Supporting Information

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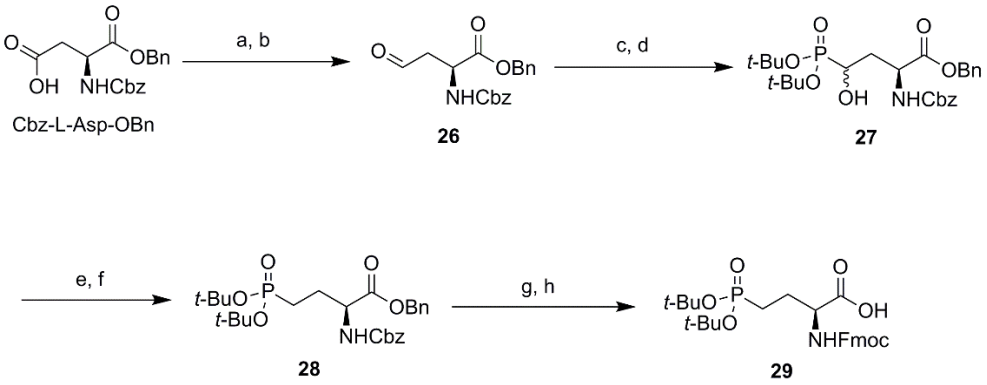
Supporting Figures and Schemes



Scheme S1. *Reagents and conditions*: a) Isobutyl chloroformate (1.1 equiv.), TEA (1.5 equiv.), THF, 0 °C, 10 min., then $NaBH_4$ (3 equiv.), H_2O, 0 °C, 10 min.; b) pyridinium chlorochromate (PCC, 1.5 equiv.), CH_2Cl_2, rt, 2 h, 51% (2 steps) c) di-*tert*-butyl phosphite (1.5 equiv.), TMS-Cl (1.5 equiv.), TEA (2.0 equiv.), CH_2Cl_2, 3 h, rt; d) 20% aq. citric acid (w/v), MeOH, 16 h, rt, 71% (2 steps); e) *O*-phenyl thiochloroformate (3 equiv.), DIEA (4 equiv.), DMAP (0.2 equiv.), CH_2Cl_2, rt, 3 h; f) tributyltin hydride (3.0 equiv.), azoisobutylnitrile (AIBN, 1.0 equiv.), toluene, reflux, 20 min, 52% (2 steps); g) 1 atm H_2, Pd/C (10% w/w, 0.2 equiv.), MeOH, 3 h, rt; h) Fmoc-OSu (1.5 equiv.), $NaHCO_3$ (5.0 equiv.), THF/H_2O (1:1), 16 h, rt, 80% (2 steps).

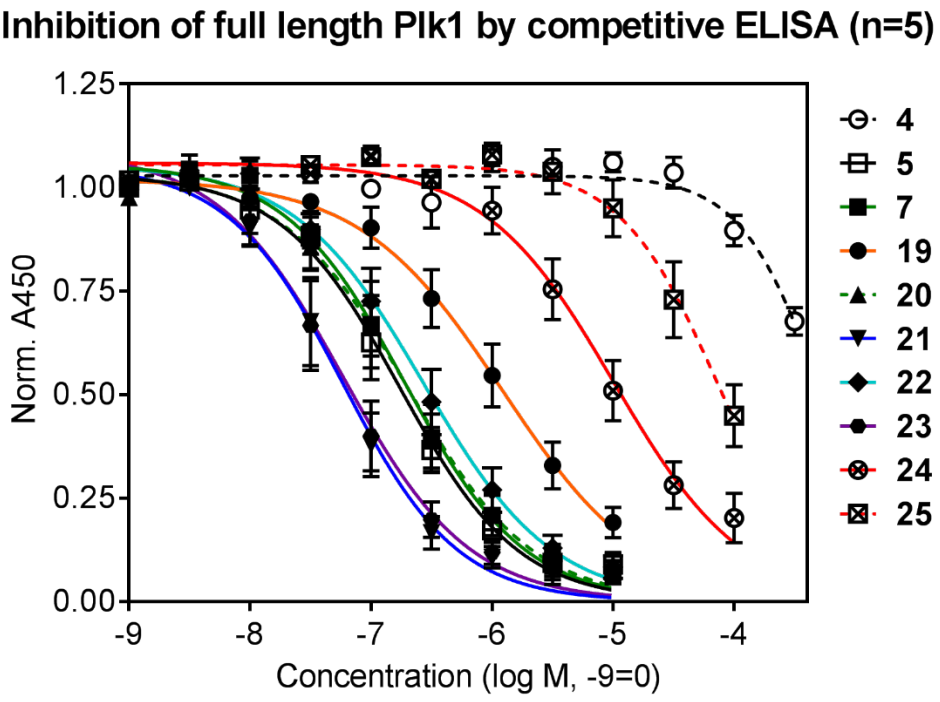


Figure S1. ELISA-based competitive inhibition of full length Plk1. Data points represent average ± SEM of normalized absorbance from five independent experiments and fit using non-linear regression in GraphPad Prism 6.

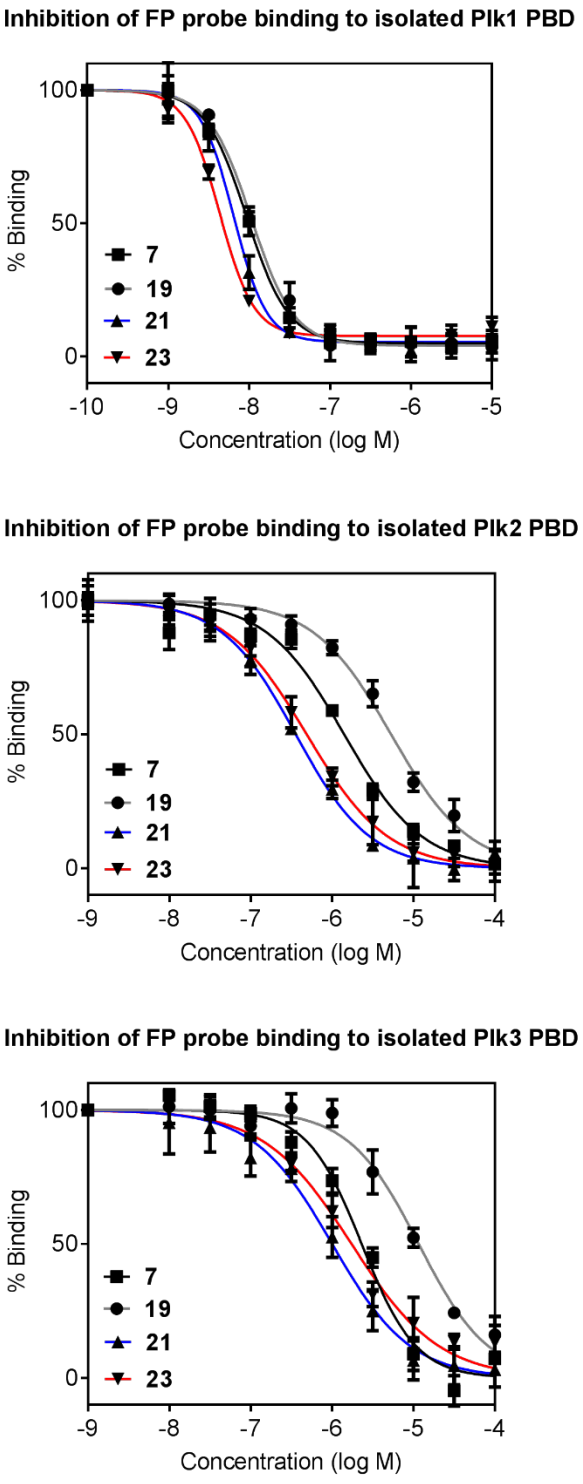


Figure S2. Fluorescence polarization assay for competitive inhibition of the isolated PBDs of Plks 1 – 3 by **7**, **19**, **21**, and **23**. Data points represent average ± SEM of % binding (normalized FP signal) from triplicate data points and fit using non-linear regression in GraphPad Prism 6.

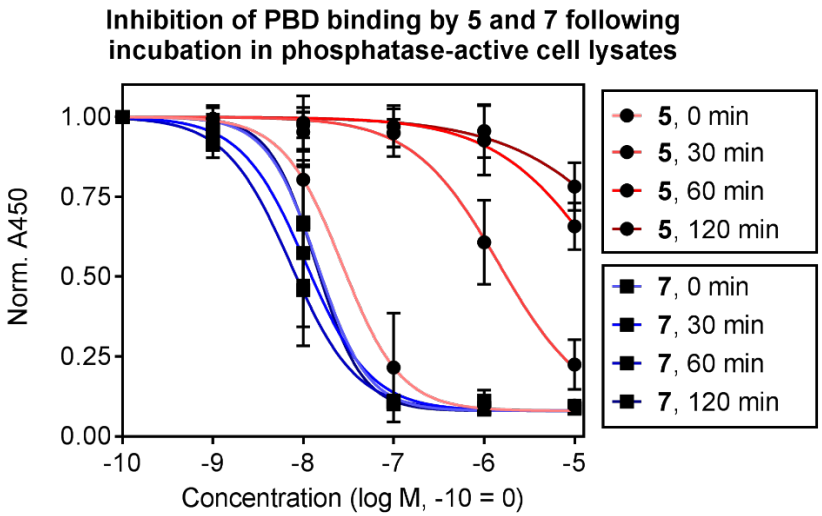


Figure S3. ELISA-based competitive inhibition of isolated PBD by **5** and **7** following incubation in crude cell lysates containing active phosphatases. Data points represent average ± SEM from three independent experiments and fit using non-linear regression in GraphPad Prism 6.

General Methods

All experiments involving moisture-sensitive compounds were conducted under anhydrous conditions (positive argon pressure) using standard syringe, cannula, and septa apparatus. Commercial reagents were purchased from Sigma, TCI America, Acros, or Chem-Impex. Fmoc-Ser(Trt)-OH, Fmoc-His(Mtt)-OH, Fmoc-Leu-OH, Fmoc-Pro-OH and Fmoc-Thr[PO(OH)((OBn)]-OH were purchased from Chem-Impex. The intermediates ethyl ((benzyloxy)carbonyl)-tosylglycinate (**8**)[1], ethyl (tert-butoxycarbonyl)-tosylglycinate (**14**)[1], Fmoc-His[N(π)-$(CH_2)_8$-Ph]-OH[2] and benzyl (S)-2-(((benzyloxy)carbonyl)amino)-4-oxobutanoate (**26**)[3] were synthesized as previously described. The final peptides PLHSpT (**4**) and PLH*SpT (**5**) were synthesized by SPPS as previously described to >95% purity.[4] All solvents were purchased in anhydrous form (Aldrich) and used directly. HPLC-grade hexanes, EtOAc, DCM, and MeOH were used in chromatography. Analytical TLCs were performed using Analtech precoated plates (Uniplate, silica gel GHLF, 250 nm) containing a fluorescence indicator. Silica column chromatography employed a Telodyne CombiFlash Rf 200i insturment with either hexane/EtOAc or DCM/MeOH gradients. NMR spectra were recorded using a Varian Inova 400 MHz spectrometer. Coupling constants are reported in Hertz, and peak shifts are reported in δ (ppm) relative to $CDCl_3$ (1H 7.26 ppm, 13C 77.16 ppm). Optical rotation was measured on a Jasco P-1010 polarimeter. Low-resolution mass spectra (ESI) were measured with either an Agilent 260 1200 LC/MSD-SL system or a Shimadzu 2020 LC/MS system. High resolution mass spectra (HRMS) were obtained by positive ion, ESI analysis on a Thermo Scientific LTQ-XL Orbitrap mass spectrometer with HPLC sample introduction using a short narrow-bore C1s reversed-phase column with CH_3CN - H_2O gradients. Preparative HPLC of final peptides was performed using a Waters 2545 binary pump (MeCN/water gradient) with a Phenomenex Gemini-C18 (5 µm, 250 x 21 mm) preparative column and UV detection at 210 nm. Analytical HPLC of final peptides was performed using an Agilent 1200 series quaternary pump (MeCN/water gradient) with a Phenomenex Kinetix-C18 (5 µm, 250 x 4 mm) analytical column and UV detection at 210 nm.

Synthetic Procedures

General Procedure A. Procedure similar to the original published procedure.[5] The aldehyde (R-CHO, 1.2 - 2 equiv.) was dissolved in chloroform (0.2 M) and stirred at RT. (S)-2-(bis(3,5-bis(trifluoromethyl)phenyl)((trimethylsilyl)oxy)methyl) pyrrolidine (**9**, 0.1 equiv., Sigma) was added and allowed to stir for 5 minutes. Ethyl ((benzyloxy) carbonyl)-tosylglycinate (1 equiv.; synthesized by a literature procedure[1]) and potassium fluoride (5 equiv.) were then added and the reaction was allowed to stir at RT for 24 - 96 h. Following completion, the reaction mixture was filtered through Celite and the eluent concentrated. Purification by silica column chromatography (hexane/EA gradient) afforded the corresponding product. Average integration of the aldehyde (9.5-10.0 ppm) and α-(CH) (4.2-4.7 ppm) protons in the ^{1}H NMR spectrum was used to determine anti/syn diastereomeric ratio (d.r.).

General Procedure B. Di-alkyl phosphite (1.5 equiv.) and triethylamine (2 equiv.) were added to DCM (0.2 M) in a round bottom flask at 0 °C. Chlorotrimethylsilane (1.5 equiv.) was then added and allowed to stir at 0 °C for an additional 5 minutes to generate a white precipitate. The corresponding aldehyde (1 equiv.) from General Procedure A was added and the reaction was

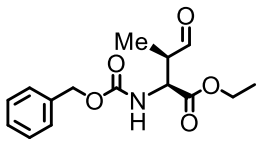
further stirred for 3 – 6 h at RT. Following completion, the reaction was diluted with DCM, washed with brine, and the aqueous layer extracted with additional DCM. The organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was re-dissolved in MeOH (0.05 M), then 20% aqueous citric acid (30% v/v) was added and stirred at RT overnight. Following removal of the TMS-group by TLC, the reaction was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated. Purification by silica column chromatography (hexane/EA gradient) afforded the corresponding product as a mixture of diastereomers.

General Procedure C. The secondary alcohol from General Procedure B was dissolved in DCM (0.1 – 0.2 M) and placed in a round-bottom flask with stirring at RT. Diisopropylethylamine (DIEA, 4 equiv.) and N,N-dimethylaminopyridine (DMAP, 0.2 equiv.) were added, followed by O-phenyl chlorothionoformate (3 equiv.). The reaction was allowed to stir for 3 – 16 h and followed by TLC. Following completion, water was added and the reaction was allowed to quench for 30 minutes with stirring at RT. The mixture was then diluted with DCM and water and the aqueous layer was extracted with DCM. The organic layers were dried over Na_2SO_4 and concentrated. Purification by silica column chromatography (hexane/EA gradient) afforded the corresponding phenylthiocarbonate as a mixture of diastereomers. The phenylthiocarbonate was dissolved in toluene (0.1 M) and placed in a round-bottom flask with stirring. Azobis-isobutylnitrile (AIBN, 1 equiv.) and tributyltin hydride (3 equiv.) were added and the reaction was heated to 110 °C for 20 minutes. Following removal of the thiocarbonate by TLC, the reaction was cooled to RT and the toluene was removed under vacuum. The resulting residue was directly purified by silica column chromatography (hexane/EA gradient) to afford the corresponding product.

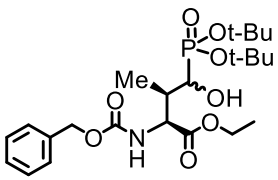
General Procedure D. The resulting Cbz-protected ethyl ester product of General Procedure D was dissolved in THF (0.1 M) and added to a round-bottom flask at RT. Lithum hydroxide (3 equiv.) was dissolved in water (25% v/v) and added to the THF solution. The reaction was allowed to stir overnight at RT. Following saponification, the THF was removed under vacuum and the aqueous solution was diluted with 0.5 M HCl and extracted into EtOAc twice. The combined organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was dissolved in MeOH (0.05 M) and degassed with argon for several minutes. Palladium on carbon (0.2 equiv., 10% w/w) was added with stirring. The reaction was placed under a blanket of hydrogen gas and stirred at RT for 3 h to remove the Cbz group. Once completed, the reaction mixture was filtered through Celite and concentrated. The resulting residue was dissolved in 1:1 THF/water (0.05 M). Sodium bicarbonate (5 equiv.) was added, followed by Fmoc-OSu (1.5 equiv.) and the reaction was allowed to stir overnight. Once completed, the THF is removed under vacuum and the aqueous mixture diluted with 0.5 M HCl. The aqueous solution was extracted twice with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated. Purification by silica column chromatography (DCM/MeOH gradient) afforded the corresponding Fmoc-protected amino acid product.

General Solid-Phase Peptide Synthesis (SPPS) procedure. NovaSyn TG Sieber resin is pre-swollen in DMF (4 mL) for 1 h with shaking. The resin is Fmoc- deprotected using 20% piperidine in DMF (4 mL) 2 times for 10 minutes each. Fmoc-protected amino acids (2 – 4

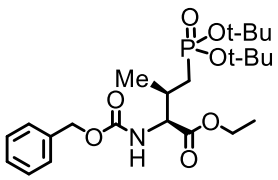
equivalents based on resin) are dissolved in DMF (3-4 mL) containing 4% DIEA and pre-activated by the addition of HATU (0.95 mol equivatents relative to the amino acid) for 5 minutes with gentle agitation. The resin is washed 4 times with DMF (6-8 mL), and the HATU-activated amino acid solution is added to the washed resin. Coupling reactions are shaken at room temperature and allowed to proceed from 3-16 hours depending on the equivalents used and steric bulk of each amino acid. Coupling reactions are routinely checked for completion using Kaiser test. Once completed, the resin is filtered and washed 4 times with DMF (6-8 mL), followed by Fmoc-deprotection using 20% piperidine in DMF (4 mL, 2x 10 minutes each). Cleavage from Sieber resin and global deprotection is done using 33% TFA with 2% triisopropylsilane (TIPS) in DCM. Crude peptides were purified using preparative reverse-phase HPLC with gradient elution (89.9/10/0.1 water/acetonitrile/TFA to 99.9/0.1 acetonitrile/TFA over 30 minutes).



Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-methyl-4-oxobutanoate (10a). Propionaldehyde (1.3 mL, 17.9 mmol, 2 equiv.) is reacted with ethyl ((benzyloxy)carbonyl)-tosylglycinate (**8**) (3.4 g, 8.94 mmol) via General Procedure A to provide **10a** (2.26 g, 86%, 12:1 anti/syn) as a white foam. $[\alpha]_D^{20}$ = +27.6 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 9.64 (s, 1H), 7.45 – 7.28 (m, 5H), 5.58 (d, J = 8.3 Hz, 1H), 5.13 (s, 2H), 4.70 (dd, J = 8.4, 3.8 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 3.18 (dd, J = 7.3, 3.8 Hz, 1H), 1.24 (t, J = 7.1 Hz, 3H), 1.18 (d, J = 7.4 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 201.58, 170.34, 156.39, 136.21, 128.68, 128.37, 128.18, 67.33, 62.15, 54.20, 48.84, 14.16, 9.61; HR-MS (ESI+) calculated for $C_{15}H_{19}NO_5$: 294.1336 [M+H+]; found: 294.1342.

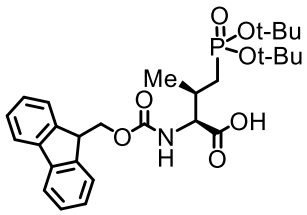


Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-methylbutanoate (11a). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-methyl-4-oxobutanoate (**10a**) (2.1 g, 7.16 mmol) was reacted with di-tert-butyl phosphite via General Procedure B to provide **11a** (2.88 g, 83%) as a mixture of diastereomers. $[\alpha]_D^{20}$ = -1.6 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.41 – 7.28 (m, 5H), 6.18 (d, J = 9.8 Hz, 0.67H), 5.66 (d, J = 8.2 Hz, 0.33H), 5.15 – 5.08 (m, 2H), 4.50 – 4.30 (m, 1H), 4.28 – 4.10 (m, 2H), 3.86 – 3.66 (m, 1H), 2.85 – 2.45 (m, 1H), 1.59 – 1.37 (m, 18H), 1.26 (t, J = 6.9 Hz, 3H), 1.22 – 1.05 (m, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 172.02, 156.92, 136.57, 128.58, 128.33, 128.20, 83.70, 70.67, 69.02, 67.01, 61.47, 59.75, 59.58, 35.94, 30.61, 14.31, 11.59; HR-MS (ESI+) calculated for $C_{23}H_{38}NO_8P$: 488.2408 [M+H+]; found: 488.2404.

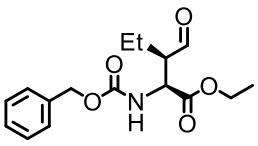


Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-3-methylbutanoate (12a). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-methylbutanoate (**11a**) (2.18 g, 5.13 mmol) was reacted via General Procedure C to provide **12a** (1.3 g, 61% over 2 steps) as a white foam. $[\alpha]_D^{20}$ = +6.6 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.27 (m, 5H), 5.78 (d, *J* = 8.5 Hz, 1H), 5.16 – 5.05 (m, 2H), 4.27 (dd, *J* = 8.3, 5.3 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 2.47 – 2.31 (m, 1H), 1.82 – 1.67 (m, 1H), 1.59 – 1.50 (m, 1H), 1.50 – 1.42 (m, 18H), 1.27 (t, *J* = 7.1

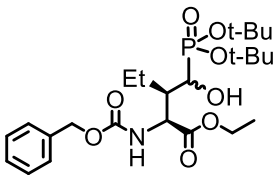
Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.72, 156.35, 136.47, 128.62, 128.24, 128.21, 82.23, 67.08, 61.55, 59.56, 33.89, 32.44, 30.52, 17.58, 14.34; HR-MS (ESI+) calculated for $C_{23}H_{38}NO_7P$: 472.2459 [M+H+]; found: 472.2474.



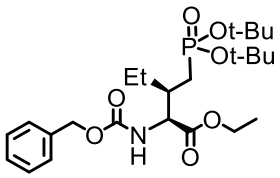
(2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-3-methylbutanoic acid (13a). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-methylbutanoate (**12a**) (1.28 g, 2.71 mmol) was reacted via General Procedure D to provide **13a** (1.25 g, 87%) as a white foam. $[\alpha]_D^{20}$ = +30.3 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.76 (d, J = 7.5 Hz, 2H), 7.63 – 7.58 (m, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.1 Hz, 2H), 5.85 (d, J = 7.3 Hz, 1H), 4.76 (dd, J = 7.0, 4.7 Hz, 1H), 4.37 (d, J = 7.1 Hz, 2H), 4.22 (t, J = 7.1 Hz, 1H), 2.61 – 2.48 (m, 1H), 1.93 – 1.83 (m, 1H), 1.74 – 1.64 (m, 1H), 1.54 (d, J = 22.8 Hz, 18H), 1.04 (d, J = 6.9 Hz, 3H). 13C NMR (101 MHz, $CDCl_3$) δ 171.83, 155.51, 143.91, 141.43, 127.82, 127.17, 125.32, 120.10, 83.96, 67.11, 56.97, 47.28, 30.55, 25.52, 16.98; HR-MS (ESI+) calculated for $C_{28}H_{38}NO_7P$: 532.2459 [M+H+]; found: 532.2461.



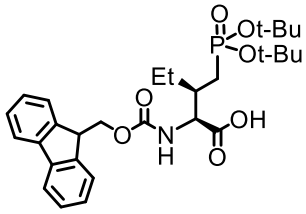
Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-ethyl-4-oxobutanoate (10b). Butyraldehyde (184 µL, 2.04 mmol, 2 equiv.) is reacted with ethyl ((benzyloxy)carbonyl)-tosylglycinate (**8**) (400 mg, 1.02 mmol) via General Procedure A to provide **10b** (290 mg, 92%, 11:1 anti/syn) as a white foam. Spectral data similar to previously reported.[5a] $[\alpha]_D^{20}$ = +30.4 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 9.77 (s, 1H), 7.59 – 7.42 (m, 5H), 5.67 (d, J = 9.5 Hz, 1H), 5.35 – 5.20 (m, 2H), 4.80 (dd, J = 9.5, 3.7 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.25 – 3.12 (m, 1H), 2.08 – 1.90 (m, 1H), 1.76 – 1.57 (m, 1H), 1.39 (t, J = 7.1 Hz, 3H), 1.27 (t, J = 7.4 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 202.49, 171.04, 156.70, 136.29, 128.67, 128.33, 128.12, 67.31, 62.06, 55.56, 52.45, 18.72, 14.18, 12.31; HR-MS (ESI+) calculated for $C_{16}H_{21}NO_5$: 308.1492 [M+H+]; found: 308.1505.



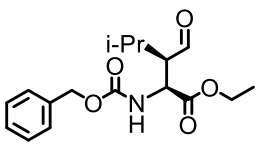
Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-ethylbutanoate (11b). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-ethyl-4-oxobutanoate (**10b**) (235 mg, 0.765 mmol) was reacted with di-tert-butyl phosphite via General Procedure B to provide **11b** (295 mg, 77%) as a mixture of diastereomers. $[\alpha]_D^{20}$ = -8.6 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.41 – 7.27 (m, 5H), 6.38 (d, J = 8.5 Hz, 1H), 5.18 – 5.02 (m, 2H), 4.59 – 4.50 (m, 1H), 4.28 – 4.06 (m, 2H), 3.81 (dd, J = 10.5, 6.0 Hz, 1H), 3.51 – 3.44 (m, 1H), 2.35 – 2.19 (m, 1H), 1.96 – 1.76 (m, 1H), 1.55 – 1.40 (m, 18H), 1.27 (t, J = 6.9 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 172.62, 156.93, 136.69, 128.61, 128.54, 128.14, 83.77, 71.48, 69.85, 66.89, 61.45, 55.93, 42.30, 30.60, 30.46, 17.87, 14.35, 12.09; HR-MS (ESI+) calculated for $C_{24}H_{40}NO_8P$: 502.2564 [M+H+]; found: 502.2583.



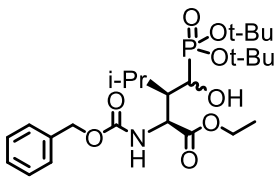
Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-3-ethylbutanoate (12b). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-ethylbutanoate (**11b**) (100 mg, 0.157 mmol) was reacted via General Procedure C to provide **12b** (60 mg, 43% over 2 steps) as a white foam. $[\alpha]_D^{20}$ = -4.0 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.36 – 7.26 (m, 5H), 6.35 (d, J = 8.7 Hz, 1H), 5.18 – 5.03 (m, 2H), 4.43 (dd, J = 8.6, 5.5 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 2.30 – 2.11 (m, 1H), 1.73 – 1.52 (m, 4H), 1.47 (d, J = 5.0 Hz, 18H), 1.26 (t, J = 7.1 Hz, 3H), 0.99 (t, J = 7.3 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 172.39, 156.69, 136.69, 128.52, 128.12, 128.09, 82.48, 66.88, 61.46, 56.88, 38.39, 31.38, 30.48, 24.61, 14.30, 11.43; HR-MS (ESI+) calculated for $C_{24}H_{40}NO_7P$: 486.2615 [M+H+]; found: 486.2633.



