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Caged *O*-phosphorothioyl amino acids as building blocks for Fmoc-based solid phase peptide synthesis

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Abstract—The synthesis of 1-(2-nitrophenylethyl) caged *O*-phosphorothioylserine, -threonine, and -tyrosine derivatives is reported. These amino acid building blocks can be directly incorporated into peptides by Fmoc-based solid phase synthesis as their pentafluorophenyl esters or as symmetric anhydrides. Upon irradiation with UV light, the thiophosphate group, representing a hydrolysis resistant phosphate analog, is revealed.

1. Introduction

The phosphorylation of serine, threonine, and tyrosine residues in proteins is a central mechanism of cellular regulation.[1,2] Phosphorylation can profoundly modulate the role of the protein in a biological system, by altering either the activity of the isolated protein or the interactions with other proteins. The phosphorylation state of a protein is dynamic and determined by the interplay of kinases, which append the phosphoryl group, and phosphatases, which remove it.[3] The use of synthetic phosphopeptides has allowed the characterization of phosphopeptide peptide/protein interactions in vitro,[4] however, application in cell free systems or in vivo can be limited by the quick hydrolysis by cellular phosphatases. Replacement of the phosphate group by a non-hydrolyzable analog modulates the interference of phosphatases on biological studies.

Thiophosphates are known to be inhibitors or poor substrates for a number of phosphatases.[5–8] The most common method for their introduction into serine, threonine, and tyrosine residues is by incubation of the peptide or protein of interest with a kinase and ATP-γ-S.[9,10] Non-enzymatic preparation of thiophosphorylated peptides is achieved either by on-resin phosphitylation and subsequent sulfurization[11–13] or by the introduction of suitably protected amino-acid building blocks through solid phase synthesis.[12,14]

Precise temporal and spatial control over the release of effector molecules can be achieved by the use of caging groups. A caged compound includes a photocleavable protecting group that masks an essential functionality; upon removal by photolysis, the functionality is revealed, generating a biologically active molecule.[15,16] This concentration burst of a single species allows the monitoring of downstream effects without disrupting other aspects of a system.

The most common photolabile groups implemented in biological experiments are derived from the 2-nitrobenzyl group.[17–19] Their use for caging phosphate groups in peptides or proteins has been recently demonstrated[20–23] and building blocks suitable for the solid phase synthesis of caged phosphopeptides have been reported.[24]

So far, only few examples of caged thiophosphorylated peptides have been described.[25,26] In these instances, the thiophosphate moiety is introduced enzymatically with subsequent installation of the 2-nitrobenzyl caging group via selective chemical modification. The peptides have been found to readily uncage under standard photolysis conditions.

The caged thiophosphate amino-acid building blocks reported here allow for convenient and facile synthesis of peptides containing a thiophosphate residue. Their synthesis follows the general scheme outlined for the phosphate analogs.[24] In a first step, a suitably substituted phosphite is installed on the side-chain hydroxyl group of the amino acid via phosphoramidite chemistry. The sulfur atom is then introduced by using a sulfurization reagent such as elemental sulfur[27] or phenylacetyl disulfide[11] instead of an oxidizing reagent as for the synthesis of phosphates.

2. Results and discussion

2.1. Building block synthesis

The intermediate phosphites **3a–c**[24] formed from phosphoramidite **1** and the Fmoc-amino acids **2a–c** were sulfurized by

