), 4.28–4.15 (m, 2 H, β-CH$_2$ Ser), 4.12 (q, 4 H, $J = 7.4$ Hz, 2 × POCH$_2$), 1.46 (s, 9 H, C(CH$_3$)$_3$), 1.41 (d, 3 H, $J = 7.2$ Hz, β-CH$_3$ Ala), 1.33 (t, 6 H, $J = 7.1$ Hz, 2 × POCH$_2$C*H*$_3$).

^{13}C NMR (125.6 MHz, CD_3OD): $\delta = 175.4$, 170.3 (CO acid, CO amide), 157.5 (CO urethane), 81.0 (*C*(CH$_3$)$_3$), 68.1 (d, $^2J_{C,P} = 5.6$ Hz, β-CH$_2$ Ser), 65.6 (d, $^2J_{C,P} = 6.0$ Hz, 2C, 2 × POCH$_2$), 55.7 (α-CH Ser), 49.4 (α-CH Ala), 28.6 (3C, C(*C*H$_3$)$_3$), 17.8 (β-CH$_3$ Ala), 16.3 (d, $^3J_{C,P} = 6.5$ Hz, 2C, 2 × POCH$_2$*C*H$_3$).

HRMS: calcd: 413.1688, found: 413.1630

$C_{15}H_{29}N_2O_9P$ (412.38) calcd C 43.69 H 7.08 N 6.79
found 43.72 7.07 6.60

N-tert-Butyloxycarbonyl-L-*alanyl-(O)-diethylphosphato*-L-*serine* (**7c**): colorless oil, yield 78%, $[\alpha]_D^{20} = -6.4$ ($c = 3.88$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.67$ (d, 1 H, $J = 2.1$ Hz, α-CH Ser), 4.47–4.27 (m, 2 H, β-CH$_2$ Ser), 4.15–4.06 (m, 5 H, 2 × POCH$_2$, α-CH Ala), 1.44 (s, 9 H, C(CH$_3$)$_3$), 1.37–1.24 (m, 9 H, 2 × POCH$_2$C*H*$_3$, β-CH$_3$ Ala).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 175.2$, 171.6 (CO acid, CO amide), 157.6 (CO urethane), 80.6 (*C*(CH$_3$)$_3$), 68.1 (d, $^2J_{C,P} = 5.3$ Hz, β-CH$_2$ Ser), 65.6 (d, $^2J_{C,P} = 5.9$ Hz, 2C, 2 × POCH$_2$), 54.1 (d, $^3J_{C,P} = 8.2$ Hz, α-CH Ser), 51.5 (α-CH Ala), 28.7 (3C, C(*C*H$_3$)$_3$), 18.3 (β-CH$_3$ Ala), 16.4 (d, $^3J_{C,P} = 6.0$ Hz, 2C, 2 × POCH$_2$*C*H$_3$).

HRMS: calcd: 412.1610, found: 412.1597

$C_{15}H_{29}N_2\ O_9P$ (412.38) calcd C 43.69 H 7.08 N 6.79
found 44.00 7.19 6.51

N-Allyloxycarbonyl-(O)-diethylphosphato-L-*serylglycine* (**7d**): yellow oil, yield 90%, $[\alpha]_D^{20} = -0.2$ ($c = 0.46$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 5.99$–5.91 (m, 1 H, C*H*=CH$_2$), 5.33 (dd, 1 H, $J_{trans} = 17.2$ Hz, $J_{gem} = 1.4$ Hz, CH=C*H*$_{2trans}$), 5.20 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.4$ Hz, CH=C*H*$_{2cis}$), 4.61–4.56 (m, 2 H, C*H*$_2$ CH=CH$_2$), 4.54–4.52 (m, 1 H, α-CH Ser), 4.34–4.24 (m, 2 H, β-CH$_2$ Ser), 4.15–4.07 (m, 4 H, 2 × POCH$_2$), 3.93 (brs, 2 H, α-CH$_2$ Gly), 1.34–1.31 (m, 6 H, 2 × POCH$_2$C*H*$_3$).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 172.5$, 171.2 (CO acid, CO amide), 158.1 (CO urethane), 134.3 (*C*H=CH$_2$), 117.8 (CH=*C*H$_2$), 68.0 (d, $^2J_{C,P} = 4.9$ Hz, β-CH$_2$ Ser), 66.9 (*C*H$_2$ CH=CH$_2$), 65.7 (d, $^2J_{C,P} = 6.0$ Hz, 2C, POCH$_2$), 56.2 (d, $^3J_{C,P} = 7.9$ Hz, α-CH Ser), 41.8 (α-CH$_2$ Gly), 16.3 (d, $^3J_{C,P} = 6.5$ Hz, 2C, POCH$_2$*C*H$_3$).

