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

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1.1 Phosphoramidites

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

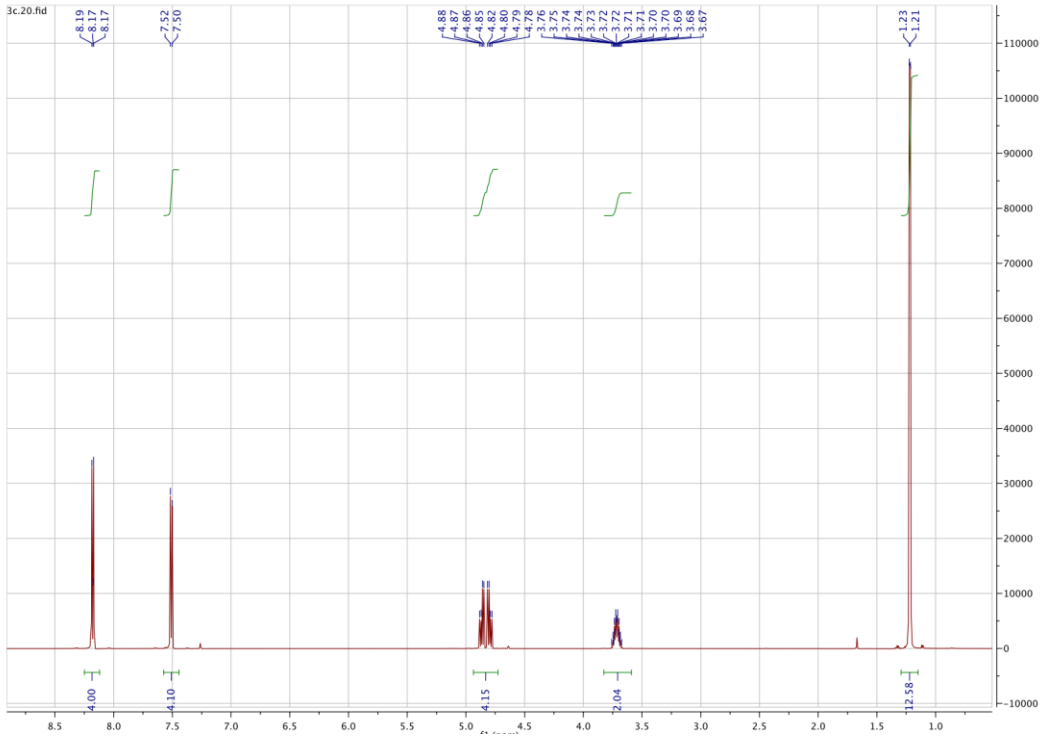


Figure S1. ^{1}H-NMR spectrum of bis(*p*-nitrobenzyl) diisopropylphosphoramidite (**3c**) (600 MHz, $CDCl_3$).

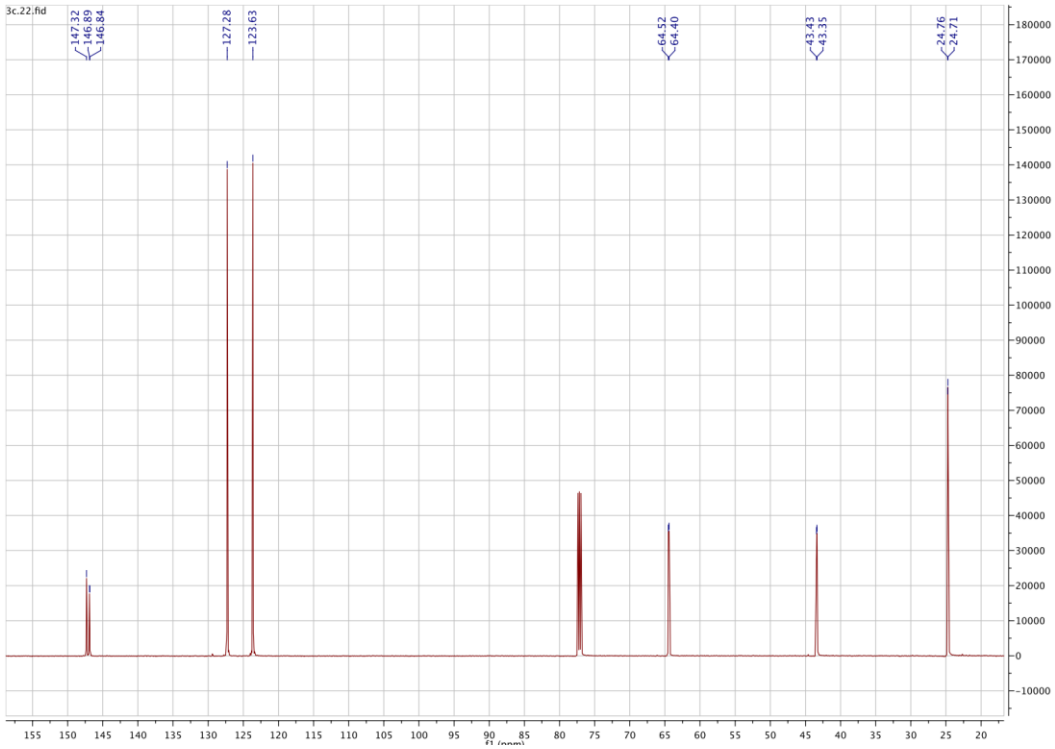


Figure S2. ^{13}C-NMR spectrum of bis(*p*-nitrobenzyl) diisopropylphosphoramidite (**3c**) (151 MHz, $CDCl_3$).

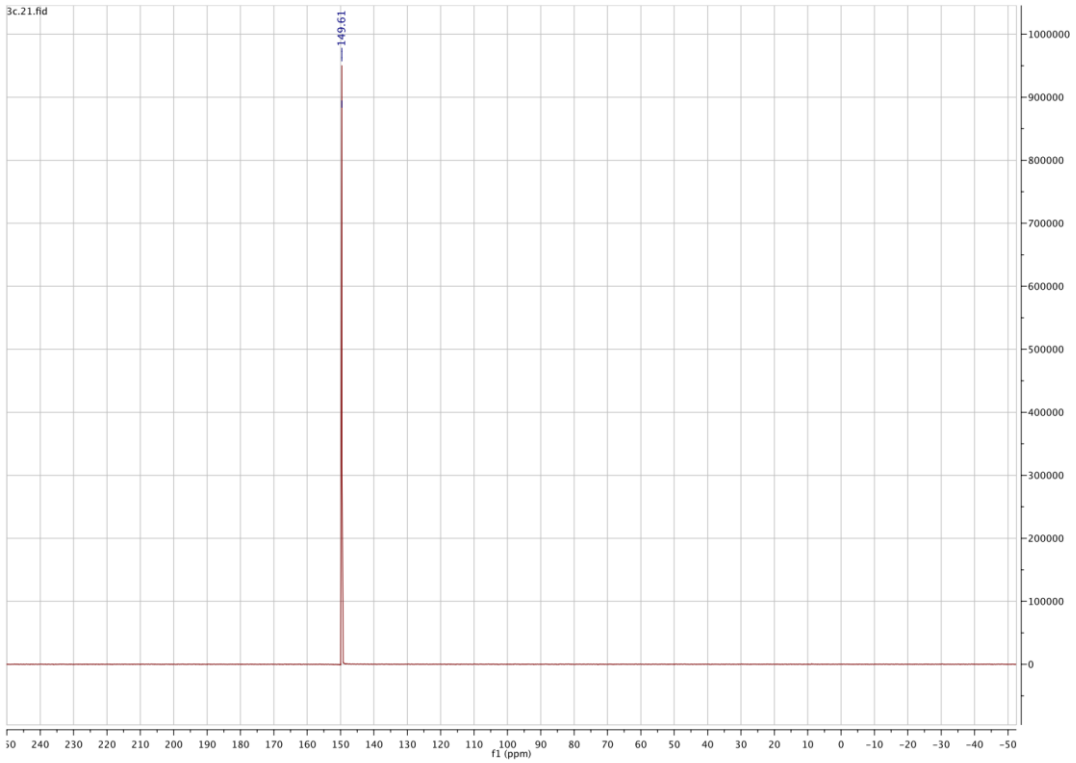


Figure S3. ^{31}P-NMR spectrum of bis(*p*-nitrobenzyl) diisopropylphosphoramidite (**3c**) (243 MHz, $CDCl_3$).

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

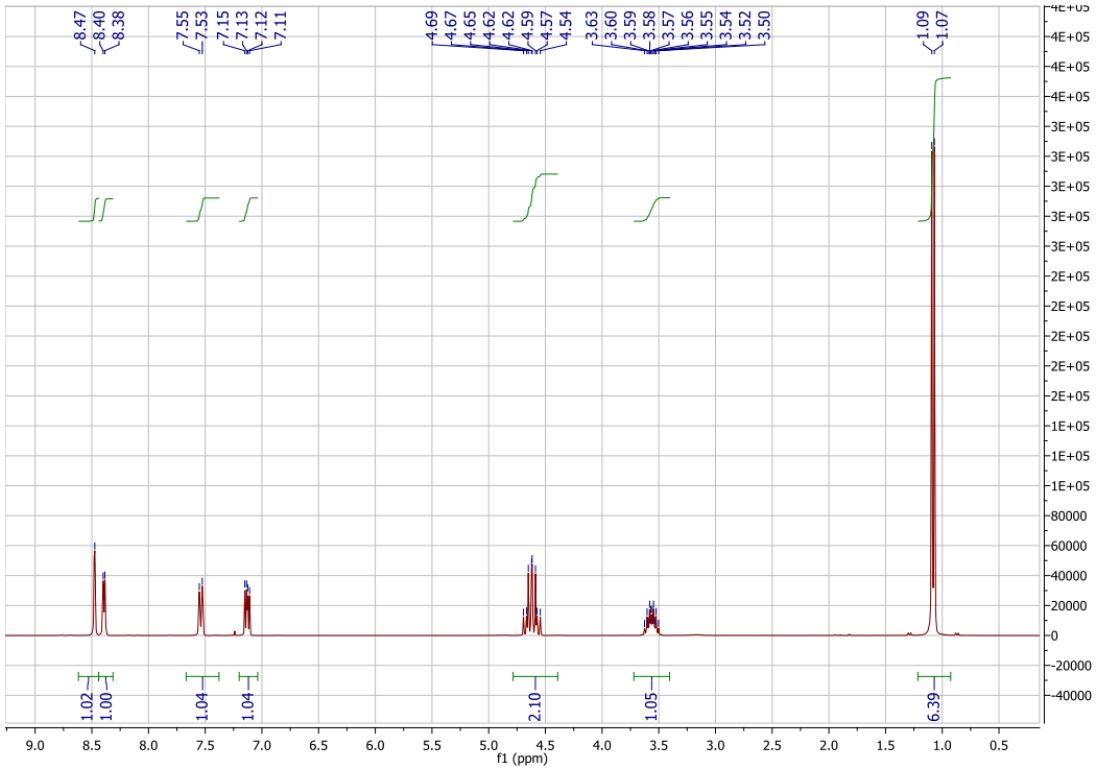


Figure S4. ^{1}H-NMR spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (**3e**) (300 MHz, $CDCl_3$).

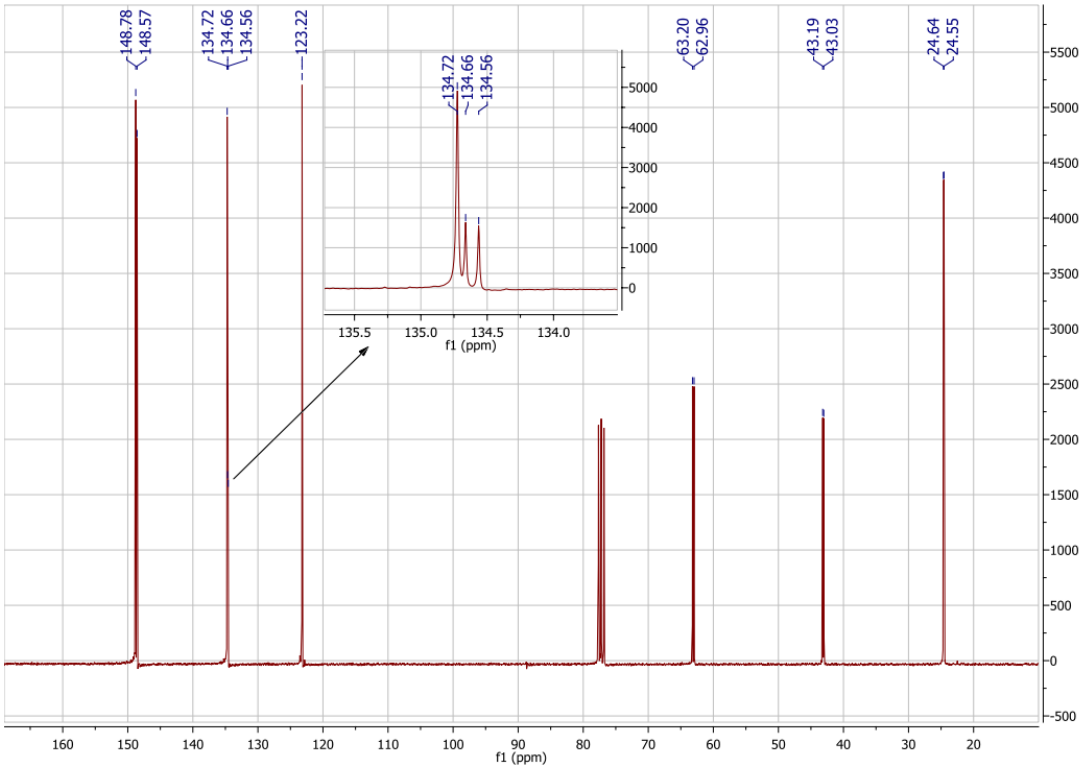


Figure S5. ^{13}C-NMR spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (**3e**) (75 MHz, $CDCl_3$).