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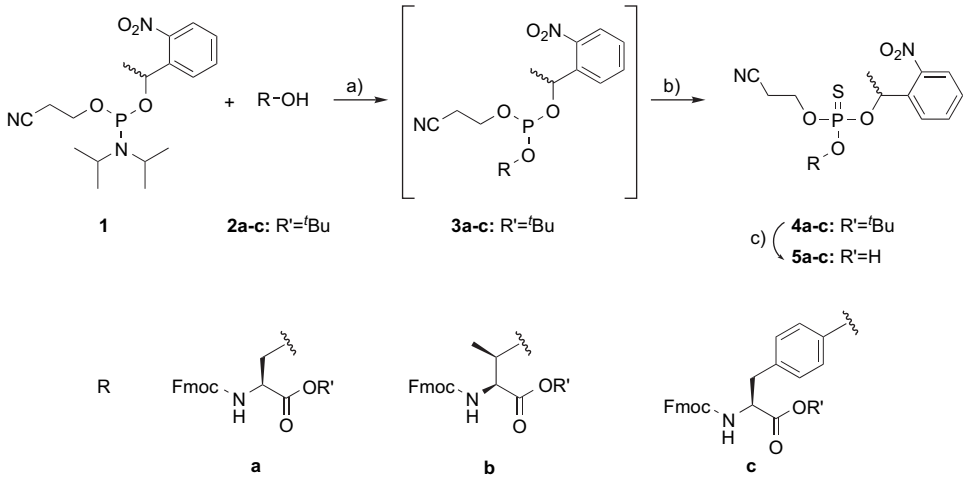


Scheme 1. Reagents and conditions: (a) 4,5-dicyanoimidazole; (b) S_8, pyridine or phenylacetyl disulfide (see text); (c) TFA, TIPS.

excess sulfur in pyridine[27] for the Ser (**4a**) and Tyr (**4c**) derivatives (Scheme 1). Purification of the Thr derivative **4b** obtained by this method was hampered by the presence of an inseparable impurity as detected by ^{31}P NMR. In this case, sulfurization by phenylacetyl disulfide[11] avoided the formation of the side product. The free carboxylic acids **5a–c** were then obtained by treatment with 50% TFA in dichloromethane.

The NPE caging group was introduced as a racemate at the benzylic methine and sulfurization of the intermediate phosphites **3a–c** resulted in an additional asymmetric phosphorus center within the amino-acid building block. Together with the strong peak splitting property of phosphorus, the ^{1}H and ^{13}C NMR spectra appear very complex. The presence of four diastereoisomers is clearly manifested in the ^{31}P NMR spectra, where four peaks are observed. The diastereoisomeric mixtures were generally found to be inseparable by flash chromatography or HPLC; only in case of the Thr derivative **5b** one isomer could be partially separated as indicated by the presence of a single peak in the ^{31}P NMR spectrum. Since uncaging would ultimately afford unique amino-acid derivatives, all three building blocks were used as diastereoisomeric mixtures in the synthesis of polypeptides.

In contrast to the phosphate analogs the asymmetry at the phosphorus center is not lost upon cleavage of the 2-cyanoethyl ester during peptide synthesis, but persists until the thiophosphate group is revealed by uncaging. The presence of diastereoisomers does not seem to have any effect on the viability of the building block itself as uncaging is unaffected.

2.2. Peptide synthesis

Three short peptides **6a–c** (Fig. 1), based on 14-3-3 binding sequences,[20] were targeted for assembly to demonstrate the practical use of the new building blocks. Proteinogenic amino acids were manually coupled by standard methods (*N*,*N*-dimethylformamide (DMF) as solvent, benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate (PyBOP) for activation, *N*,*N*-diisopropylethylamine (DIPEA) as a base, and 20% piperidine in DMF for Fmoc-deprotection). These conditions failed for the phosphorothioyl amino acids **5a** and **5b** (the tyrosine derivative **5c** was not examined) and numerous side products were observed and only a minor amount of the target peptide could be isolated. The variation of the activating reagent (HBTU/HOBt, HATU/HOAt, PyBroP) did not increase the yield. Therefore, a more detailed investigation of the side products was carried out. This study indicated that β-elimination of the thiophosphate moiety had occurred to afford a dehydroalaninyl peptide, which was then subjected to nucleophilic addition of piperidine during the subsequent Fmoc-deprotection step.