$C_{13}H_{23}N_2O_9P$ (382.30) calcd C 40.84 H 7.32 N 6.06
found 40.61 7.41 5.96

N-Allyloxycarbonyl-(O)-diethylphosphato-L-*seryl*-L-*leucine* (**7e**): yellow oil, yield 90%, $[\alpha]_D^{20} = -11.0$ ($c = 0.85$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 5.98$–5.90 (m, 1 H, C*H*=CH$_2$), 5.32 (dd, 1 H, $J_{trans} = 17.2$ Hz, $J_{gem} = 1.5$ Hz, CH=C*H*$_{2trans}$), 5.19 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.4$ Hz, CH=C*H*$_{2cis}$), 4.57–4.56 (m, 2 H, C*H*$_2$ CH=CH$_2$), 4.54–4.44 (m, 2 H, α-CH Ser, α-CH Leu), 4.31–4.27 (m, 1 H, β-CH$_{2a}$ Ser), 4.23–4.19 (m, 1 H, β-CH$_{2b}$ Ser), 4.15–4.09 (m, 4 H, 2 × POCH$_2$), 1.74–1.68 (m, 1 H, γ-CH Leu), 1.66–1.62 (m, 2 H, β-CH$_2$ Leu), 1.34–1.31 (m, 6 H, 2 × POCH$_2$C*H*$_3$), 0.96 (d, 3 H, $J = 6.4$ Hz, δ-CH$_3$ Leu), 0.92 (d, 3 H, $J = 6.4$ Hz, δ-CH$_3$ Leu).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 175.4$, 170.9 (CO acid, CO amide), 158.0 (CO urethane), 134.1 (*C*H=CH$_2$), 117.8 (CH=*C*H$_2$), 67.9 (d, $^2J_{C,P} = 5.2$ Hz, β-CH$_2$ Ser), 66.8 (*C*H$_2$ CH=CH$_2$), 65.7 (d, $^2J_{C,P} = 5.9$ Hz, 2C, 2 × POCH$_2$), 56.2 (α-CH Ser), 52.2 (α-CH Leu), 41.7 (β-CH$_2$ Leu), 25.8 (γ-CH Leu), 23.3 (δ-CH$_3$ Leu), 21.8 (δ-CH$_3$ Leu), 16.3 (d, $^3J_{C,P} = 6.6$ Hz, 2C, 2 × POCH$_2$*C*H$_3$).

HRMS: calcd: 439.1845, found: 439.1914

$C_{17}H_{31}N_2O_9P$ (438.41) calcd C 46.57 H 7.12 N 6.39
found 46.67 7.07 6.95

N-Allyloxycarbonyl-(O)-diethylphosphato-L-*seryl*-L-*valine* (**7f**): yellow oil, yield 89%, $[\alpha]_D^{20} = -6.0$ ($c = 0.1$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.14$ (d, 1 H, $J = 8.3$ Hz, NH amide), 5.98–5.90 (m, 1 H, C*H*=CH$_2$), 5.32 (dd, 1 H, $J_{trans} = 17.2$ Hz, $J_{gem} = 1.4$ Hz, CH≡C*H*$_{2trans}$), 5.19 (dd, 1 H, $J_{cis} = 10.5$ Hz, $J_{gem} = 1.2$ Hz, CH=C*H*$_{2cis}$), 4.57 (d, 2 H, $J = 5.2$ Hz, C*H*$_2$ CH=CH$_2$), 4.55–4.54 (m, 1 H, α-CH Ser), 4.37–4.35 (m, 1 H, α-CH Val), 4.30–4.20 (m, 2 H, β-CH$_2$ Ser), 4.14–4.08 (m, 4 H, 2 × POCH$_2$), 2.22–2.15 (m, 1 H, β-CH Val), 1.38–1.27 (m, 6 H, 2 × POCH$_2$C*H*$_3$), 0.98–0.96 (m, 6 H, 2 × γ-CH$_3$ Val).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 174.2$, 170.9 (CO acid, CO amide), 158.0 (CO urethane), 134.1 (*C*H=CH$_2$), 117.8 (CH=*C*H$_2$), 67.9 (d, $^2J_{C,P} = 4.8$ Hz, β-CH$_2$ Ser), 66.8 (*C*H$_2$ CH=CH$_2$), 65.7 (d, $^2J_{C,P} = 5.9$ Hz, 2C, 2 × POCH$_2$), 59.0 (α-CH Ser), 56.1 (α-CH Val), 31.9 (β-CH Val), 19.5, 18.2 (2 × γ-CH$_3$ Val), 16.3 (d, $^3J_{C,P} = 6.4$ Hz, 2C, 2 × POCH$_2$CH$_3$).