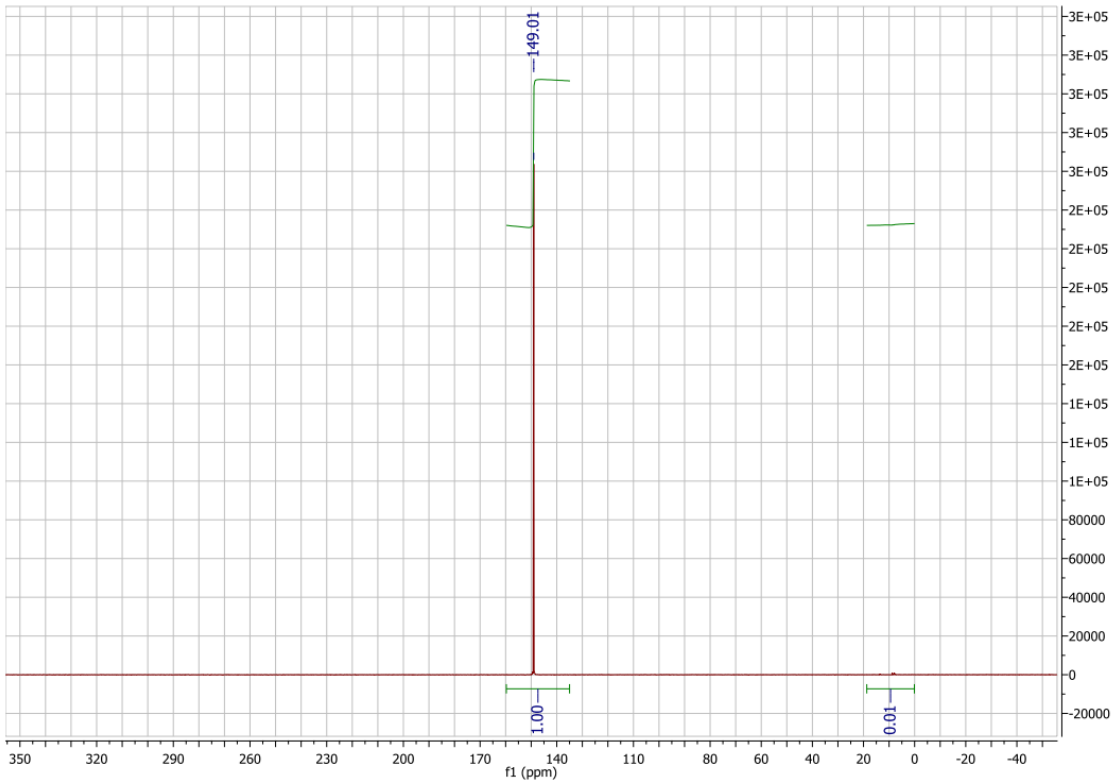


Figure S6. ^{31}P-NMR spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (**3e**) (122 MHz, $CHCl_3$).

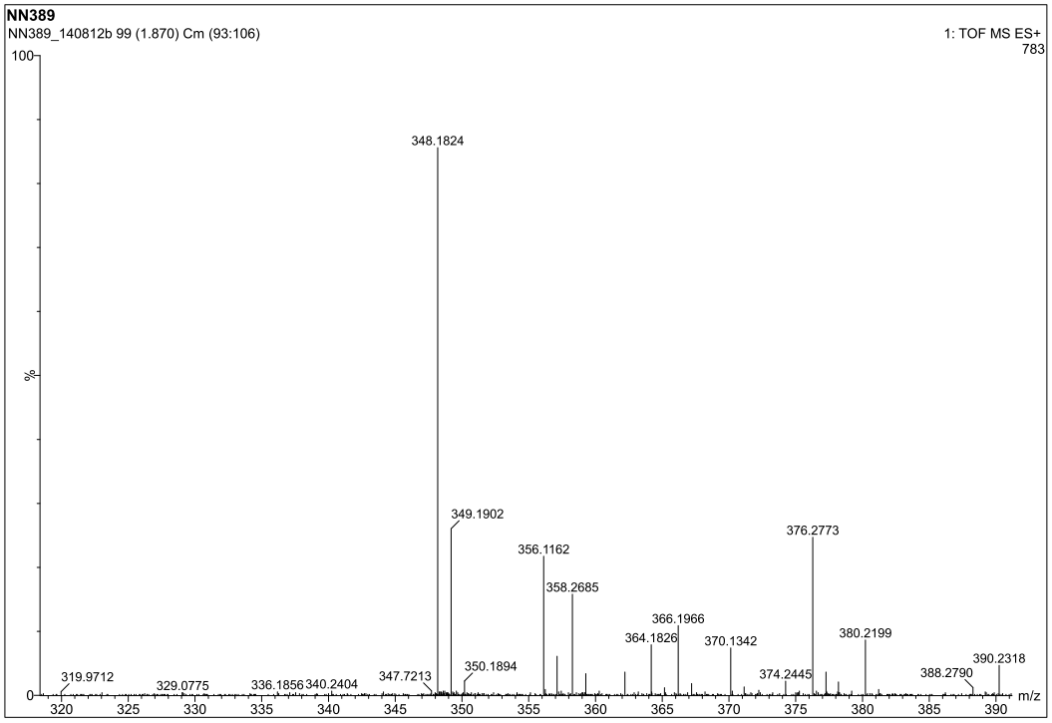


Figure S7. ESI-MS spectrum of bis(pyridin-3-ylmethyl) diisopropylphosphoramidite (**3e**).

1.2 Phosphites

1.2.1 Dibenzyl butyl phosphite (**4a**)

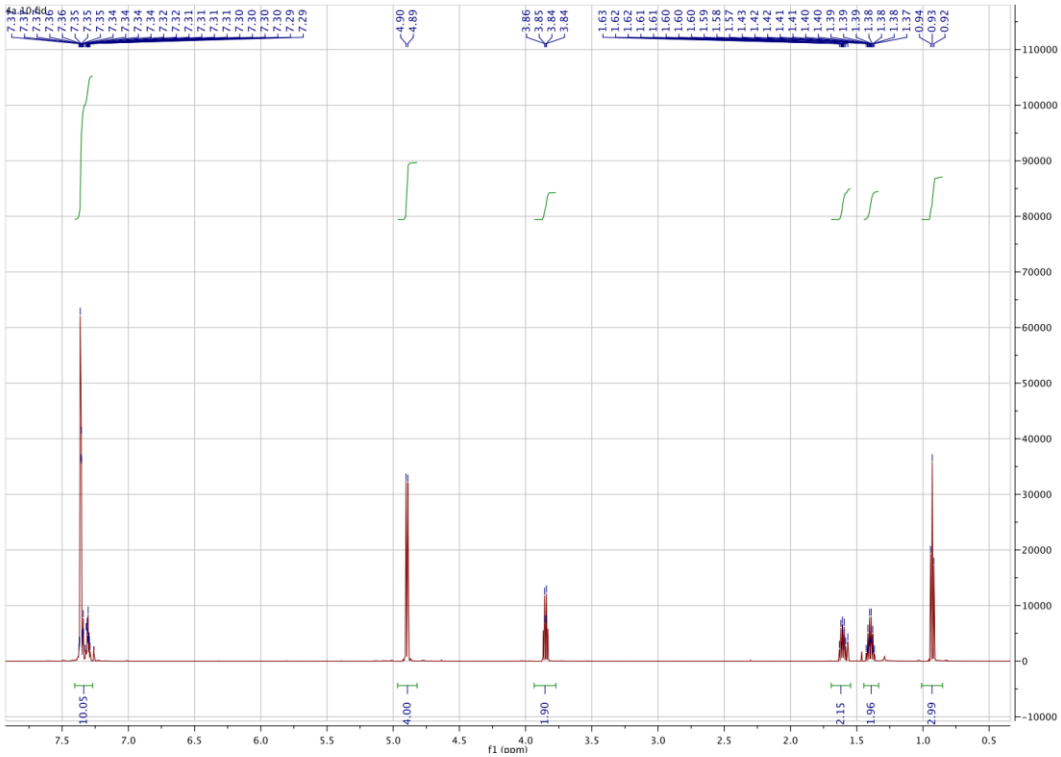


Figure S8. ^{1}H-NMR spectrum of dibenzyl butyl phosphite (**4a**) (600 MHz, $CDCl_3$).

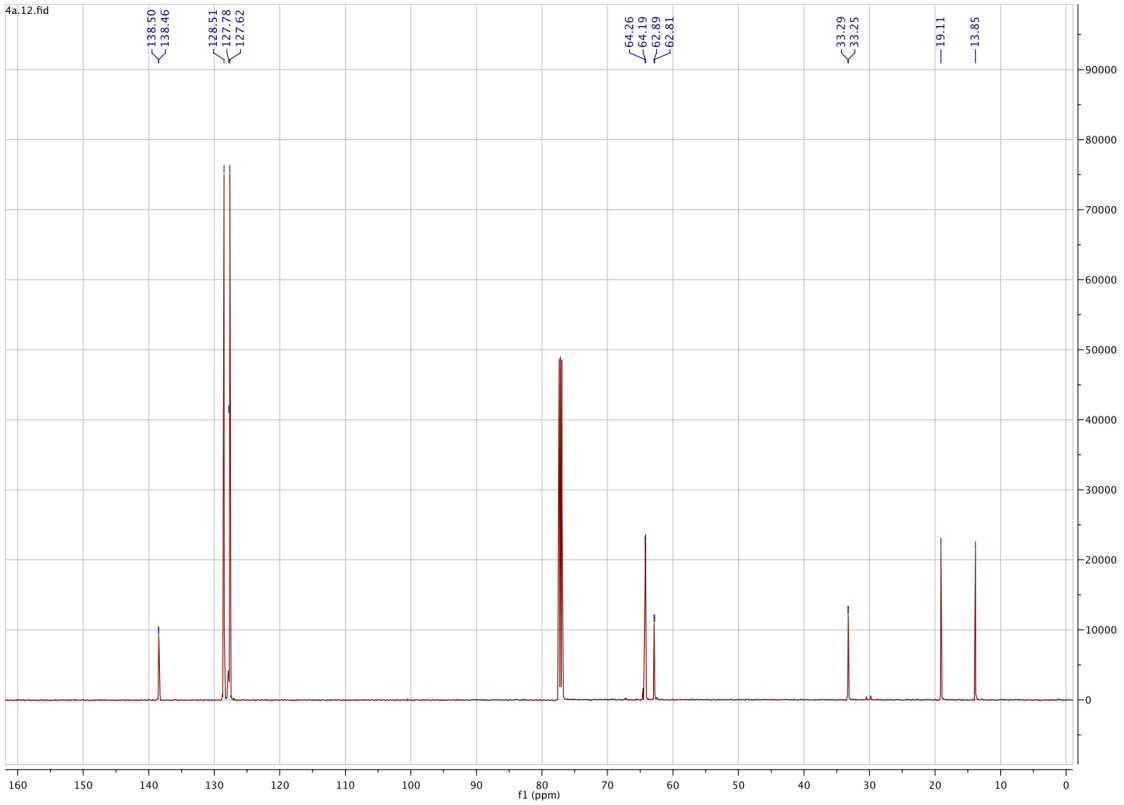


Figure S9. ^{13}C-NMR spectrum of dibenzyl butyl phosphite (**4a**) (151 MHz, $CDCl_3$).

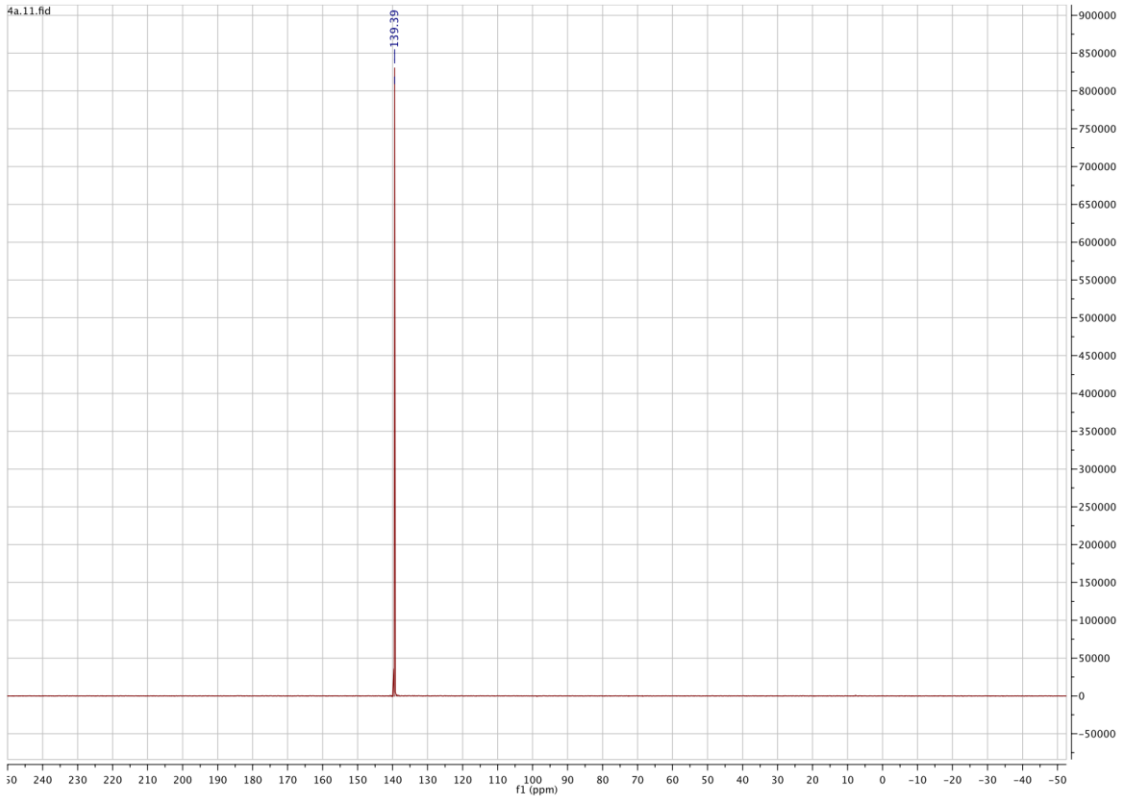


Figure S10. ^{31}P-NMR spectrum of dibenzyl butyl phosphite (**4a**) (243 MHz, $CDCl_3$).