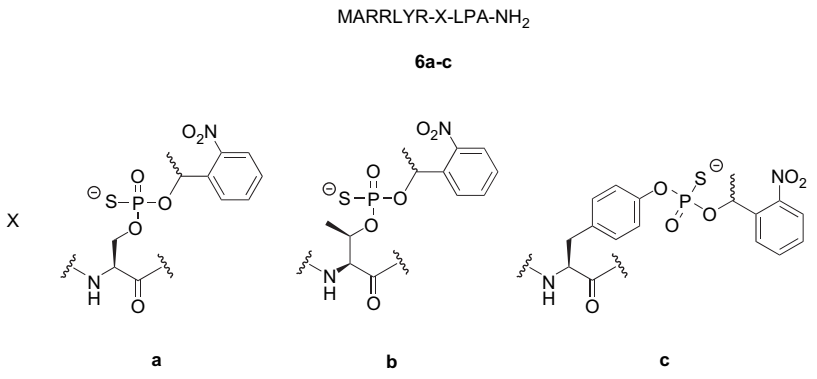


Figure 1. Target caged peptides. It should be noted that the thiolo form of the thiophosphate group depicted here is typically dominant in solution; the thiolo/thiono equilibrium, however, is dependent upon the solvent.[30]

Since β-elimination is favored by the presence of base, coupling of the phosphorothioyl residues was performed in the absence of DIPEA. Amino acids were activated by dicyclohexyl carbodiimide (DCC) mediated symmetric anhydride formation or as pentafluorophenyl (Pfp) esters. Both methods were found to produce identical coupling results but since the use of symmetrical anhydrides is relatively wasteful, Pfp-esters were the preferred method for incorporation of caged phosphorothioyl residues.

Fmoc-deprotection with piperidine did not lead to β-elimination, however, cleavage of the 2-cyanoethyl ester was observed as with the corresponding phosphate analogs.[24] The phosphodiester thus formed is much less susceptible to elimination[28] and chain elongation proceeded smoothly by the standard PyBOP/DIPEA protocol. Acidolytic cleavage of the peptides from the resin by TFA yielded the target sequences in >80% purity (Fig. 2, left column). After purification by preparative HPLC the peptides were obtained in 50–60% yield.

For all three peptides multiple peaks are observed in the HPLC chromatograms as a result of the presence of the phosphorothioyl residue as a mixture of diastereoisomers. This peak splitting is especially distinct for the Thr derivative (Fig. 2B) in accordance with the phosphate analog.[29] However, no attempts were made to isolate individual isomers since uncaging of all species results in the formation of a single, unique peptide product.

The caged phosphorothioyl residues have been found stable toward the exposure to the acidic conditions employed for HPLC analysis and purification. No detectable decomposition occurred as observed previously for thiophosphotyrosine, which had to be purified at neutral pH in order to avoid loss of the thiophosphate group.[12,13]

When exposed to long wave UV light (360–380 nm) for approximately two minutes, the NPE group was quantitatively removed from all peptides (Scheme 2), giving rise to a single peak for peptides **7a–c** in the HPLC chromatogram (Fig. 2, right column). The minor peaks observed could not be attributed to distinct chemical species with the exception of a minor peak having a mass increase of 16 units compared to the target peptide, which is most likely the result of a methionine oxidation. The remaining species are most likely to be low molecular weight side products derived from nitrosoacetophenone formed during the photochemical reaction since