HRMS: calcd: 425.1689, found: 425.1583

$C_{16}H_{29}N_2O_9P$ (424.39) calcd C 45.28 H 6.88 N 6.60
found 45.36 6.92 6.62

N-Allyloxycarbonyl-(O)-diethylphosphato-L-*seryl*-L-*tyrosine* (**7g**): yellow oil, yield 56%, $[\alpha]_D^{20} = +10.0$ ($c = 0.08$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 7.02$ (d, 2 H, $J = 8.3$ Hz, CH-2/6 Tyr), 6.69 (d, 2 H, $J = 8.3$ Hz, CH-3/5 Tyr), 5.98–5.90 (m, 1 H, C*H*=CH$_2$), 5.32 (d, 1 H, $J_{trans} = 17.1$ Hz, CH=C*H*$_{2trans}$), 5.19 (d, 1 H, $J_{cis} = 10.5$ Hz, CH=C*H*$_{2cis}$), 4.63–4.60 (m, 1 H, α-CH Tyr), 4.59–4.52 (m, 2 H, C*H*$_2$ CH=CH$_2$), 4.46 (br, 1 H, α-CH Ser), 4.25–4.21 (m, 1 H, β-CH$_{2a}$ Ser), 4.12–4.05 (m, 1 H, β-CH$_{2b}$ Ser), 4.12–4.05 (m, 4 H, 2 × POCH$_2$), 3.09 (dd, 1 H, $J = 14.0$, 5.5 Hz, β-CH$_{2a}$ Tyr), 2.93 (dd, 1 H, $J = 14.0$, 7.7 Hz, β-CH$_{2b}$ Tyr), 1.31 (t, 6 H, 2 × POCH$_2$C*H*$_3$).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 174.1$. 170.5 (CO acid, CO amide), 158.0 (C-4 Tyr), 157.3 (CO urethane), 134.1 (CH=CH$_2$), 131.3 (2C, CH-2/6 Tyr), 128.6 (C-1 Tyr), 117.9 (CH=CH$_2$), 116.2 (2C, CH-3/5 Tyr), 67.8 (d, $^2J_{C,P} = 4.8$ Hz, β-CH$_2$ Ser), 66.9 (*C*H$_2$ CH=CH$_2$), 65.7 (d, $^2J_{C,P} = 6.0$ Hz, 2C, 2 × POCH$_2$), 56.1 (d, $^3J_{C,P} = 8.0$ Hz, α-CH Ser), 55.3 (α-CH Tyr), 37.5 (β-CH$_2$ Tyr), 16.3 (d, $^3J_{C,P} = 6.5$ Hz, 2C, 2 × POCH$_2$C*H*$_3$).

HRMS: calcd: 489.1638, found: 489.1700

$C_{20}H_{29}N_2O_{10}P \cdot 0.5\,H_2O$ (488.43) calcd C 48.29 H 6.10 N 5.63
found 48.07 6.13 5.15

N-Allyloxycarbonyl-(O)-diethylphosphato-L-*seryl*-L-*phenylalanine* (**7h**): wax, yield 78%, $[\alpha]_D^{20} = +13.4$ ($c = 0.67$, MeOH).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 7.31$–7.18 (m, 5 H, 5 × CH$_{arom}$ Phe), 5.97–5.90 (m, 1 H, C*H*=CH$_2$), 5.32 (d, 1 H, $J_{trans} = 17.2$ Hz, CH=C*H*$_{2trans}$), 5.19 (d, 1 H, $J_{cis} = 10.5$ Hz, CH=C*H*$_{2cis}$), 4.69–4.66 (m, 1 H, α-CH Phe), 4.58–4.51 (m, 2 H, C*H*$_2$ CH=CH$_2$), 4.46–4.44