(2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-3-ethylbutanoic acid (13b). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-ethylbutanoate (**12b**) (50 mg, 0.103 mmol) was reacted via General Procedure D to provide **13b** (45 mg, 80%) as a white foam. $[\alpha]_D^{20}$ = +40.6 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.75 (d, J = 7.6 Hz, 2H), 7.64 – 7.55 (m, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 6.01 (d, J = 7.4 Hz, 1H), 4.81 (dd, J = 7.2, 4.2 Hz, 1H), 4.36 (d, J = 7.2, 2.1 Hz, 2H), 4.22 (t, J = 7.2 Hz, 1H), 2.34 – 2.20 (m, 1H), 2.03 – 1.87 (m, 1H), 1.70 – 1.40 (m, 19H), 1.33 – 1.18 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 172.62, 155.75, 143.92, 141.41, 127.80, 127.16, 125.32, 120.08, 83.84, 67.11, 55.92, 53.56, 47.28, 38.43, 30.55, 23.83, 11.80; HR-MS (ESI+) calculated for $C_{29}H_{40}NO_7P$: 546.2615 [M+H+]; found: 546.2634.

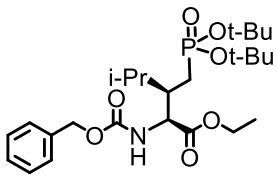


Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-isopropyl-4-oxobutanoate (10c). Isovaleraldehyde (0.22 mL, 2.04 mmol, 2 equiv.) is reacted with ethyl ((benzyloxy)carbonyl)-tosylglycinate (**8**) (400 mg, 1.02 mmol) via General Procedure A to provide **10c** (250 mg, 76%, 10:1 anti/syn) as a white foam. Spectral data similar to previously reported.[5a] $[\alpha]_D^{20}$ = +26.4 (c 0.8, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 9.80 (s, 1H), 7.42 – 7.27 (m, 5H), 5.63 (d, J = 10.1 Hz, 1H), 5.14 (q, J = 12.3 Hz, 2H), 4.75 – 4.58 (m, 1H), 4.17 (q, J = 7.3 Hz, 2H), 2.98 (dd, J = 8.2, 3.6 Hz, 1H), 2.16 – 2.06 (m, 1H), 1.24 (t, J = 7.2 Hz, 3H), 1.18 (d, J = 6.8 Hz, 3H), 1.10 (d, J = 6.7 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 204.86, 171.57, 156.73, 136.39, 128.65, 128.29, 128.08, 67.26, 62.01, 59.19, 52.46, 27.63, 21.59, 20.65, 14.17; HR-MS (ESI+) calculated for $C_{17}H_{23}NO_5$: 322.1649 [M+H+]; found: 322.1665.

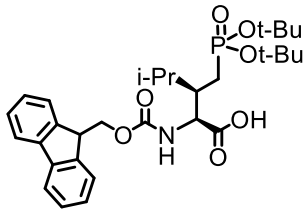


Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-isopropylbutanoate (11c). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-isopropyl-4-oxobutanoate (**10c**) (200 mg, 0.622 mmol) was reacted with di-tert-butyl phosphite via General Procedure B to provide **11c** (165 mg, 52%) as a mixture of diastereomers. $[\alpha]_D^{20}$ = -5.2 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.41 – 7.27 (m, 5H), 6.70 (d, J = 8.1 Hz, 0.33H), 5.89 (d, J = 10.3 Hz, 0.66H), 5.18 – 5.05 (m, 2H), 4.65 – 4.49 (m, 0.66H), 4.22 – 4.05 (m, 2H), 4.03 – 3.95 (m, 0.33H), 3.81 – 3.72 (m, 0.66H), 3.27 –

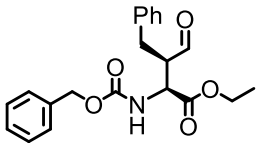
2.92 (m, 0.66H), 2.71 – 2.57 (m, 0.66H), 2.47 – 2.33 (m, 0.33H), 2.32 – 2.24 (m, 0.33H), 2.19 – 2.06 (m, 0.66H), 1.59 – 1.41 (m, 18H), 1.31 – 1.15 (m, 3H), 1.13 – 1.00 (m, 4H), 0.86 (d, J = 6.9 Hz, 2H); 13C NMR (101 MHz, $CDCl_3$) δ 173.55, 173.08, 157.02, 156.58, 136.78, 128.57, 128.12, 128.05, 84.08, 83.68, 71.61, 69.99, 69.16, 67.54, 66.98, 66.83, 61.41, 54.25, 54.12, 51.77, 51.64, 47.49, 46.11, 30.60, 27.02, 22.94, 21.71, 20.00, 16.67, 14.29, 14.21; HR-MS (ESI+) calculated for $C_{25}H_{42}NO_8P$: 516.2721 [M+H+]; found: 516.2742.



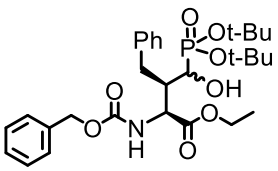
Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-3-isopropylbutanoate (12c). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-isopropylbutanoate (**11c**) (100 mg, 0.194 mmol) was reacted via General Procedure C to provide **12c** (45 mg, 46% over 2 steps) as a clear residue on glass. $[\alpha]_D^{20}$ = -23.0 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.54 (d, J = 7.7 Hz, 1H), 7.37 – 7.22 (m, 5H), 5.20 – 5.00 (m, 2H), 4.25 – 4.08 (m, 3H), 2.26 – 2.11 (m, 1H), 1.96 – 1.78 (m, 1H), 1.73 – 1.62 (m, 1H), 1.46 (d, J = 14.5 Hz, 19H), 1.25 (t, J = 7.1 Hz, 3H), 0.91 (dd, J = 13.0, 6.8 Hz, 6H); 13C NMR (101 MHz, $CDCl_3$) δ 172.58, 156.72, 136.94, 128.25, 127.91, 127.71, 82.51, 66.40, 61.04, 57.50, 40.76, 30.35, 28.76, 28.03, 26.59, 20.81, 17.05, 14.13; HR-MS (ESI+) calculated for $C_{25}H_{42}NO_7P$: 500.2772 [M+H+]; found: 500.2792.



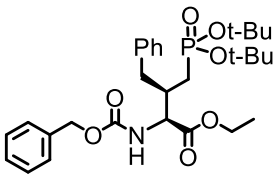
(2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-3-isopropylbutanoic acid (13c). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxy-3-isopropylbutanoate (**12c**) (25 mg, 0.050 mmol) was reacted via General Procedure D to provide **13c** (22 mg, 79%) as a clear residue on glass. $[\alpha]_D^{20}$ = +21.4 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.74 (d, J = 7.5 Hz, 2H), 7.68 – 7.59 (m, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.32 – 7.25 (m, 2H), 7.00 – 6.90 (m, 1H), 4.55 (t, J = 6.9 Hz, 1H), 4.42 – 4.28 (m, 2H), 4.26 – 4.18 (m, 1H), 2.40 – 2.26 (m, 1H), 2.12 – 2.00 (m, 1H), 1.89 – 1.62 (m, 2H), 1.52 (s, 18H), 0.92 (dd, J = 23.4, 6.7 Hz, 6H); 13C NMR (101 MHz, $CDCl_3$) δ 174.28, 156.43, 143.99, 141.38, 127.75, 127.20, 125.42, 120.00, 83.51, 67.21, 47.32, 41.26, 30.53, 28.37, 27.67, 26.24, 21.48, 17.79; HR-MS (ESI+) calculated for $C_{30}H_{42}NO_7P$: 560.2772 [M+H+]; found: 560.2799.



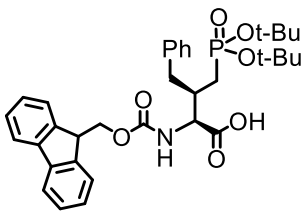
Ethyl (2S,3R)-3-benzyl-2-(((benzyloxy)carbonyl)amino)-4-oxobutanoate (10d). Hydrocinnamaldehyde (539 mg, 4.02 mmol, 2 equiv.) is reacted with ethyl ((benzyloxy)-carbonyl)-tosylglycinate (**8**) (786 mg, 2.01 mmol) via General Procedure A to provide **10d** (425 mg, 57%, 12:1 anti/syn) as a white foam. Spectral data similar to previously reported.[5a] $[\alpha]_D^{20}$ = +32.2 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 9.65 (s, 1H), 7.44 – 7.12 (m, 10H), 5.55 (d, J = 9.4 Hz, 1H), 5.15 (s, 2H), 4.60 (dd, J = 9.5, 3.1 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.49 (td, J = 8.1, 3.5 Hz, 1H), 3.10 (dd, J = 14.1, 6.8 Hz, 1H), 2.80 (dd, J = 14.1, 8.4 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 201.97, 170.73, 156.62, 137.77, 136.34, 129.17, 129.00, 128.69, 128.38, 128.19, 127.08, 67.35, 62.17, 55.59, 52.98, 31.79, 14.17; HR-MS (ESI+) calculated for $C_{21}H_{23}NO_5$: 370.1649 [M+H+]; found: 370.1655.



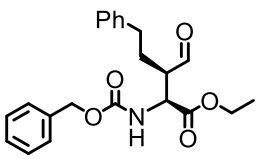
Ethyl (2S,3R)-3-benzyl-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxybutanoate (11d). Ethyl (2S,3R)-3-benzyl-2-(((benzyloxy)carbonyl)amino)-4-oxobutanoate (**10d**) (400 mg, 1.083 mmol) was reacted with di-tert-butyl phosphite via General Procedure B to provide **11d** (400 mg, 66%) as a mixture of diastereomers. $[\alpha]_D^{20}$ = -14.8 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.44 – 7.14 (m, 10H), 6.56 – 6.28 (m, 1H), 5.17 – 5.10 (m, 2H), 4.27 – 4.21 (m, 1H), 4.21 – 4.08 (m, 2H), 3.99 (d, J = 8.9 Hz, 1H), 3.68 – 3.55 (m, 0.33H), 3.29 (d, J = 9.0Hz, 1H), 3.20 – 2.85 (m, 0.67H), 2.82 – 2.38 (m, 2H), 1.58 – 1.46 (m, 18H), 1.25 – 1.16 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.37, 156.66, 139.47, 136.86, 129.28, 128.76, 128.64, 128.55, 128.25, 128.13, 126.53, 84.07, 71.38, 69.74, 66.87, 61.43, 56.18, 56.01, 42.46, 31.16, 30.71, 14.29; HR-MS (ESI+) calculated for $C_{29}H_{42}NO_8P$: 564.2721 [M+H+]; found: 564.2721.



Ethyl (2S,3R)-3-benzyl-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl) butanoate (12d). Ethyl (2S,3R)-3-benzyl-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxybutanoate (**11d**) (380 mg, 0.674 mmol) was reacted via General Procedure C to provide **12d** (216 mg, 58% over 2 steps) as a white foam. $[\alpha]_D^{20}$ = -2.3 (c 1.00, $CHCl_3$); ^1H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.14 (m, 10H), 6.19 (d, *J* = 8.0 Hz, 1H), 5.22 – 5.04 (m, 2H), 4.40 (d, *J* = 8.0 Hz, 1H), 4.15 (q, *J* = 6.7 Hz, 2H), 3.01 – 2.82 (m, 1H), 2.76 – 2.56 (m, 2H), 1.81 – 1.64 (m, 2H), 1.53 – 1.37 (m, 18H), 1.24 (t, *J* = 7.0 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 172.07, 156.53, 139.44, 136.66, 129.53, 128.60, 128.56, 128.17, 128.16, 126.51, 82.57, 66.97, 61.56, 56.51, 38.97, 37.77, 30.50, 29.85, 14.31; HR-MS (ESI+) calculated for $C_{29}H_{42}NO_7P$: 548.2772 [M+H+]; found: 548.2784.

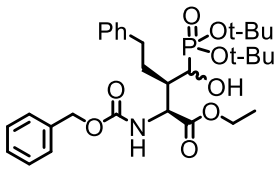


(2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-benzyl-4-(di-tert-butoxyphosphoryl)butanoic acid (13d). Ethyl (2S,3R)-3-benzyl-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl) butanoate (**12d**) (200 mg, 0.365 mmol) was reacted via General Procedure D to provide **13d** (180 mg, 81%) as a white foam. $[\alpha]_D^{20}$ = +40.3 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.76 (d, J = 7.5 Hz, 2H), 7.62 (t, J = 6.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.34 – 7.07 (m, 7H), 5.98 (d, J = 7.0 Hz, 1H), 4.97 – 4.92 (m, 1H), 4.39 (d, J = 7.3 Hz, 2H), 4.23 (t, J = 7.3 Hz, 1H), 3.10 – 3.04 (m, 1H), 2.82 – 2.69 (m, 1H), 2.31 (dd, J = 13.8, 10.1 Hz, 1H), 1.88 – 1.75 (m, 1H), 1.62 – 1.40 (m, 19H). 13C NMR (101 MHz, CDCl3) δ 171.82, 155.23, 143.76, 141.30, 139.05, 129.10, 128.65, 127.70, 127.08, 126.46, 125.18, 119.96, 83.91, 67.04, 55.76, 47.12, 38.28, 37.03, 30.39, 25.38; HR-MS (ESI+) calculated for $C_{34}H_{42}NO_7P$: 608.2772 [M+H+]; found: 608.2771.

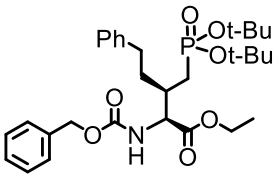


Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-formyl-5-phenylpentanoate (10e). 4-Phenylbutanal (320 mg, 2.159 mmol, 2 equiv.) was reacted with ethyl ((benzyloxy)carbonyl)-tosylglycinate (**8**) (786 mg, 2.01 mmol) via General Procedure A to provide **10e** (365 mg, 88%, 11:1 anti/syn) as a white foam. $[\alpha]_D^{20}$ = +45.3 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 9.57 (s, 1H), 7.47 – 7.08 (m, 10H), 5.55 (d, J = 9.4 Hz, 1H), 5.15 (s, 2H), 4.74 (dd, J = 9.5, 3.7 Hz, 1H), 4.18 (q, J = 6.4, 5.7 Hz, 2H), 3.19 – 3.12 (m, 1H),

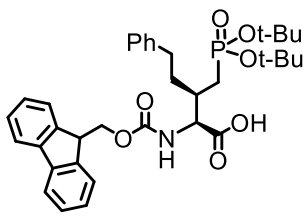
2.94 – 2.70 (m, 2H), 2.14 – 1.97 (m, 1H), 1.83 – 1.71 (m, 1H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.08, 170.81, 156.69, 140.74, 136.30, 128.75, 128.70, 128.62, 128.36, 128.15, 126.48, 67.36, 62.13, 53.28, 52.43, 33.48, 26.97, 14.18; HR-MS (ESI+) calculated for $C_{22}H_{25}NO_5$: 384.1805 [M+H+]; found: 384.1813.



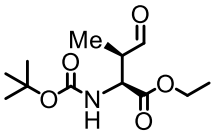
Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-((di-tert-butoxyphosphoryl)(hydroxy) methyl)-5-phenylpentanoate (11e). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-formyl-5-phenyl-pentanoate (**10e**) (260 mg, 0.678 mmol) was reacted with di-tert-butyl phosphite via General Procedure B to provide **11e** (302 mg, 77%) as a mixture of diastereomers. $[\alpha]_D^{20}$ = -7.7 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.41 – 7.09 (m, 10H), 6.44 (d, J = 8.6 Hz, 0.67H), 5.71 (d, J = 9.9 Hz, 0.33H), 5.17 – 5.05 (m, 2H), 4.78 – 4.62 (m, 1H), 4.27 – 4.05 (m, 2H), 3.88 – 3.77 (m, 0.67H), 3.77 – 3.67 (m, 0.67H), 3.51 – 3.40 (m, 0.33H), 3.27 – 3.19 (m, 0.33H), 2.96 – 2.51 (m, 2H), 2.49 – 2.13 (m, 1H), 2.02 – 1.85 (m, 1H), 1.50 – 1.38 (m, 18H), 1.32 – 1.22 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.73, 172.36, 157.61, 156.96, 141.93, 141.67, 136.70, 128.83, 128.67, 128.61, 128.55, 128.41, 128.17, 128.11, 125.94, 125.86, 84.05, 83.66, 82.95, 71.42, 69.77, 66.93, 61.47, 60.52, 56.37, 56.19, 53.55, 42.84, 39.74, 33.58, 33.33, 30.51, 26.39, 21.18, 14.31; HR-MS (ESI+) calculated for $C_{30}H_{44}NO_8P$: 578.2877 [M+H+]; found: 578.2877.



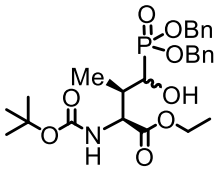
Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-((di-tert-butoxyphosphoryl)methyl)-5-phenylpentanoate (12e). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-((di-tert-butoxyphosphoryl)(hydroxy) methyl)-5-phenylpentanoate (**11e**) (240 mg, 0.415 mmol) was reacted via General Procedure C to provide **12e** (105 mg, 45% over 2 steps) as a white foam. $[\alpha]_D^{20}$ = -4.8 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.44 – 7.04 (m, 10H), 6.23 (d, J = 8.9 Hz, 1H), 5.23 – 5.02 (m, 2H), 4.60 (dd, J = 8.7, 4.5 Hz, 1H), 4.28 – 4.07 (m, 2H), 2.87 – 2.57 (m, 2H), 2.49 – 2.27 (m, 1H), 2.05 – 1.92 (m, 1H), 1.85 – 1.52 (m, 4H), 1.44 (d, J = 9.0 Hz, 18H), 1.27 (t, J = 7.1 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 172.22, 156.73, 141.84, 136.63, 128.64, 128.58, 128.54, 128.45, 128.17, 125.92, 82.58, 67.01, 61.62, 56.52, 36.50, 33.41, 33.12, 31.59, 30.49, 14.30; HR-MS (ESI+) calculated for $C_{30}H_{44}NO_7P$: 562.2928 [M+H+]; found: 562.2937.



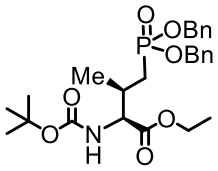
(2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((di-tert-butoxyphosphoryl) methyl)-5-phenylpentanoic acid (13e). Ethyl (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-((di-tert-butoxy phosphoryl) methyl)-5-phenylpentanoate (**12e**) (100 mg, 0.178 mmol) was reacted via General Procedure D to provide **13e** (82 mg, 74%) as a white foam. $[\alpha]_D^{20}$ = +30.4 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.75 (d, J = 7.2 Hz, 2H), 7.61 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 2H), 7.32 – 7.12 (m, 7H), 5.95 (d, J = 7.3 Hz, 1H), 4.87 (dd, J = 7.1, 4.2 Hz, 1H), 4.35 (d, J = 7.2 Hz, 2H), 4.21 (t, J = 7.1 Hz, 1H), 2.76 – 2.56 (m, 2H), 2.50 – 2.36 (m, 1H), 2.06 – 1.86 (m, 2H), 1.72 – 1.47 (m, 19H); 13C NMR (101 MHz, $CDCl_3$) δ 172.31, 155.60, 144.11, 143.92, 141.42, 128.55, 128.51, 127.82, 127.18, 126.12, 125.33, 120.10, 84.02, 67.17, 55.75, 47.26, 36.42, 33.51, 32.50, 30.53, 25.52; HR-MS (ESI+) calculated for $C_{35}H_{44}NO_7P$: 622.2928 [M+H+]; found: 622.2938.



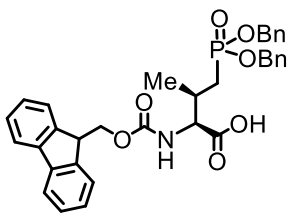
Ethyl (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methyl-4-oxobutanoate (15). Propionaldehyde (0.4 mL, 5.60 mmol, 2 equiv.) is reacted with ethyl (tert-butoxycarbonyl)- tosylglycinate (**14**) (1 g, 2.80 mmol) via General Procedure A to provide **15** (620 mg, 85%, 10:1 anti/syn) as a white foam. Spectral data similar to previously reported.[5a] $[\alpha]_D^{20}$ = +38.4 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 9.64 (s, 1H), 5.29 (d, J = 7.6 Hz, 1H), 4.64 (dd, J = 8.6, 3.7 Hz, 1H), 4.27 – 4.14 (m, 2H), 3.19 – 3.06 (m, 1H), 1.45 (s, 9H), 1.24 (t, J = 7.1 Hz, 3H), 1.17 (d, J = 7.4 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 201.74, 170.70, 155.80, 80.36, 62.03, 53.77, 49.03, 28.42, 14.21, 9.59; HR-MS (ESI+) calculated for $C_{12}H_{21}NO_5$: 260.1492 [M+H+]; found: 260.1490.



Ethyl (2S,3R)-4-(bis(benzyloxy)phosphoryl)-2-((tert-butoxy-carbonyl)amino)-4-hydroxy-3-methylbutanoate (16). Ethyl (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methyl-4-oxobutanoate (**15**) (250 mg, 0.964 mmol) was reacted with dibenzyl phosphite via General Procedure B to provide **16** (440 mg, 88%) as a mixture of diastereomers. $[\alpha]_D^{20}$ = +7.2 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.36 – 7.29 (m, 10H), 5.45 – 5.31 (m, 1H), 5.15 – 4.98 (m, 4H), 4.25 – 4.13 (m, 3H), 3.85 – 3.74 (m, 0.67H), 3.38 – 3.30 (m, 0.33H), 2.83 – 2.22 (m, 1H), 1.48 – 1.38 (m, 9H), 1.26 – 1.20 (m, 3H), 1.19 – 1.03 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.99, 171.26, 156.65, 156.22, 136.27, 135.69, 128.85, 128.70, 128.15, 80.68, 69.01, 68.43, 68.22, 67.46, 61.49, 60.51, 53.55, 52.05, 38.69, 37.94, 28.37, 21.17, 14.59, 14.22, 11.34; HR-MS (ESI+) calculated for $C_{26}H_{36}NO_8P$: 522.2251 [M+H+]; found: 522.2235.

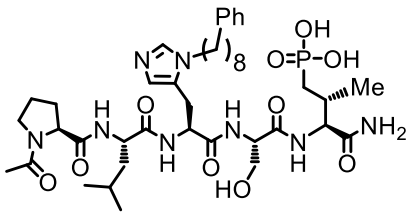


Ethyl (2S,3R)-4-(bis(benzyloxy)phosphoryl)-2-((tert-butoxycarbonyl) amino)-3-methylbutanoate (17). Ethyl (2S,3R)-4-(bis(benzyloxy)phosphoryl)-2-((tert-butoxycarbonyl)amino)-4-hydroxy-3-methylbutanoate (**16**) (290 mg, 0.633 mmol) was reacted via General Procedure C to provide **17** (164 mg, 58% over 2 steps) as a white foam. $[\alpha]_D^{20}$ = +13.3 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.43 – 7.28 (m, 10H), 5.15 (d, J = 7.9 Hz, 1H), 5.08 – 4.87 (m, 4H), 4.26 – 4.09 (m, 3H), 2.44 – 2.28 (m, 1H), 1.97 – 1.83 (m, 1H), 1.72 – 1.60 (m, 1H), 1.43 (s, 9H), 1.22 (t, J = 7.1 Hz, 3H), 1.08 (d, J = 6.8 Hz, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 171.66, 155.61, 136.44, 136.38, 128.73, 128.56, 128.11, 128.08, 80.11, 67.37, 61.56, 58.69, 32.03, 29.81, 28.43, 17.56, 14.30; HR-MS (ESI+) calculated for $C_{26}H_{36}NO_7P$: 506.2302 [M+H+]; found: 506.2288.

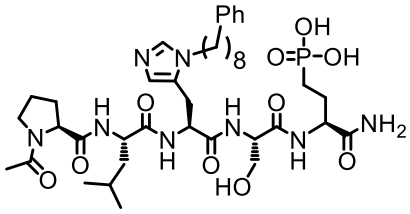


(2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(bis(benzyloxy)phosphoryl)-3-methylbutanoic acid (18). Ethyl (2S,3R)-4-(bis(benzyloxy)phosphoryl)-2-((tert-butoxycarbonyl)amino)-3-methylbutanoate (**17**) (135 mg, 0.267 mmol) was was dissolved in THF (3 mL) and added to a round-bottom flask. Lithum hydroxide (34 mg, 0.80 mmol) was dissolved in water (1 mL) and added to the THF solution. The reaction was allowed to stir overnight at RT. Following saponification, the THF was removed under vacuum and the aqueous solution was diluted with 0.5 M HCl and extracted into EtOAc twice. The combined organic layers were dried over Na_2SO_4 and concentrated. The resulting residue was dissolved in DCM (2 mL) and trifluoroacetic acid (0.5 mL) was added to

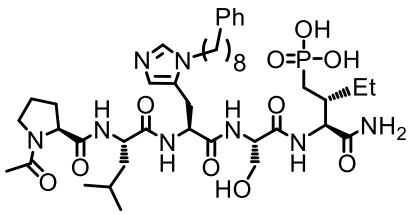
remove the Boc group. The reaction was stirred for 2 h at RT, then concentrated to dryness. The resulting residue was dissolved in 1:1 THF/water (4 mL). Sodium bicarbonate (67 mg, 0.80 mmol) was added, followed by Fmoc-OSu (135 mg, 0.40 mmol) and the reaction was allowed to stir overnight. The THF was removed under vacuum and the aqueous mixture diluted with 0.5 M HCl. The aqueous solution was extracted twice with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated. Purification by silica column chromatography (0 – 10% MeOH in DCM gradient) afforded **18** (128 mg, 80%) as a white foam. $[\alpha]_D^{20}$ = +19.8 (c 1.00, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.75 (d, J = 7.4 Hz, 2H), 7.64 – 7.46 (m, 2H), 7.44 – 7.22 (m, 14H), 5.77 (d, J = 8.1 Hz, 1H), 5.14 – 4.87 (m, 4H), 4.52 (dd, J = 7.6, 3.7 Hz, 1H), 4.38 (d, J = 6.9 Hz, 2H), 4.21 (t, J = 7.0 Hz, 1H), 2.62 – 2.49 (m, 1H), 2.07 – 1.74 (m, 2H), 1.07 (d, 3H); 13C NMR (101 MHz, $CDCl_3$) δ 172.70, 156.26, 143.81, 141.43, 135.85, 128.79, 128.32, 128.22, 127.85, 127.22, 125.25, 120.11, 68.17, 67.27, 58.36, 47.28, 31.45, 25.50, 17.56; HR-MS (ESI+) calculated for $C_{34}H_{34}NO_7P$: 600.2146 [M+H+]; found: 600.2132.



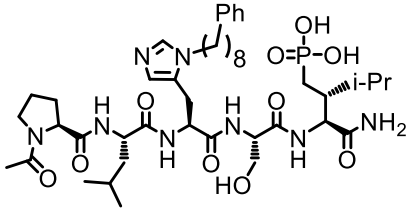
N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Seryl-((2S,3R)-2-amino-3-methyl-4-phosphonobutanoic acid)-$CONH_2$ (7). The peptide Ac-PLH*S(Pmab)-NH_2 was synthesized on 0.1 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **7** (49 mg, 57% overall) with ≥95% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{41}H_{65}N_8O_{10}P$: 861.5 [M+H+]; found: 861.7.



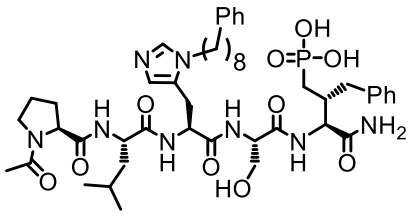
N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Seryl-((2S)-2-amino-4-phosphonobutanoic acid)-$CONH_2$ (19). The peptide Ac-PLH*S(Pab)-NH_2 was synthesized on 0.033 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **19** (6 mg, 24% overall) with ≥95% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{40}H_{63}N_8O_{10}P$: 847.4 [M+H+]; found: 847.9.