1.2.2 Bis(*o*-nitrobenzyl) butyl phosphite (**4b**)

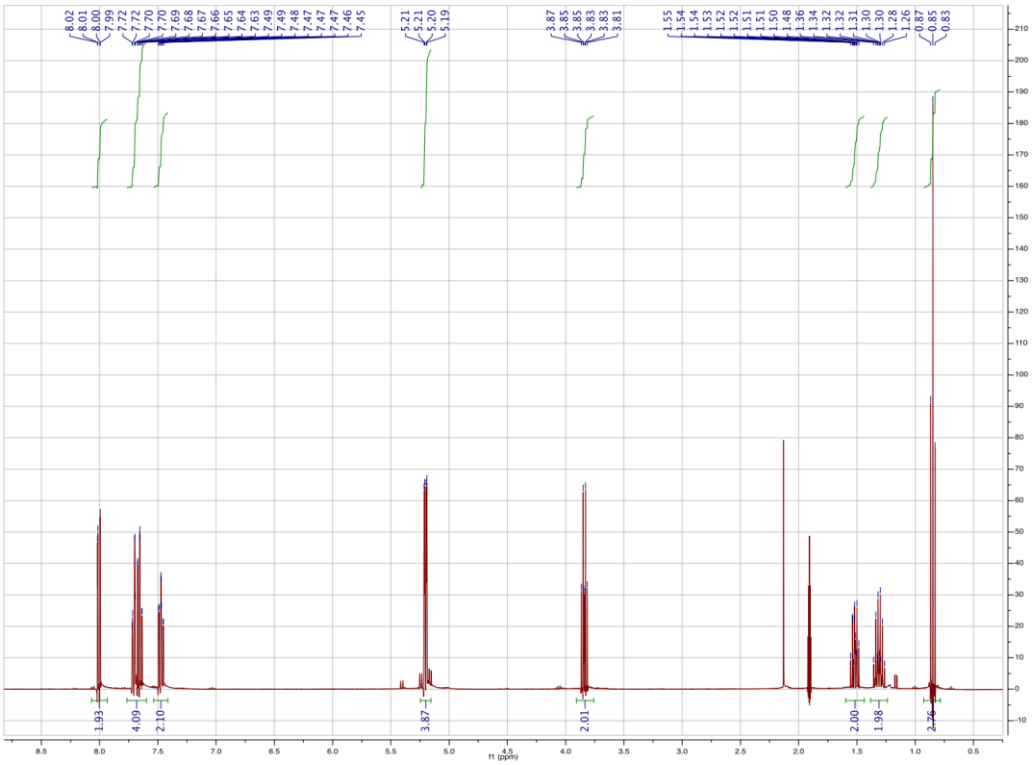


Figure S11. ^{1}H-NMR spectrum of bis(*o*-nitrobenzyl) butyl phosphite (**4b**) (300 MHz, CD_3CN).

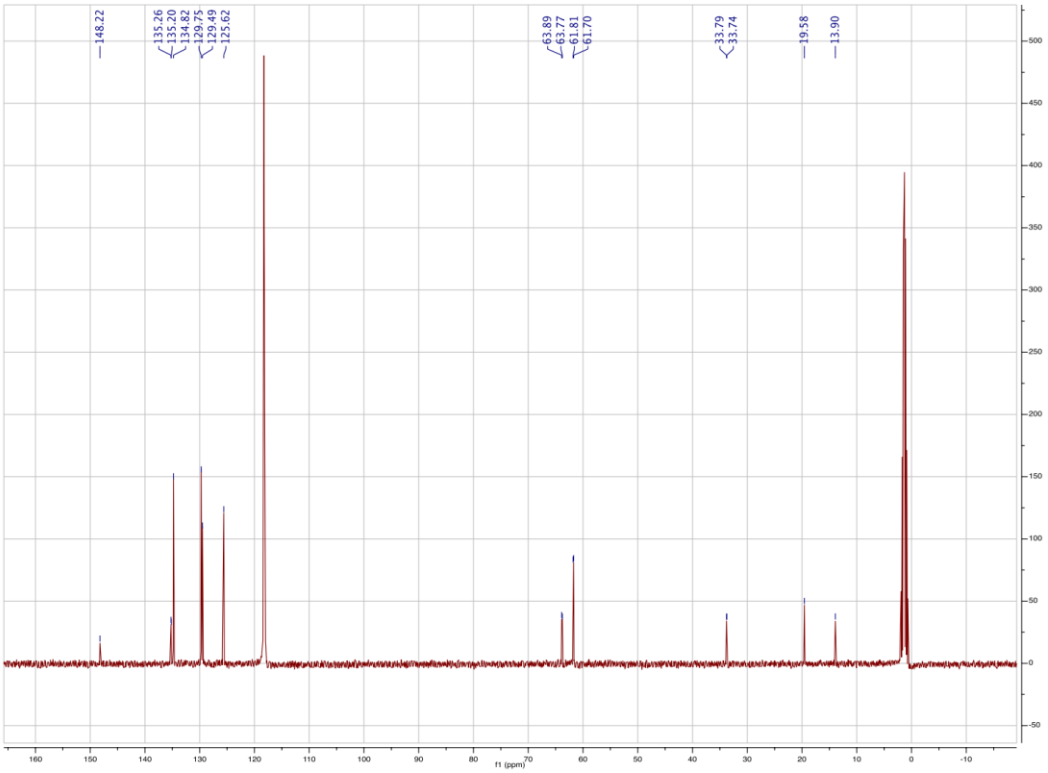


Figure S12. ^{13}C-NMR spectrum of bis(*o*-nitrobenzyl) butyl phosphite (**4b**) (75 MHz, CD_3CN).

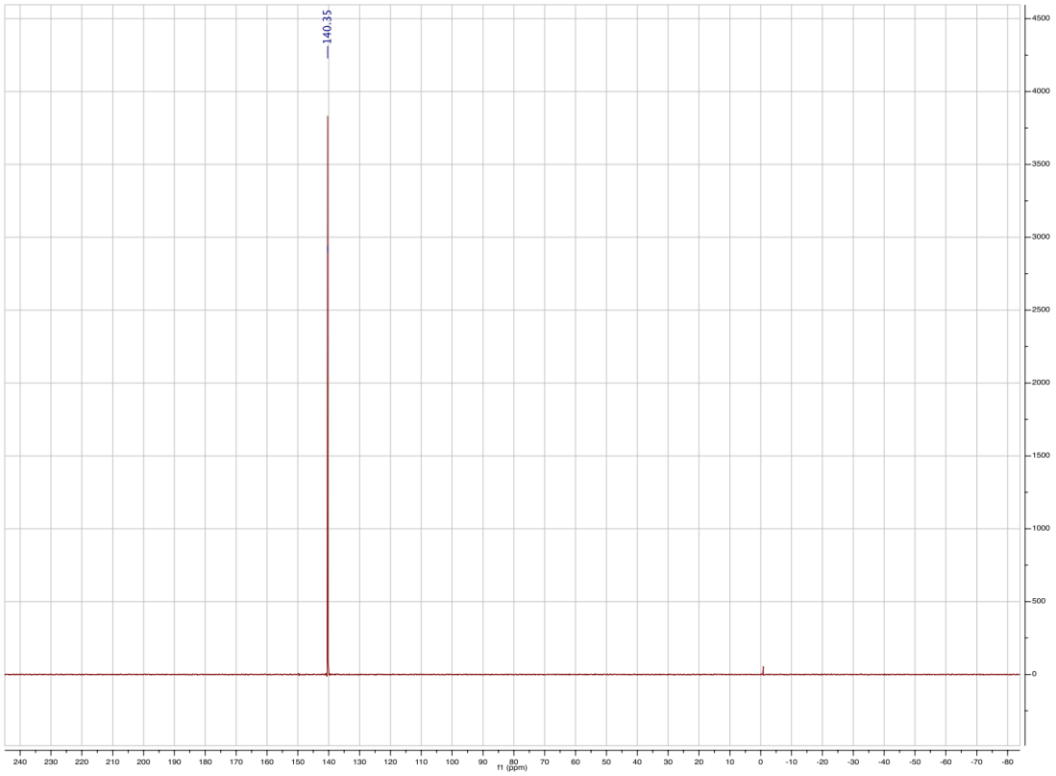


Figure S13. ^{31}P-NMR spectrum of bis(*o*-nitrobenzyl) butyl phosphite (**4b**) (122 MHz, CD_3CN).

1.2.3 Bis(*p*-nitrobenzyl) butyl phosphite (**4c**)

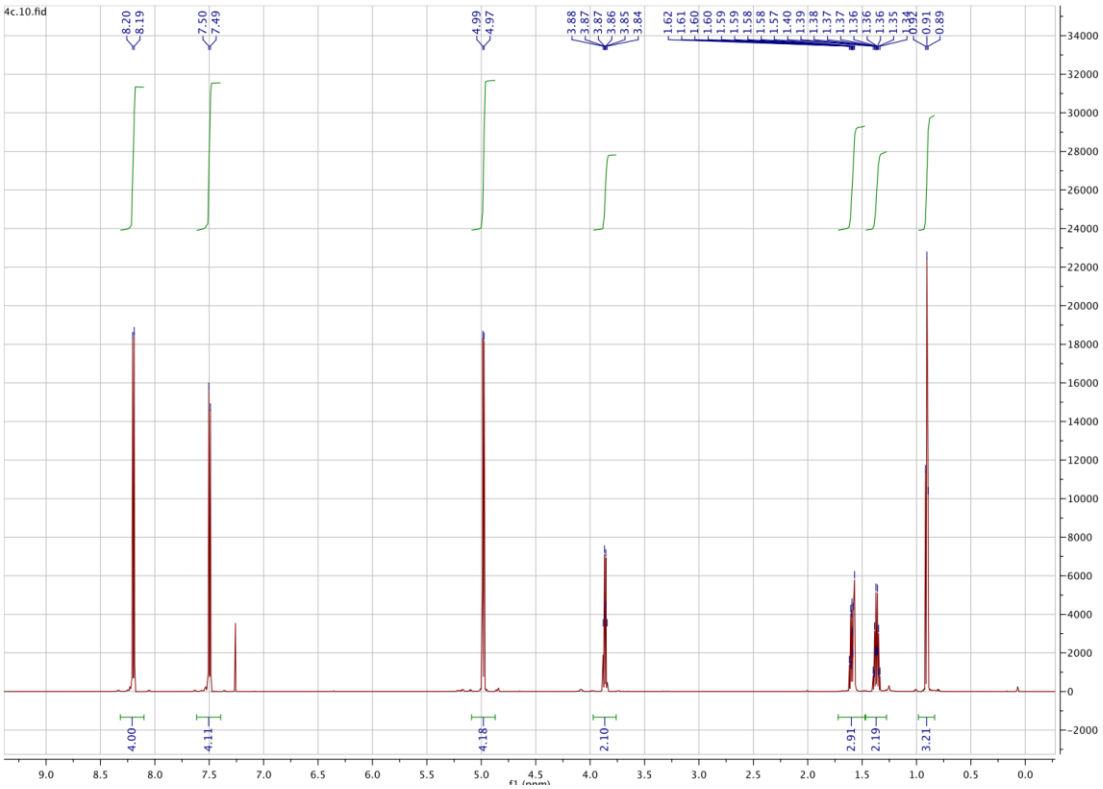


Figure S14. ^{1}H-NMR spectrum of bis(*p*-nitrobenzyl) butyl phosphite (**4c**) (600 MHz, $CDCl_3$).

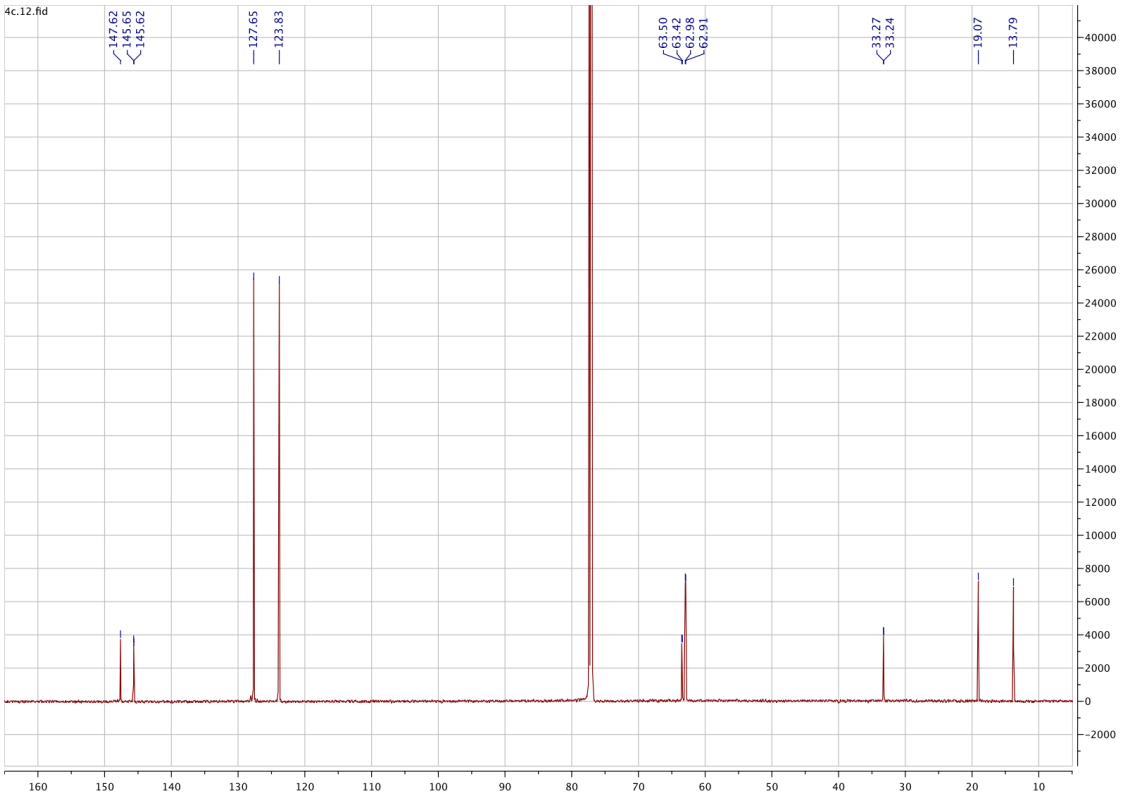


Figure S15. ^{13}C-NMR spectrum of bis(*p*-nitrobenzyl) butyl phosphite (**4c**) (151 MHz, $CDCl_3$).