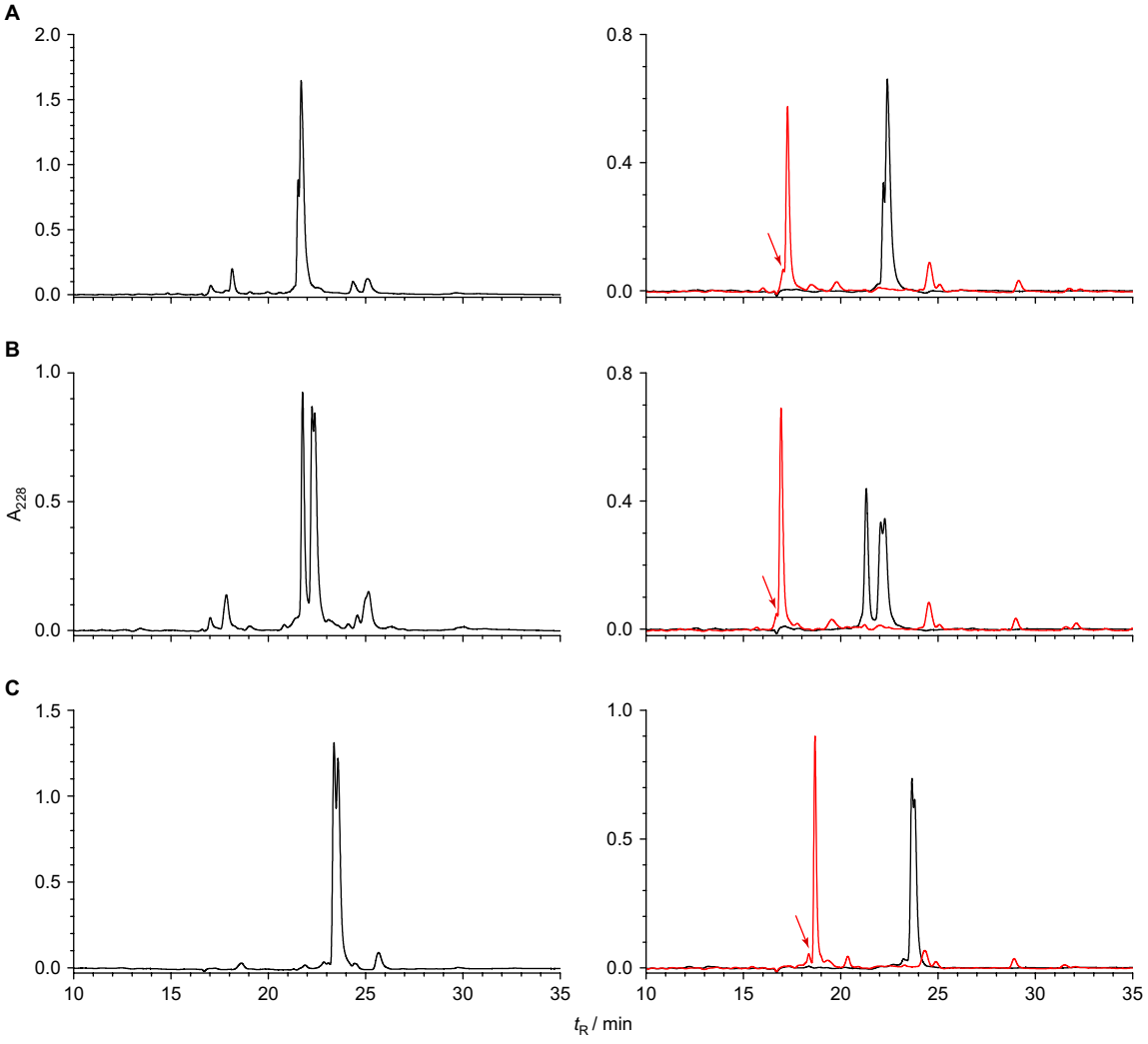
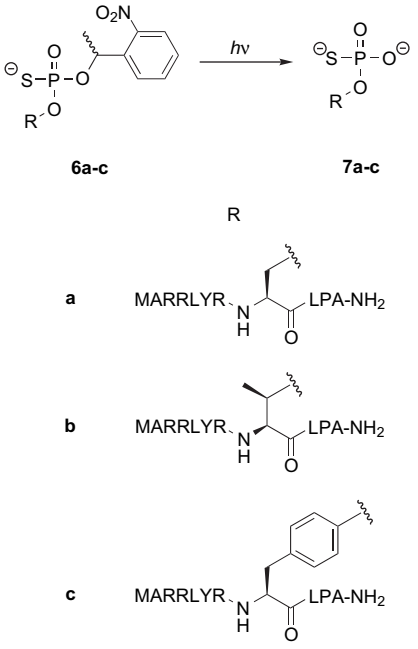


Figure 2. HPLC chromatograms for (A) **6a**, (B) **6b**, and (C) **6c**. Left: crude reaction product after cleavage from the resin. Right: purified peptide before (—) and after (—) irradiations. The arrow points to a presumed methionine oxidation product.

their retention time and distribution pattern seem to be independent of the identity of the parent peptide.