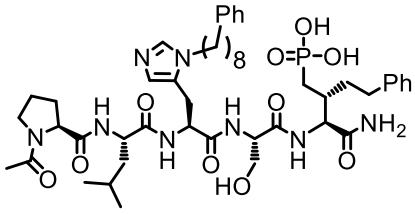
N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Seryl-((2S,3R)-2-amino-3-ethyl-4-phosphonobutanoic acid)-$CONH_2$ (20). The peptide Ac-PLH*S(C3-Et-Pmab)-NH_2 was synthesized on 0.066 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **20** (38 mg, 66% overall) with ≥95% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{42}H_{67}N_8O_{10}P$: 875.5 [M+H+]; found: 875.9.



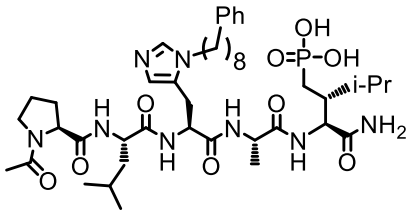
N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Seryl-((2S,3R)-2-amino-3-isopropyl-4-phosphonobutanoic acid)-$CONH_2$ (21). The peptide Ac-PLH*S(C3-iPr-Pmab)-NH_2 was synthesized on 0.033 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **21** (10 mg, 34% overall) with ≥90% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{43}H_{69}N_8O_{10}P$: 889.5 [M+H+]; found: 889.8.



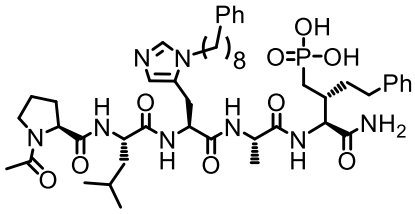
N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Seryl-((2S,3R)-2-amino-3-benzyl-4-phosphonobutanoic acid)-$CONH_2$ (22). The peptide Ac-PLH*S(C3-Bn-Pmab)-NH_2 was synthesized on 0.05 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **22** (14 mg, 30% overall) with ≥90% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{47}H_{69}N_8O_{10}P$: 937.5 [M+H+]; found: 937.8.



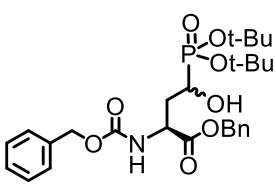
N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Seryl-((2S,3R)-2-amino-3-(ethylphenyl)-4-phosphonobutanoic acid)-$CONH_2$ (23). The peptide Ac-PLH*S(C3-Phenylethyl-Pmab)-NH_2 was synthesized on 0.05 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **23** (22 mg, 46% overall) with ≥95% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{48}H_{71}N_8O_{10}P$: 951.5 [M+H+]; found: 951.8.



N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Alanyl-((2S,3R)-2-amino-3-isopropyl-4-phosphonobutanoic acid)-$CONH_2$ (24). The peptide Ac-PLH*A(C3-iPr-Pmab)-NH_2 was synthesized on 0.033 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **24** (10 mg, 34% overall) with ≥95% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{43}H_{69}N_8O_9P$: 873.5 [M+H+]; found: 874.0.

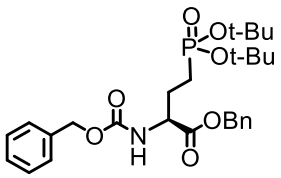


N-Acetyl-L-Prolyl-L-Leucyl-L-Histidyl[N(π)-$(CH_2)_8$-Ph]-L-Alanyl-((2S,3R)-2-amino-3-(ethylphenyl)-4-phosphonobutanoic acid)-$CONH_2$ (25). The peptide Ac-PLH*A(C3-Phenylethyl-Pmab)-NH_2 was synthesized on 0.05 mmol scale using the general SPPS procedure. Purification by preparative RP-HPLC afforded **25** (22 mg, 46% overall) with ≥95% purity by analytical HPLC. LR-MS (ESI+) calculated for $C_{48}H_{71}N_8O_9P$: 935.5 [M+H+]; found: 936.1.

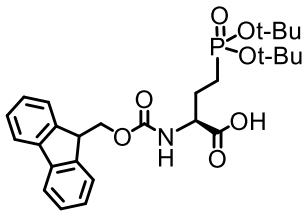


Benzyl (2S)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxybutanoate (27). Benzyl (S)-2-(((benzyloxy)carbonyl)amino)-4-oxobutanoate (**26**), synthesized as previously described[3], (210 mg, 0.615 mmol) was reacted with di-tert-butyl phosphite via General Procedure B to provide **27** (235 mg, 71%) as a mixture of diastereomers. 1H NMR (400 MHz, Chloroform-d) δ 7.41 – 7.26 (m, 10H), 5.95 (d, J = 8.2 Hz, 1H), 5.23 – 5.05 (m, 4H), 4.69 – 4.43 (m, 1H), 3.87 – 3.68 (m, 2H), 2.36 – 2.02 (m, 2H), 1.53 – 1.36 (m, 18H); 13C NMR (101 MHz, $CDCl_3$) δ 172.06, 156.82, 136.32, 135.42,

128.73, 128.66, 128.64, 128.53, 128.42, 128.28, 83.31, 67.39, 67.25, 65.46, 52.06, 34.54, 30.59; HR-MS (ESI+) calculated for $C_{27}H_{38}NO_8P$: 536.2408 [M+H+]; found: 536.2399.



Benzyl (S)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxy-phosphoryl)butanoate (28). Benzyl (2S)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)-4-hydroxybutanoate (**27**) (170 mg, 0.317 mmol) was reacted via General Procedure C to provide **28** (78 mg, 52% over 2 steps) as a white foam. 1H NMR (400 MHz, Chloroform-d) δ 7.42 – 7.25 (m, 10H), 5.67 (d, J = 8.1 Hz, 1H), 5.23 – 5.03 (m, 4H), 4.52 – 4.38 (m, 1H), 2.26 – 2.06 (m, 1H), 2.01 – 1.86 (m, 1H), 1.72 – 1.50 (m, 2H), 1.44 (s, 18H); 13C NMR (101 MHz, $CDCl_3$) δ 171.67, 155.93, 136.26, 135.19, 128.65, 128.51, 128.28, 128.16, 128.09, 81.90, 67.28, 66.98, 54.26, 30.38, 26.63, 25.16; HR-MS (ESI+) calculated for $C_{27}H_{38}NO_7P$: 520.2459 [M+H+]; found: 520.2446.



(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)butanoic acid (29). Benzyl (S)-2-(((benzyloxy)carbonyl)amino)-4-(di-tert-butoxyphosphoryl)butanoate (**28**) (50 mg, 0.096 mmol) was dissolved in MeOH (0.05 M) and degassed with argon for several minutes. Palladium on carbon (10% w/w, 10 mg, 0.010 mmol) was added with stirring. The reaction was placed under a blanket of hydrogen gas and stirred at RT for 3 h to remove the Cbz and benzyl groups. Once completed, the reaction mixture was filtered through Celite and concentrated. The resulting residue was dissolved in 1:1 THF/water (0.05 M). Sodium bicarbonate (24 mg, 0.289 mmol) was added, followed by Fmoc-OSu (49 mg, 0.144 mmol) and the reaction was allowed to stir overnight. The THF is removed under vacuum and the aqueous mixture diluted with 0.5 M HCl. The aqueous solution was extracted twice with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated. Purification by silica column chromatography (0-10% MeOH in DCM gradient) afforded **29** (40 mg, 80%) as a white foam. 1H NMR (400 MHz, Chloroform-d) δ 7.75 (d, J = 7.5 Hz, 2H), 7.65 – 7.55 (m, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.33 – 7.25 (m, 2H), 5.90 (d, J = 7.2 Hz, 1H), 4.47 (q, J = 5.9 Hz, 1H), 4.42 – 4.29 (m, 2H), 4.21 (t, J = 7.2 Hz, 1H), 2.25 – 1.97 (m, 2H), 1.94 – 1.78 (m, 1H), 1.77 – 1.59 (m, 1H), 1.52 - 1.48 (m, 18H); 13C NMR (101 MHz, $CDCl_3$) δ 173.37, 156.04, 143.88, 141.39, 127.83, 127.21, 125.27, 120.08, 83.58, 67.24, 53.71, 47.24, 30.47, 26.60, 24.79; HR-MS (ESI+) calculated for $C_{27}H_{36}NO_7P$: 518.2302 [M+H+]; found: 518.2295.

Synthesis of 5-carboxyfluorescein (5-CF) labeled pThr sequences for FP assay

The 5-carboxyfluorescein labeled peptides used as probes for the fluorescence polarization assay were synthesized using the general SPPS procedure described above. However Rink amide resin was utilized rather than NovaSyn TG Sieber resin. The sequences were synthesized using standard Fmoc amino acid chemistry and capped using 5-carboxyfluorescein NHS ester (synthesized as previously described[6]). Cleavage from the resin was done in 95/2.5/2.5 TFA/TIPS/H_2O, followed by preparative RP-HPLC to afford the final probes.

FP probe for Plk1 PBD: The sequence [5-CF]-GPMQSpTPLNG-NH_2 was synthesized on 0.05 mmol scale using the described procedure to afford the Plk1 PBD probe (6 mg, 17% overall yield) with ≥95% purity by analytical HPLC. LR-MS (ESI-) calculated for $C_{62}H_{80}N_{13}O_{23}PS$: 1437.1 [M-H+]; found: 1436.5.
FP probe for Plk2 PBD: The sequence [5-CF]-GPMQTSpTPKNG-NH_2 was synthesized on 0.05 mmol scale using the described procedure to afford the Plk2 PBD probe (7 mg, 18% overall yield) with ≥95% purity by analytical HPLC. LR-MS (ESI-) calculated for $C_{66}H_{88}N_{15}O_{25}PS$: 1553.2 [M-H+]; found: 1552.6.
FP probe for Plk3 PBD: The sequence [5-CF]-GPLATSpTPKNG-NH_2 was synthesized on 0.05 mmol scale using the described procedure to afford the Plk3 PBD probe (6 mg, 16% overall yield) with ≥95% purity by analytical HPLC. LR-MS (ESI-) calculated for $C_{65}H_{87}N_{14}O_{24}P$: 1478.5 [M-H+]; found: 1477.6.

Biochemical Methods

Lysate production for ELISA-based inhibition assay against full length Plk1.[4] A plasmid encoding myc-tagged full-length Plk1 (Addgene, Plasmid #41160)[7] was transiently transfected into HEK-293T cells using the TurboFect reagent (Thermo) according to manufacturer's instructions. Following 48 h expression, cells were harvested, lysed in buffer (PBS 7.4 with 0.5% NP-40 and protease/phosphatase inhibitor cocktail) using freeze/thaw cycles (3x) and centrifuged at 10,000 xG for 10 minutes. The supernatant was removed to provide a crude cytosolic lysate containing overexpressed, myc-tagged Plk1 (total protein concentration determined by BCA assay).

Determination of inhibitory potency in an ELISA assay using full-length Plk1. The protocol for generating cell lysates containing myc-tagged full-length Plk1 can be found in the Supporting Information. A biotinylated phosphopeptide (sequence: Biotin-Ahx-PMQS(pT)PLN-NH_2) was diluted to 1 μM (from a 10 mM DMSO stock solution) in PBS pH 7.4 and loaded onto the wells of a 96-well Neutravidin-coated plate (Pierce Biotechnology) at 100 μL per well for 1 h (background control contained no biotinylated peptide). The wells were washed once with 150 μL PBST (PBS 7.4 + 0.05% Tween-20), and then 100 μL of 1% BSA in PBS 7.4 (blocking buffer) was added for 1 h. A cytosolic lysate containing transiently expressed myc-tagged Plk1 protein was diluted to 300 μg/mL in PBS 7.4 containing protease/phosphatase inhibitors (Pierce Biotechnology), mixed with competitive inhibitor (from a 10x stock in 5% DMSO/PBS), and allowed to pre-incubate for 1 h (100 μL per well in a 96-well plate, 30 μg total protein). The blocked ELISA plate is washed 2x with PBST (150 μL) and the pre-incubated lysates were added to the plate to incubate for 1 h. The wells was washed 4x with PBST (150 μL), then treated with anti-myc primary antibody (1:1,500 dilution in PBS, mouse monoclonal, Pierce Biotechnology) for 1 h. The wells were then washed 4x with PBST (150 μL), and incubated with rabbit anti-mouse HRP conjugate (1:3,000 dilution in 1% BSA (%w/v) in PBS, Pierce Biotechnology) for 1 h. The wells were then washed 5x with PBST (150 μL) and incubated with Turbo TMB-ELISA solution (Pierce Biotechnology) until the desired absorbance was reached (5 – 10 minutes). The reaction was quenched by the addition of 2 N aqueous H_2SO_4 and the absorbance was measured at 450 nm using a BioTek Synergy 2 96-well plate reader.

Absorbance was plotted versus concentration (log M) and fit to a non-linear regression analysis using GraphPad Prism 6 software (model: log(inhibitor) vs. response -- Variable slope (four parameters)) to provide IC_{50} values. IC_{50} values from multiple independent experiments were normalized and averaged to provide values ± standard error of the mean (SEM).

Determination of binding selectivity against the PBDs of Plks 1 – 3 using fluorescence polarization. Procedures for immunoprecipitation of the isolated PBDs of Plks 1 – 3 and synthesis of the fluorescent PBD-binding peptides are provided in the Supporting Information. Isolated PBD protein was diluted to a 2x working dilution in assay buffer (HEPES-buffered saline with 0.05% Tween-20, 1 mM DTT, and 1 mM EDTA). The following final protein concentrations were used: 100 nM for Plk1 PBD, 200 nM for Plk2 PBD, and 500 nM for Plk3 PBD. These concentrations represent the approximate K_d values determined for the respective fluorescence polarization (FP) probe sequences. Inhibitors were serially diluted to generate 4x working dilutions in assay buffer containing 4% DMSO. 20 µL of 2_X PBD solution was added to each well of a 384-well plate (0% binding controls received 20 µL of assay buffer). 10 µL of the 4x inhibitor solution (or DMSO blank) was added to corresponding wells and allowed to pre-incubated at RT for 30 minutes with shaking. The following sequences were utilized as fluorescent probes: 5CF-GPMQSpTPLNG-NH_2 for Plk1 PBD, 5CF-GPMQTSpTPKNG-NH_2 for Plk2 PBD, and 5CF-PLATSpTPKNG-NH_2 for Plk3 PBD.[8] Fluorescent probes were diluted to 40 nM (4x) in assay buffer, then 10 µL was added to each well. The plate was allowed to equilibrate at RT for 30 minutes with shaking. The FP was read using a BioTek Synergy 2 plate reader with 485/20 excitation and 528/20 emission. The FP values were obtained in triplicate and normalized to 100% (no inhibitor) and 0% binding (no protein) controls. Normalized values were plotted versus concentration and analyzed using non-linear regression in GraphPad Prism 6 [log(inhibitor) vs response – variable slope (four parameter) model]. IC_{50} values represent average ± std. dev.

*Cell lysate assay for phosphatase inactivation of pThr-containing peptide **17**.* Non-transfected HEK-293T cells (~20M) were harvested, lysed in 500 µL of lysis buffer *without* phosphatase inhibitors (PBS 7.4 + 0.5% NP-40 + protease inhibitor cocktail (Sigma)) using 3 freeze/thaw cycles, and centrifuged at 10,000 xG for 10 minutes. The supernatant containing active phosphatases was isolated and the total protein concentration was determined by a BCA assay. Peptides **17** and **19a** were serially diluted as 100_X stocks in PBS plus 5% DMSO, then spiked into aliquots of the concentrated cell lysate (3 mg/mL total protein) to produce a 10x working reactions. The phosphatase inactivation was allowed to proceed for 0, 30, 60, or 120 minutes at 37 °C, and was halted by spiked addition of phosphatase inhibitor (from 100x stock, Sigma) and placed on ice. Inhibition of binding of the isolated Plk1 PBD was then measured for the 10x reaction aliquots by ELISA as described above.

Expression and immunoprecipitation of isolated PBDs of Plks 1 – 3 for FP assays. A plasmid encoding myc-tagged Plk1 PBD was purchased from Addgene (Plasmid #41162)[9]. Plasmids encoding the myc-tagged PBDs of Plk2 and Plk3 were a generous gift from Prof. Erich Nigg (Univ. of Basel, Switzerland).[9] ~20 M HEK-293T cells (2 x 15 cm plates) were transfected with each plasmid using TurboFect reagent. Following 24h expression, cells are harvested, lysed in buffer (HEPES buffered saline (HBS) containing 0.05% Tween-20, 1 mM EDTA, 1 mM DTT,

0.5% NP-40, and protease/phosphatase inhibitor cocktail) using freeze/thaw cycles (3x) and centrifuged at 10,000 xG for 10 minutes. The supernatant containing expressed protein is diluted into 8 mL of HBS containing 0.05% Tween-20, 1 mM EDTA, 1 mM DTT, and protease/phosphatase inhibitor cocktail. This protein solution is added to a 1 mL bed of myc-agarose resin (Thermo) and allowed to bind for 3 h at 4 °C with gentle rotation. The lysate is eluted and the resin is washed 5x with HBS + 1 mM DTT and 1 mM EDTA. The bound PBD protein is then eluted with a 1 mg/mL solution of myc peptide (EQKLISEEDL) in HBS + 1 mM DTT and 1 mM EDTA. The purified PBD protein is dialyzed 4x with HBS + 1 mM DTT and 1 mM EDTA using a 10 kDa MWCO filter (Sigma, fixed angle rotor at 7,500 xG, 4 °C). The concentration of the final protein solution is determined by absorbance at 280 nm and purity is determined by SDS-PAGE with Coomassie staining.

Molecular Modeling

Molecular docking to generate Figure 2 was accomplished using ICM Chemist Pro software running on a MacIntosh computer (OSX v10.10.5) using default parameters and procedures.[1,2] In summary, the previously reported crystal structure of Plk1 PBD with peptide **5** bound (PDB accession code 3RQ7) was loaded and converted to ICM format with optimization of His/Pro/Asn/Gln and Cys residues and retention of all waters. The wa40 and wa105 waters were removed, since these occlude the binding region proximal to the C3 methyl group of **5**. A protein electrostatic surface was generated using a 5-step gradation from red (negative charge) to blue (positive) with neutral shown as white. The bound **5** was set up as the "Ligand" with automatic assignment of charge (pH 7.00). The phosphate oxygen was replaced with a methylene group using Ligand Edit tools. The "Receptor" was defined from protein residues by making a box around the Ligand, which encompassed all residues proximal to the Ligand. Ligand positional restraints were placed on the phosphate oxygens as well as the Ser sidechain oxygen. The Ligand was Re-docked in the Receptor using a rigid docking protocol and default parameters. A Table of docking results was generated and from a selection made from among the highest scoring poses and displayed as docked **7** (panel A, Figure 2). The Ligand C3 methyl substituent was converted to an iPr using standard Ligand Edit procedures and the resulting Ligand re-docked in the Current Receptor as before. Docked **21** was selected from among the highest scoring poses and displayed in Figure 2, panel B. Finally, the Ligand was edited to form the C3-CH_2CH_2Ph group and re-docking was performed as before to generate docked **23** (Figure 2, panel C).

1H and 13C NMR Spectra for New Compounds

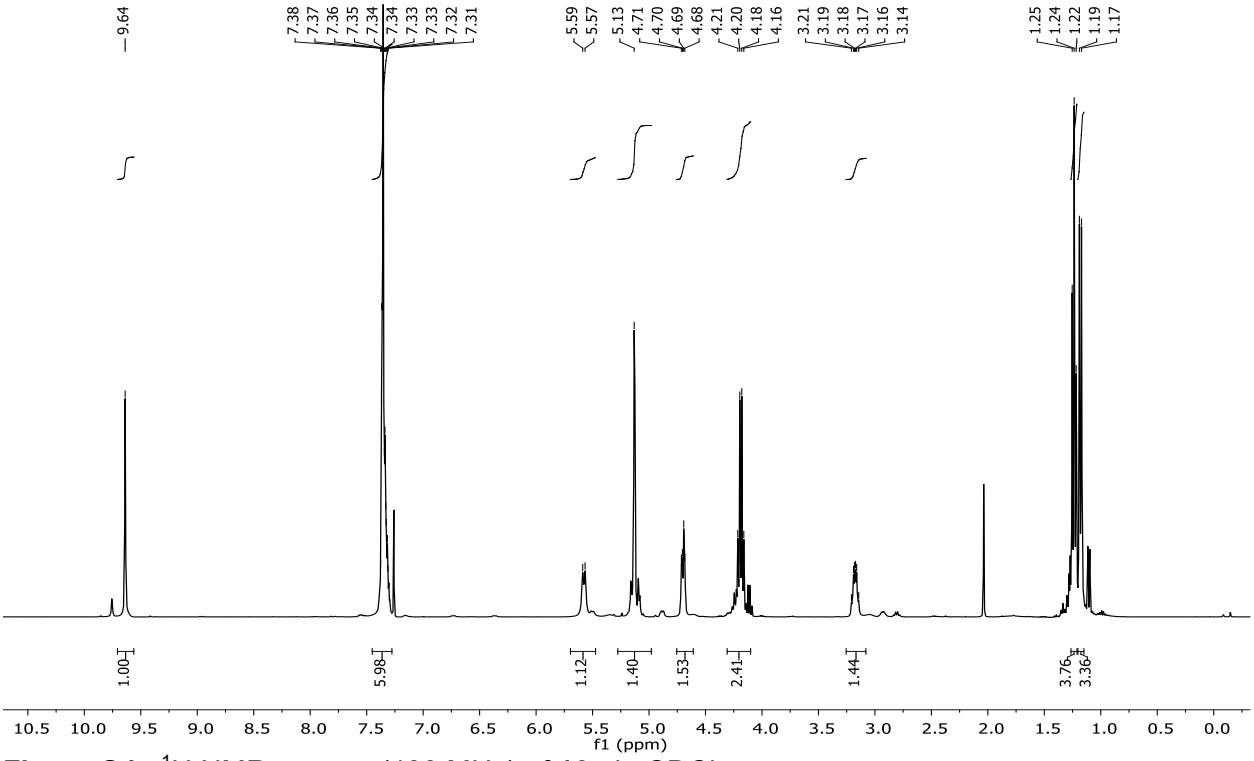


Figure S4. ^{1}H NMR spectra (400 MHz) of **10a** in $CDCl_3$.

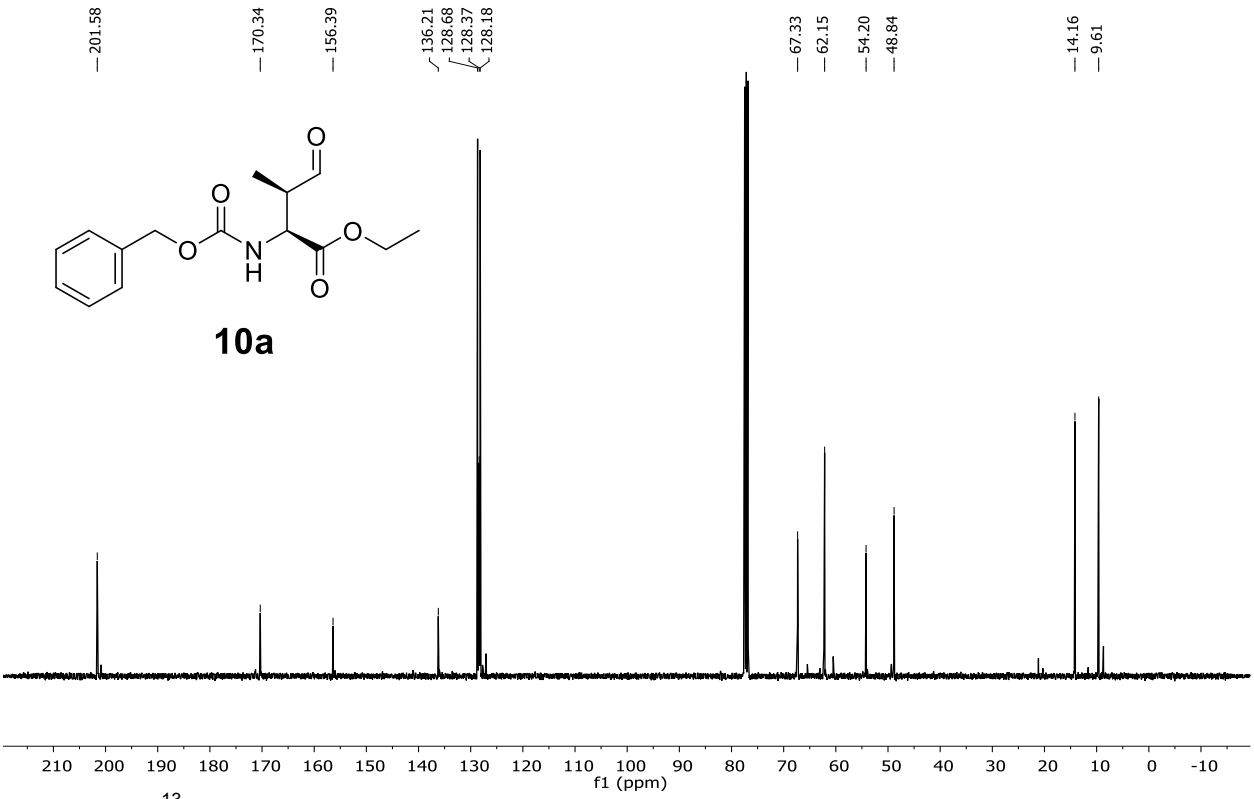


Figure S5. ^{13}C NMR spectra (100 MHz) of **10a** in $CDCl_3$.

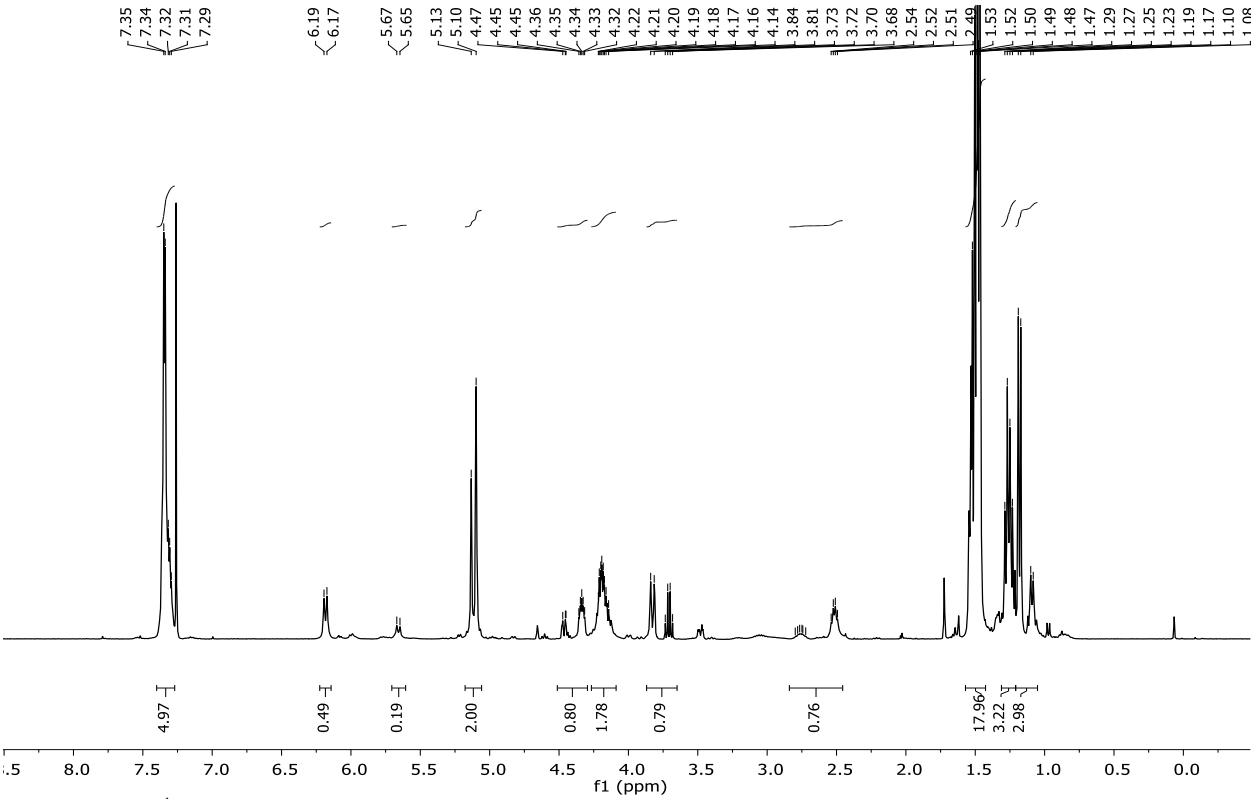


Figure S6. ^{1}H NMR spectra (400 MHz) of **11a** in $CDCl_3$.