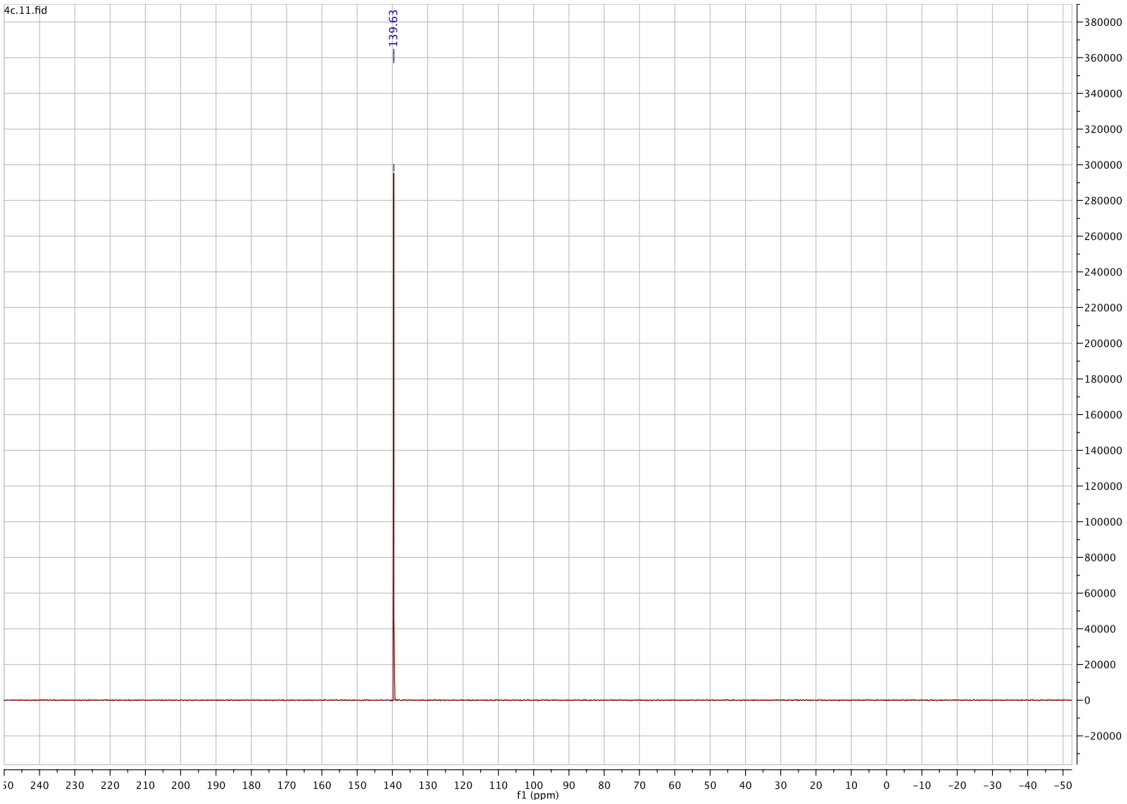


Figure S16. ^{31}P-NMR spectrum of bis(*p*-nitrobenzyl) butyl phosphite (**4c**) (243 MHz, $CDCl_3$).

1.2.4 Bis(*p*-methoxy) butyl phosphite (**4d**)

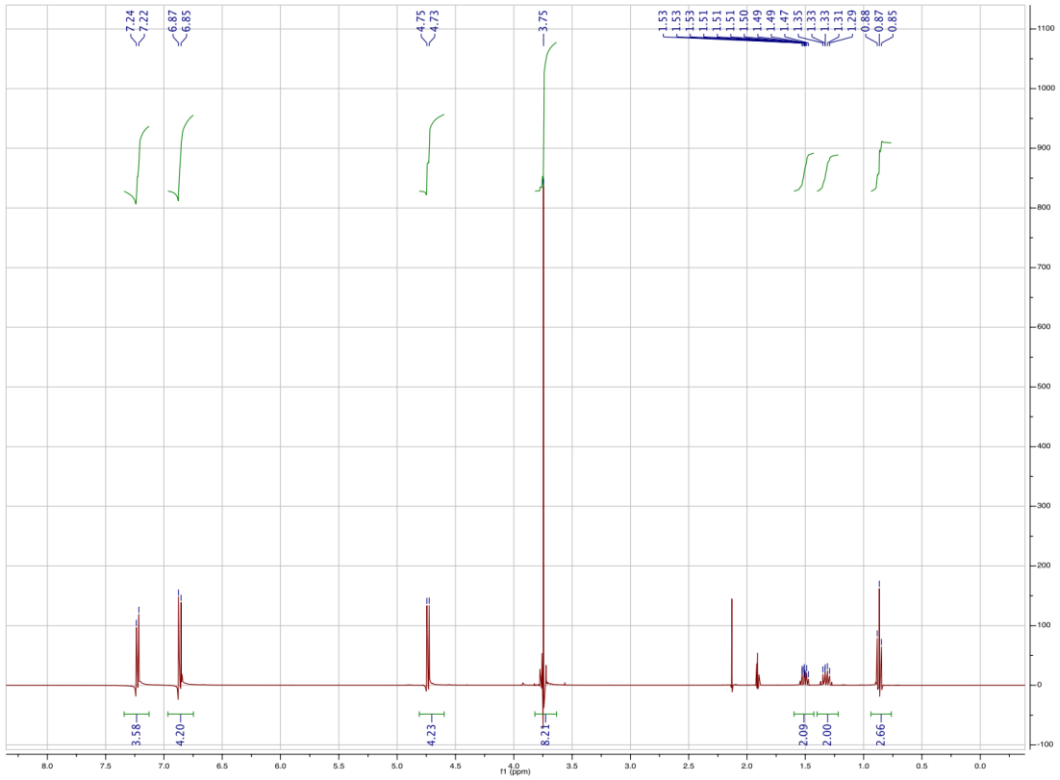


Figure S17. ^{1}H-NMR spectrum of bis(*p*-methoxy) butyl phosphite (**4d**) (400 MHz, CD_3CN).

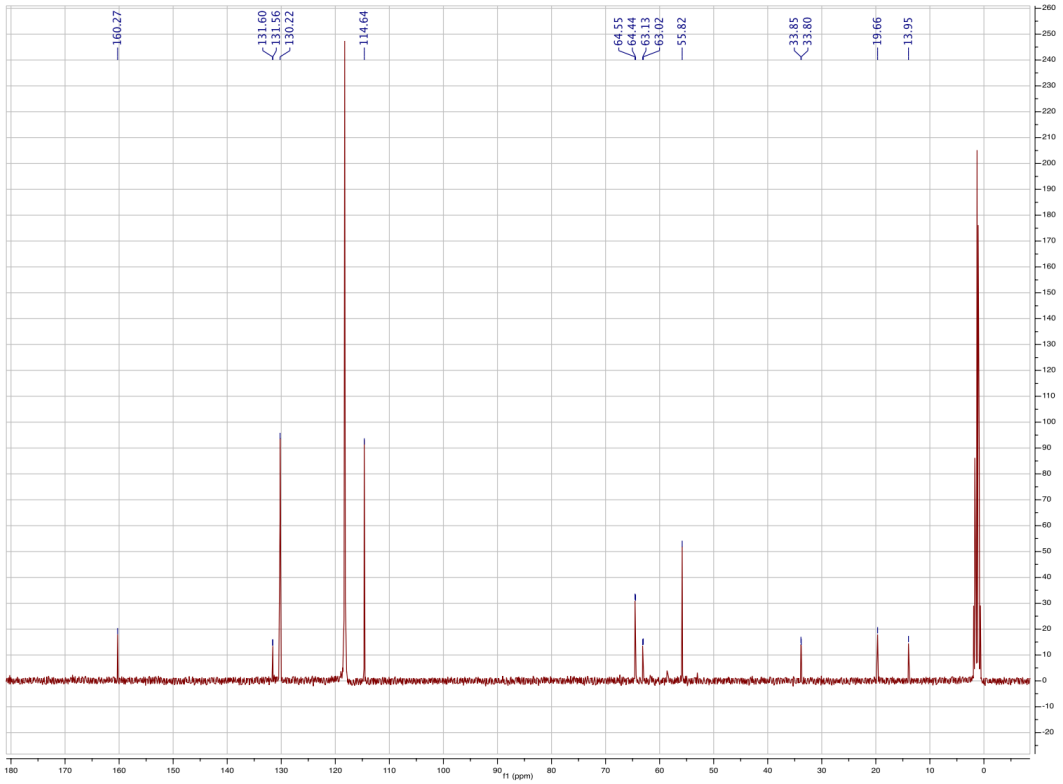


Figure S18. ^{13}C-NMR spectrum of bis(*p*-methoxy) butyl phosphite (**4d**) (100 MHz, CD_3CN).

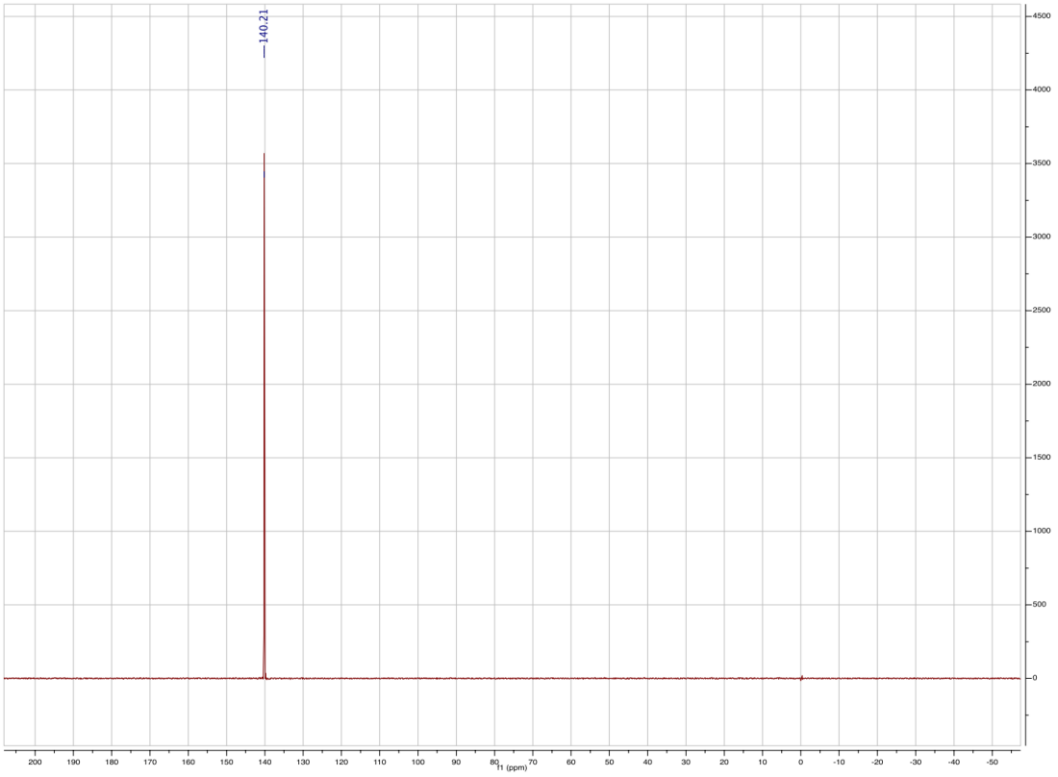


Figure S19. ^{31}P-NMR spectrum of bis(*p*-methoxy) butyl phosphite (**4d**) (162 MHz, CD_3CN).