(m, 1 H, α-CH Ser), 4.27–4.20 (m, 1 H, β-CH_{2a} Ser), 4.17–4.02 (m, 5 H, β-CH_{2b} Ser, 2 × $POCH_2$), 3.20 (dd, 1 H, J = 13.9, 5.3 Hz, β-CH_{2a} Phe), 3.02 (dd, 1 H, J = 13.9, 8.0 Hz, β-CH_{2a} Phe), 1.32–1.29 (m, 6 H, 2 × $POCH_2CH_3$).

^{13}C NMR (125.7 MHz, CD_3OD): δ = 173.9, 170.5 (CO acid, CO amide), 158.0 (CO urethane), 152.1 (ipso-C Phe), 138.0 ($CH{=}CH_2$), 130.3, 130.1, 129.9, 129.4, 127.8 (5 × CH_{arom}), 117.8 ($CH{=}CH_2$), 67.8 (d, $^2J_{C,P}$ = 5.4 Hz, β-CH_2 Ser), 66.8 (CH_2 $CH{=}CH_2$), 64.4 (d, $^2J_{C,P}$ = 6.0 Hz, 2C, 2 × $POCH_2$), 56.1 (d, $^3J_{C,P}$ = 7.0 Hz, α-CH Ser), 55.0 (α-CH Phe), 38.3 (β-CH_2 Phe), 16.3 (d, $^3J_{C,P}$ = 6.5 Hz, 2C, 2 × $POCH_2CH_3$).

HRMS: calcd: 473.1688, found: 473.1581

$C_{20}H_{29}N_2O_9P$ (472.43)	calcd	C 50.84	H 6.19	N 5.93
	found	50.18	6.20	7.16

*N-tert-Butyloxycarbonyl-(O)-diethylphosphato-*L*-seryl-*L*-alanyl-*L*-seryl-*L*-alanine* (**7i**): yellow oil, yield 57%, $[\alpha]_D^{20}$ = +2.5 (c = 0.16, MeOH).

^{1}H NMR (500 MHz, CD_3OD): δ = 4.51–4.41 (m, 4 H, 2 × α-CH Ala, 2 × α-CH Ser), 4.35–4.20 (m, 2 H, β-CH_2 Ser), 4.15–4.01 (m, 4 H, 2 × $POCH_2$), 3.93–3.79 (m, 2 H, β-CH_2 Ser), 1.43 (s, 9 H, $C(CH_3)_3$), 1.38 (d, 3 H, J = 5.6 Hz, β-CH_3 Ala), 1.36 (d, 3 H, J = 6.4 Hz, β-CH_3 Ala), 1.30–1.21 (m, 6 H, 2 × $POCH_2CH_3$).

^{13}C NMR (125.6 MHz, CD_3OD): δ = 174.8, 172.1, 171.7, 171.3 (CO acid, 3 × CO amide), 156.7 (CO urethane), 80.1 ($C(CH_3)_3$), 68.9 (β-CH_2 Ser), 66.2 (d, $^2J_{C,P}$ = 5.6 Hz, 2C, 2 × $POCH_2$), 63.5 (β-CH_2 Ser), 56.4, 56.0 (2 × α-CH Ser), 50.6, 49.1 (2 × α-CH Ala), 28.6 (3C, $C(CH_3)_3$), 18.8, 17.9 (2 × β-CH_3 Ala), 16.7 (d, $^3J_{C,P}$ = 6.7 Hz, 2C, 2 × $POCH_2CH_3$).

MALDI-TOF-MS (DHB): 456 [M − phosphate + K]$^+$, 440 [M − phosphate + Na]$^+$, 192 [phosphate + K]$^+$, 177 [phosphate + Na]$^+$.

$C_{21}H_{39}N_4O_{12}P$ (570.53).