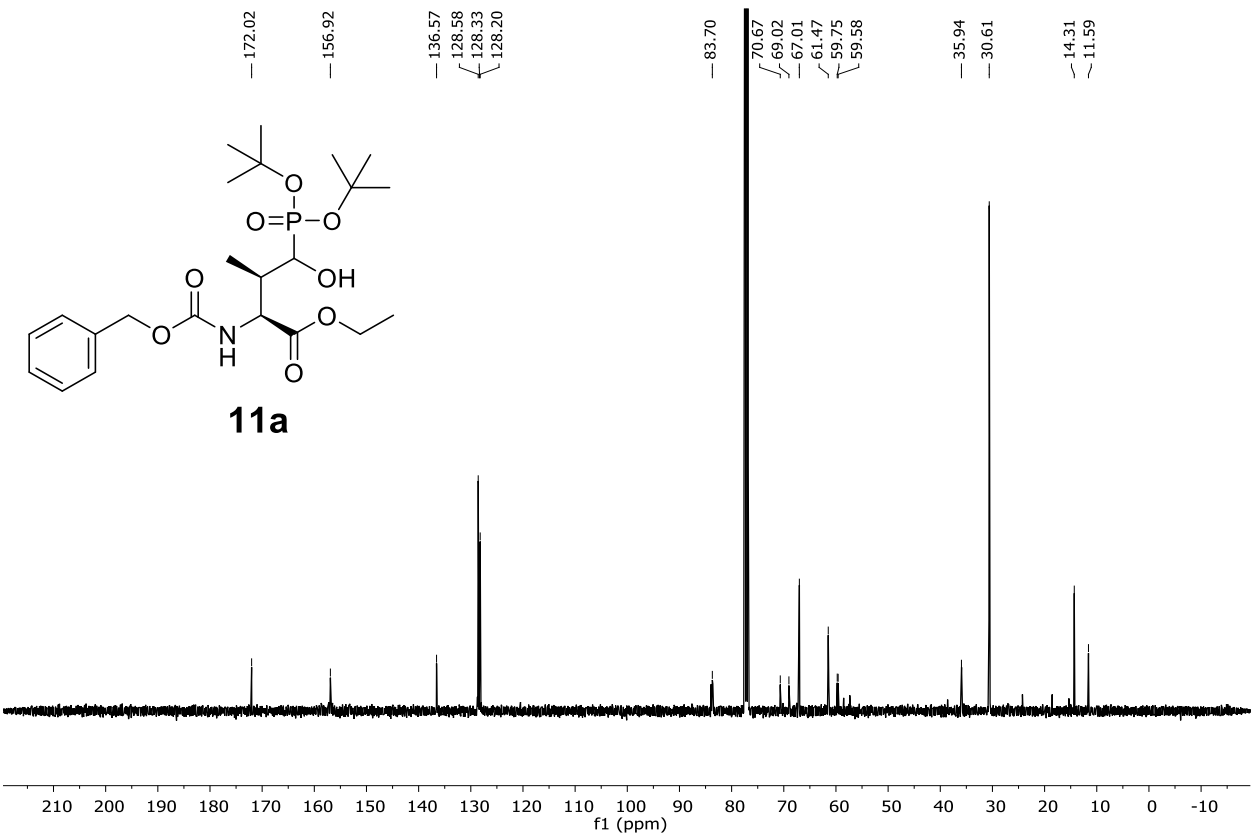


Figure S7. ^{13}C NMR spectra (100 MHz) of **11a** in $CDCl_3$.

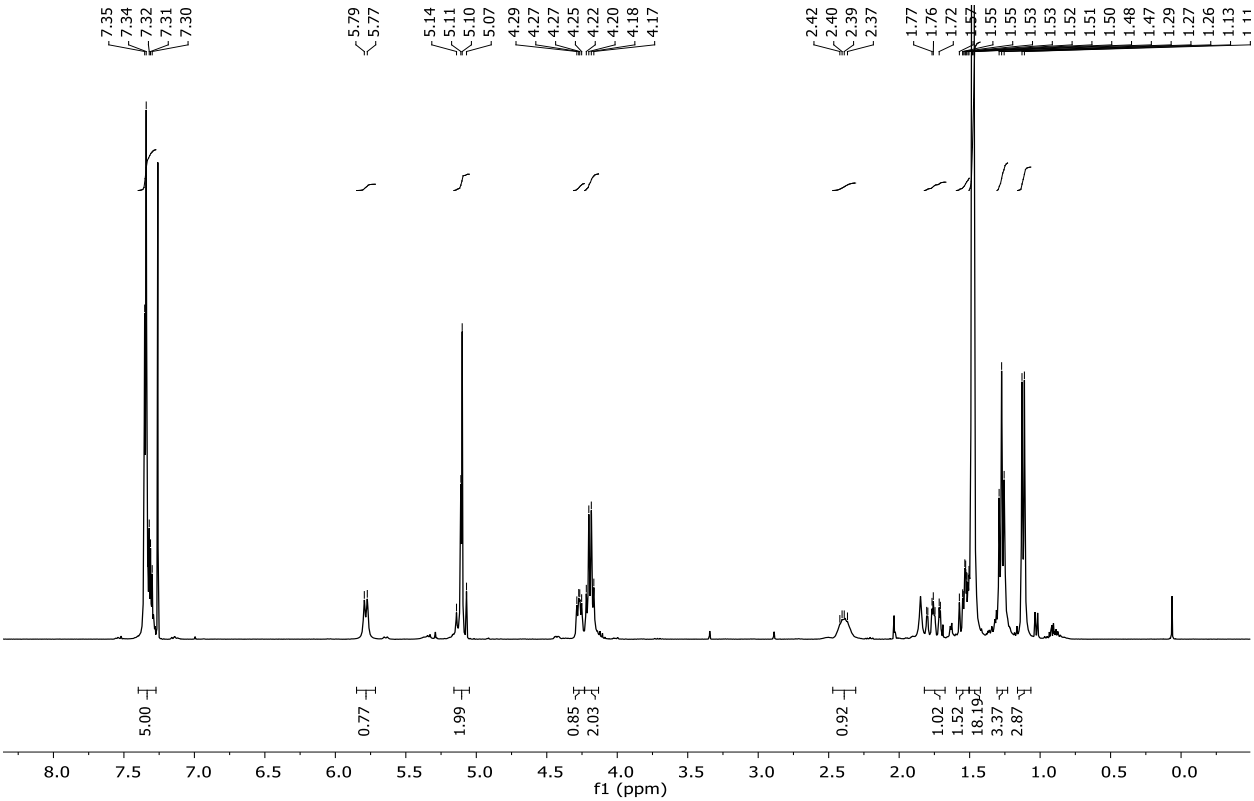


Figure S8. ^{1}H NMR spectra (400 MHz) of **12a** in $CDCl_3$.

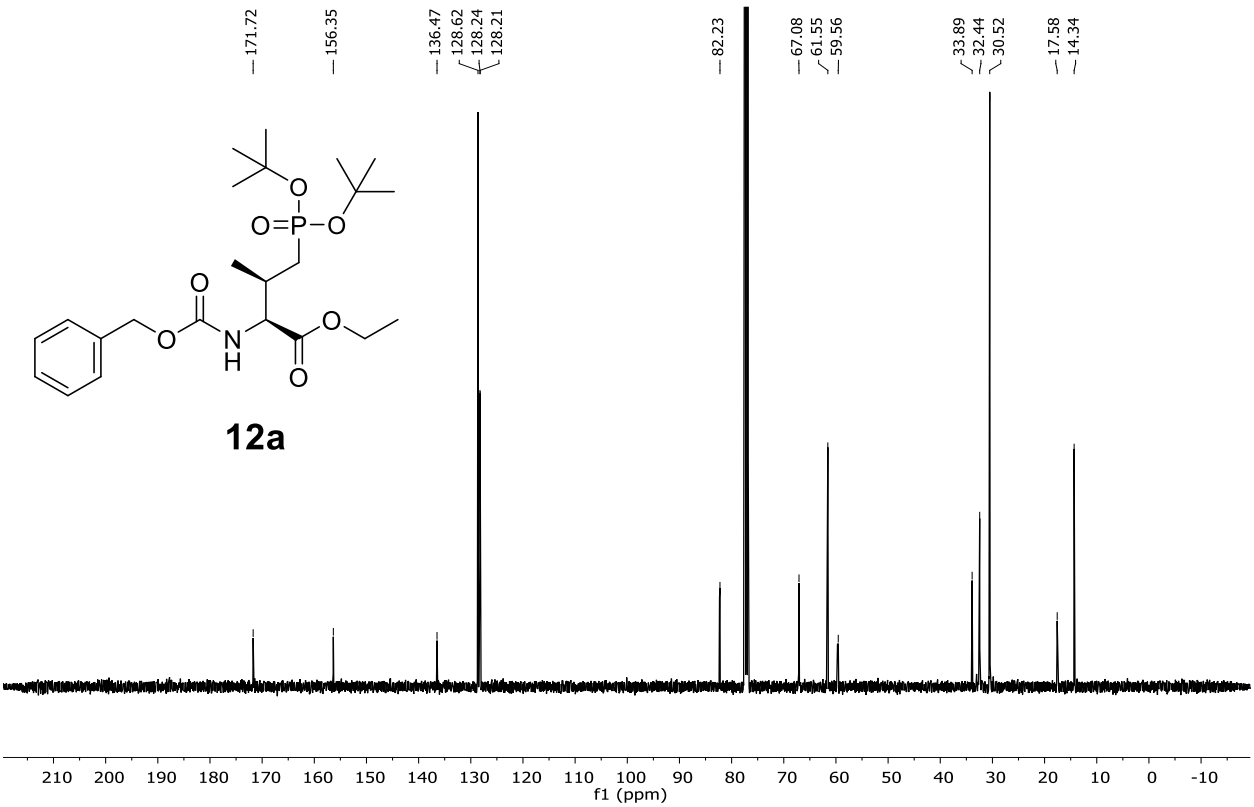


Figure S9. ^{13}C NMR spectra (100 MHz) of **12a** in $CDCl_3$.

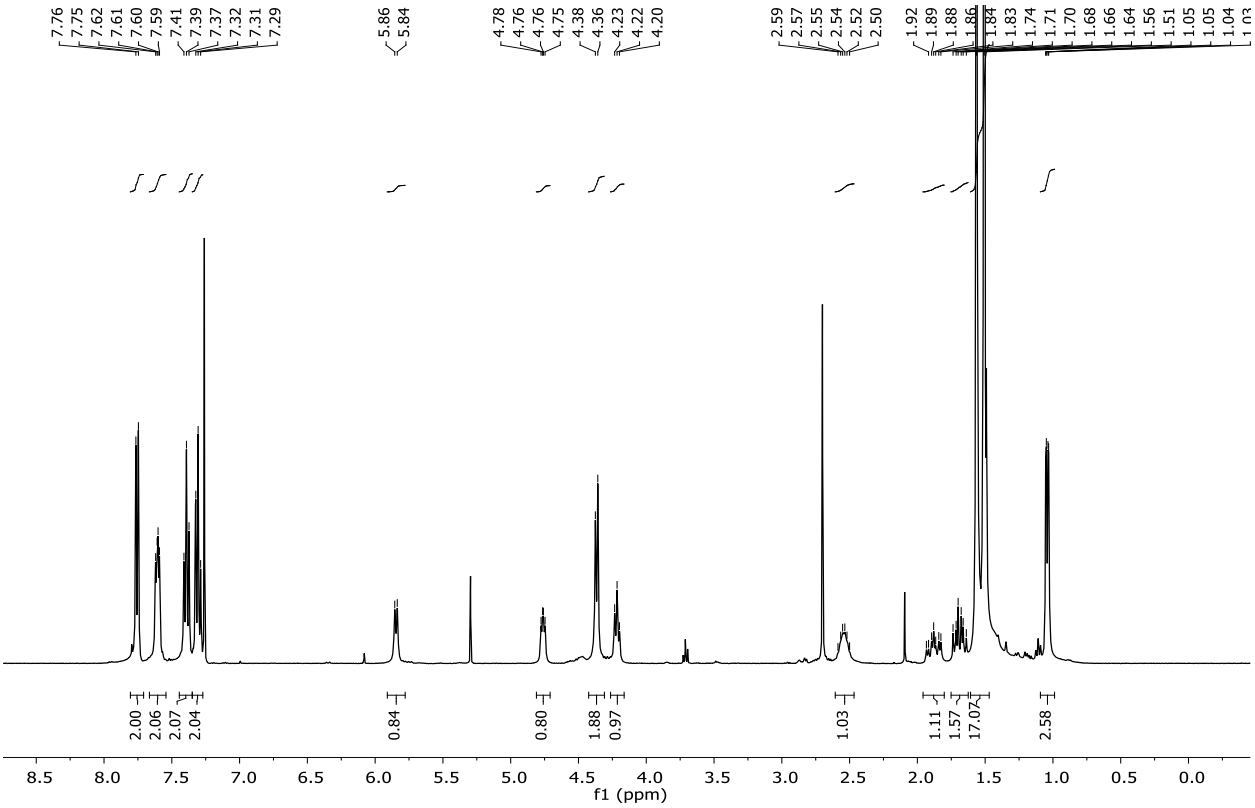


Figure S10. ^{1}H NMR spectra (400 MHz) of **13a** in $CDCl_3$.

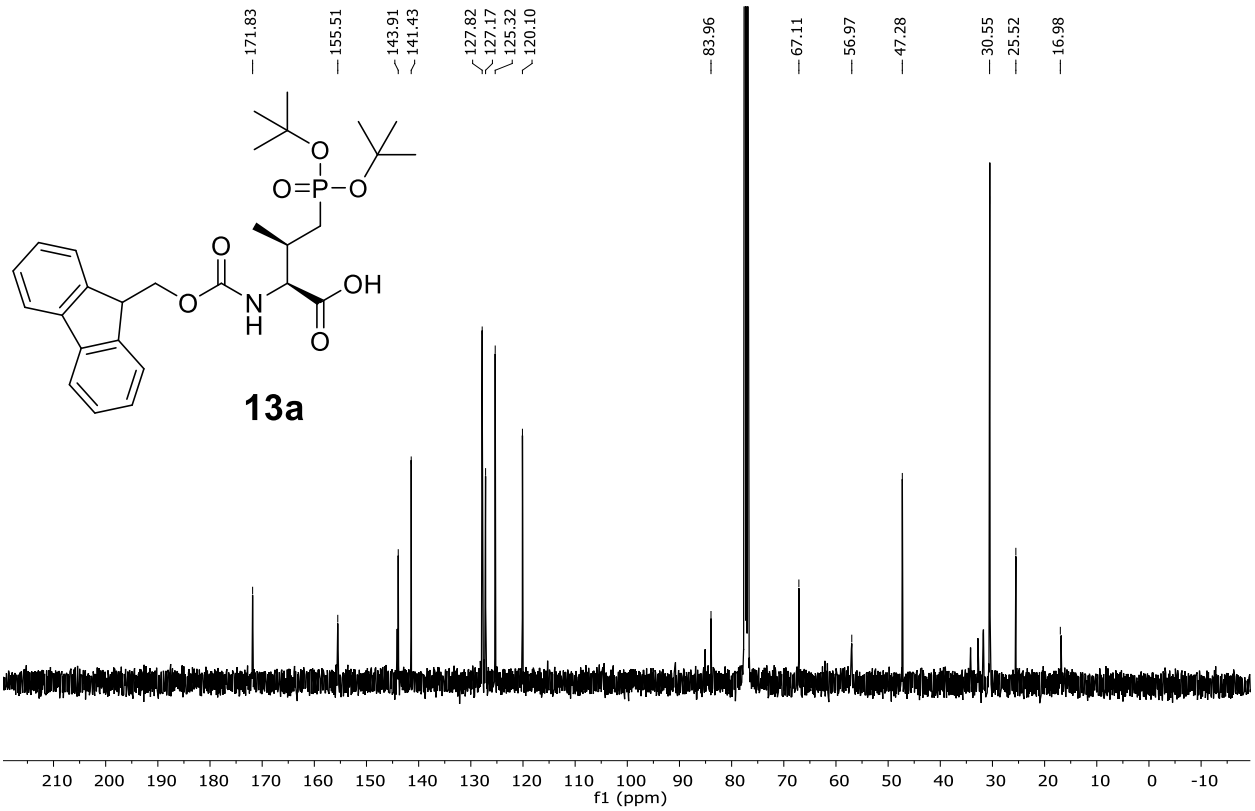


Figure S11. ^{13}C NMR spectra (100 MHz) of **13a** in $CDCl_3$.

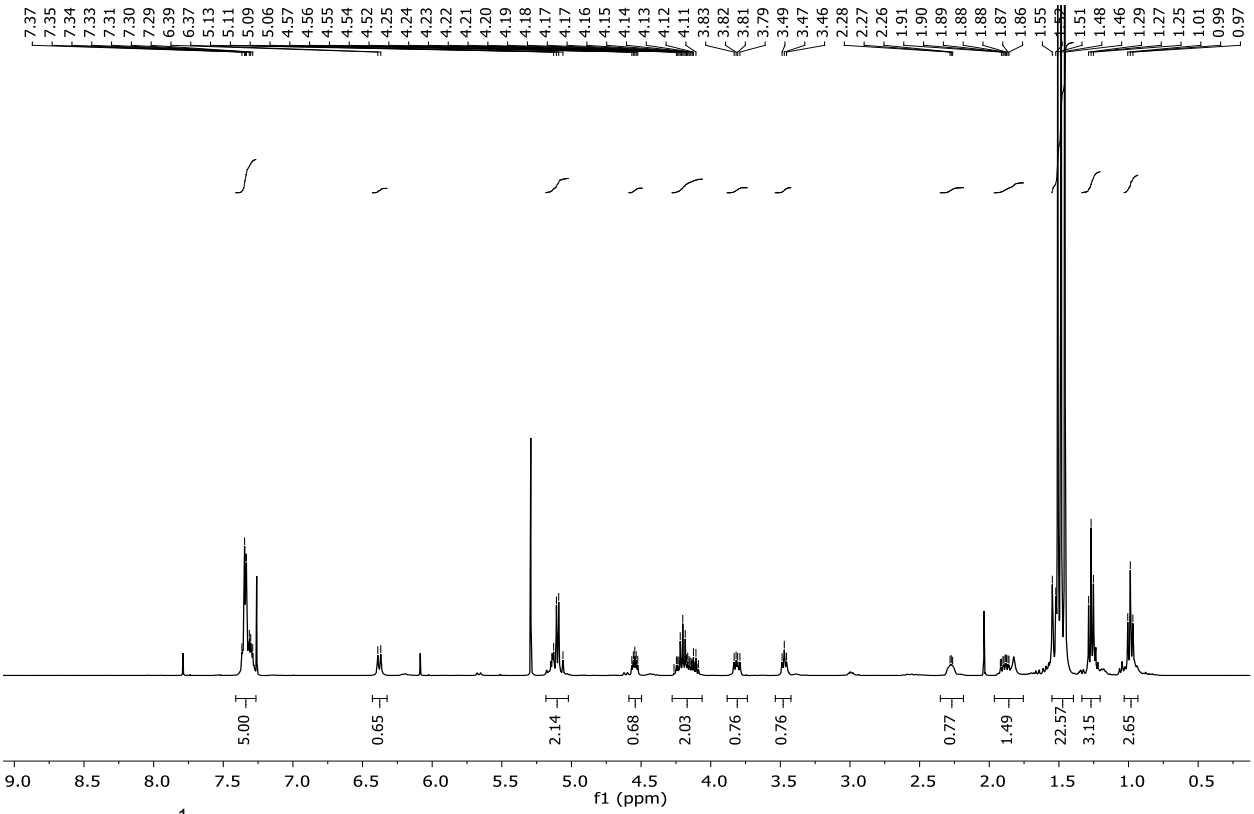


Figure S12. ^{1}H NMR spectra (400 MHz) of **11b** in $CDCl_3$.

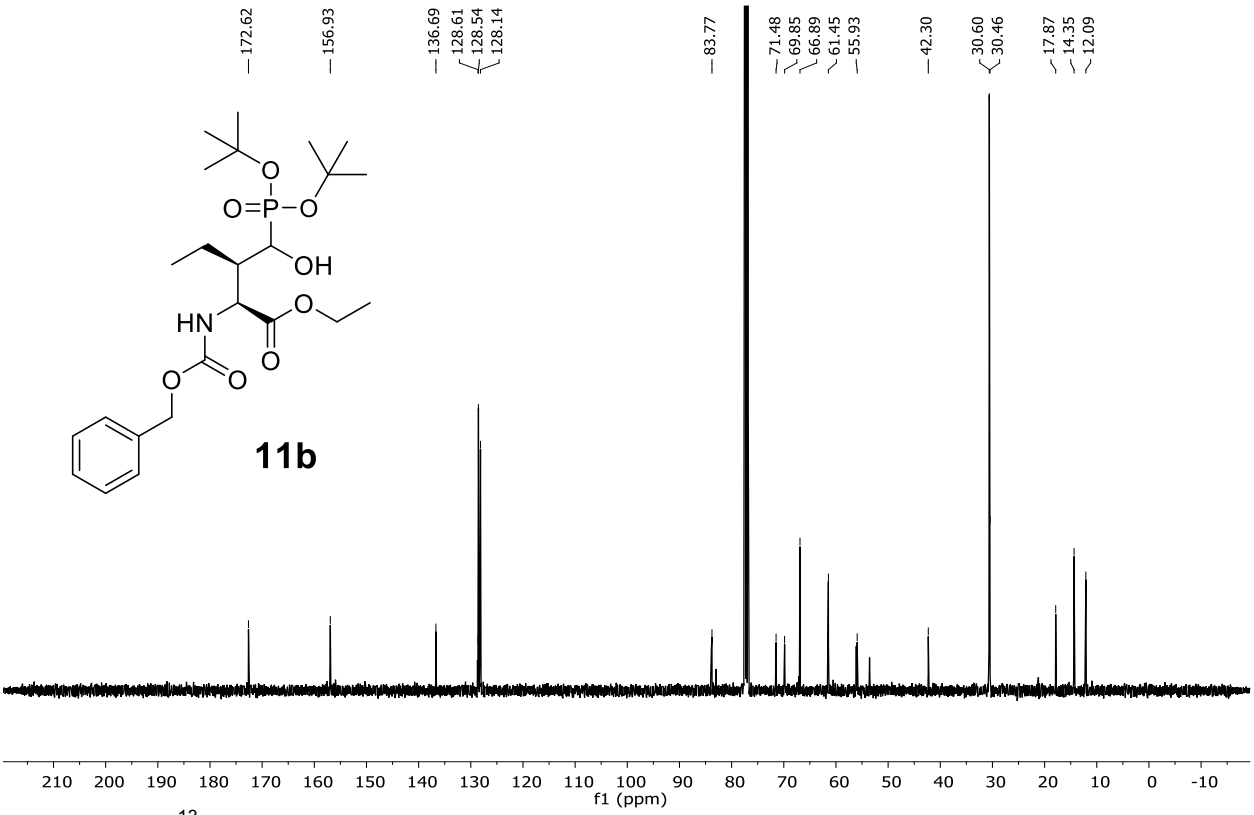


Figure S13. ^{13}C NMR spectra (100 MHz) of **11b** in $CDCl_3$.

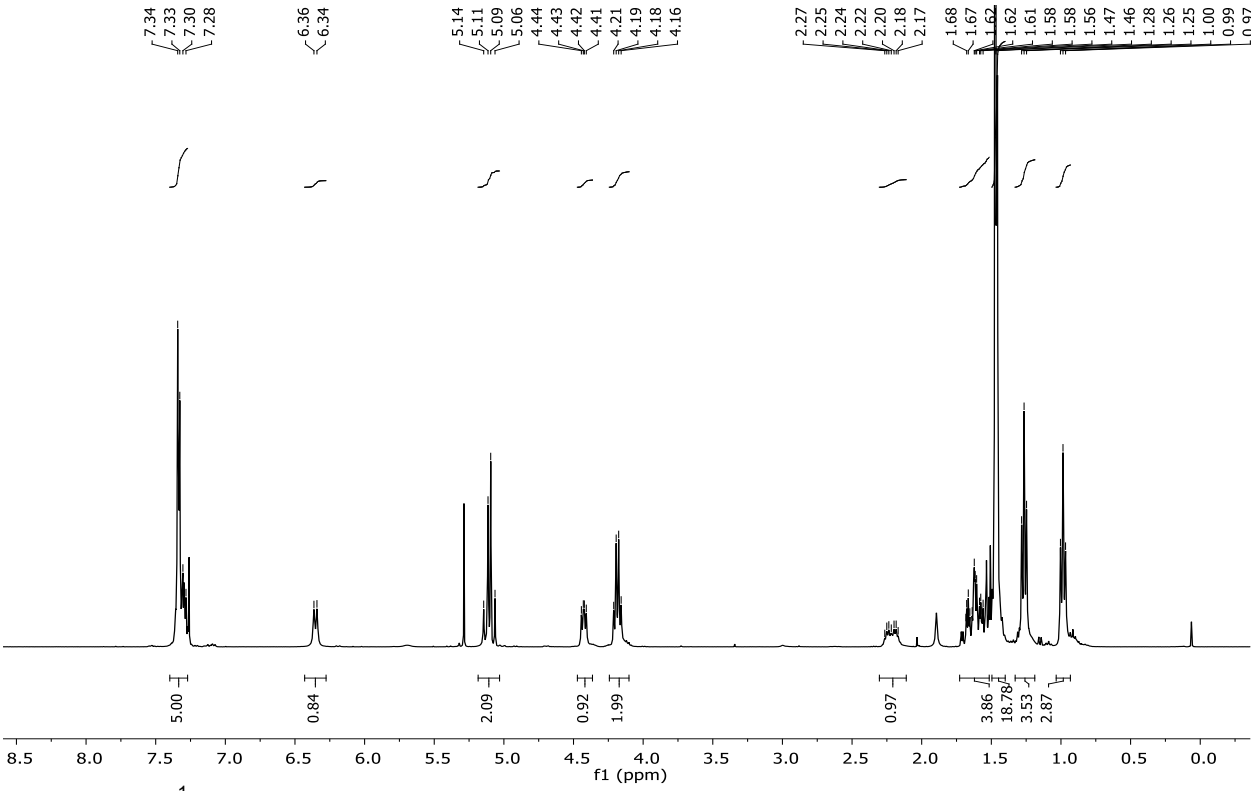


Figure S14. ^{1}H NMR spectra (400 MHz) of **12b** in $CDCl_3$.

