Bis(arylmethyl) substituted unsymmetrical phosphites for the synthesis of lipidated peptides via Staudinger-phosphite reactions.

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[1]
1 NMR-Spectra of Phosphorous Compounds

1.1 Phosphoramidites

1.1.1 Bis(p-nitrobenzyl) diisopropylphosphoramidite (3c)

Figure S1. $^1$H-NMR spectrum of bis(p-nitrobenzyl) diisopropylphosphoramidite (3c) (600 MHz, CDCl₃).

Figure S2. $^{13}$C-NMR spectrum of bis(p-nitrobenzyl) diisopropylphosphoramidite (3c) (151 MHz, CDCl₃).
Figure S3. $^{31}$P-NMR spectrum of bis(p-nitrobenzyl) diisopropylphosphoramidite (3c) (243 MHz, CDCl$_3$).

1.1.2 Bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (3e)

Figure S4. $^1$H-NMR spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (3e) (300 MHz, CDCl$_3$).
Figure S5. $^{13}$C-NMR spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (3e) (75 MHz, CDCl$_3$).

Figure S6. $^{31}$P-NMR spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (3e) (122 MHz, CHCl$_3$).
Figure S7. ESI-MS spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (3e).

1.2 Phosphites

1.2.1 Dibenzyl butyl phosphite (4a)

Figure S8. $^1$H-NMR spectrum of dibenzyl butyl phosphite (4a) (600 MHz, CDCl$_3$).
Figure S9. $^{13}$C-NMR spectrum of dibenzyl butyl phosphite (4a) (151 MHz, CDCl$_3$).

Figure S10. $^{31}$P-NMR spectrum of dibenzyl butyl phosphite (4a) (243 MHz, CDCl$_3$).
1.2.2 Bis(o-nitrobenzyl) butyl phosphite (4b)

**Figure S11.** $^1$H-NMR spectrum of bis(o-nitrobenzyl) butyl phosphite (4b) (300 MHz, CD$_3$CN).

**Figure S12.** $^{13}$C-NMR spectrum of bis(o-nitrobenzyl) butyl phosphite (4b) (75 MHz, CD$_3$CN).
1.2.3 Bis(\(\rho\)-nitrobenzyl) butyl phosphite (4c)

Figure S13. \(^{31}\text{P}\)-NMR spectrum of bis(\(\omega\)-nitrobenzyl) butyl phosphite (4b) (122 MHz, CD\(_3\)CN).

Figure S14. \(^1\text{H}\)-NMR spectrum of bis(\(\rho\)-nitrobenzyl) butyl phosphite (4c) (600 MHz, CDCl\(_3\)).
Figure S15. $^{13}$C-NMR spectrum of bis(p-nitrobenzyl) butyl phosphite (4c) (151 MHz, CDCl$_3$).

Figure S16. $^{31}$P-NMR spectrum of bis(p-nitrobenzyl) butyl phosphite (4c) (243 MHz, CDCl$_3$).
1.2.4 Bis(\(\rho\)-methoxy) butyl phosphite (4d)

**Figure S17.** \(^1\)H-NMR spectrum of bis(\(\rho\)-methoxy) butyl phosphite (4d) (400 MHz, CD\(_3\)CN).

**Figure S18.** \(^13\)C-NMR spectrum of bis(\(\rho\)-methoxy) butyl phosphite (4d) (100 MHz, CD\(_3\)CN).
Figure S19. $^{31}$P-NMR spectrum of bis(p-methoxy) butyl phosphite (4d) (162 MHz, CD$_3$CN).

1.2.5 Bis(pyridin-3-ylmethyl) butyl phosphite (4e)

Figure S20. $^1$H-NMR spectrum of bis(pyridin-3-ylmethyl) butyl phosphite (4e) (600 MHz, CDCl$_3$).
Figure S21. $^{13}$C-NMR spectrum of bis(pyridin-3-ylmethyl) butyl phosphite (4e) (151 MHz, CDCl$_3$).

Figure S22. $^{31}$P-NMR spectrum of bis(pyridin-3-ylmethyl) butyl phosphite (4e) (243 MHz, CDCl$_3$).