*N-Allyloxycarbonyl-(O)-diallylphosphato-*L*-serine Heptyl Ester* (**9**):

Compound **9** was synthesized according to the procedure described above for the phosphates **4**; colorless oil, yield 96%, $[\alpha]_D^{20}$ = +11.5 (c = 0.8, $CHCl_3$).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.03–5.87 (m, 3 H, 3 × $CH{=}CH_2$), 5.81 (d, 1 H, J = 6.7 Hz, NH urethane), 5.41 (d, 3 H, J_{trans} = 17.1 Hz, 3 × $CH{=}CH_{2trans}$), 5.21 (d, 3 H, J_{cis} = 10.4 Hz, 3 × $CH{=}CH_{2cis}$), 4.56–4.53 (m, 6 H, 3 × CH_2 $CH{=}CH_2$), 4.49 (brs, 1 H, α-CH Ser), 4.38 (brs, 1 H, β-CH_{2a} Ser), 4.19–4.13 (m, 1 H, β-CH_{2b} Ser), 4.12–4.05 (m, 2 H, CO_2CH_2), 1.68–1.63 (m, 2 H, $CO_2CH_2CH_2$), 1.45–1.28 (m, 8 H, 4 × CH_2 Hep), 0.89 (t, 3 H, J = 6.6 Hz, CH_3 Hep).

^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 168.9 (CO ester), 155.9 (CO urethane), 132.5 ($CH{=}CH_2$), 132.2 (2C, $CH{=}CH_2$), 118.6 ($CH{=}CH_2$), 117.9 (2C, $CH{=}CH_2$), 68.5 ($^2J_{C,P}$ = 5.6 Hz, 2C, CH_2 $CH{=}CH_2$), 66.7 (d, $^2J_{C,P}$ = 5.3 Hz, β-CH_2 Ser), 66.5 (CH_2, $CH{=}CH_2$), 66.3 (CO_2CH_2), 54.8 (d, $^3J_{C,P}$ = 7.1 Hz, α-CH Ser), 31.6, 28.8, 28.4, 25.7, 22.5 (5 × CH_2 Hep), 14.0 (CH_3 Hep).

HRMS: calcd: 447.2022, found: 447.2010

$C_{20}H_{34}NO_8P$ (447.47)	calcd	C 53.68	H 7.66	N 3.13
	found	53.31	7.54	2.99

*N-Allyloxycarbonyl-(O)-diallylphosphato-*L*-serine* (**11**):

Compound **11** was synthesized according to the procedure described above for the carboxylic acids **7**; yellow oil, yield 73%, $[\alpha]_D^{20}$ = +27.2 (c = 0.05, CH_2Cl_2).

^{1}H NMR (500 MHz, CD_3OD): δ = 6.01–5.90 (m, 3 H, 3 × $CH{=}CH_2$), 5.41–5.37 (m, 2 H, 2 × $CH{=}CH_{2trans}$), 5.37–5.34 (m, 1 H, $CH{=}CH_{2trans}$), 5.28–5.25 (m, 2 H, 2 × $CH{=}CH_{2cis}$), 5.19 (d, 1 H, J_{cis} = 10.5 Hz, $CH{=}CH_{2cis}$), 4.58–4.48 (m, 6 H, CH_2 $CH{=}CH_2$), 4.42–4.40 (m, 1 H, α-CH Ser), 4.38–4.33 (m, 2 H, β-CH_2 Ser).

^{13}C NMR (62.8 MHz, CD_3OD): δ = 171.9 (CO acid), 158.1 (CO urethane), 134.1 ($CH{=}CH_2$), 133.7 (2C, 2 × $CH{=}CH_2$), 118.9 (2C, 2 × $CH{=}CH_2$), 117.7 ($CH{=}CH_2$), 69.8 (d, $^2J_{C,P}$ = 5.6 Hz, 2C, 2 × CH_2 $CH{=}CH_2$), 68.5 (d, $^2J_{C,P}$ = 5.2 Hz, β-CH_2 Ser), 66.7 (CH_2 $CH{=}CH_2$), 55.6 (d, $^3J_{C,P}$ = 8.0 Hz, α-CH Ser).

HRMS: calcd: 349.0926, found: 349.0976

$C_{13}H_{20}NO_8P$ (349.28).