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

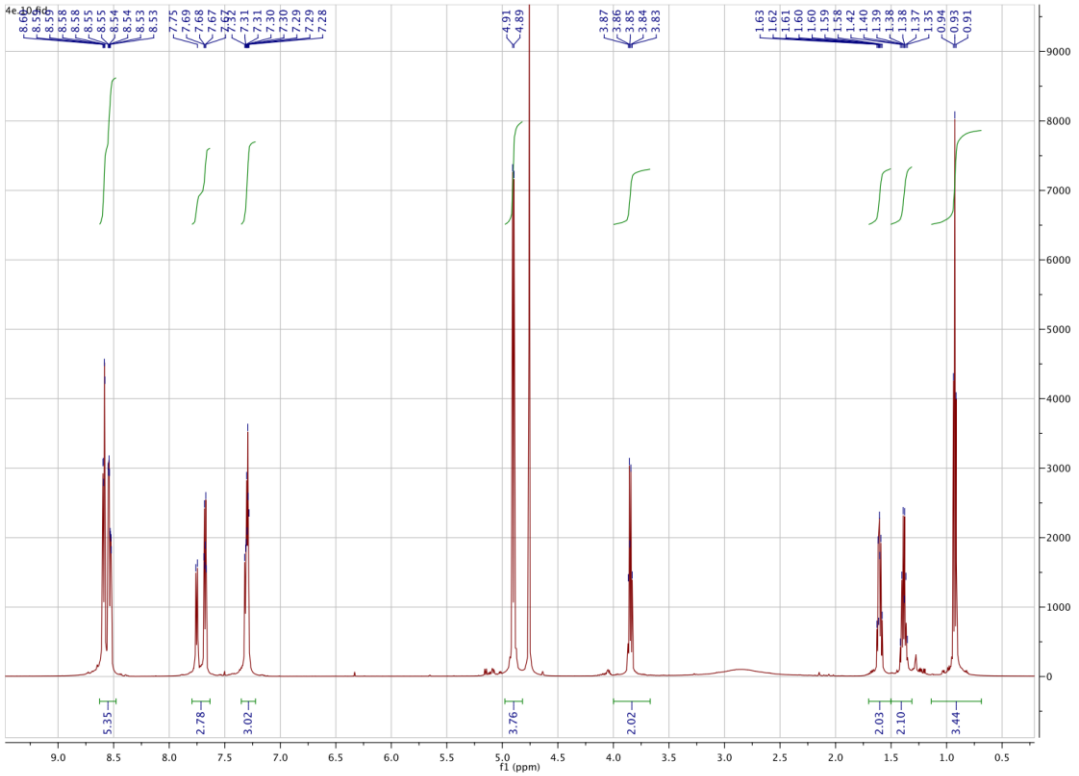


Figure S20. ^{1}H-NMR spectrum of bis(pyridin-3-ylmethyl) butyl phosphite (**4e**) (600 MHz, $CDCl_3$).

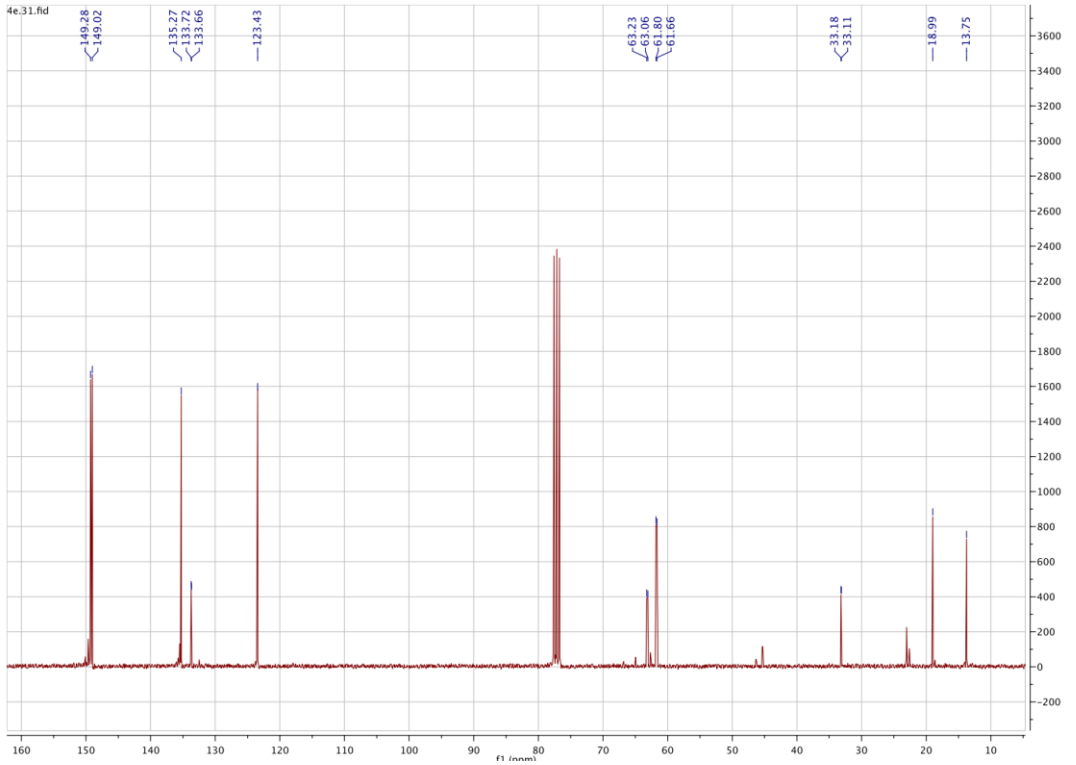


Figure S21. ^{13}C-NMR spectrum of bis(pyridin-3-ylmethyl) butyl phosphite (**4e**) (151 MHz, $CDCl_3$).

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**)

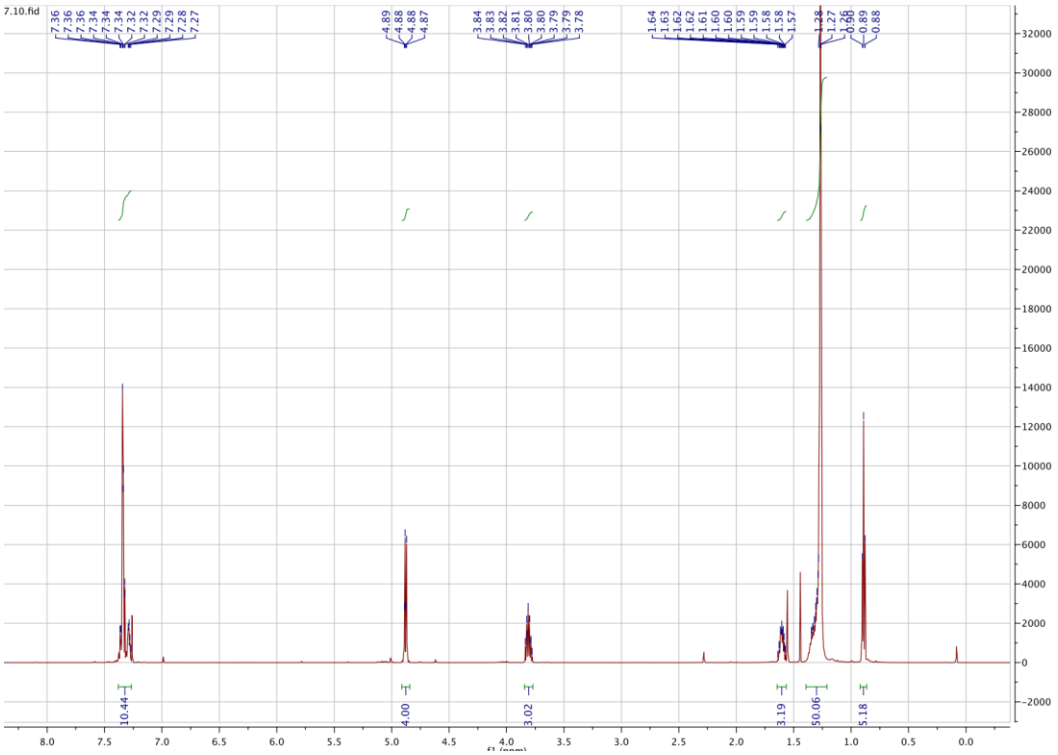


Figure S23. ^{1}H-NMR spectrum of octadecyl dibenzyl phosphite (**7**) (600 MHz, $CDCl_3$).

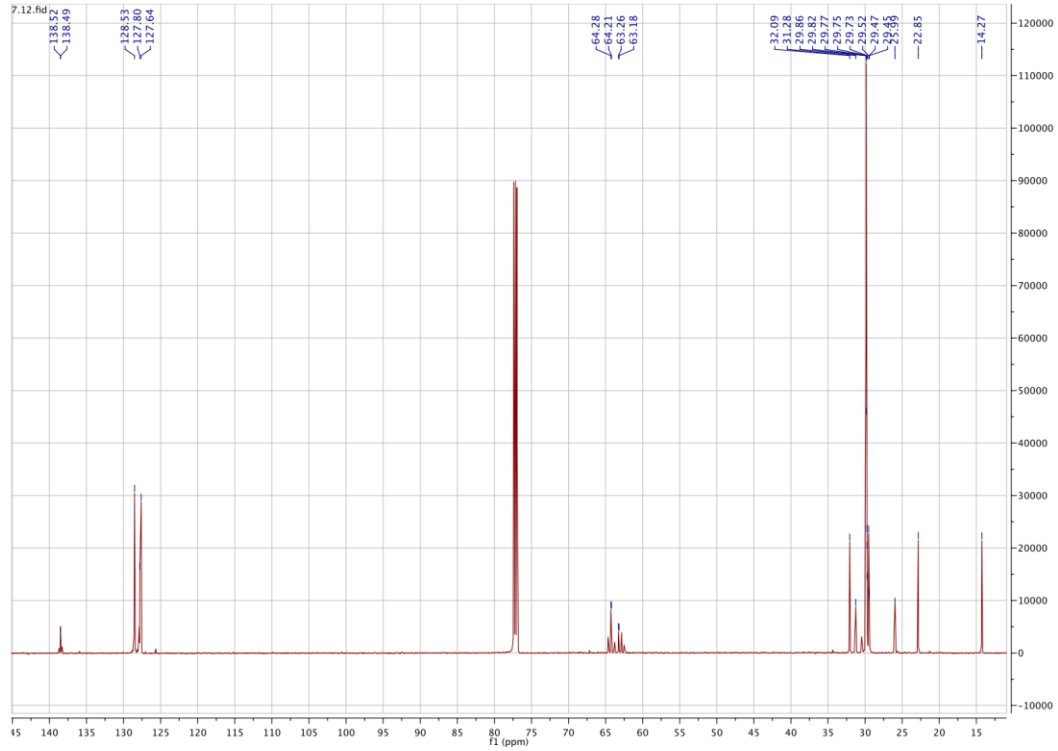


Figure S24. ^{13}C-NMR spectrum of octadecyl dibenzyl phosphite (**7**) 151 MHz, $CDCl_3$).

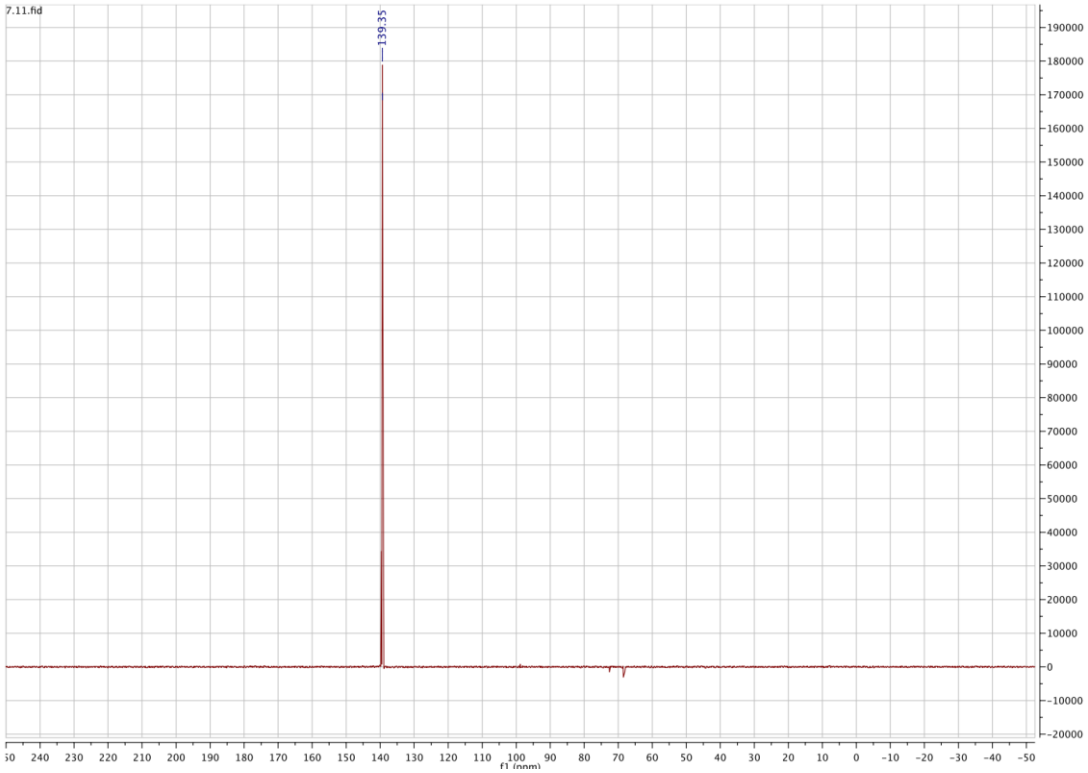


Figure S25. ^{31}P-NMR spectrum of octadecyl dibenzyl phosphite (**7**) (243 MHz, $CDCl_3$).