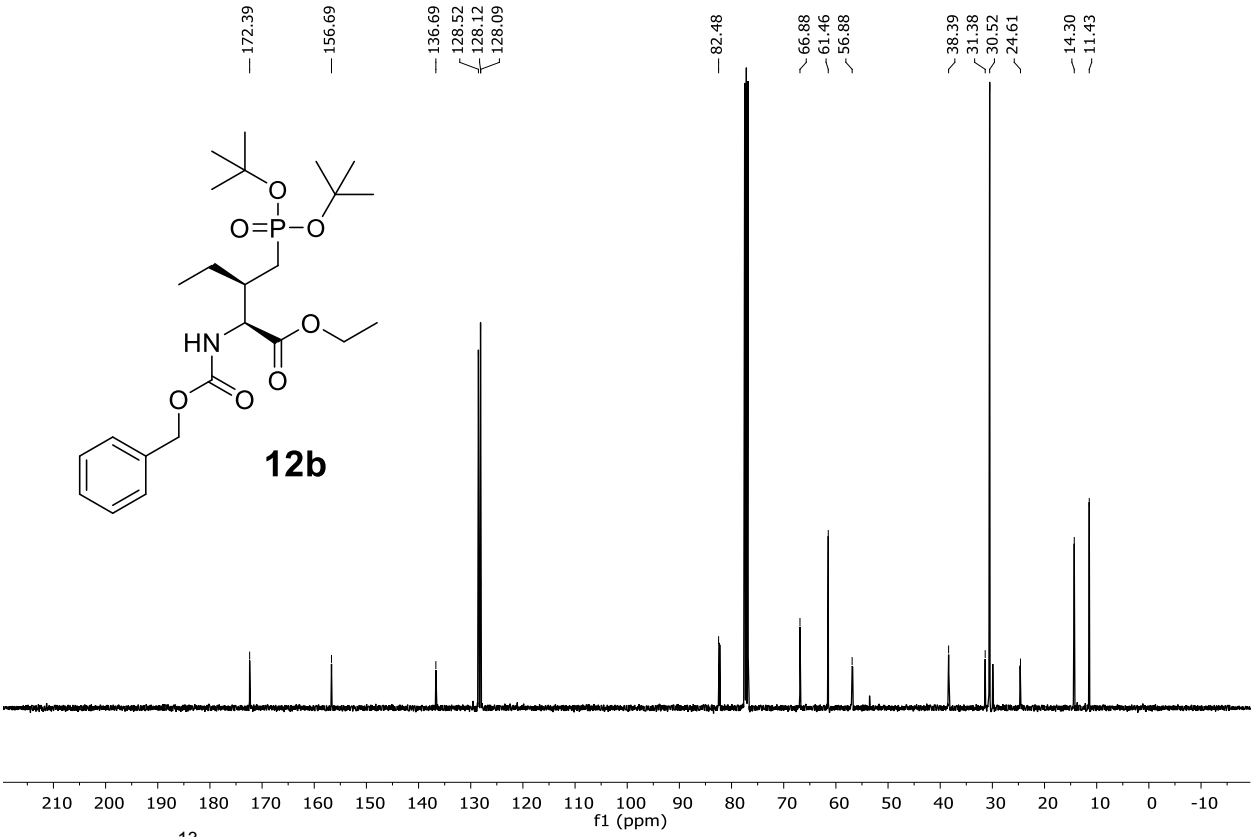


Figure S15. ^{13}C NMR spectra (100 MHz) of **12b** in $CDCl_3$.

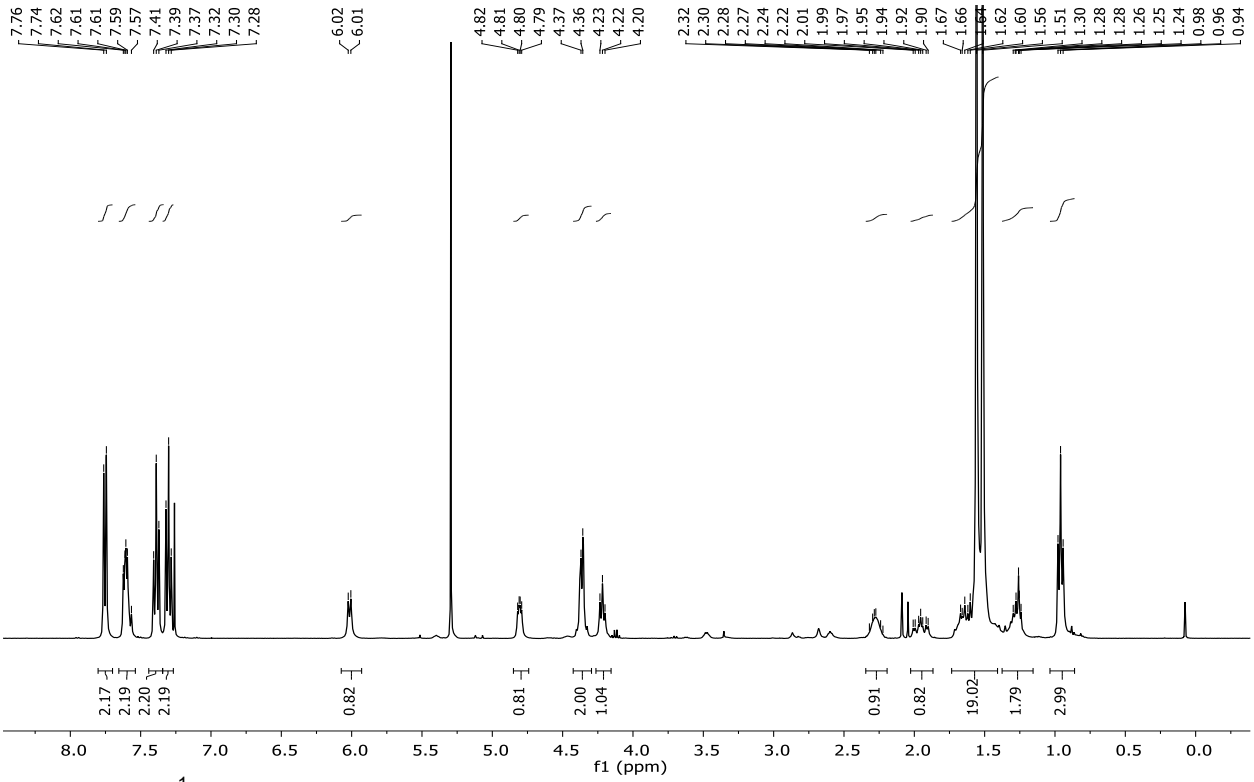


Figure S16. ^{1}H NMR spectra (400 MHz) of **13b** in $CDCl_3$.

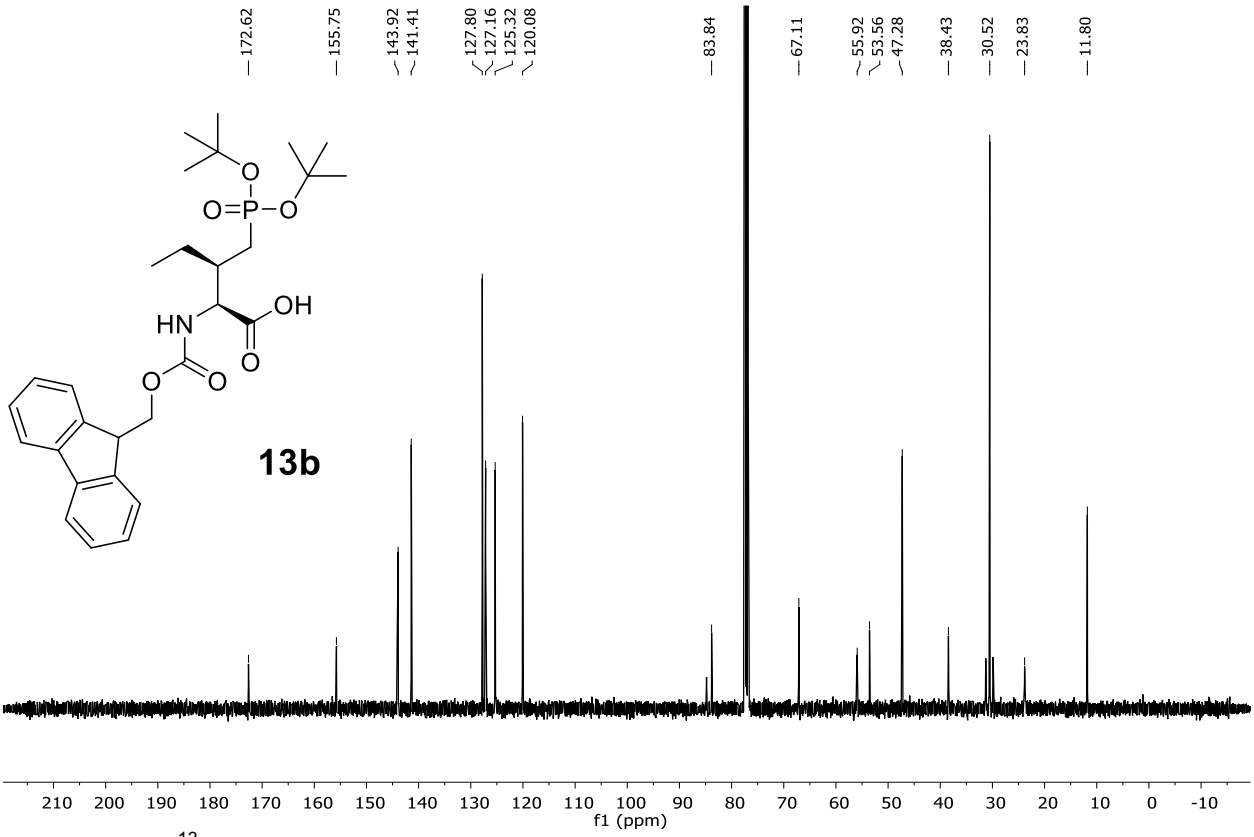


Figure S17. ^{13}C NMR spectra (100 MHz) of **13b** in $CDCl_3$.

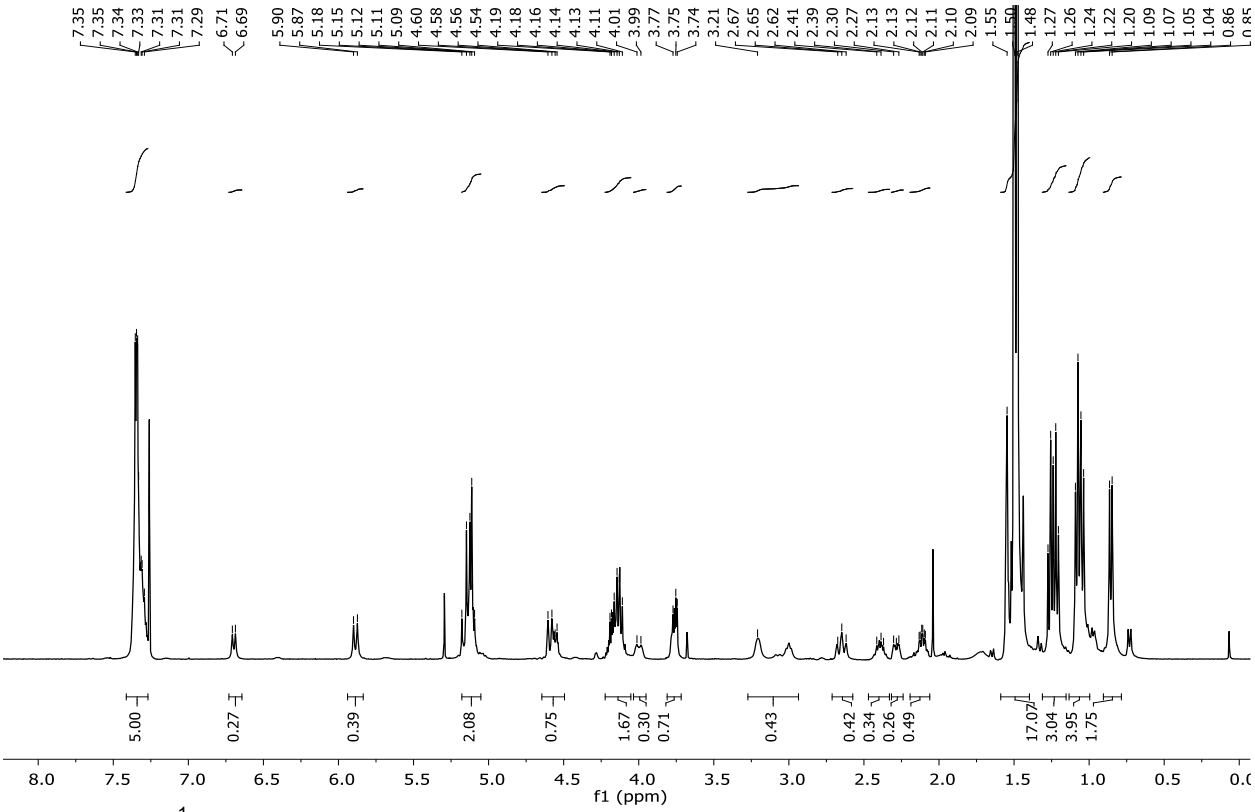


Figure S18. ^{1}H NMR spectra (400 MHz) of **11c** in $CDCl_3$.

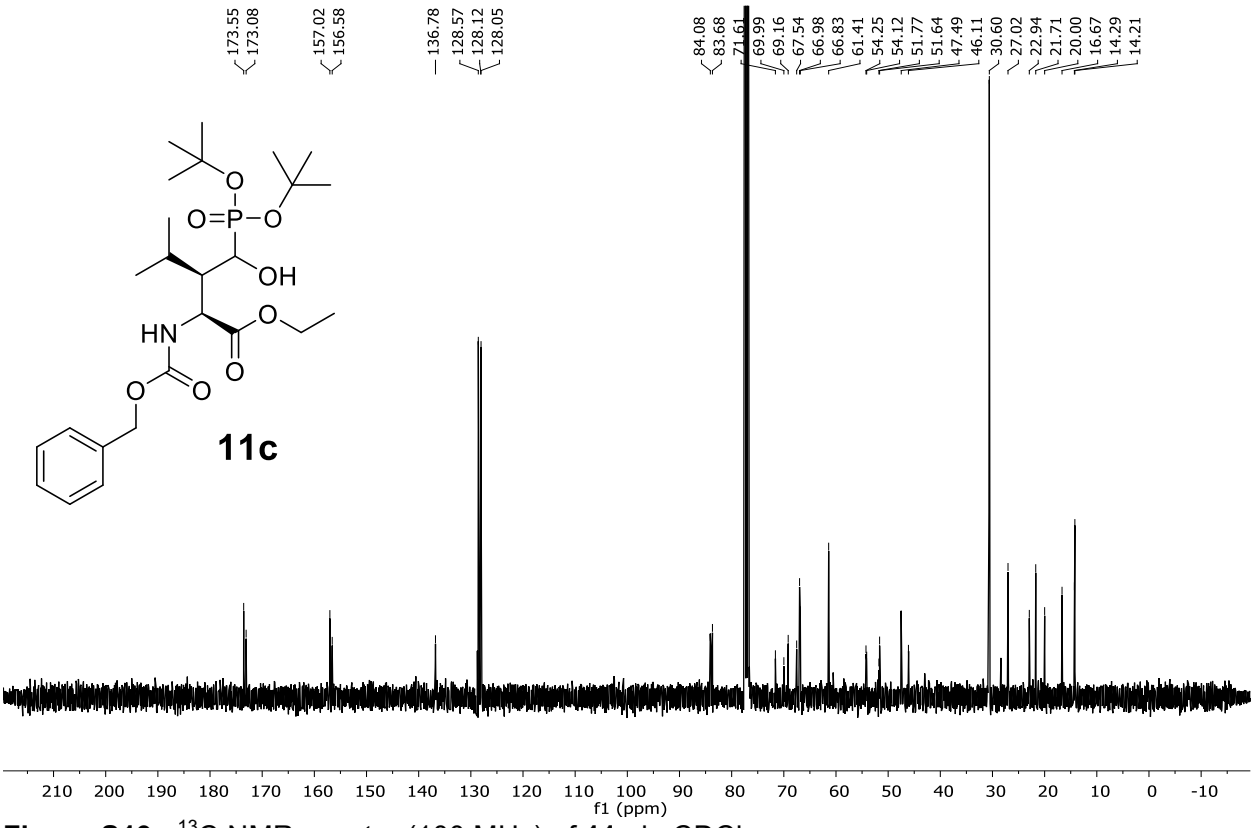


Figure S19. ^{13}C NMR spectra (100 MHz) of **11c** in $CDCl_3$.

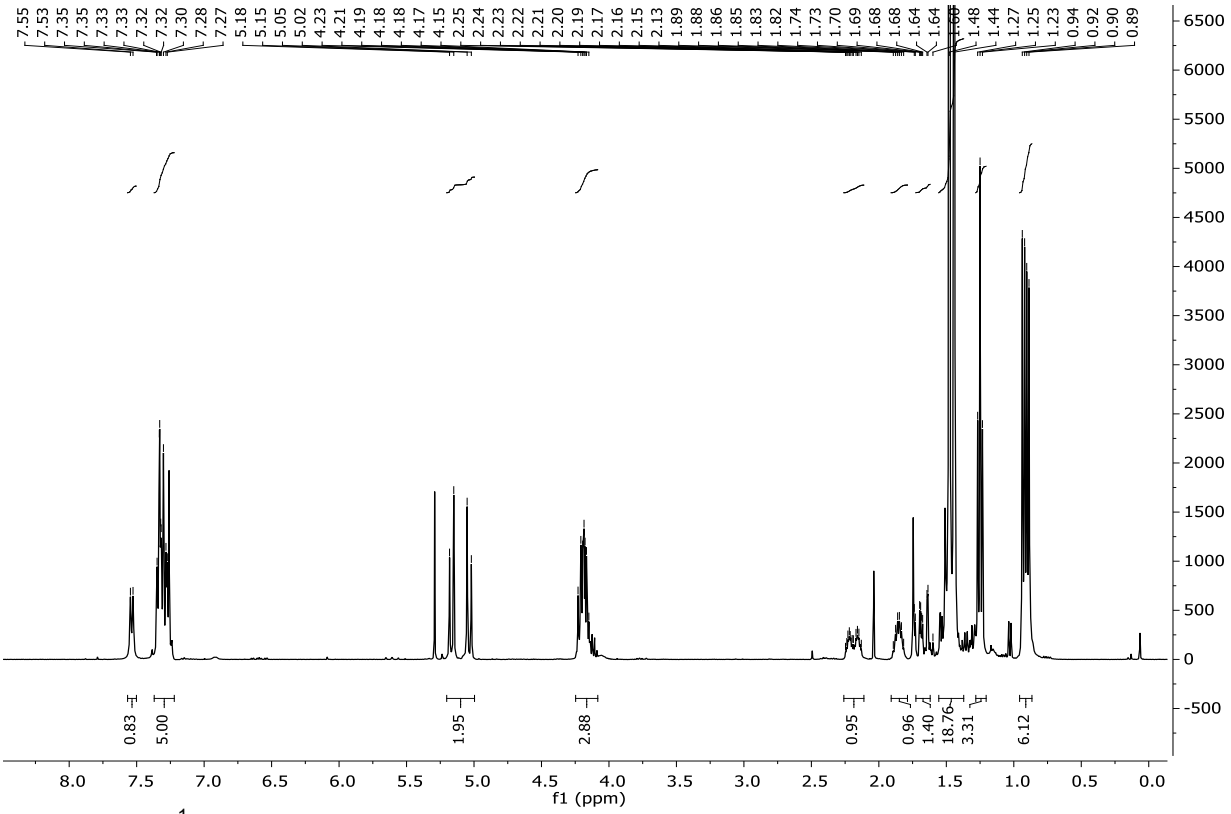


Figure S20. ^{1}H NMR spectra (400 MHz) of **12c** in $CDCl_3$.

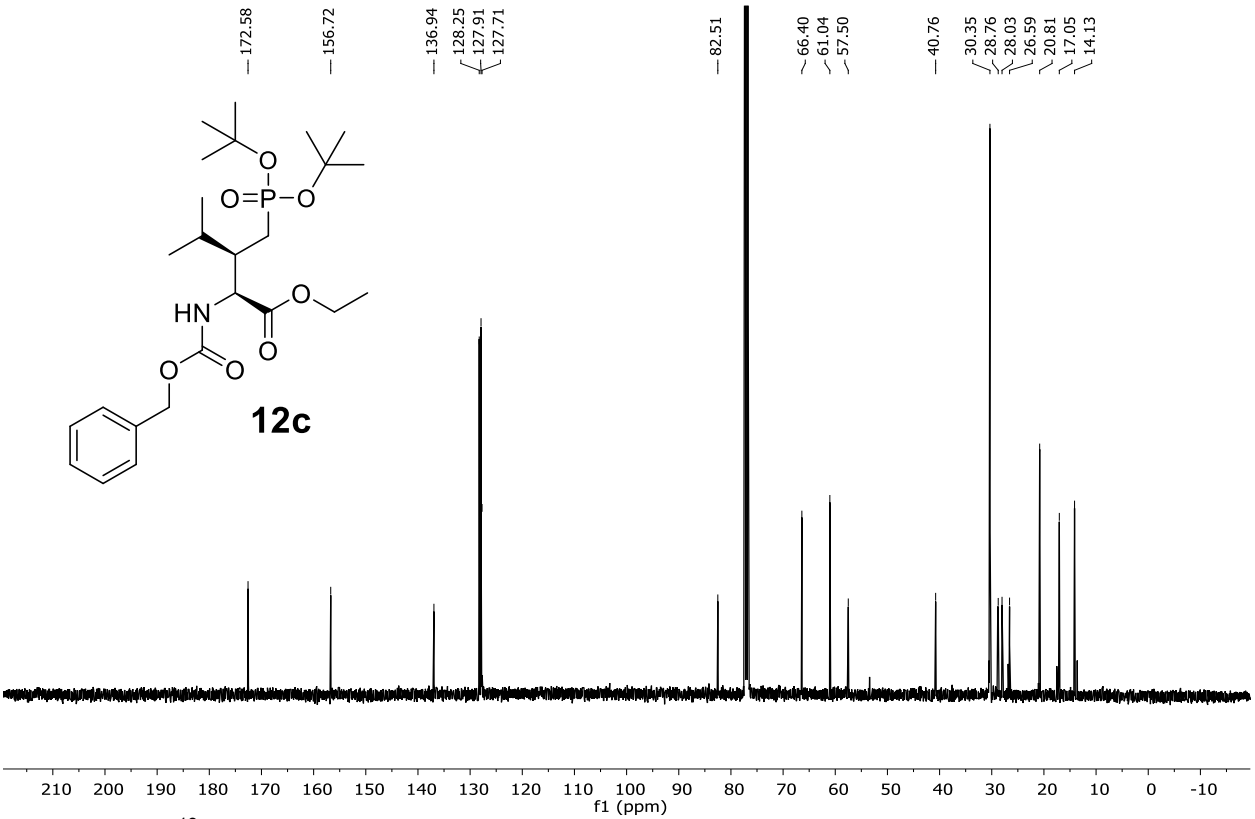


Figure S21. ^{13}C NMR spectra (100 MHz) of **12c** in $CDCl_3$.

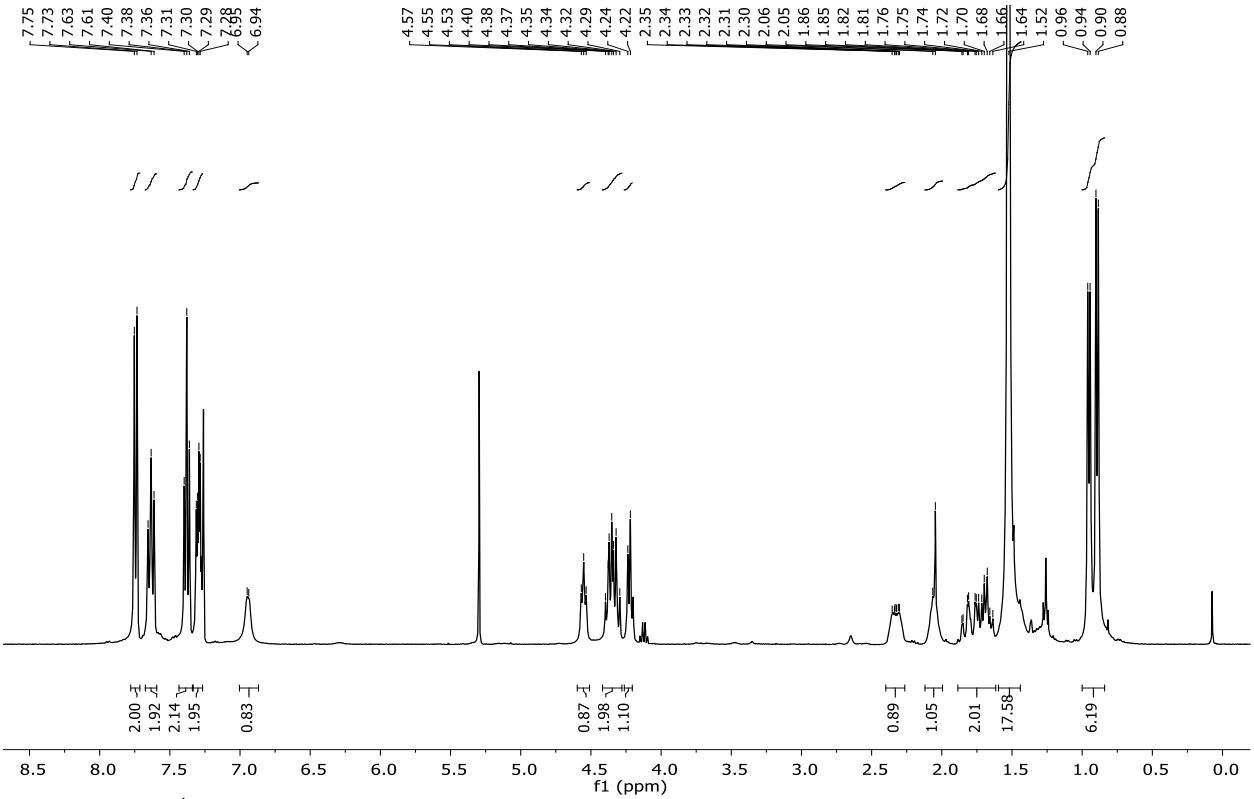


Figure S22. ^{1}H NMR spectra (400 MHz) of **13c** in $CDCl_3$.