N-Allyloxycarbonyl-(O)-diallylphosphato-L-seryl-L-threonyl-L-serine Heptyl Ester (13):

To a solution of **11** (75 mg, 2.14 mmol), EDC (83 mg, 4.28 mmol, 2 equiv) and 7-aza-*N*-hydroxybenzotriazole (HOAt) (59 mg, 4.28 mmol, 2 equiv) in anhyd CH_2Cl_2 (10 mL) was added at 0 °C a solution of L-threonyl-L-serine heptyl ester hydrochloride **12** (73 mg, 2.14 mmol, 1 equiv) and Et_3N (22 mg, 2.14 mmol, 1 equiv) in anhyd CH_2Cl_2 (5 mL). After stirring for 1 h at this temperature, the mixture was left overnight at r.t. and filtered. The filtrate was washed with 0.5 M HCl (3 × 10 mL), dried and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane = 4/1 (v/v)).

Colorless amorphous solid, yield 126 mg (93%), $[\alpha]_D^{20}$ = −21.6 (c = 0.06, CH_2Cl_2).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.61 (d, 1 H, J = 7.6 Hz, NH amide), 7.47 (d, 1 H, J = 8.1 Hz, NH amide), 6.38 (d, 1 H, J = 6.7 Hz, NH urethane), 5.95–5.87 (m, 3 H, 3 × $CH{=}CH_2$), 5.37 (dd, 3 H, J_{trans} = 17.1 Hz, J_{gem} = 1.2 Hz, 2 × $CH{=}CH_{2trans}$), 5.27 (d, 2 H, J_{cis} = 10.4 Hz, 2 × $CH{=}CH_{2cis}$), 5.21 (dd, 1 H, J_{cis} = 10.4 Hz, J_{gem} = 1.0 Hz, $CH{=}CH_{2cis}$), 4.61–4.52 (m, 9 H, 2 × α-CH Ser, α-CH Thr, 3 × CH_2 $CH{=}CH_2$), 4.48–4.46 (m, 1 H, β-CH_{2a} Ser), 4.44–4.39 (m, 1 H, β-CH Thr), 4.31–4.28 (m, 1 H, β-CH_{2b} Ser), 4.16–4.12 (m, 2 H, CO_2CH_2), 3.95–3.89 (m, 2 H, β-CH_2 Ser), 1.66–1.61 (m, 2 H, $CO_2CH_2CH_2$), 1.31–1.27 (m, 8 H, 4 × CH_2 Hep), 1.17 (d, 3 H, J = 6.4 Hz, γ-CH_3 Thr), 0.88 (t, 3 H, J = 6.6 Hz, CH_3 Hep).

^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 170.6, 170.1, 169.3 (CO ester, 2 × CO amide), 156.2 (CO urethane), 132.1 (d, $^3J_{C\text{-}P}$ = 6.3 Hz, 2C, 2 × $CH{=}CH_2$), 131.9 ($CH{=}CH_2$), 119.0 ($CH{=}CH_2$), 118.9 (d, $^4J_{C\text{-}P}$ = 4.4 Hz, 2C, 2 × $CH{=}CH_2$), 68.9 (d, $^2J_{C\text{-}P}$ = 5.0 Hz, 2C, 2 × CH_2 $CH{=}CH_2$), 66.9 (CH_2 $CH{=}CH_2$), 66.7 (β-CH Thr), 66.3 (CO_2CH_2), 65.9, 62.3 (2 × β-CH_2 Ser), 57.8 (α-CH Thr), 55.2 (2 × α-CH Ser), 31.6, 28.8, 28.4, 25.7, 22.5 (5 × CH_2 Hep), 18.6 (γ-CH_3 Thr), 14.4 (CH_3 Hep).

HRMS: calcd: 636.2897, found: 636.2974

$C_{27}H_{46}N_3O_{12}P$ (635.65)	calcd	C 51.01	H 7.29	N 6.61
	found	51.20	7.17	6.81

N-Allyloxycarbonyl-(O)-diallylphosphato-L-seryl-L-threonyl-L-serine (14):

To a solution of lipase from *Aspergillus niger* (80 mg) in phosphate buffer (40 mL, 0.2 M, pH 7) was added a solution of ester **13** (44 mg, 70 μmol) in acetone (2 mL) at 37 °C. The mixture was shaken at 37 °C for 48 h, saturated with NaCl, and the pH was adjusted to 4.5 with conc. HCl. The solution was extracted with EtOAc (5 × 100 mL), the combined organic layers were dried ($MgSO_4$) and concentrated to dryness. The crude product was purified by flash chromatography (EtOAc/hexane = 10/3 and 1–2 vol% CH_3CO_2H).