1.2.7 Octadecyl bis(pyridin-3-ylmethyl) phosphite (**8**)

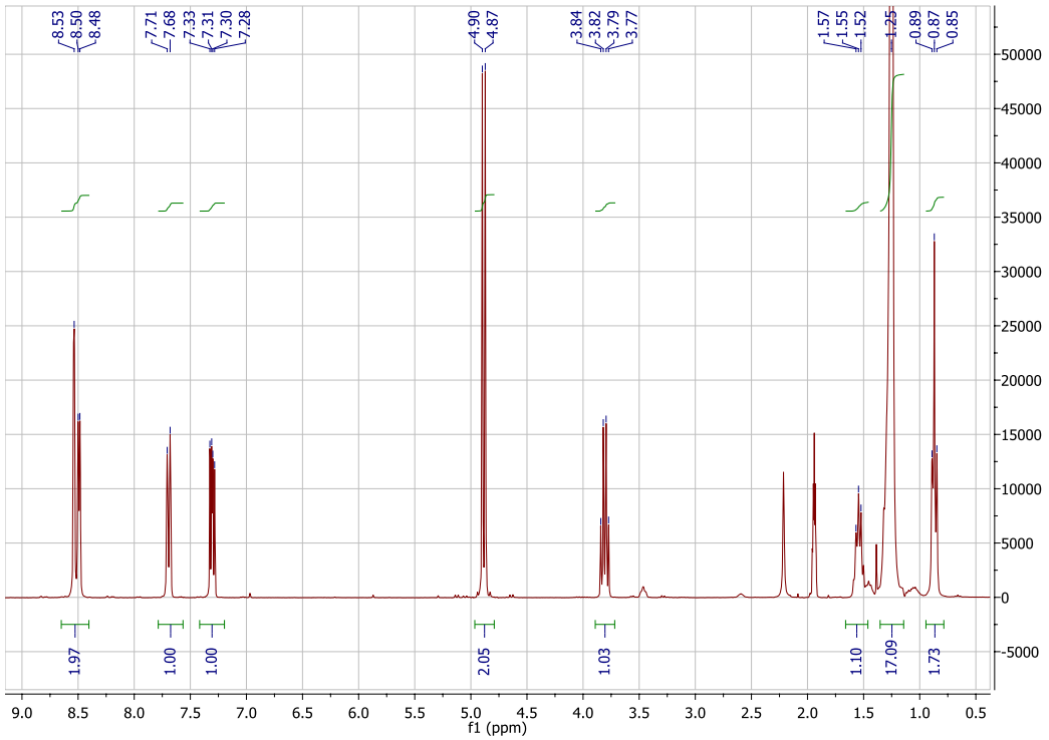


Figure S26. ^{1}H-NMR spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (**8**) (300 MHz, CD_3CN).

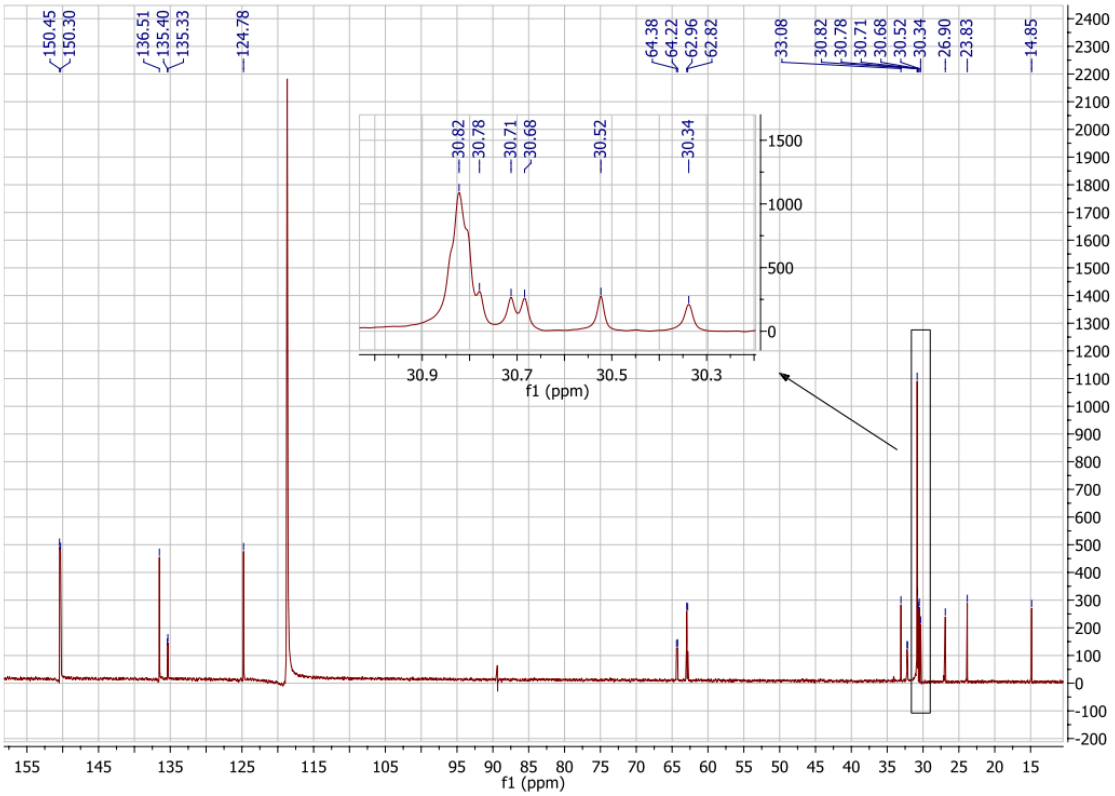


Figure S27. ^{13}C-NMR spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (**8**) (75 MHz, CD_3CN).

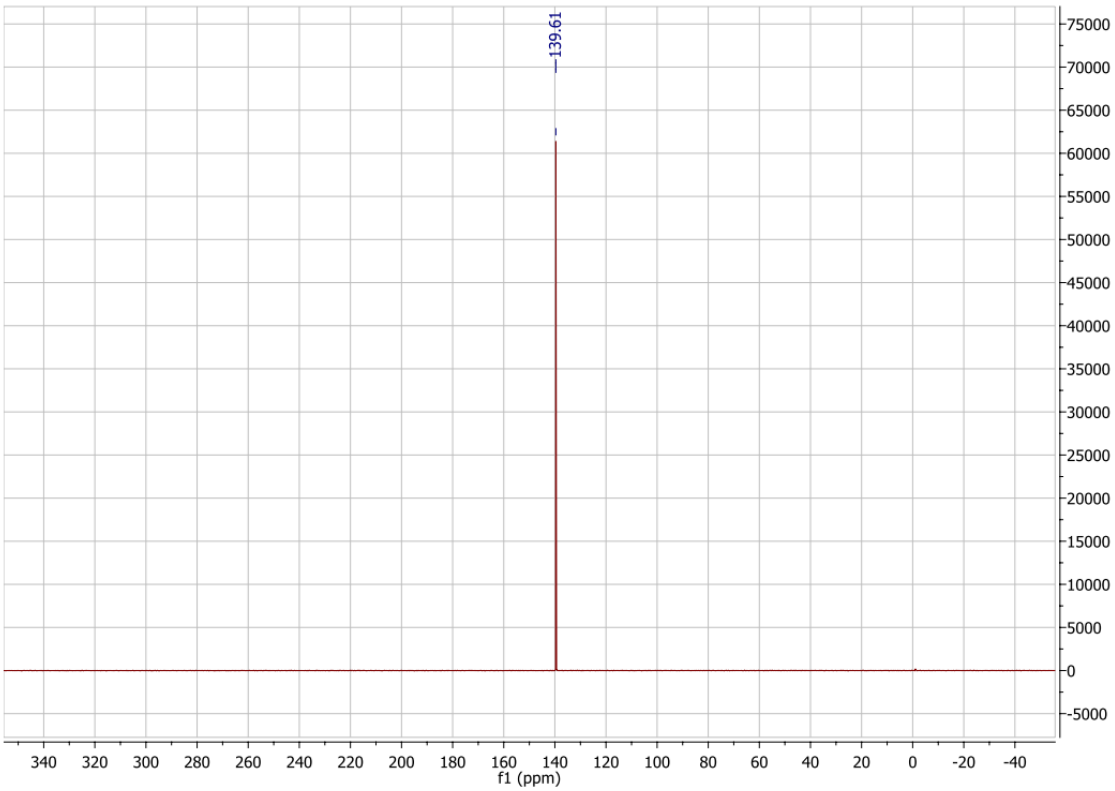


Figure S28. ^{31}P-NMR spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (**8**) (122 MHz, CD_3CN).

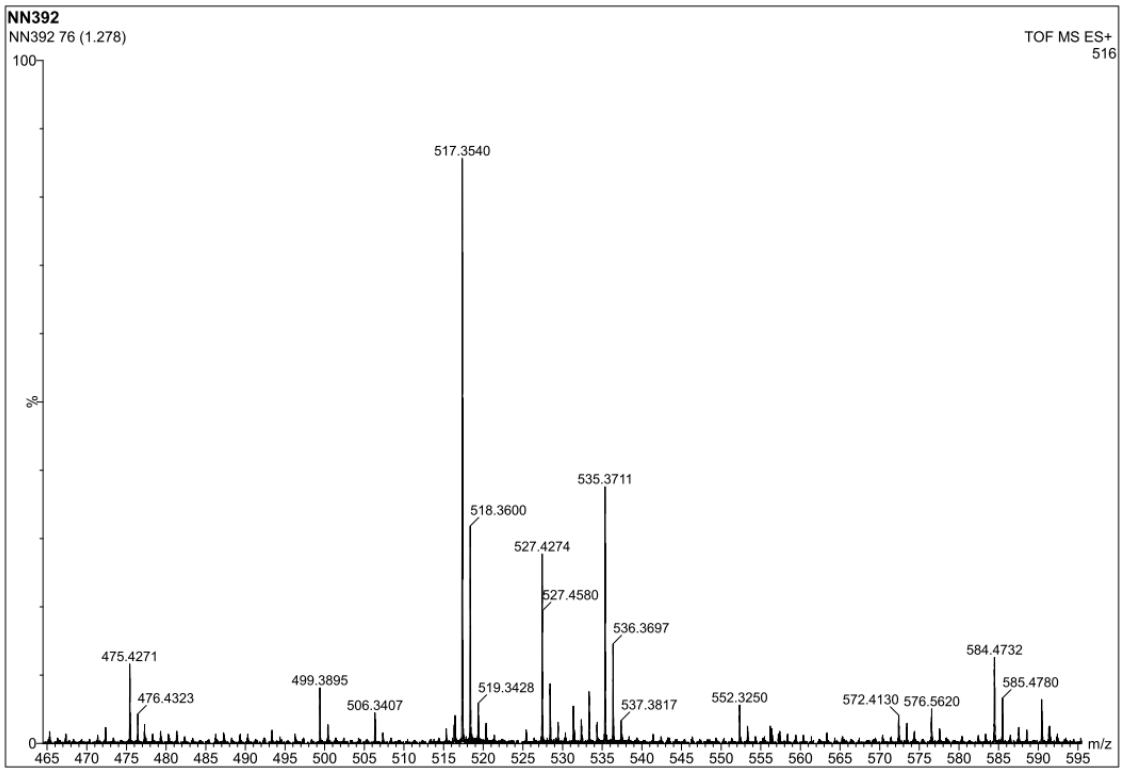


Figure S29. ESI-MS spectrum of octadecyl bis(pyridin-3-ylmethyl) phosphite (**8**).

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)

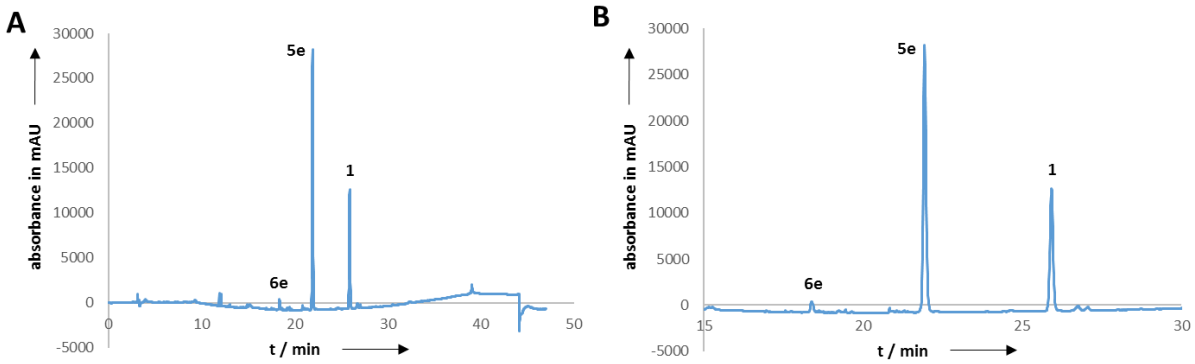


Figure S30. HPLC-UV chromatograms of the reaction of **1** with 10 eq. of **4a**, (**A**) full, (**B**) zoom in.

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

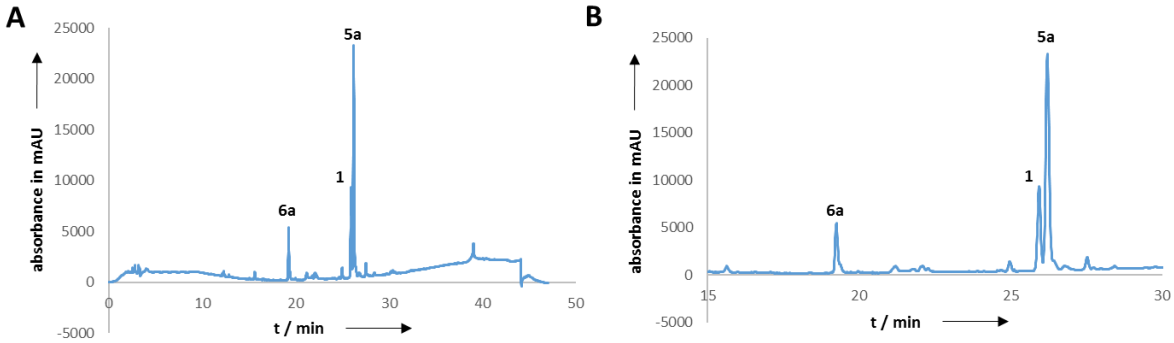
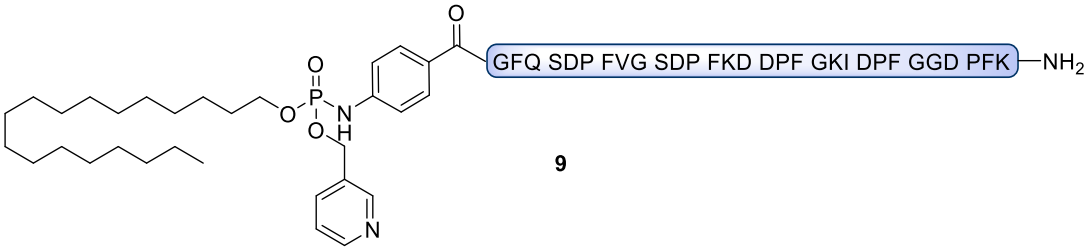


Figure S31. HPLC-UV chromatograms of the reaction of **1** with 10 eq. of **4e**, (**A**) full, (**B**) zoom in.