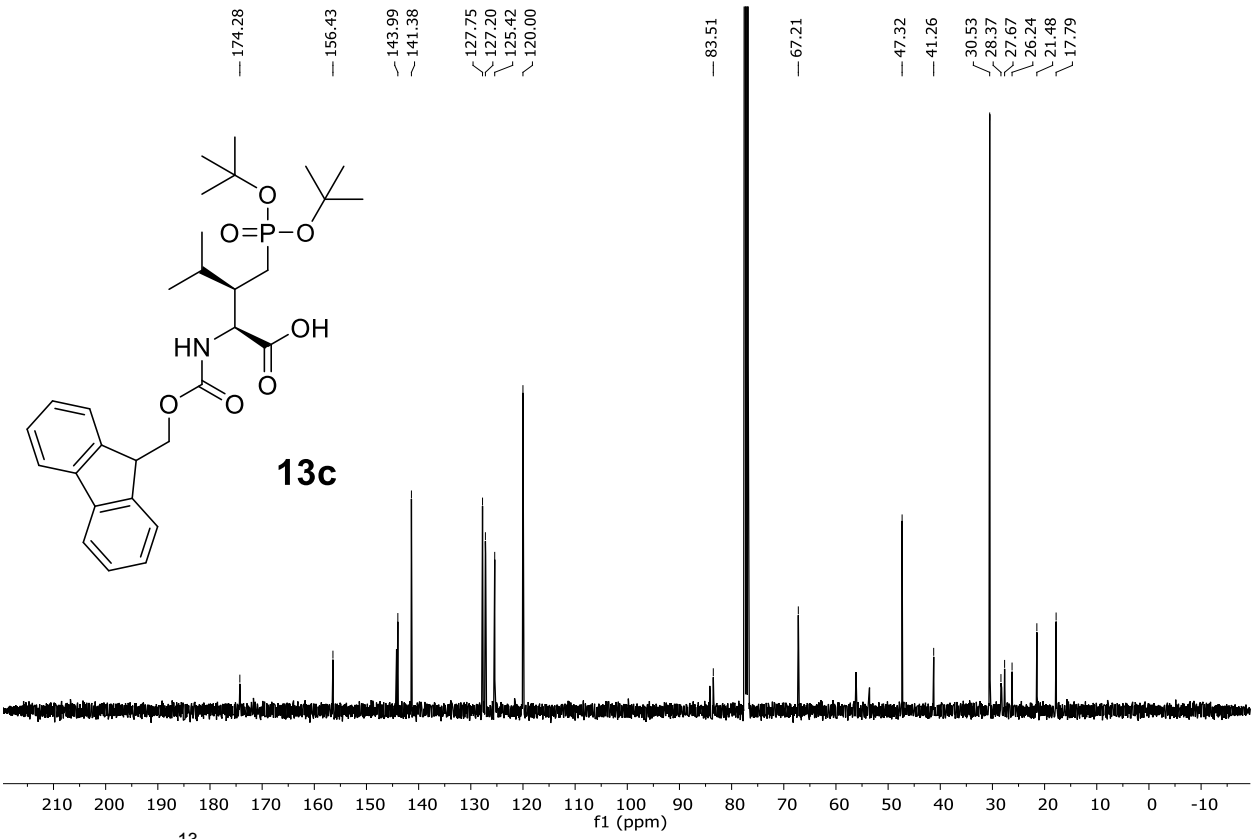


Figure S23. ^{13}C NMR spectra (100 MHz) of **13c** in $CDCl_3$.

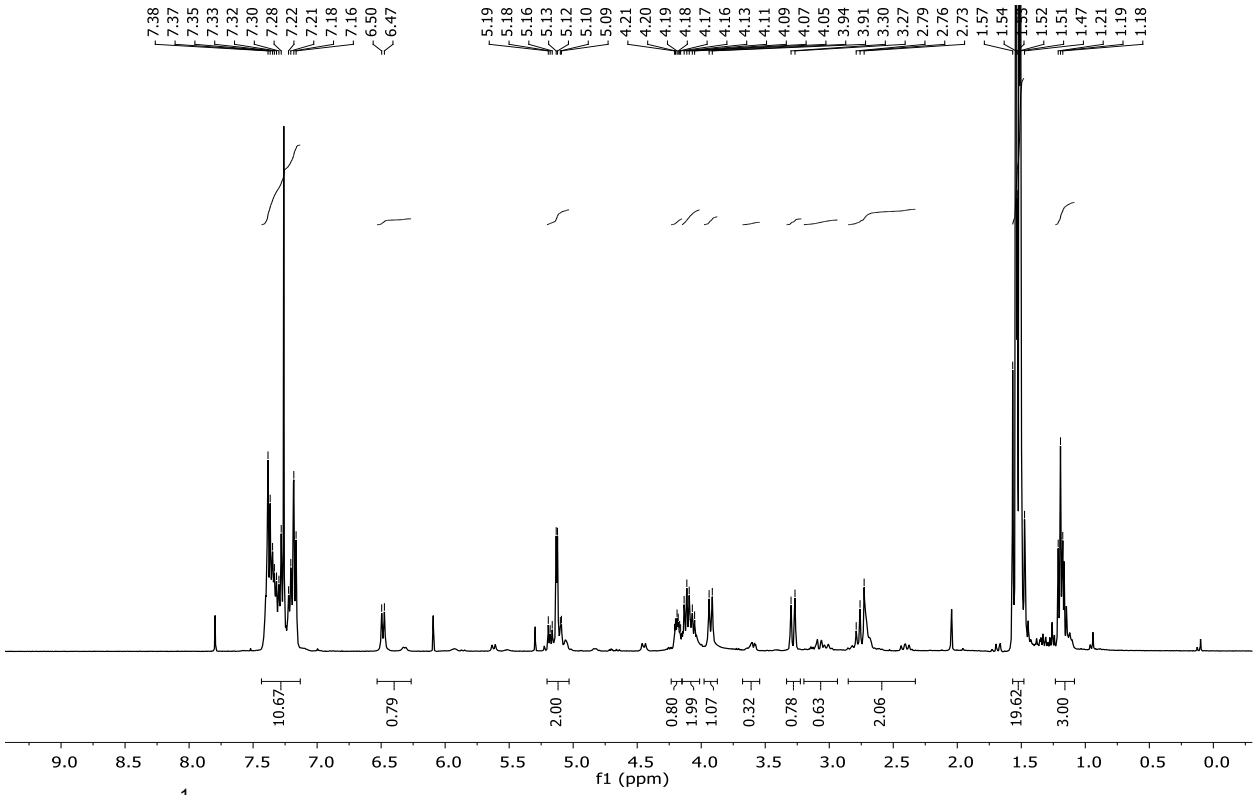


Figure S24. ^{1}H NMR spectra (400 MHz) of **11d** in $CDCl_3$.

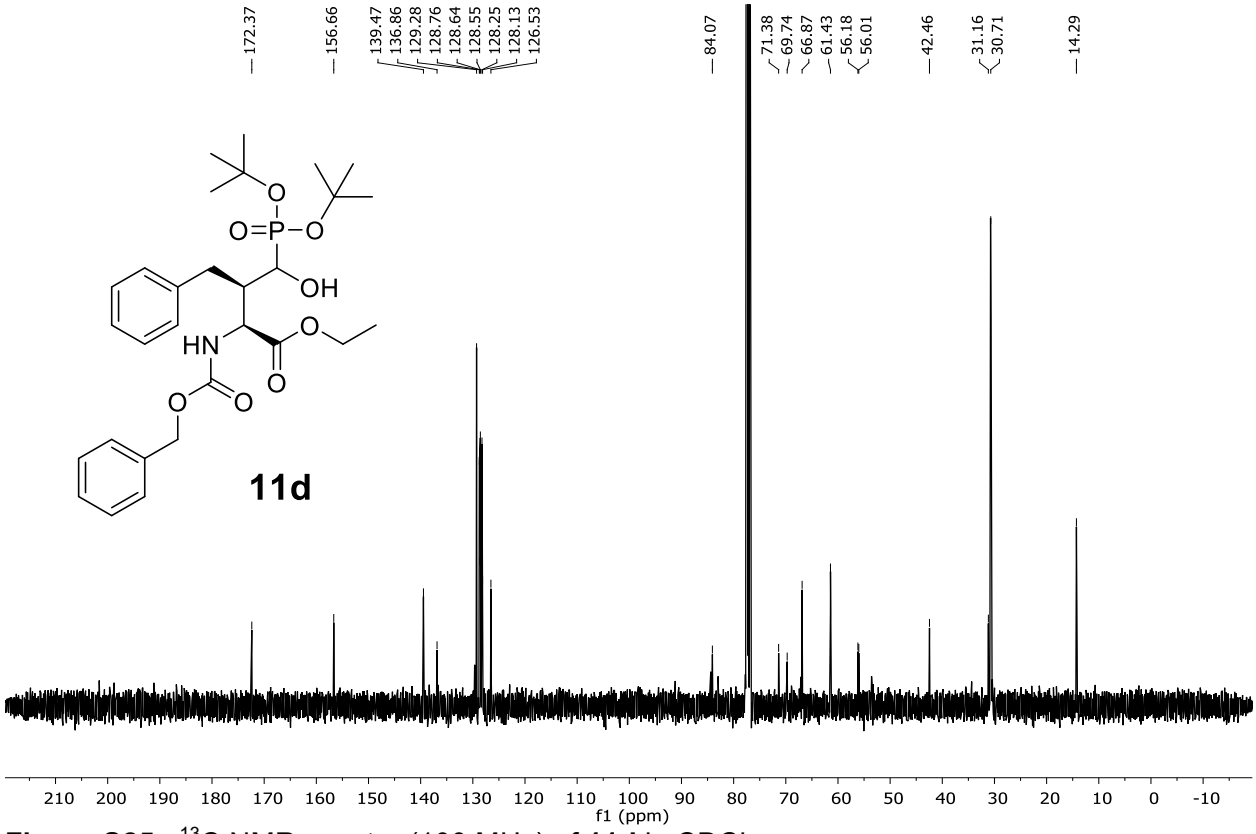


Figure S25. ^{13}C NMR spectra (100 MHz) of **11d** in $CDCl_3$.

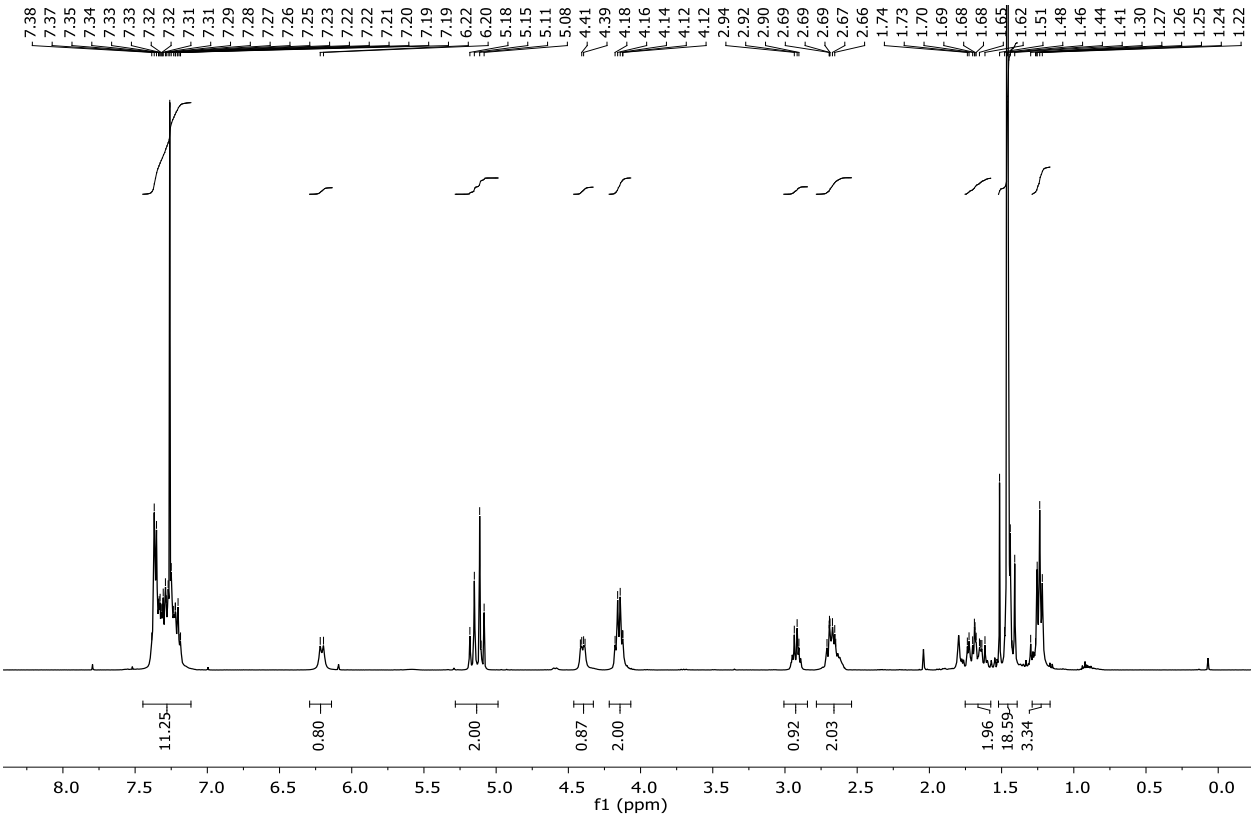


Figure S26. ^{1}H NMR spectra (400 MHz) of **12d** in $CDCl_3$.

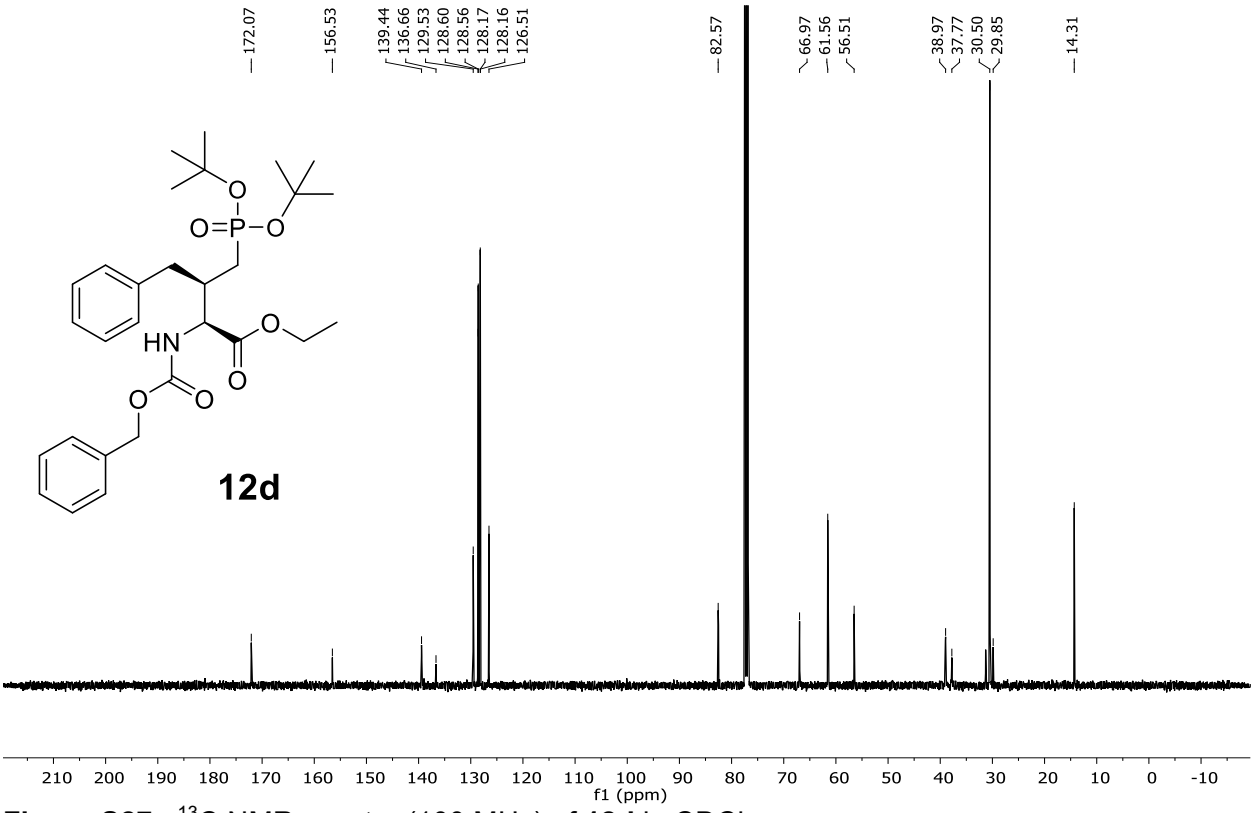


Figure S27. ^{13}C NMR spectra (100 MHz) of **12d** in $CDCl_3$.

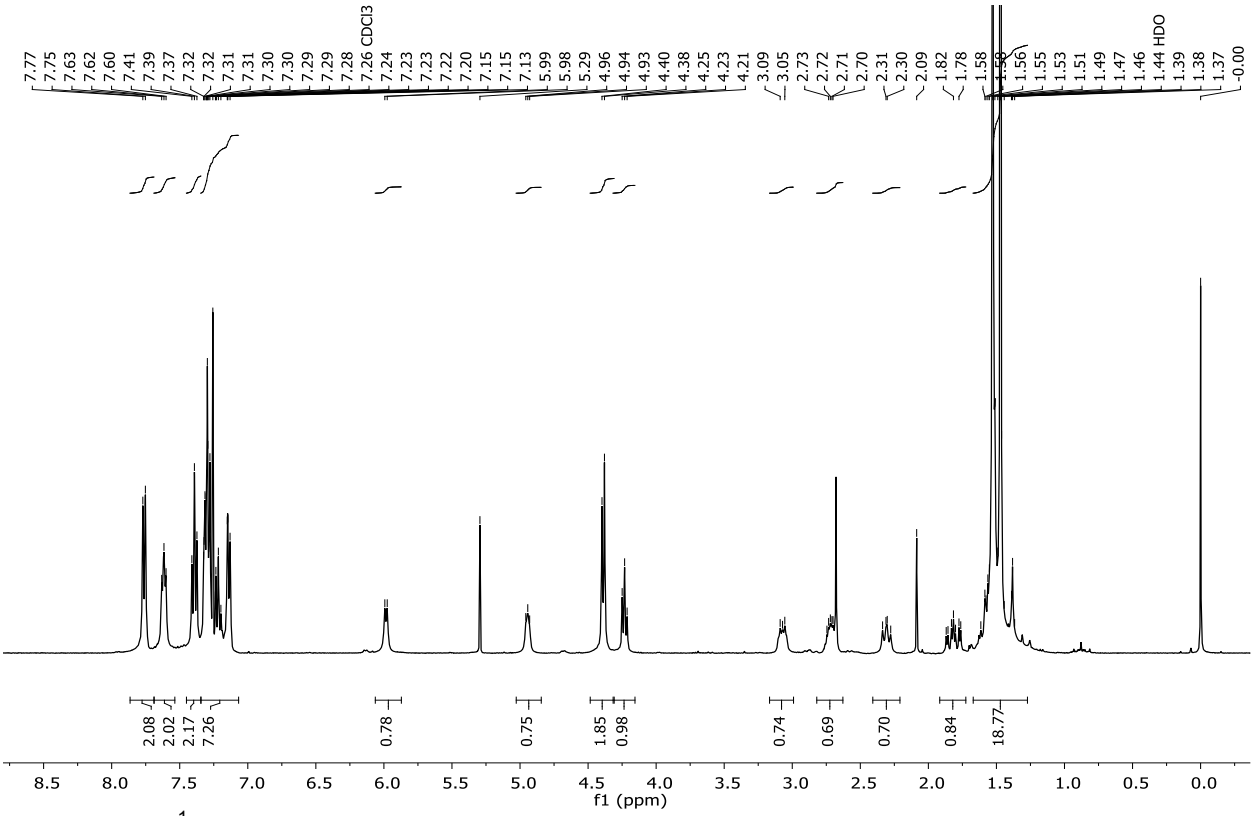


Figure S28. ^{1}H NMR spectra (400 MHz) of **13d** in $CDCl_3$.

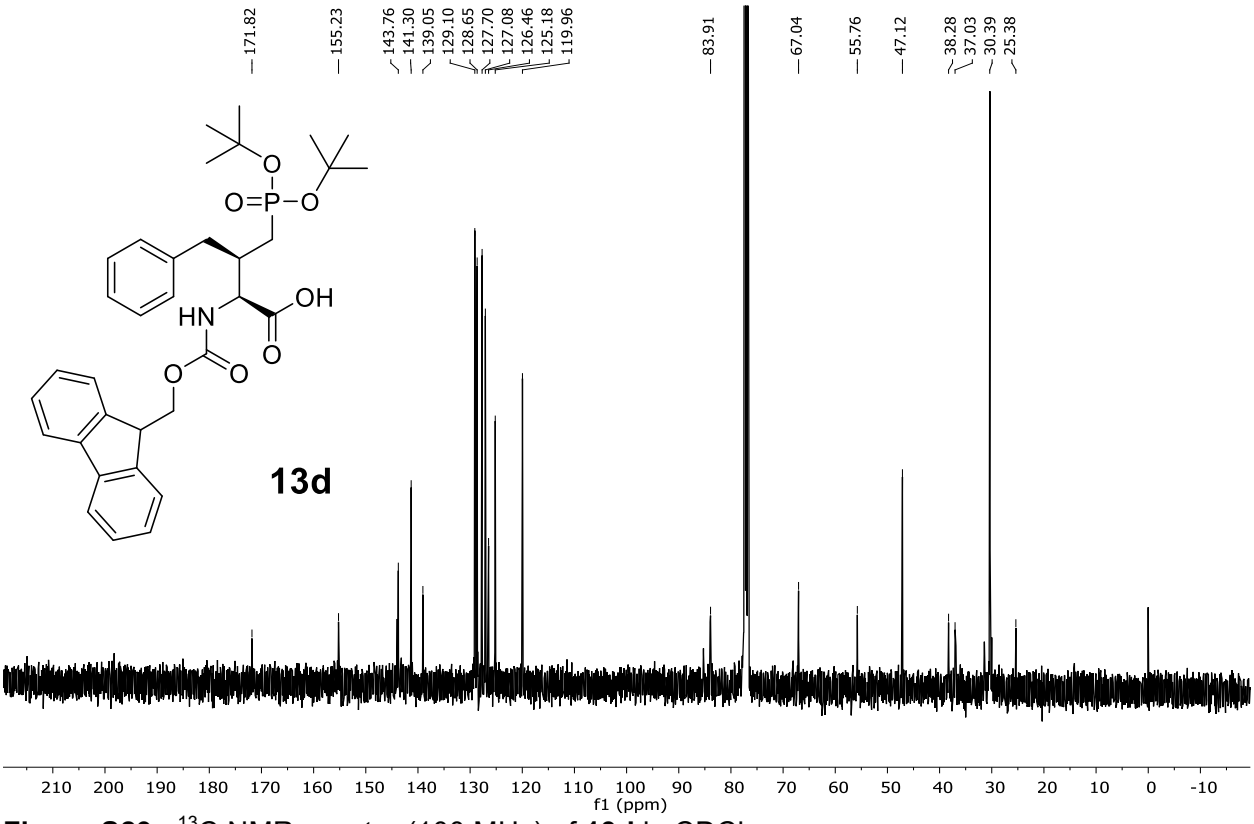


Figure S29. ^{13}C NMR spectra (100 MHz) of **13d** in $CDCl_3$.

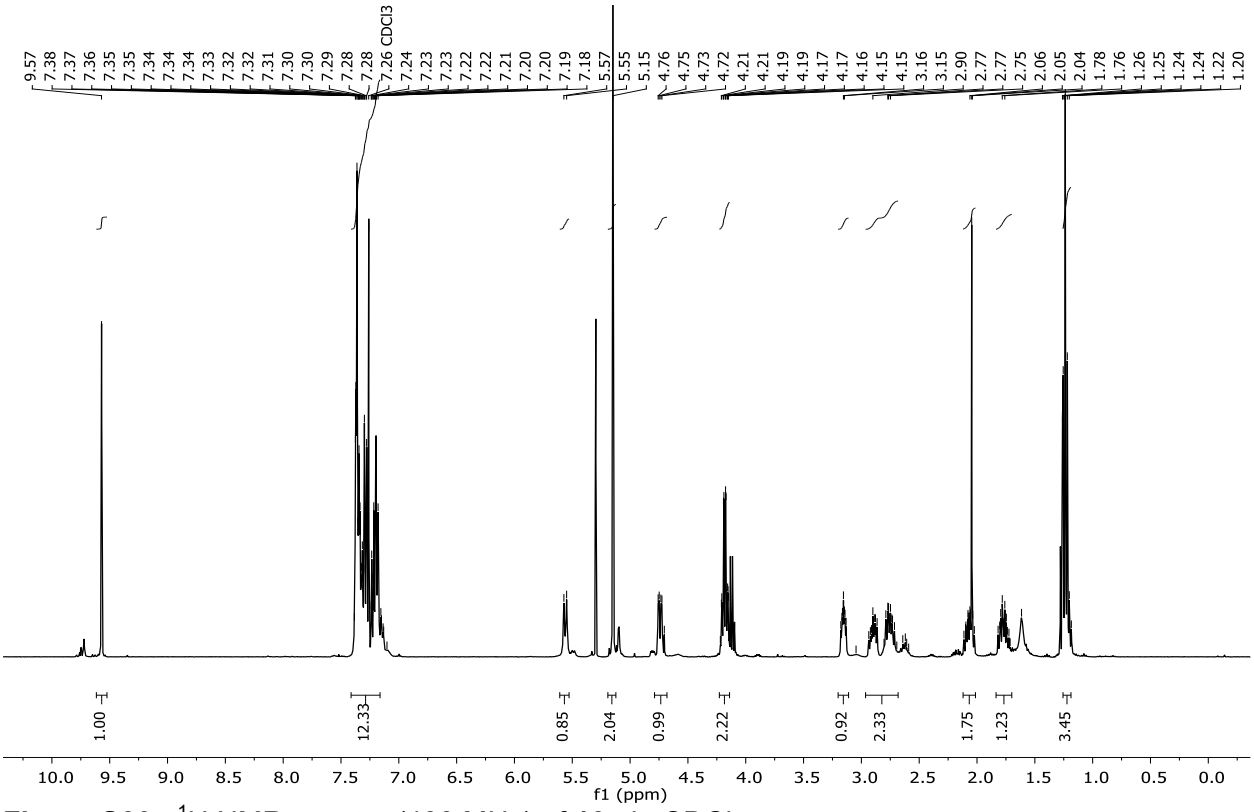


Figure S30. ^{1}H NMR spectra (400 MHz) of **10e** in $CDCl_3$.