Scheme 2.

3. Conclusion

Thiophosphoamino acids provide an interesting alternative to the corresponding phospho analogs for the investigation of complex kinase pathways. The caged *O*-phosphorothioyl building blocks presented herein allow for the straightforward incorporation of such residues into peptide sequences by standard solid phase synthetic methods. Irradiation with UV light reveals the thiophospho group in a clean and quantitative manner. These peptides are expected to allow for the clear manifestation of downstream effects of phosphorylation events in vivo.

4. Experimental

4.1. General

All commercially available reagents were used as received. Non-volatile oils and solids were dried under vacuum at 0.01 Torr. Reactions were monitored by thin-layer chromatography on precoated E. Merck silica gel 60 F_{254} plates (0.25 mm) and visualized by short wave UV. Flash chromatography was performed on Merck silica gel 60. Chemical shifts are expressed in parts per million relative to tetramethylsilane as internal standard (^{1}H and ^{13}C) or 85% phosphoric acid as external standard (^{31}P). Signals in the ^{13}C NMR spectra for compounds **4a**–**c** and **5a**–**c** typically consist of several close peaks ($\Delta\delta \approx 0.05$ ppm) due to the presence of diastereoisomers. For clarity, an average shift is reported.

4.2. General method A

To a solution of phosphite **3a** or **3c** (synthesized according to the literature[24]) in anhydrous pyridine (3 mL/mmol) was added finely powdered sulfur (10 equiv). The suspension was stirred for 12 h at room temperature in the dark. The sulfur was filtered off through Celite and the filtrate was concentrated. The oily suspension was dissolved in ethyl acetate and filtered again through Celite to remove residual sulfur. The filtrate was washed three times with 1 M HCl, then with satd $NaHCO_3$ followed by brine. After drying over $MgSO_4$, the solvent was evaporated and the residue purified by flash chromatography (ethyl acetate/hexane 1:2).

4.2.1. *tert*-Butyl *O*-{(2-cyanoethoxy)[1-(2-nitrophenyl)-ethoxy]phosphorothioyl}-*N*-[(9*H*-fluoren-9-ylmethoxy)-carbonyl]-L-serinate (4a). Yield 61%. Off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98–7.90 (m, 1H), 7.78–7.62 (m, 6H), 7.48–7.26 (m, 5H), 6.22–6.18 (m, 1H), 5.64 (d, J=7.6 Hz, 0.2H), 5.59 (d, J=7.8 Hz, 0.2H), 5.46 (d, J=7.8 Hz, 0.3H), 5.42 (d, J=8.1 Hz, 0.3H), 4.48–3.98 (m, 8H), 2.71–2.64 (m, 1H), 2.61–2.47 (m, 1H), 1.72—1.69 (m, 3H), 1.50–1.43 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.7, 155.7, 147.0, 143.8, 141.3, 137.3, 133.9, 128.9, 128.0, 127.8, 127.2, 125.2, 124.5, 120.1, 116.4, 83.5, 73.9, 68.2, 67.2, 62.4, 54.5, 47.1, 28.0, 24.1, 19.4; ^{31}P NMR (202 MHz, $CDCl_3$) δ 67.83, 67.80, 67.71, 67.68; HRMS (ESI) *m*/*z* calcd for $C_{33}H_{36}N_3NaO_9PS^+$ $[M+Na]^+$: 704.1802, found: 704.1821.