Yellow oil, yield 30 mg (81%), $[\alpha]_D^{20}$ = +1.0 (c = 1.0, MeOH).

^{1}H NMR (500 MHz, CD_3OD): δ = 5.99–5.91 (m, 3 H, 3 × $CH{=}CH_2$), 5.39 (dd, 2 H, J_{trans} = 17.1 Hz, J_{gem} = 1.4 Hz, 2 × $CH{=}CH_{2trans}$), 5.33 (dd, 1 H, J_{trans} = 15.7 Hz, J_{gem} = 1.6 Hz, $CH{=}CH_{2trans}$), 5.26 (d, 2 H, J_{cis} = 10.4 Hz, J_{gem} = 1.2 Hz, 2 × $CH{=}CH_{2cis}$), 5.20 (dd, 1 H, J_{cis} = 10.4 Hz, J_{gem} = 1.4 Hz, $CH{=}CH_{2cis}$), 4.58–4.55 (m, 6 H, β-CH_2 Ser, 2 × CH_2 $CH{=}CH_2$), 4.54–4.47 (m, 1 H, α-CH Ser), 4.45–4.42 (m, 1 H, α-CH Thr), 4.39–4.33 (m, 3 H, CH_2 $CH{=}CH_2$, α-CH Ser), 4.24–4.19 (m, 1 H, β-CH Thr), 3.94–3.91 (m, 1 H, β-CH_{2a} Ser), 3.86–3.83 (m, 1 H, β-CH_{2b} Ser), 1.20 (d, 3 H, J = 6.4 Hz, γ-CH_3 Thr).

^{13}C NMR (125.7 MHz, CD_3OD): δ = 173.0, 171.9, 171.0 (CO acid, 2 × CO amide), 158.3 (CO urethane), 134.0 ($CH{=}CH_2$), 133.6 (d, $^3J_{C\text{-}P}$ = 6.5 Hz, 2C, 2 × $CH{=}CH_2$), 118.9 (2C, 2 × $CH{=}CH_2$), 117.9 ($CH{=}CH_2$), 69.8 (m, $^2J_{C\text{-}P}$ = 4.5 Hz, β-CH_2 Ser), 68.4 (β-CH Thr), 68.1 (CH_2 $CH{=}CH_2$), 67.0 (2C, 2 × CH_2 $CH{=}CH_2$), 62.8 (β-CH_2 Ser), 59.7, 56.4 (2 × α-CH Ser), 56.2 (α-CH Thr), 19.7 (γ-CH_3 Thr).

HRMS: calcd: 538.1802 found: 538.1884

$C_{20}H_{32}N_3O_{12}P$ (537.46).

N-Allyloxycarbonyl-(O)-diallylphosphato-L-seryl-L-threonyl-L-seryl-L-threonyl-L-proline Allyl Ester (16):
To a solution of **14** (25 mg, 46.5 μmol), EDC (18 mg, 92 μmol, 2 equiv) and N-hydroxybenzotriazole (HOBt) (13 mg, 92 μmol, 2 equiv) in anhyd CH_2Cl_2 (10 mL) was added at 0 °C a solution of hydrochloride **15** (14 mg, 46.5 μmol, 1 equiv) and Et_3N (5 mg, 46.5 μmol, 1 equiv) in anhyd CH_2Cl_2 (5 mL). After stirring for 1 h at this temperature, the mixture was left overnight at r.t. and filtered. The organic layer was extracted with 0.5 M HCl (3 × 5 mL), dried and concentrated under reduced pressure. The crude product was purified by flash chromatography [$CHCl_3$/EtOH = 20/1 (v/v)]; colorless oil, yield 26 mg (71 %), $[\alpha]_D^{20} = -37.2$ ($c = 0.09$, CH_2Cl_2).