3 UV-Chromatograms and MS-Spectra of Synthetic Peptides

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



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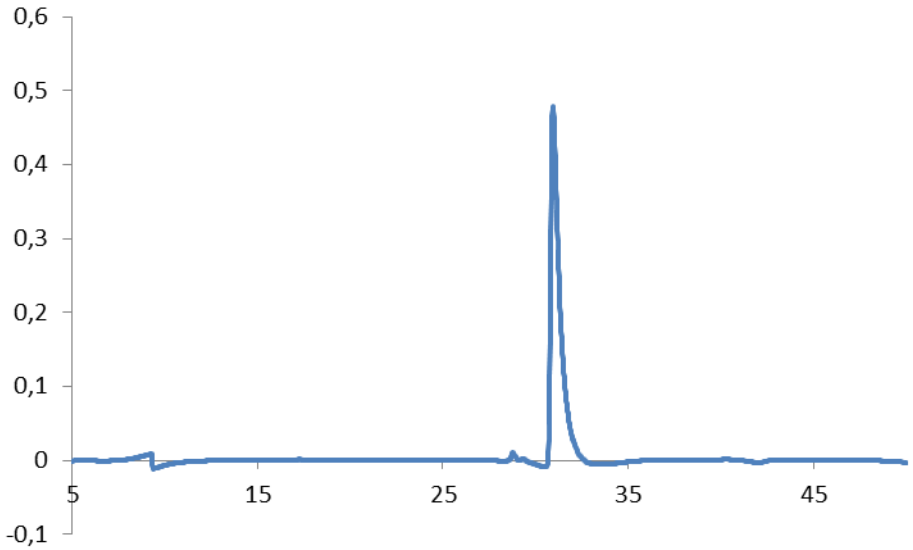


Figure S32. HPLC-UV chromatogram of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (**9**).

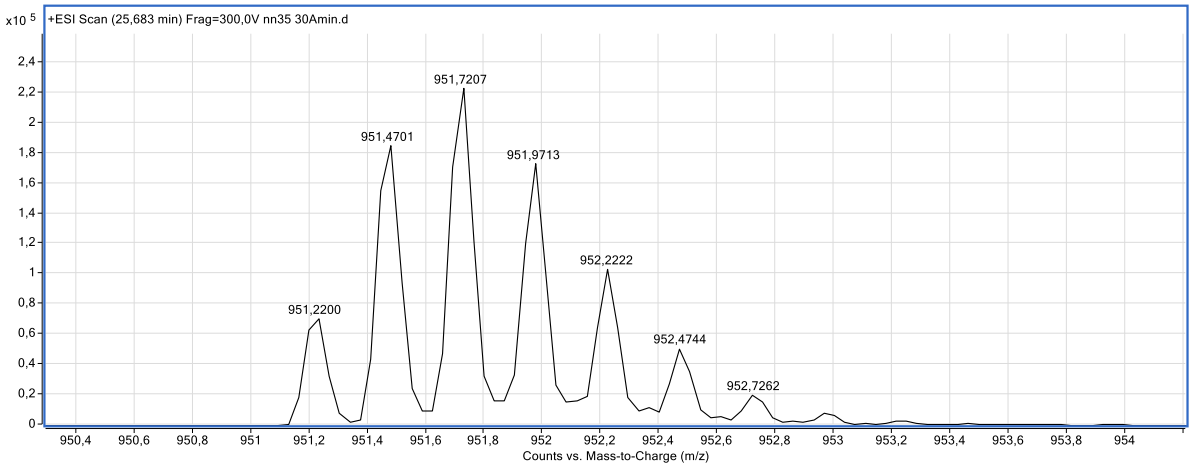


Figure S33. ESI-MS spectrum of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (**9**).

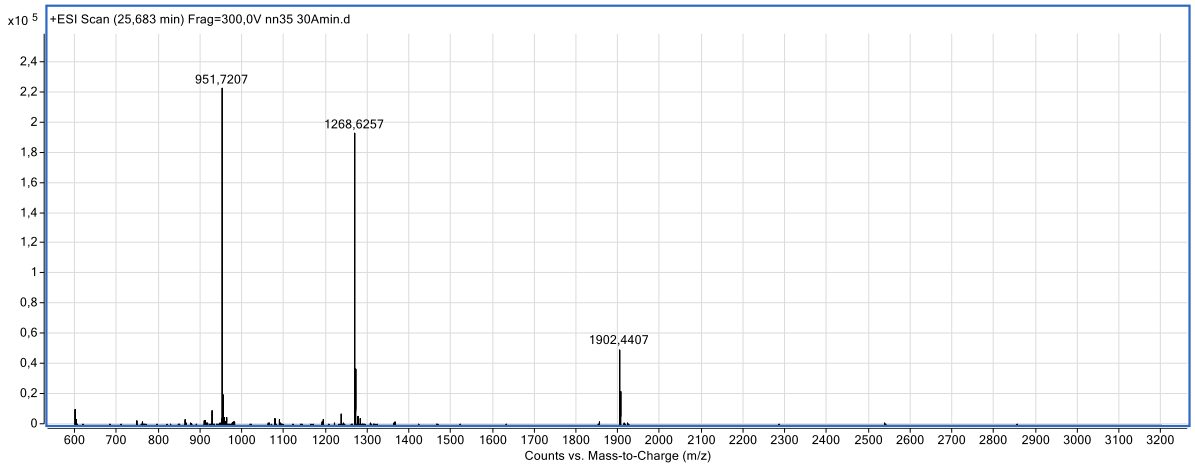


Figure S34. ESI-MS spectrum of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (**9**).

3.2 Azidobenzoic acetylated EPS15-peptide (**11**)

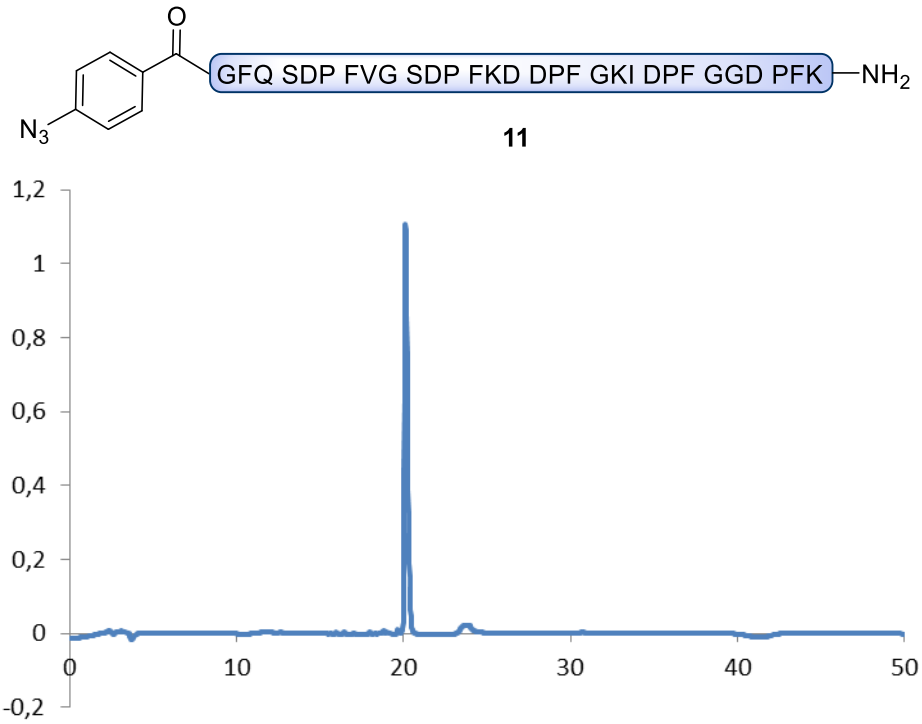


Figure S35. HPLC-UV chromatogram of azidobenzoic acetylated EPS15-peptide (**11**).

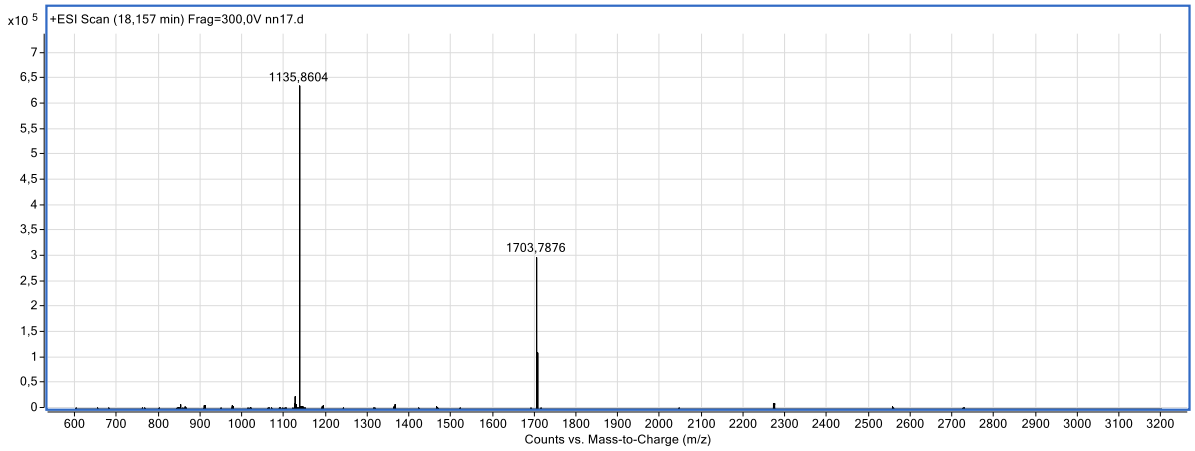


Figure S36. ESI-MS spectrum of azidobenzoic acetylated EPS15-peptide (**11**).

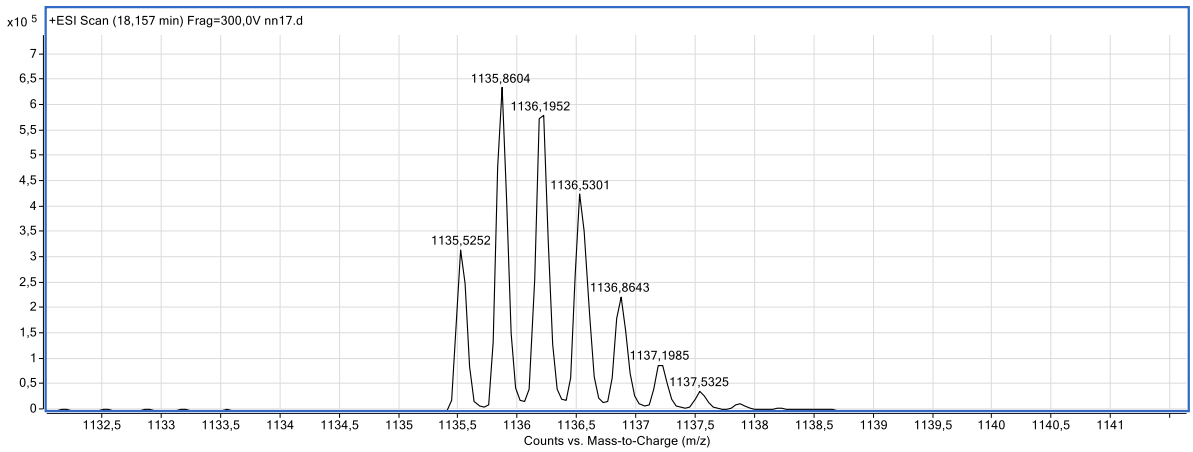
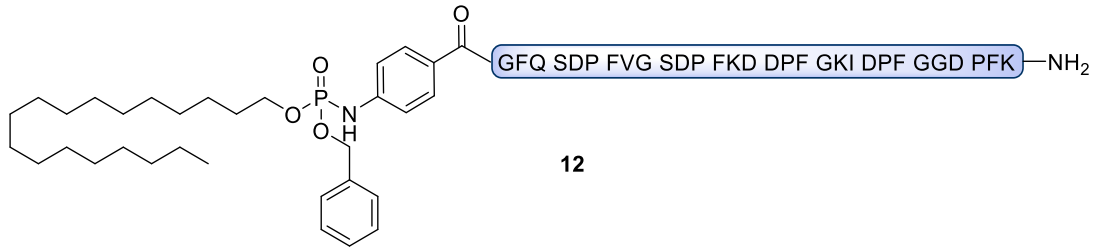


Figure S37. ESI-MS spectrum of azidobenzoic acetylated EPS15-peptide (**11**).