4.2.2. *tert*-Butyl *O*-{(2-cyanoethoxy)[1-(2-nitrophenyl)-ethoxy]phosphorothioyl}-*N*-[(9*H*-fluoren-9-ylmethoxy)-carbonyl]-L-tyrosinate (4c). Yield 88%. Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97–7.96 (m, 0.8H), 7.82–7.57 (m, 6.2H), 7.49–7.30 (m, 5H), 7.14–6.92 (m, 4H), 6.33–6.26 (m, 1H), 5.35–5.29 (m, 1H), 4.54–4.05 (m, 6H), 3.07–3.02 (m, 2H), 2.70–2.65 (m, 1H), 2.59–2.49 (m, 1H), 1.77–1.71 (m, 3H), 1.41–1.39 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 155.5, 149.1, 146.9, 143.8, 141.3, 137.1, 133.8, 130.7, 128.9, 128.1, 127.7, 127.1, 125.1, 124.4, 120.9, 120.7, 120.0, 116.2, 82.6, 74.2, 66.8, 62.7, 55.1, 47.2, 37.7, 28.0, 24.1, 19.3; ^{31}P NMR (202 MHz, $CDCl_3$) δ 62.81, 62.72, 62.46, 62.39; HRMS (ESI) *m*/*z* calcd for $C_{39}H_{40}N_3NaO_9PS^+$ $[M+Na]^+$: 780.2115, found: 780.2115.

4.2.3. *tert*-Butyl *O*-{(2-cyanoethoxy)[1-(2-nitrophenyl)-ethoxy]phosphorothioyl}-*N*-[(9*H*-fluoren-9-ylmethoxy)-carbonyl]-L-threoninate (4b). To the reaction mixture obtained in the synthesis of **3b**[24] was added 2.5 equiv (with respect to **2b**) phenylacetyl disulfide in one portion and the mixture was stirred for 1 h at room temperature. The solvent was evaporated and the yellow residue was dissolved in ethyl acetate. The solution was washed with satd $NaHCO_3$, water, and brine, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash chromatography (ethyl acetate/hexane 1:2) to obtain **4b** as an off-white solid in 79% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–7.93 (m, 1H), 7.81–7.55 (m, 6H), 8.48–7.35 (m, 5H), 6.21–6.16 (m, 1H), 5.57 (d, J=9.6 Hz, 0.3H), 5.51 (d, J=9.5 Hz, 0.3H), 5.39 (d, J=9.5 Hz, 0.1H), 5.14–5.00 (m, 1H), 4.92 (d, J=9.6 Hz, 0.3H), 4.49–3.96 (m, 6H), 2.73–2.67 (m, 1H), 2.64–2.46 (m, 1H), 1.74–1.70 (m, 3H), 1.51–1.41 (m, 9H), 1.34–1.31 (m, 1.8H), 1.18 (d, J=6.3 Hz, 0.9H), 1.01 (d, J=6.7 Hz, 0.3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2, 156.5, 147.0, 143.7, 141.3, 137.2, 133.8, 128.9, 128.1, 127.8, 127.1, 125.2, 124.3, 120.0, 116.5, 83.2, 73.6, 67.3, 67.0, 62.1, 58.6, 47.1, 27.9, 24.2, 19.3, 18.4; ^{31}P NMR (202 MHz, $CDCl_3$) δ 66.82, 66.71, 66.47, 66.24; HRMS (ESI) *m*/*z* calcd

for $C_{34}H_{38}N_3NaO_9PS^+$ $[M+Na]^+$: 718.1959, found: 718.1939.

4.3. General method B

A solution of **4a–c** and triisopropylsilane (5 equiv) in CH_2Cl_2 (10 mL/mmol) was cooled to 0 °C and trifluoroacetic acid (10 mL/mmol) was added. The mixture was stirred at room temperature in the dark until TLC showed consumption of the starting material (1–2 h). The solvents were evaporated and the residue purified by flash chromatography (CH_2Cl_2/ethanol/acetic acid 94:5:1).