^{1}H NMR (500 MHz, CD_3OD): $\delta = 6.01-5.89$ (m, 4 H, 4 × C*H*=CH_2), 5.39 (dd, 1 H, $J_{trans} = 17.1$ Hz, $J_{gem} = 1.5$ Hz, CH=C*H*$_{2trans}$), 5.33 (dd, 2 H, $J_{trans} = 17.2$ Hz, $J_{gem} = 1.5$ Hz, 2 × CH=C*H*$_{2trans}$), 5.33 (d, 1 H, $J_{trans} = 17.3$ Hz, CH=C*H*$_{2trans}$), 5.27 (dd, 2 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.3$ Hz, 2 × CH=C*H*$_{2cis}$), 5.22 (dd, 1 H, $J_{cis} = 10.6$ Hz, $J_{gem} = 1.3$ Hz, CH=C*H*$_{2cis}$), 5.20 (dd, 1 H, $J_{cis} = 10.8$ Hz, $J_{gem} = 1.2$ Hz, CH=C*H*$_{2cis}$), 4.64–4.55 (m, 10 H, 2 × α-CH Thr, 4 × C*H*$_2$ CH=CH_2), 4.52–4.44 (m, 3 H, 2 × α-CH Ser, α-CH Pro), 4.38–4.33 (m, 2 H, β-CH_2 Ser), 4.23 (q, 1 H, $J = 3.8$ Hz, β-CH Thr), 4.04–3.99 (m, 1 H, β-CH Thr), 3.91–3.55 (m, 4 H, β-CH_2 Ser, δ-CH_2 Pro), 2.33–2.24 (m, 1 H, β-CH_{2a} Pro), 2.07–1.94 (m, 3 H, β-CH_{2b} Pro, γ-CH_2 Pro), 1.20 (d, 3 H, $J = 6.4$ Hz, γ-CH_3 Thr), 1.17 (d, 3 H, $J = 6.3$ Hz, γ-CH_3 Thr).

^{13}C NMR (125.7 MHz, CD_3OD): $\delta = 173.1$, 172.1, 171.9, 171.1, 171.0 (CO ester, 4 × CO amide), 158.3 (CO urethane), 134.0, 133.7, 133.6, 133.3 (4 × *C*H=CH_2), 119.1, 118.9, 118.7, 117.9 (4 × CH=*C*H$_2$), 69.9, 69.8 (2 × *C*H$_2$ CH=CH_2), 68.7, 68.2 (2 × β-CH Thr), 68.0 (β-CH_2 Ser), 67.0, 66.7 (2 × *C*H$_2$ CH=CH_2), 60.6 (β-CH_2 Ser), 59.7, 58.6 (2 × α-CH Ser), 58.2 (α-CH Thr), 56.8 (α-CH Pro), 56.5 (α-CH Thr), 47.6 (δ-CH_2 Pro), 30.1 (β-CH_2 Pro), 25.8 (γ-CH_2 Pro), 19.7 (2C, γ-CH_3 Thr).

HRMS: calcd: 776.3119 found: 776.3197

$C_{32}H_{50}N_5O_{15}P$ (775.75)

(O)-Phosphato-L-seryl-L-threonyl-L-seryl-L-threonyl-L-proline (17):
To a solution of ester **16** (10 mg, 12.9 μmol) in anhyd THF under Ar was added 10 mol% of $Pd(PPh_3)_4$. After stirring for 5 min at r.t. a solution of formic acid (6 mg, 129 μmol, 10 equiv) in THF (0.5 mL) was added dropwise followed by a solution of butylamine (5.4 mg, 78 μmol, 6 equiv) in THF (0.5 mL). The mixture was stirred at r.t. until no starting material **16** could be detected by t.l.c. anymore (ca. 4 h). The solution was poured into $CHCl_3$ (20 mL) and extracted with H_2O (5 × 10 mL). The combined aqueous layers were lyophilized and the resulting residue was purified by size exclusion chromatography (Sephadex G10); yellow oil, yield 5.1 mg (73 %), $[\alpha]_D^{20} = -76$ ($c = 0.06$, H_2O).

^{1}H NMR (500 MHz, D_2O): $\delta = 4.47-3.48$ (m, 13 H, 2 × α-CH Thr, 2 × α-CH Ser, α-CH Pro, 2 × β-CH_2 Ser, 2 × β-CH Thr, δ-CH_2 Pro), 2.15–2.06 (m, 1 H, β-CH_{2a} Pro), 1.90–1.72 (m, 3 H, β-CH_{2b} Pro, γ-CH_2 Pro), 1.10 (d, 3 H, $J = 6.3$ Hz, γ-CH_3 Thr), 1.02 (d, 3 H, $J = 6.4$ Hz, γ-CH_3 Thr).

MALDI-TOF-MS (sinapinic acid): calcd: 571.1 found: 571.1

$C_{19}H_{34}N_5O_{13}P$ (571.48).

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