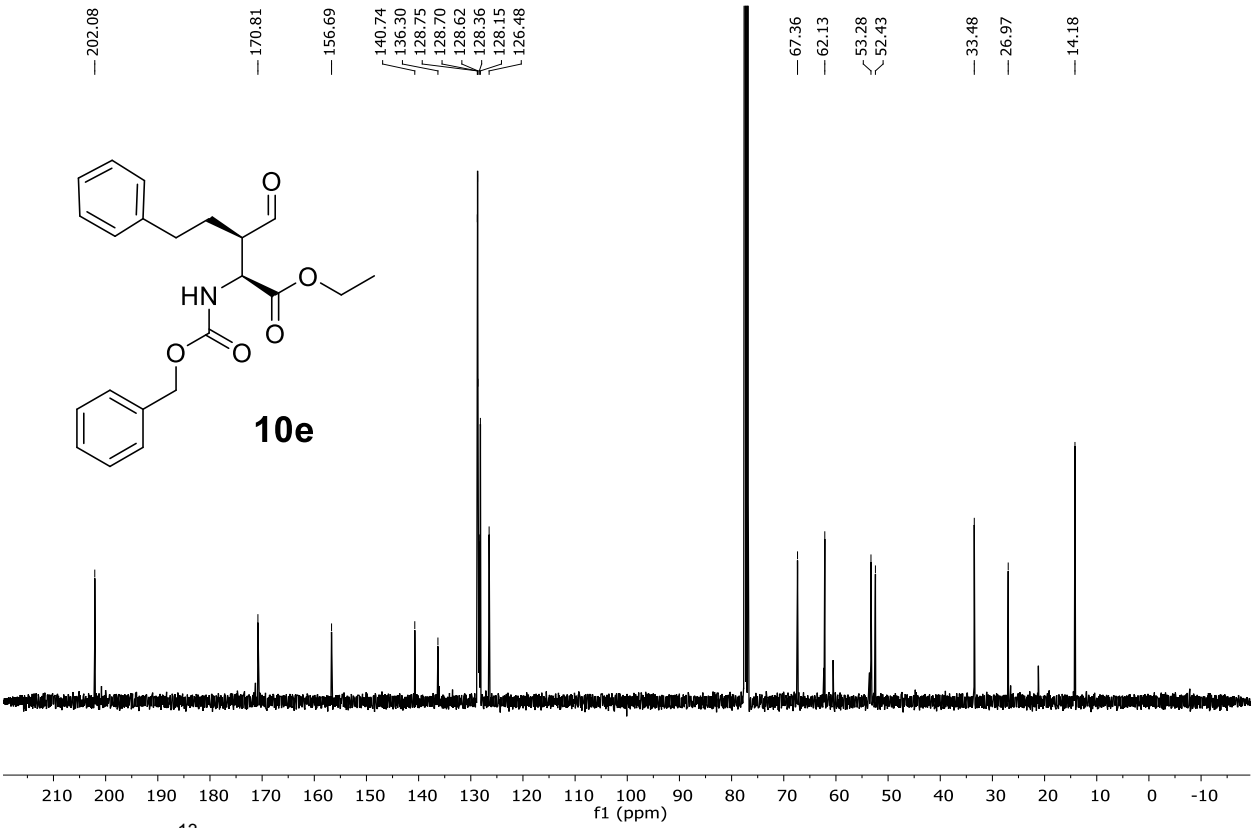


Figure S31. ^{13}C NMR spectra (100 MHz) of **10e** in $CDCl_3$.

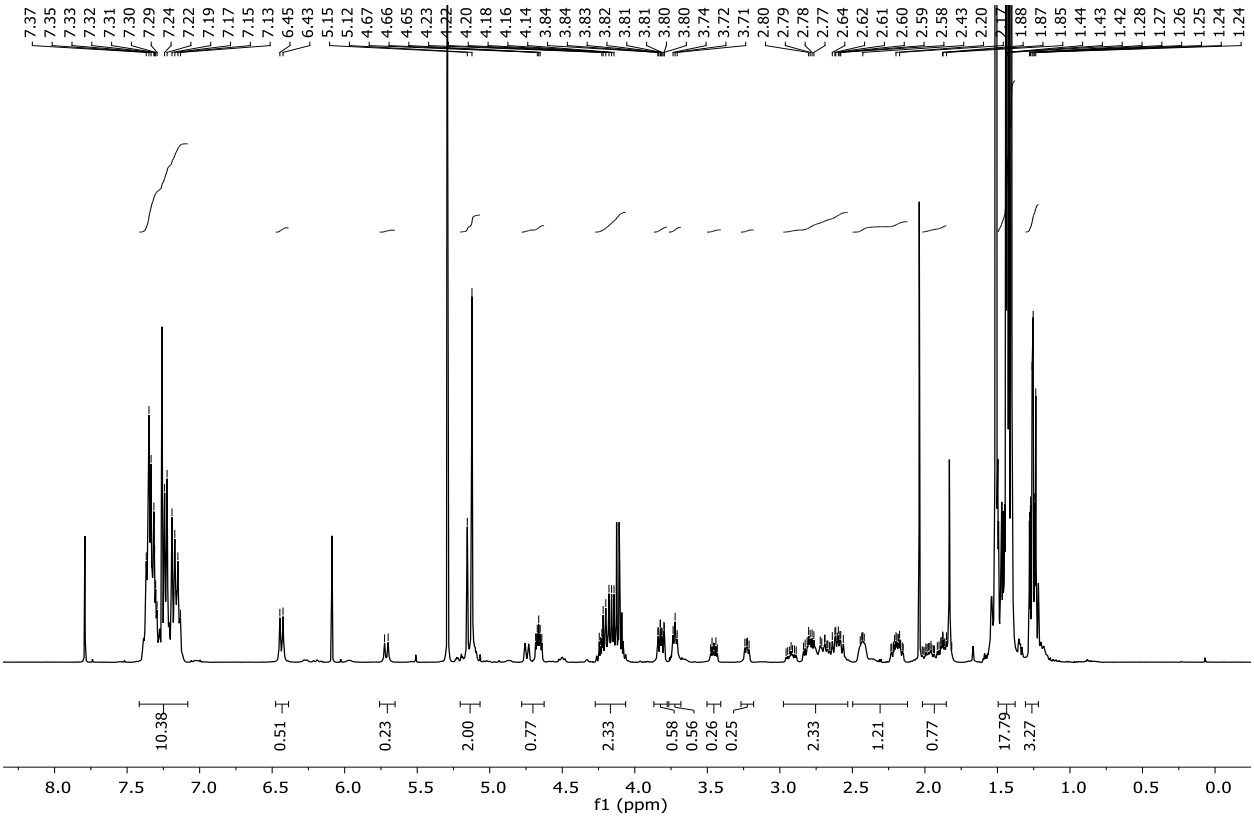


Figure S32. ^{1}H NMR spectra (400 MHz) of **11e** in $CDCl_3$.

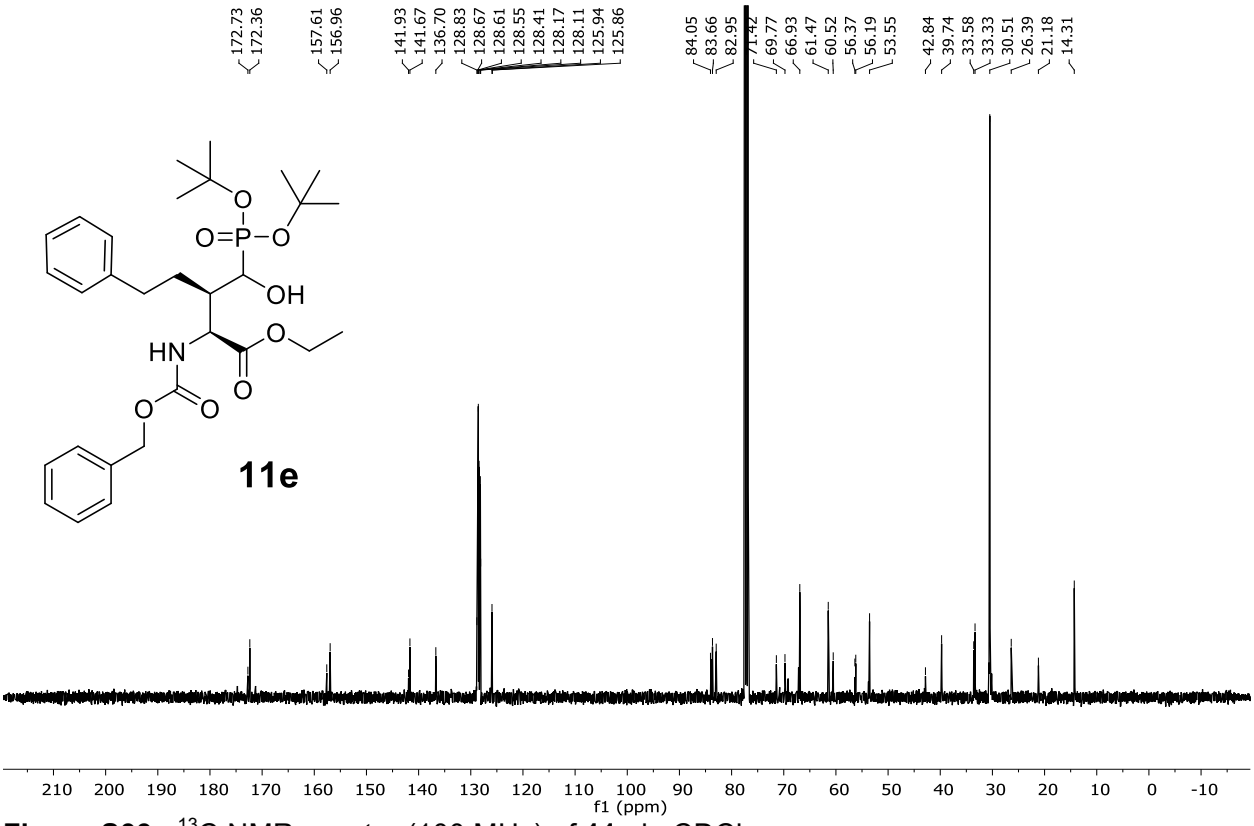


Figure S33. ^{13}C NMR spectra (100 MHz) of **11e** in $CDCl_3$.

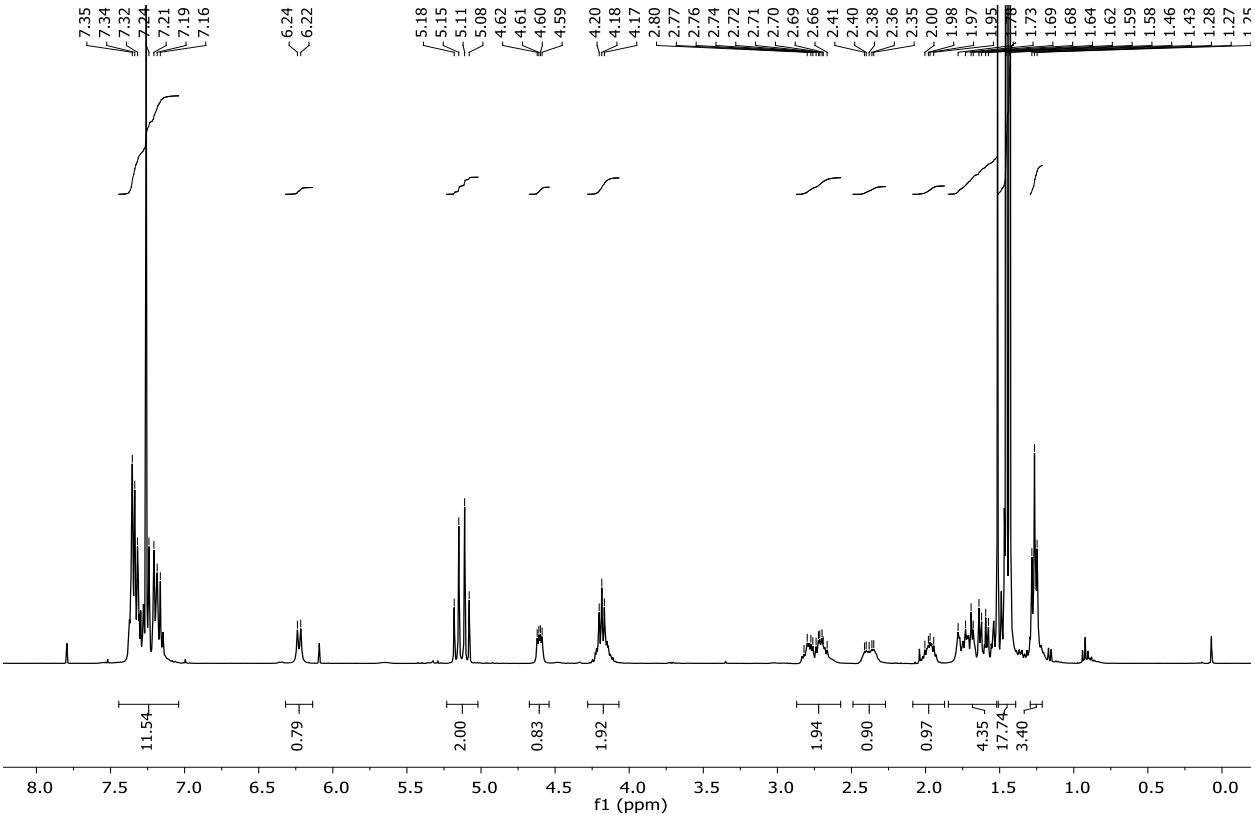


Figure S34. ^{1}H NMR spectra (400 MHz) of **12e** in $CDCl_3$.

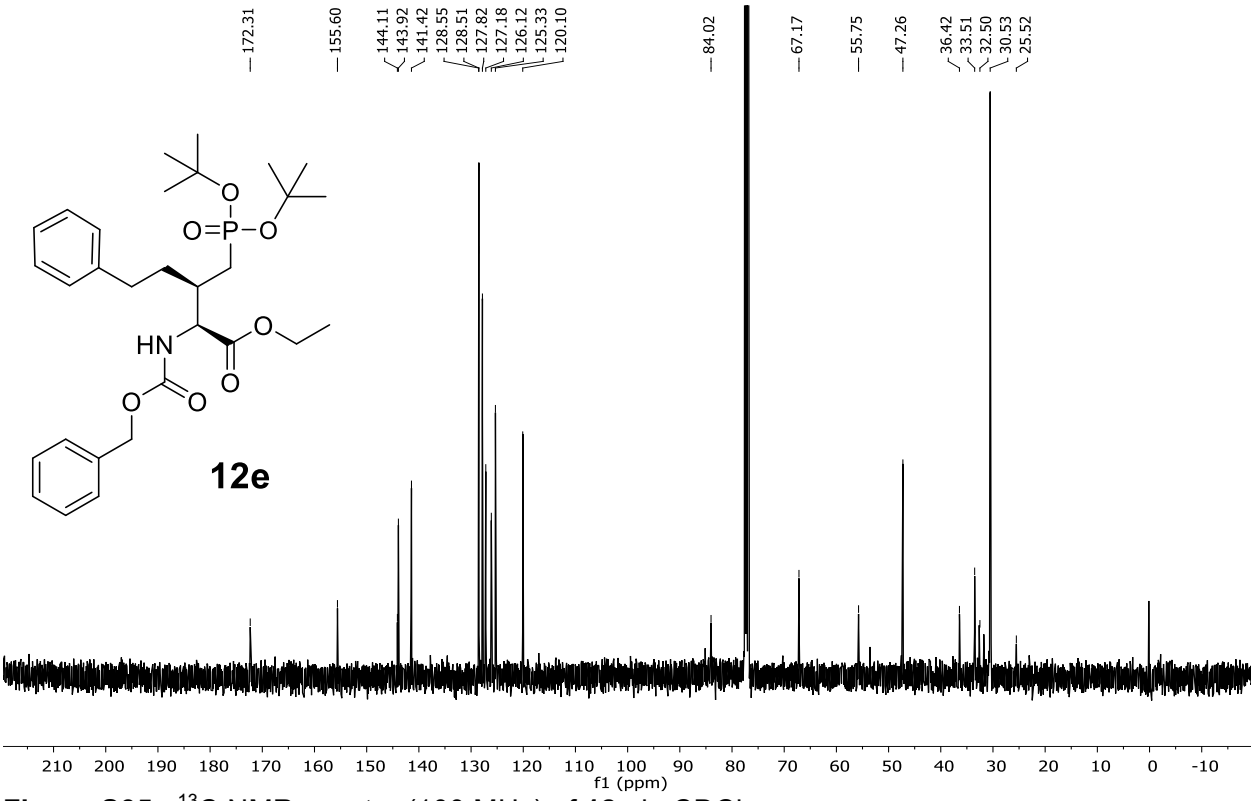


Figure S35. ^{13}C NMR spectra (100 MHz) of **12e** in $CDCl_3$.

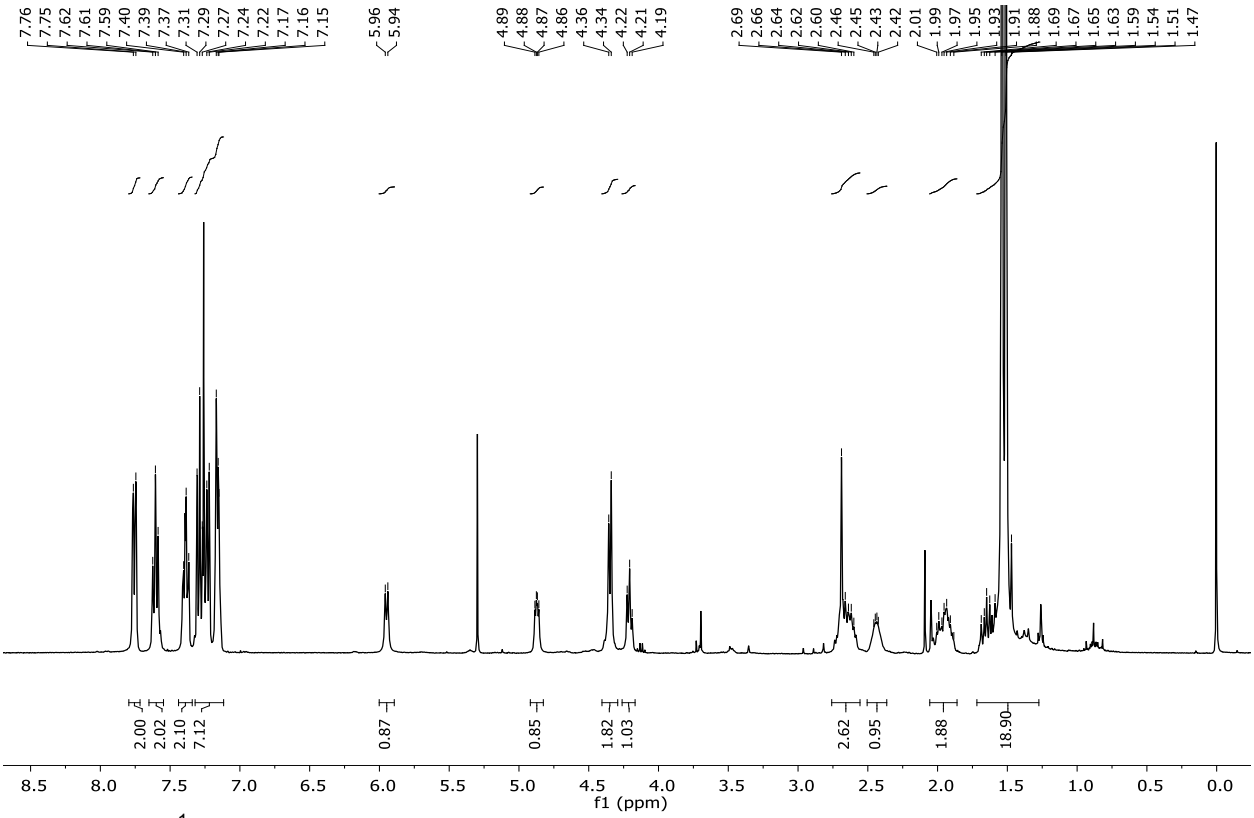


Figure S36. ^{1}H NMR spectra (400 MHz) of **13e** in $CDCl_3$.

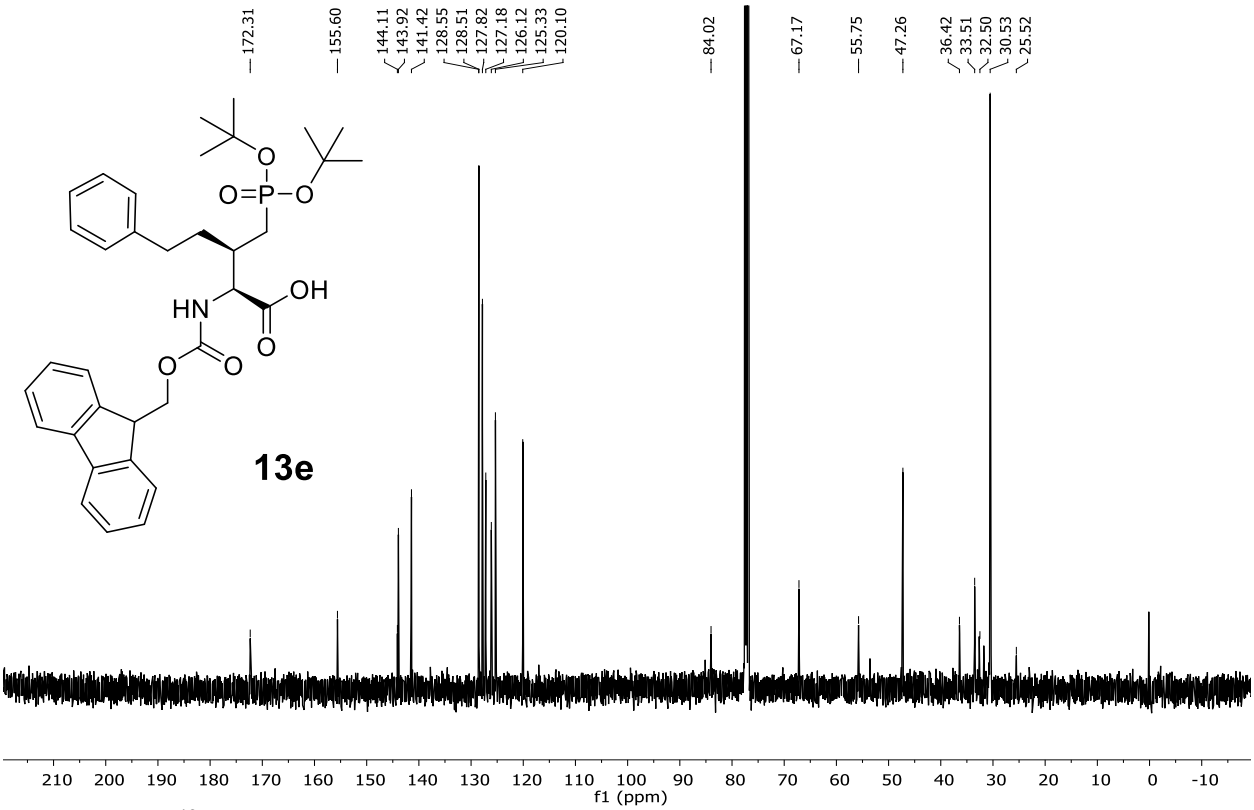


Figure S37. ^{13}C NMR spectra (100 MHz) of **13e** in $CDCl_3$.

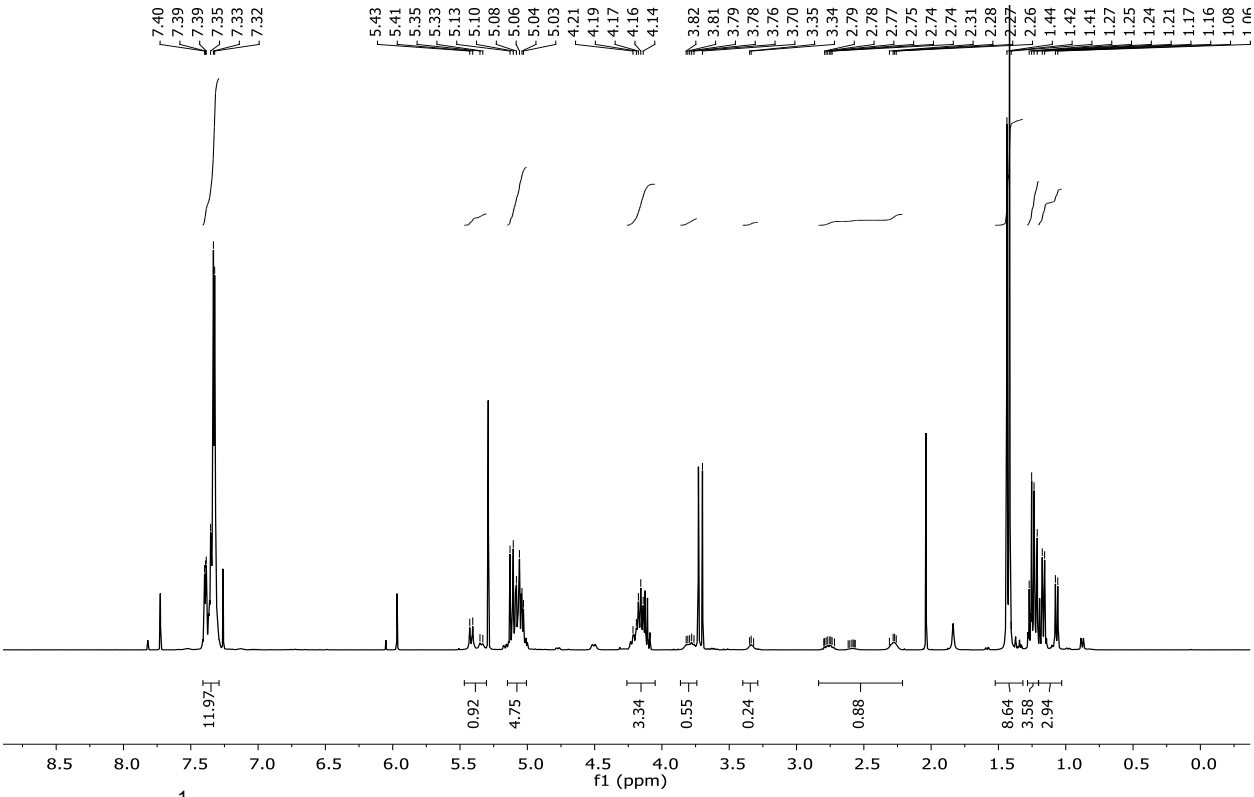


Figure S38. ^{1}H NMR spectra (400 MHz) of **16** in $CDCl_3$.

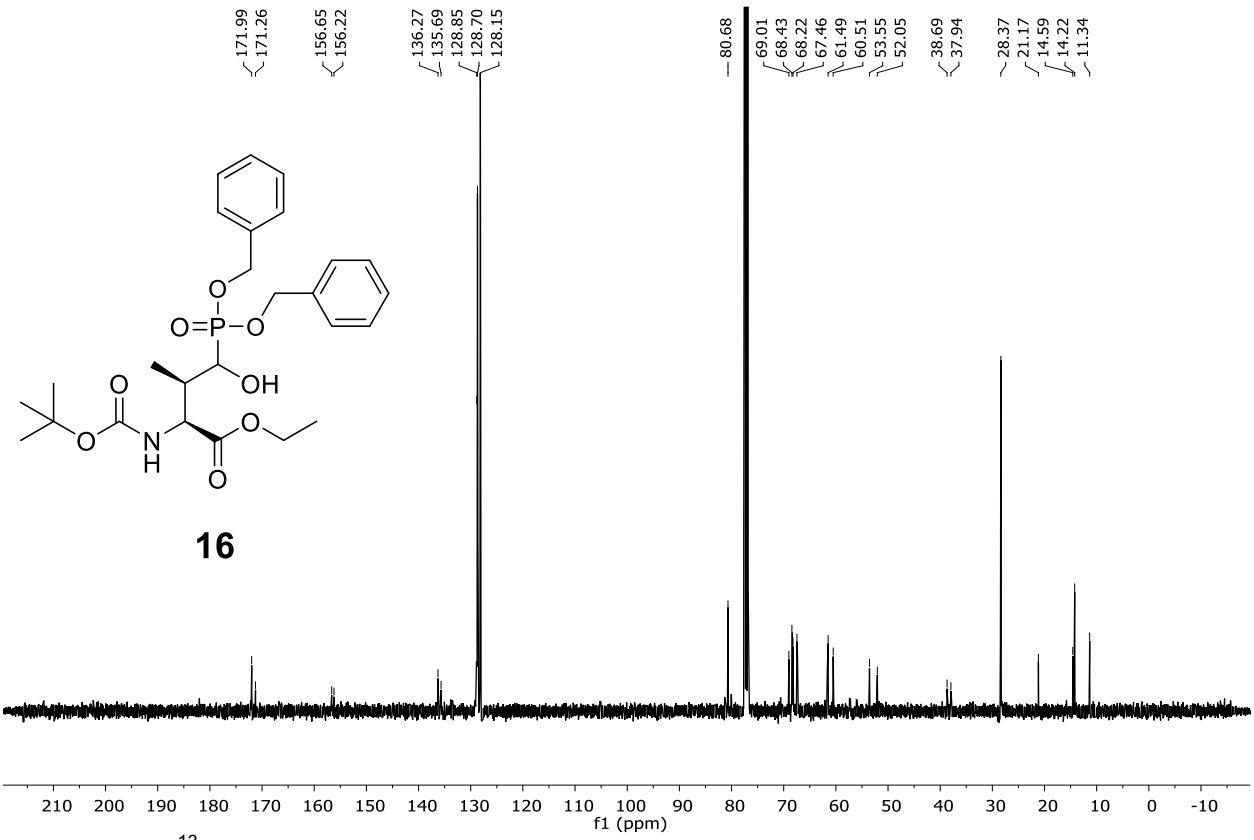


Figure S39. ^{13}C NMR spectra (100 MHz) of **16** in $CDCl_3$.