4.3.1. *O*-{(2-Cyanoethoxy)[1-(2-nitrophenyl)ethoxy]-phosphorothioyl}-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-serine (5a). Yield 71%. Pale brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (br s, 1H), 7.92–7.85 (m, 1H), 7.76–7.56 (m, 6H), 7.41–7.30 (m, 5H), 6.20–6.12 (m, 1H), 5.89 (d, J=8.1 Hz, 0.2H), 5.75–5.73 (m, 0.5H), 5.59 (d, J=8.2 Hz, 0.3H), 4.64–4.00 (m, 8H), 2.65–2.63 (m, 1H), 2.57–2.47 (m, 1H), 1.70–1.67 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 156.0, 146.9, 143.6, 141.2, 137.0, 133.9, 128.9, 128.0, 127.8, 127.1, 125.1, 124.4, 120.1, 116.6, 73.9, 67.5 (two overlapping signals), 62.4, 53.9, 46.9, 23.9, 19.3; ^{31}P NMR (202 MHz, $CDCl_3$) δ 67.57, 67.53, 67.45, 67.43; HRMS (ESI) *m/z* calcd for $C_{29}H_{29}N_3O_9PS^+$ $[M+H]^+$: 626.1357, found: 626.1364.

4.3.2. *O*-{(2-Cyanoethoxy)[1-(2-nitrophenyl)ethoxy]-phosphorothioyl}-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-threonine (5b). Yield 91%. Off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (br s, 1H), 7.94–7.26 (m, 12H), 6.17–6.05 (m, 1H), 5.53 (d, J=9.6 Hz, 0.3H), 5.44 (d, J=9.6 Hz, 0.3H), 5.33 (d, J=9.5 Hz, 0.1H), 5.18–5.01 (m, 1.3H), 4.52–4.37 (m, 3H), 4.26–3.93 (m, 3H), 2.73–2.63 (m, 1H), 2.62–2.46 (m, 1H), 1.72–1.67 (m, 3H), 1.33–1.30 (m, 1.8H), 1.13 (d, J=6.3 Hz, 0.9H), 1.04 (d, J=6.4 Hz, 0.3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 156.6, 147.0, 143.6, 141.3, 137.2, 133.8, 128.9, 128.2, 127.8, 127.1, 125.1, 124.3, 120.0, 116.8, 73.6, 67.4, 67.1, 62.3, 58.0, 47.1, 24.0, 19.3, 18.2; ^{31}P NMR (202 MHz, $CDCl_3$) δ 66.42, 66.34, 66.26 (two overlapping signals); HRMS (ESI) *m/z* calcd for $C_{30}H_{31}N_3O_9PS^+$ $[M+H]^+$: 640.1513, found: 640.1501.

4.3.3. *O*-{(2-Cyanoethoxy)[1-(2-nitrophenyl)ethoxy]-phosphorothioyl}-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-tyrosine (5c). Yield 62%. Off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.00 (br s, 1H), 7.95–7.93 (m, 0.8H), 7.80–7.28 (m, 11.2H), 7.11–6.90 (m, 4H), 6.31–6.26 (m, 1H), 5.33–5.28 (m, 1H), 4.68–4.04 (m, 6H), 3.16–3.06 (m, 2H), 2.65–2.48 (m, 2H), 1.75–1.70 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7, 155.9, 149.2, 147.0, 143.6, 141.2, 137.0, 133.9, 130.6, 128.9, 128.1, 127.8, 127.1, 125.0, 124.4, 121.0, 120.9, 120.0, 116.3, 74.2, 67.0, 62.8, 54.8, 47.0, 37.0, 24.1, 19.3; ^{31}P NMR (202 MHz, $CDCl_3$) δ 62.47, 62.46, 62.23, 62.21; HRMS (ESI) *m/z* calcd for $C_{35}H_{33}N_3O_9PS^+$ $[M+H]^+$: 702.1670, found: 702.1655.

4.4. General method C

To a solution of **5a–c** in anhydrous DMF (3 mL/mmol) were added anhydrous pyridine (1.1 equiv) and pentafluorophenyl trifluoroacetate (1.2 equiv). The mixture was stirred for 30 min at room temperature in the dark, diluted with ethyl acetate (60 mL/mmol), and extracted three times with each 1 M HCl and satd $NaHCO_3$, brine, and dried over $MgSO_4$. After evaporation of the solvent, the pentafluorophenyl ester was obtained as a pale yellow solid in typically 95% crude yield and was used without further purification for peptide synthesis. The crude reaction product could be stored at −20 °C in the dark for weeks.