1.2.6 Octadecyl dibenzyl phosphite (7)
Figure S23. $^1$H-NMR spectrum of octadecyl dibenzyl phosphite (7) (600 MHz, CDCl$_3$).

Figure S24. $^{13}$C-NMR spectrum of octadecyl dibenzyl phosphite (7) 151 MHz, CDCl$_3$).
1.2.7 Octadecyl bis(pyridin-3-ylmethyl) phosphite (8)

Figure S25. $^3$P-NMR spectrum of octadecyl dibenzyl phosphite (7) (243 MHz, CDCl$_3$).

Figure S26. $^1$H-NMR spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (8) (300 MHz, CD$_3$CN).
Figure S27. $^{13}$C-NMR spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (8) (75 MHz, CD$_3$CN).

Figure S28. $^{31}$P-NMR spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (8) (122 MHz, CD$_3$CN).
Figure S29. ESI-MS spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (8).
2 UV-Chromatograms of Staudinger-Phosphite Reactions

Staudinger-phosphite reaction of aryl azide 1 were reacted with \( n \)-butyl phosphites 4a-e to butyl phosphoramidates 5a-e and unmodified phosphoramidates 6a-e. Reaction mixtures were analyzed by HPLC/UV and product amount quantified by integration. Shown here are the chromatograms for the reactions of 4a and 4e.

2.1 \( n \)-butyl dibenzyl phosphite (4a)

**Figure S30.** HPLC-UV chromatograms of the reaction of 1 with 10 eq. of 4a, (A) full, (B) zoom in.

2.2 \( n \)-butyl bis(pyridine-3-ylmethyl) phosphite (4e)

**Figure S31.** HPLC-UV chromatograms of the reaction of 1 with 10 eq. of 4e, (A) full, (B) zoom in.
3 UV-Chromatograms and MS-Spectra of Synthetic Peptides

3.1 Octadecyl pyridin-3-ylmethyl phosphoramidate EPS15-peptide (9)

![Chemical Structure](image)

**Figure S32.** HPLC-UV chromatogram of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (9).

**Figure S33.** ESI-MS spectrum of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (9).
3.2 Azidobenzoic acetylated EPS15-peptide (11)

Figure S34. ESI-MS spectrum of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (9).

Figure S35. HPLC-UV chromatogram of azidobenzoic acetylated EPS15-peptide (11).
Figure S36. ESI-MS spectrum of azidobenzoic acetylated EPS15-peptide (11).

Figure S37. ESI-MS spectrum of azidobenzoic acetylated EPS15-peptide (11).

3.3 Benzyl octadecyl phosphoramidate EPS15-peptide (12)
Figure S38. HPLC-UV chromatogram of benzyl octadecyl phosphoramidate EPS15-peptide (12) on a C4 column with a gradient from 60 % to 100 % of MeCN in water containing 0.1 % TFA.

Figure S39. ESI-MS spectrum of benzyl octadecyl phosphoramidate EPS15-peptide (12).

Figure S40. ESI-MS spectrum of benzyl octadecyl phosphoramidate EPS15-peptide (12).
3.4 Fluorescein labelled octadecyl pyridin-3-ylmethyl phosphoramidate EPS15-peptide (13)

Scheme S1. Synthesis of C-terminally fluorophore labelled control peptides. Loading of a rink amide resin with carboxyfluorescein was performed according to a protocol demonstrated by Brock.\(^1\) Trityl-protection of the hydroxyl groups of the carboxyfluorescein was followed by removal of an orthogonally protected lysine side chain allowing subsequent SPPS. Subsequently, Staudinger-phosphite reaction resulted in the fluorescein labelled monolipidated phosphoramidate modified EPS-peptide 13.

Figure S41. HPLC-UV chromatogram of fluorescein labelled octadecyl pyridin-3-ylmethyl phosphoramidate EPS15-peptide (13) on a C4-column.
Figure S42. ESI-MS spectrum of fluorescein labelled octadecyl pyridin-3-ylmethyl phosphoramidate EPS15-peptide (13).
4 Dynamic Light Scattering

Figure S43. Size distribution of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (9).

Figure S44. Size distribution of fluorescein labelled octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (13).
5 References