3.3 Benzyl octadecyl phosphoramidate EPS15-peptide (12)



12

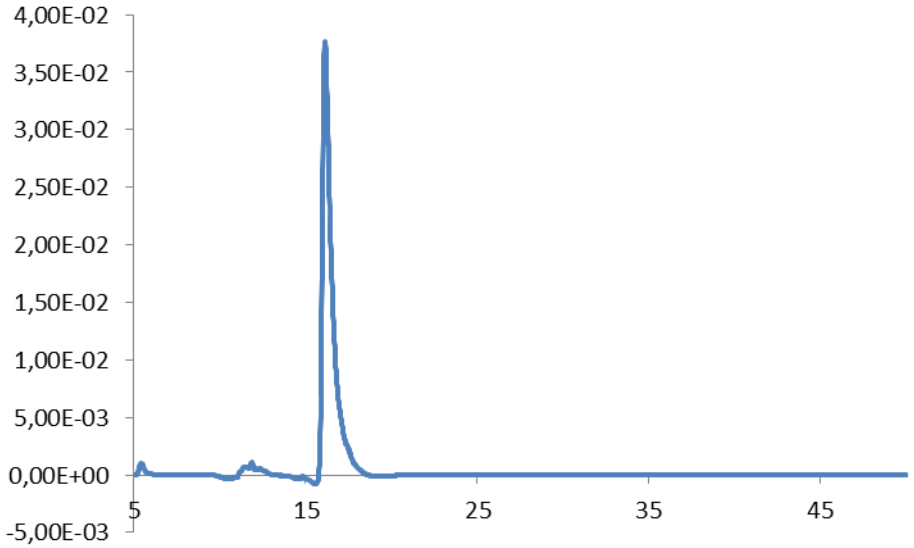


Figure S38. HPLC-UV chromatogram of benzyl octadecyl phosphoramidate EPS15-peptide (**12**) on a C4 column with a gradient from 60 % to 100 % of MeCN in water containing 0.1 % TFA

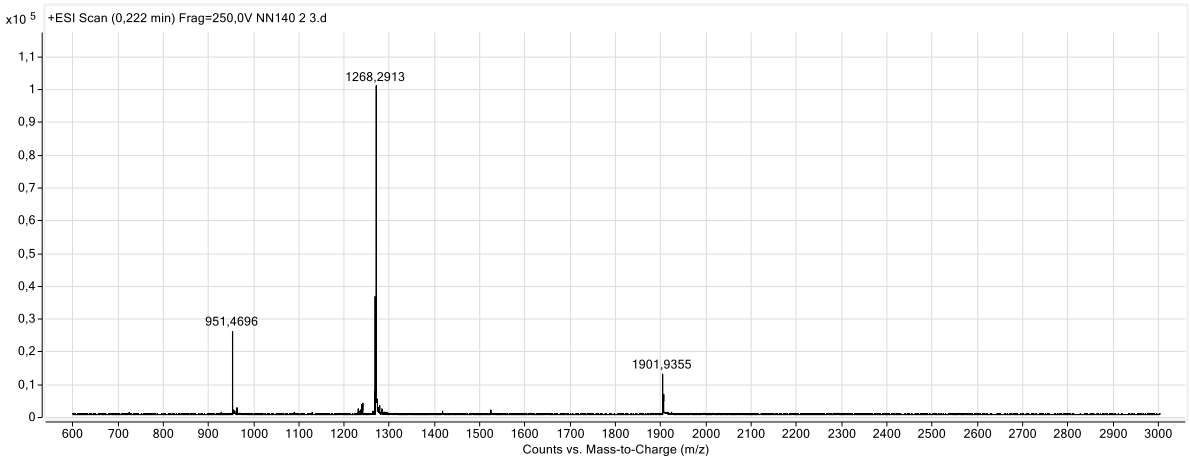


Figure S39. ESI-MS spectrum of benzyl octadecyl phosphoramidate EPS15-peptide (**12**).

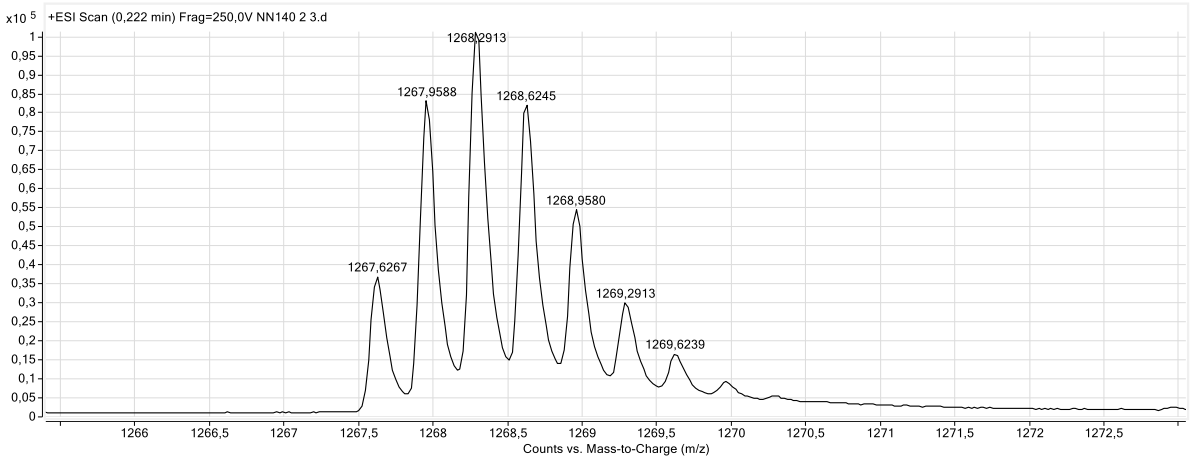
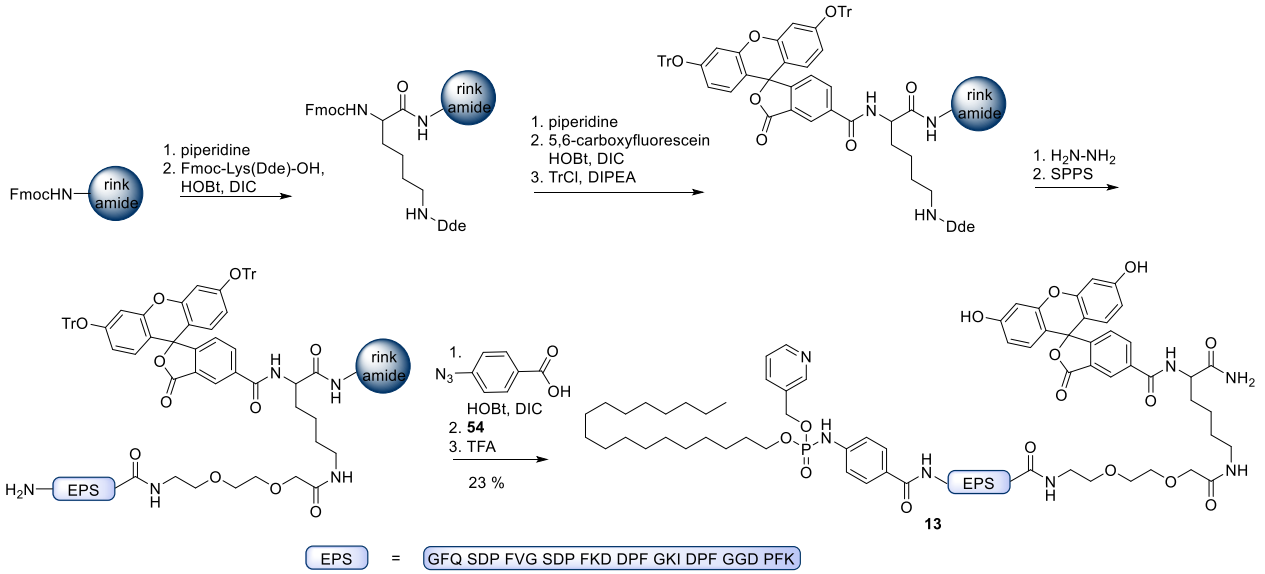


Figure S40. ESI-MS spectrum of benzyl octadecyl phosphoramidate EPS15-peptide (**12**).

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 allow subsequent SPPS. Subsequently, Staudinger-phosphite reaction resulted in the fluorescein labelled monolipidated phosphoramidate modified EPS-peptide **13**.

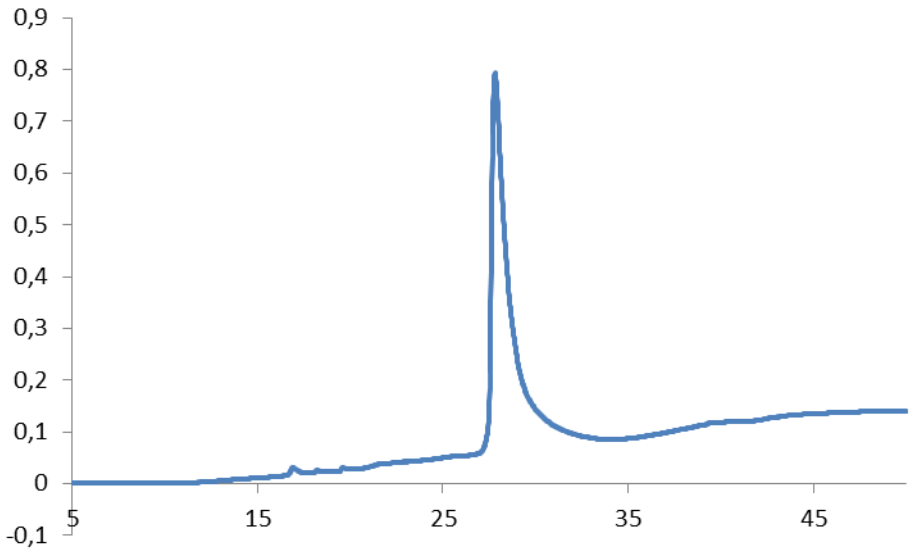


Figure S41. HPLC-UV chromatogram of fluorescein labelled octadecyl pyridin-3-ylmethyl phosphoramidate EPS15-peptide (**13**) on a C4-column.

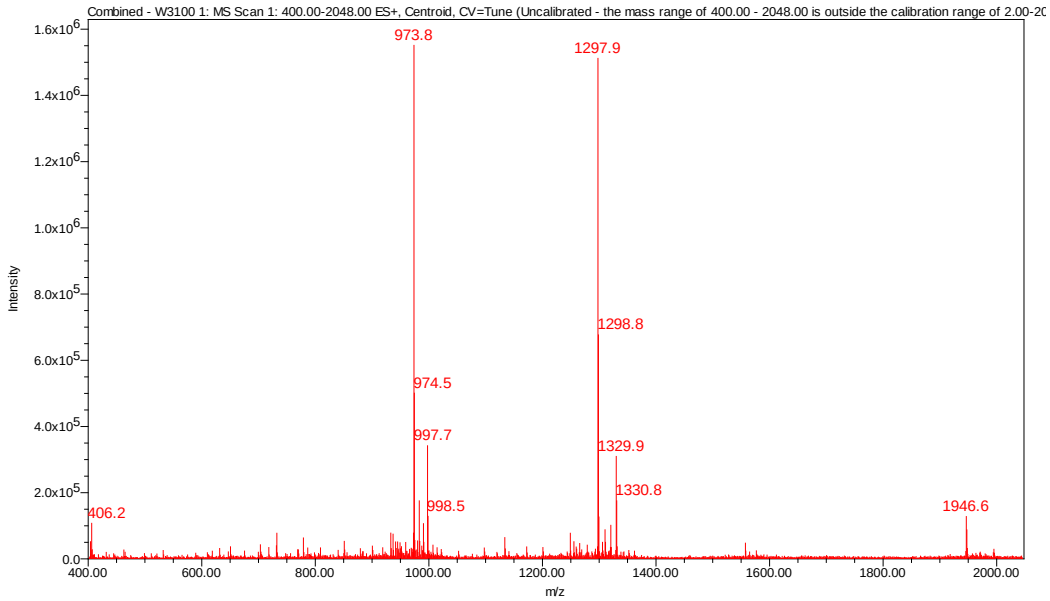


Figure S42. ESI-MS spectrum of fluorescein labelled octadecyl pyridin-3-ylmethyl phosphoramidate EPS15-peptide (**13**).

4 Dynamic Light Scattering

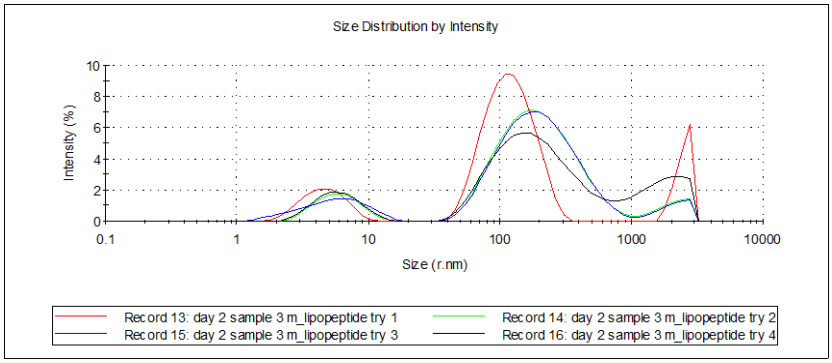


Figure S43. Size distribution of octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (**9**).

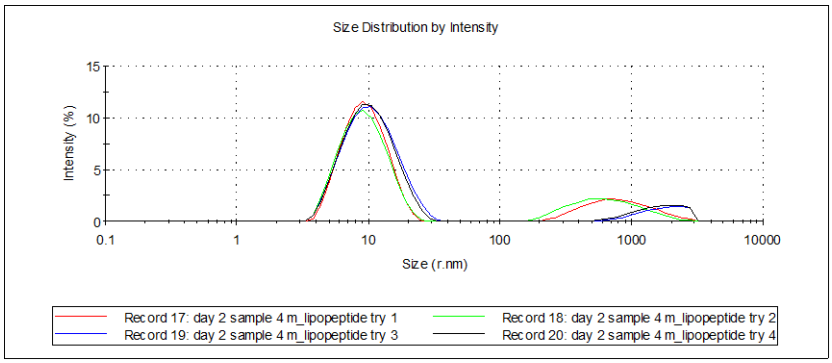


Figure S44. Size distribution of fluorescein labelled octadecyl pyridine-3-ylmethyl phosphoramidate EPS15-peptide (**13**).

5 References

(1) Fischer, R.; Mader, O.; Jung, G.; Brock, R. *Bioconjugate Chem.* **2003**, *14*, 653.