4.5. General method D

To a solution of 6 equiv (with respect to the resin substitution) Fmoc-amino acid in anhydrous CH_2Cl_2 (5 mL/mmol) was added 3 equiv DCC in anhydrous CH_2Cl_2 (5 mL/mmol) at 0 °C. The mixture was stirred for 30 min at 0 °C in the dark during which dicyclohexylurea precipitated. The solvent was removed in vacuo, the residual solid was dissolved in anhydrous DMF, and immediately used for peptide synthesis.

4.6. Peptide synthesis

Peptides were prepared by manual solid phase peptide synthesis on a 3 μmol scale. Amino acids (2 equiv with respect to the resin substitution) were coupled using standard Fmoc-based conditions (*N*,*N*-dimethylformamide (DMF) as solvent, 1.9 equiv benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) for activation, 4 equiv *N*,*N*-diisopropylethylamine (DIPEA) as a base, and 20% piperidine in DMF for Fmoc-deprotection) on a PAL–PEG–PS amide resin (substitution 0.18 mmol/g). Caged thiophosphoamino acids were introduced as preformed symmetrical anhydrides (3 equiv) or pentafluorophenyl esters (2 equiv) in DMF and allowed to react for 2 h in the dark. All subsequent reaction steps were carried out in dimmed light. The peptides were cleaved from the resin with a solution of TFA/H_2O/TIPS 95:2.5:2.5 and precipitated from ice cold diethyl ether.

Crude peptides were purified by reverse phase HPLC (linear gradient of 5–70% MeCN in H_2O with 0.1% TFA over 45 min at a flow rate of 10 mL/min on a YMC-Pack ODS-A 5/120, 20×250 mm^2 column), lyophilized, and characterized by electrospray MS. Analytical HPLC was performed on a Waters YMC ODS-A 5/120, 4.6×250 mm^2 column with a linear gradient of 5–70% MeCN in H_2O with 0.1% TFA over 30 min at a flow rate of 1 mL/min.

4.6.1. Analytical data. Compound **6a**: MS (ESI) *m/z* calcd for $C_{66}H_{111}N_{22}O_{17}PS_2^{2+}$ $[M+2H]^{2+}$: 789.4, found: 789.4.

Compound **6b**: MS (ESI) *m/z* calcd for $C_{67}H_{113}N_{22}O_{17}PS_2^{2+}$ $[M+2H]^{2+}$: 796.4, found: 796.4.

Compound **6c**: MS (ESI) *m/z* calcd for $C_{72}H_{115}N_{22}O_{17}PS_2^{2+}$ $[M+2H]^{2+}$: 827.4, found: 827.4.

Compound **7a**: MS (ESI) *m/z* calcd for $C_{58}H_{104}N_{21}O_{15}PS_2^{2+}$ $[M+2H]^{2+}$: 714.9, found: 714.9.

Compound **7b**: MS (ESI) *m/z* calcd for $C_{59}H_{106}N_{21}O_{15}PS_2^{2+}$ $[M+2H]^{2+}$: 721.9, found: 721.9.

Compound **7c**: MS (ESI) *m/z* calcd for $C_{64}H_{108}N_{21}O_{15}PS_2^{2+}$ $[M+2H]^{2+}$: 752.9, found: 752.9.

4.7. UV irradiation

Peptides **6a–c** (0.3 mg/mL) were dissolved in 10 mM degassed HEPES buffer (pH 7.2), placed in a quartz vessel, and irradiated for 2 min at 360–380 nm (2× Philips PL-S 9W/08 compact fluorescent tubes) under air cooling.

Acknowledgements

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Supplementary data

^{1}H, ^{13}C, and ^{31}P NMR spectra of compounds **4a–c** and **5a–c**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.03.023.

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