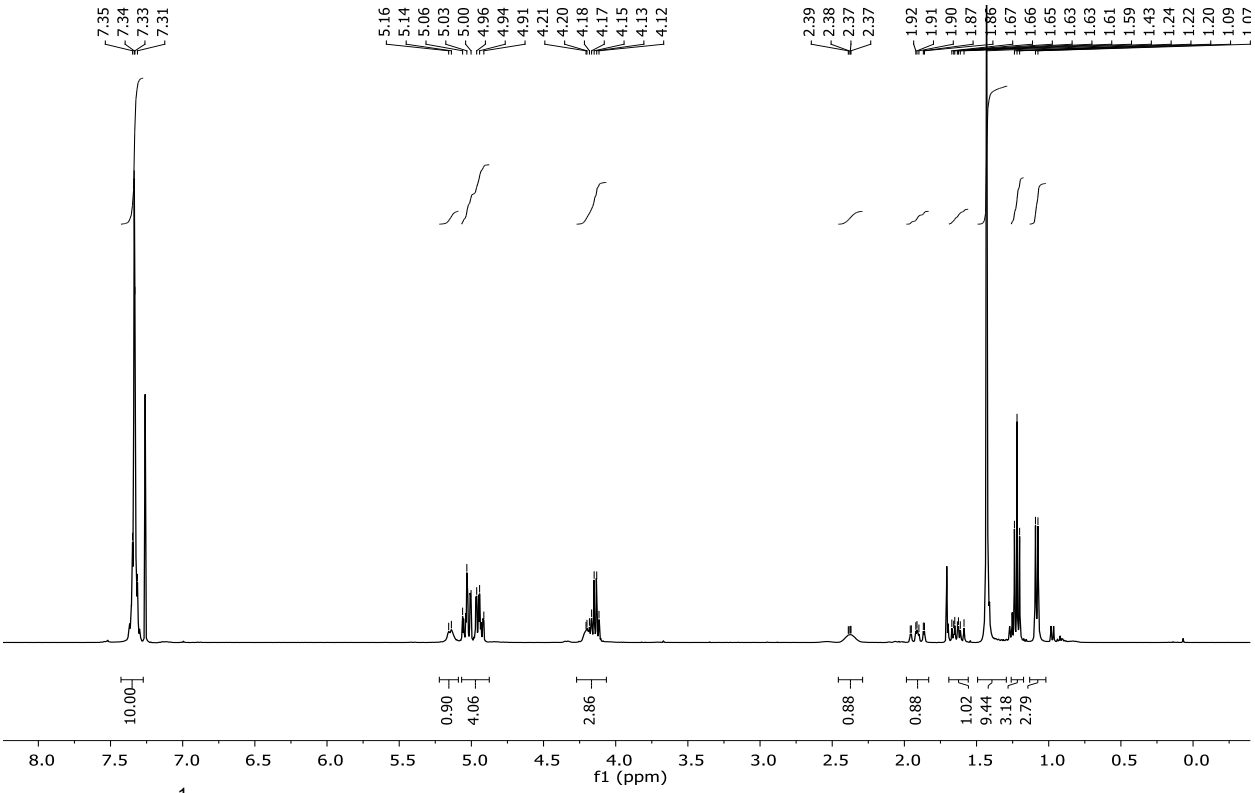


Figure S40. ^{1}H NMR spectra (400 MHz) of **17** in $CDCl_3$.

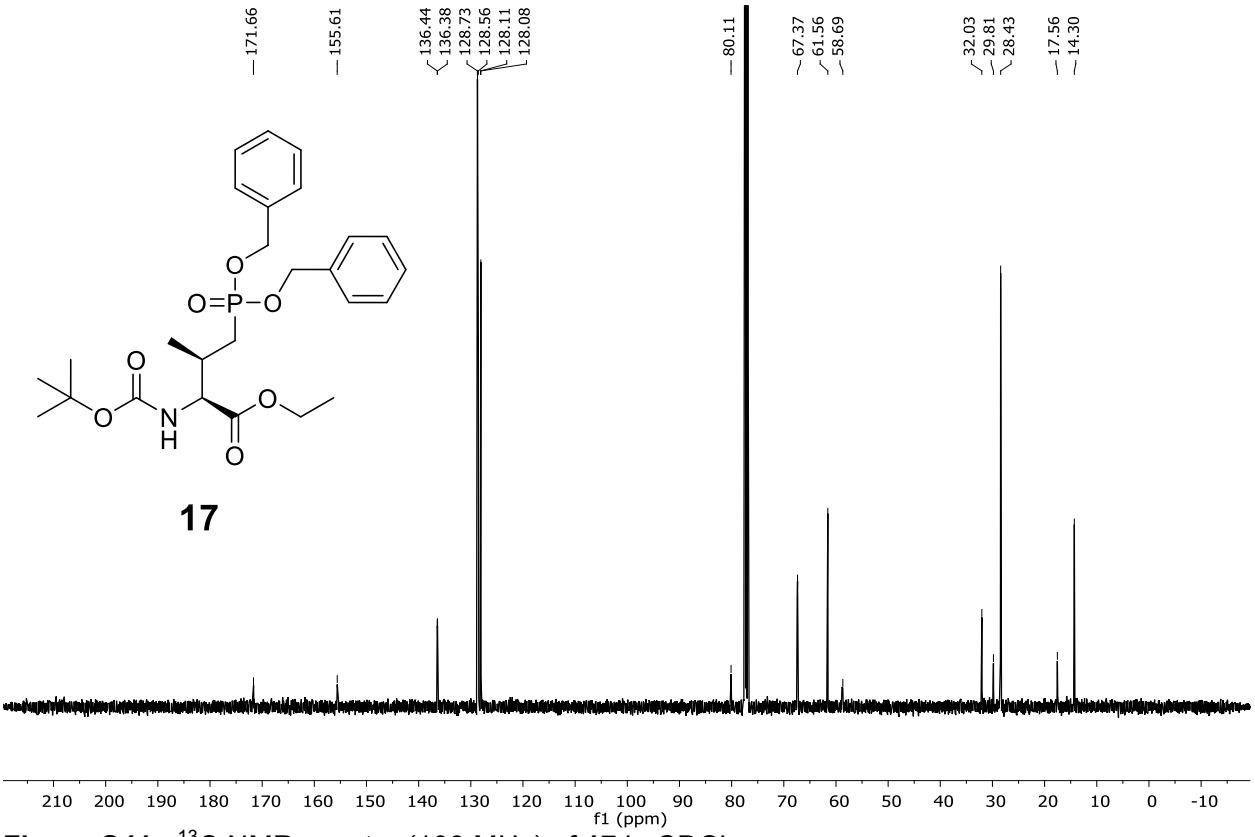


Figure S41. ^{13}C NMR spectra (100 MHz) of **17** in $CDCl_3$.

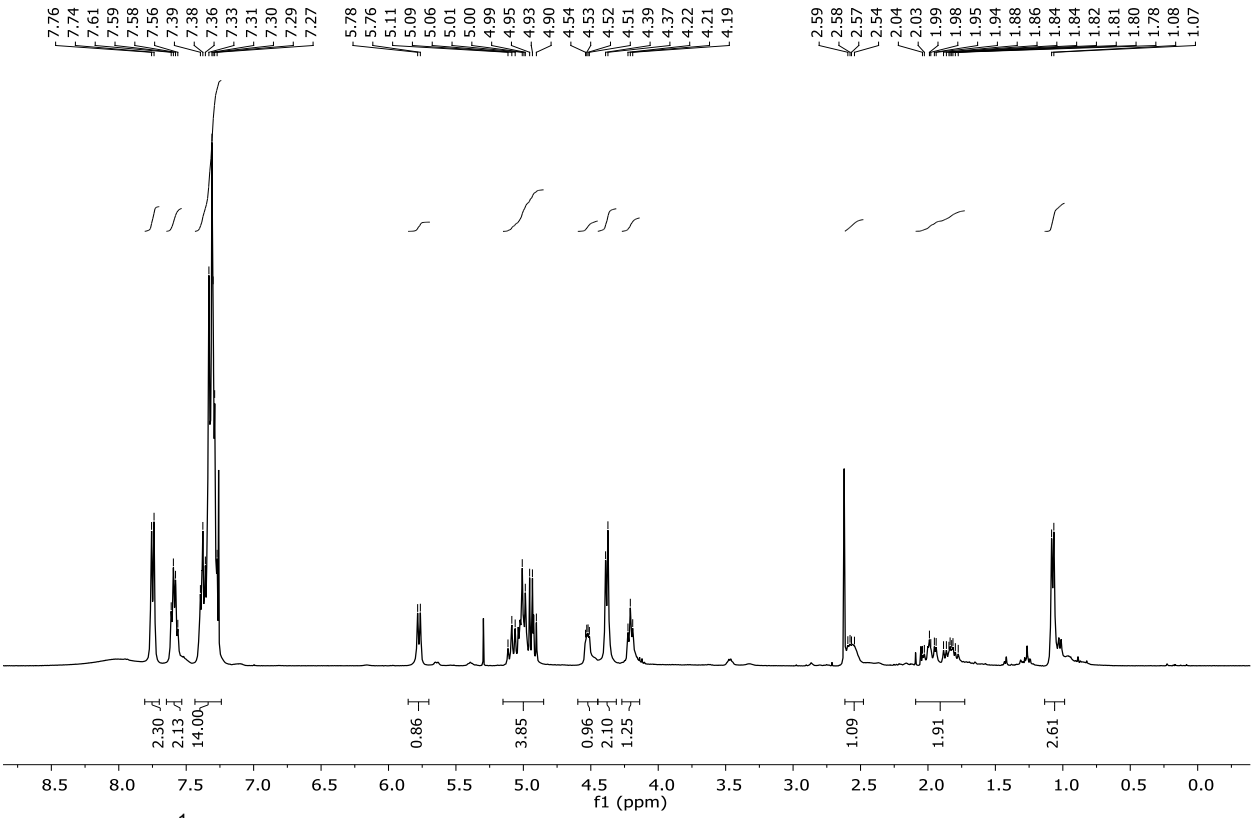


Figure S42. ^{1}H NMR spectra (400 MHz) of **18** in $CDCl_3$.

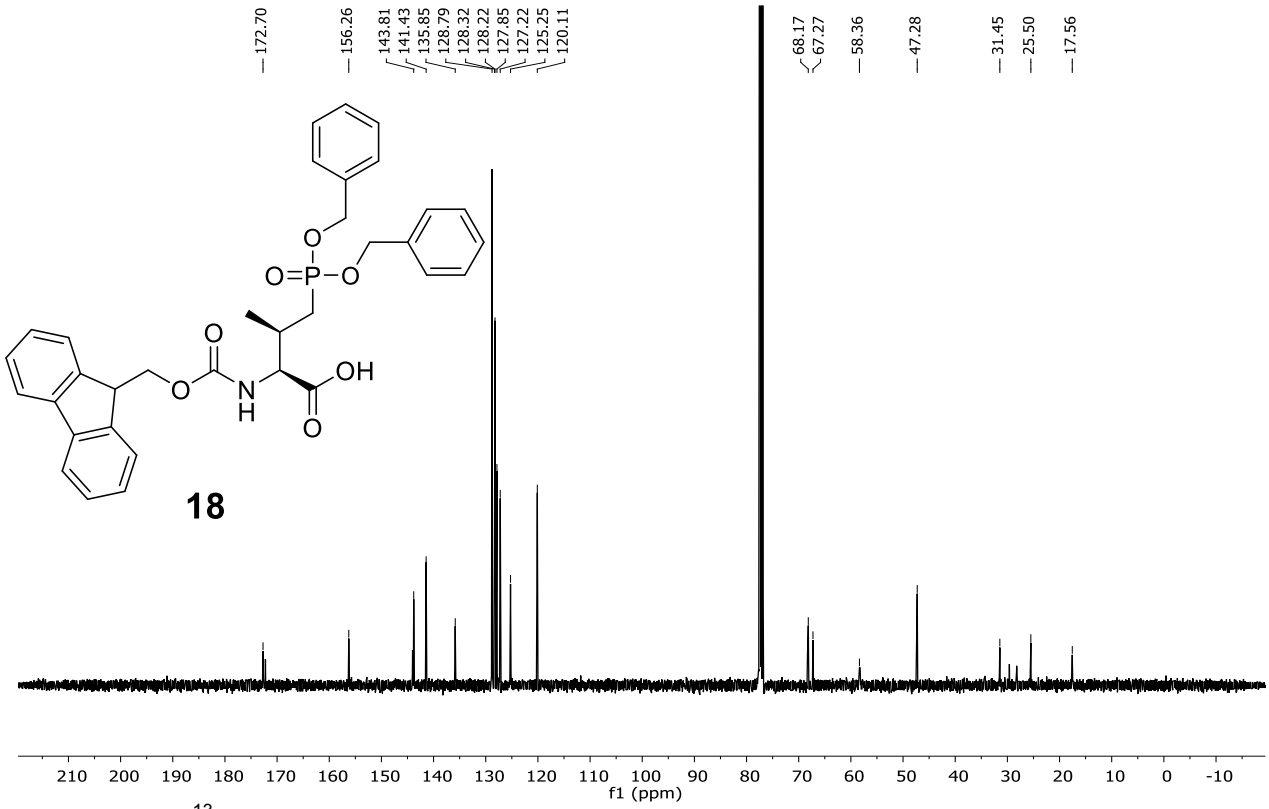


Figure S43. ^{13}C NMR spectra (100 MHz) of **18** in $CDCl_3$.

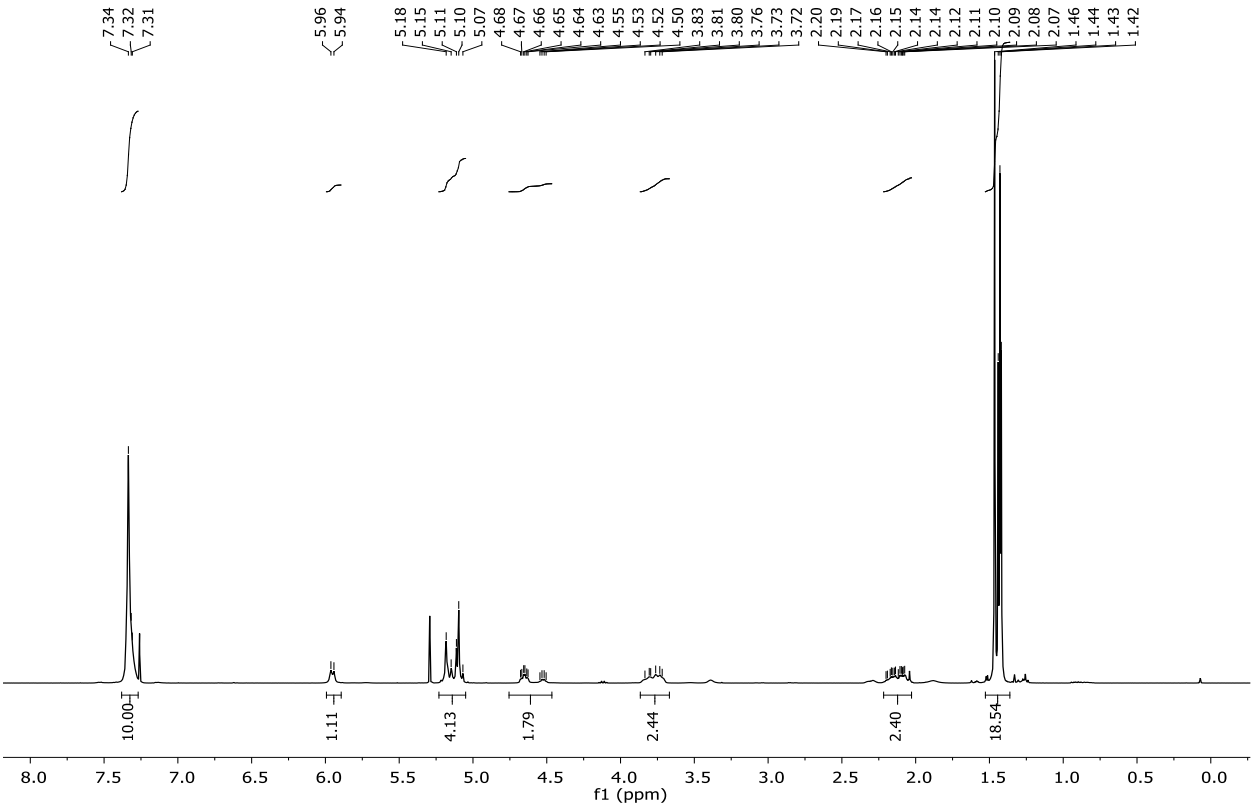


Figure S44. ^{1}H NMR spectra (400 MHz) of **27** in $CDCl_3$.

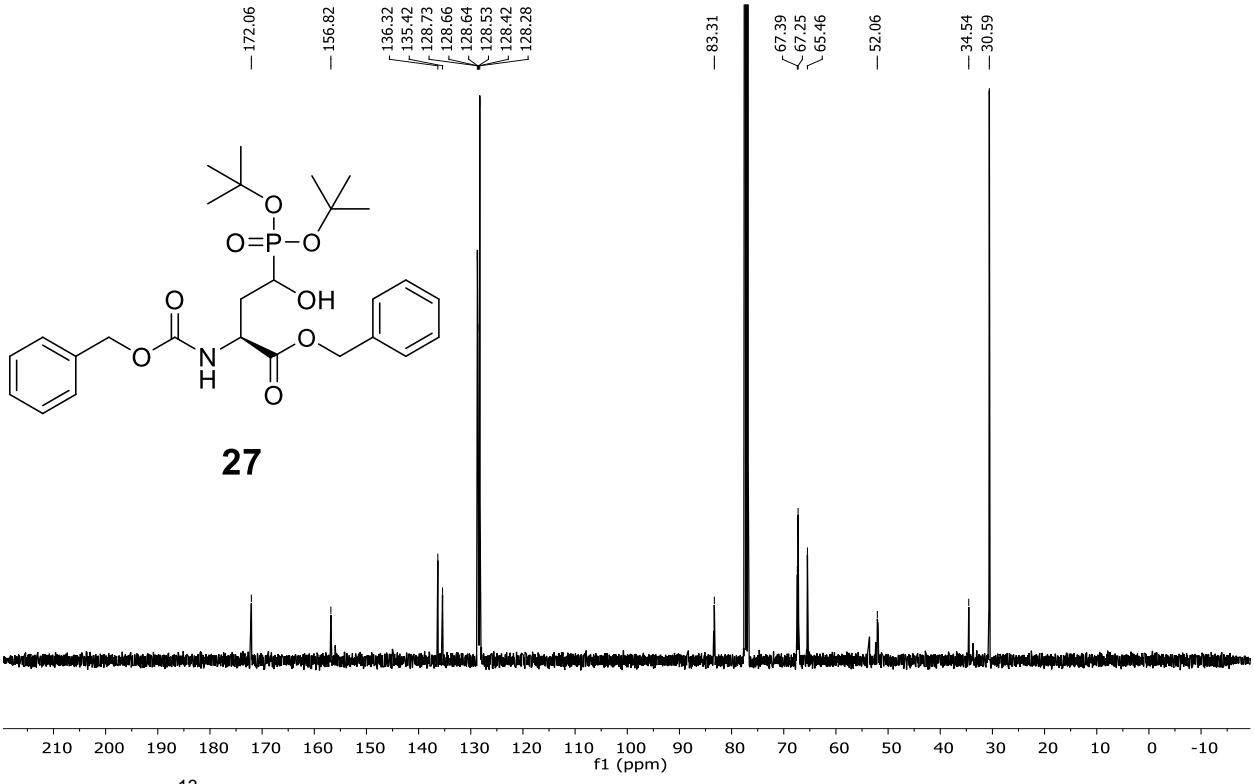


Figure S45. ^{13}C NMR spectra (100 MHz) of **27** in $CDCl_3$.

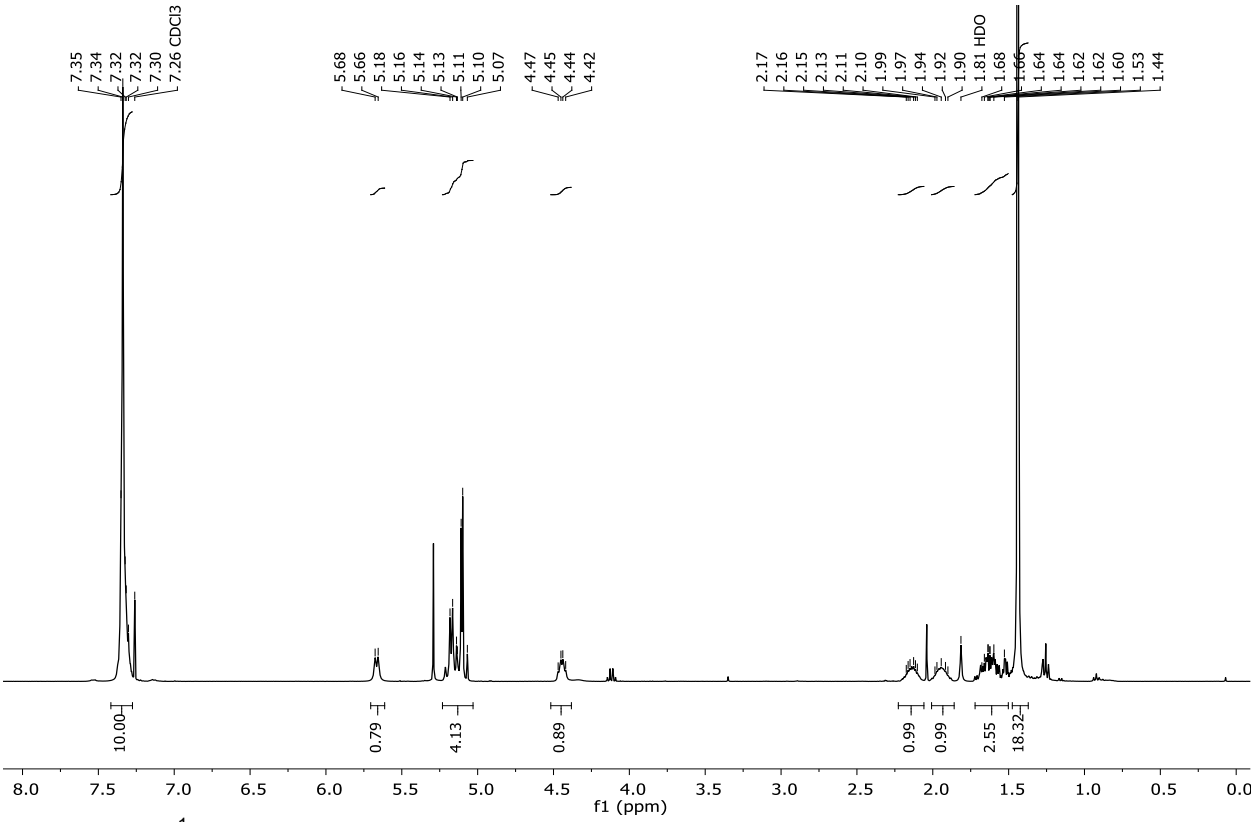


Figure S46. ^{1}H NMR spectra (400 MHz) of **28** in $CDCl_3$.

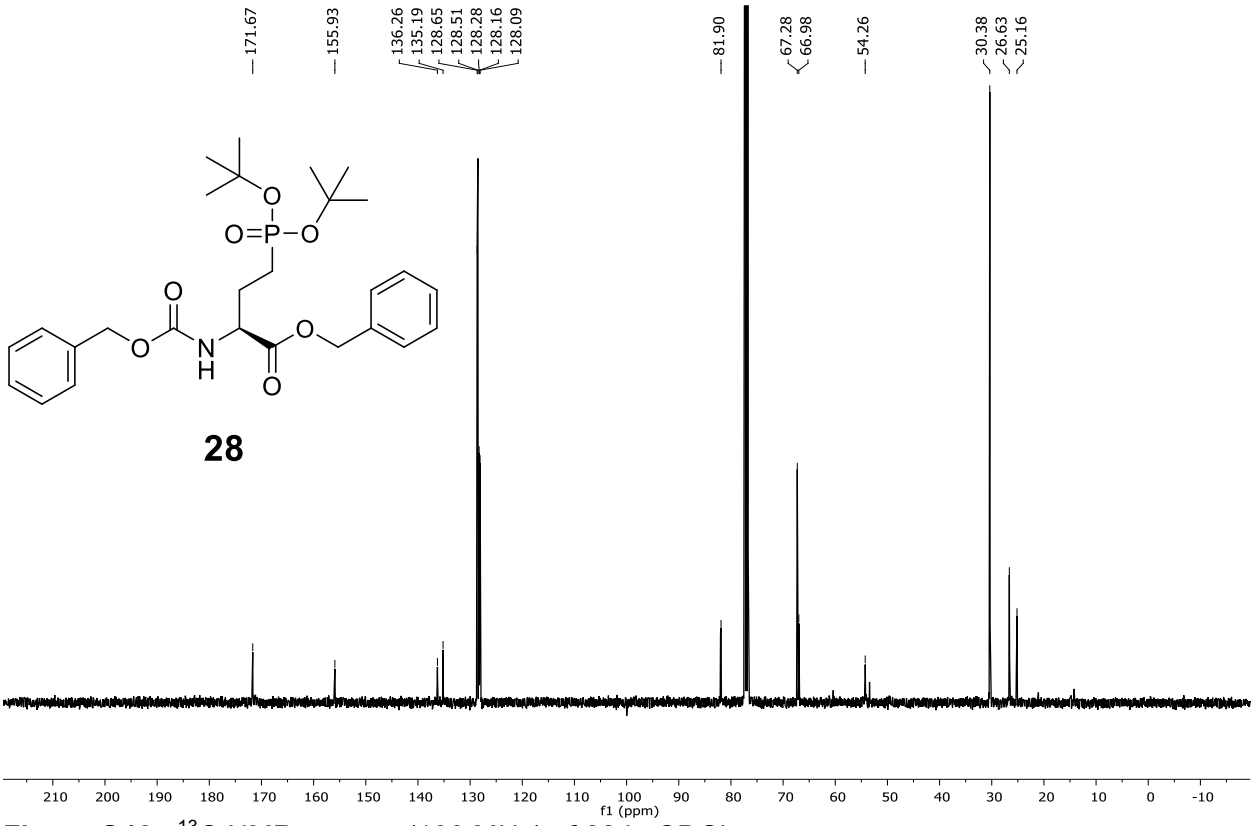


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Figure S46. ^{13}C NMR spectra (100 MHz) of **28** in $CDCl_3$.

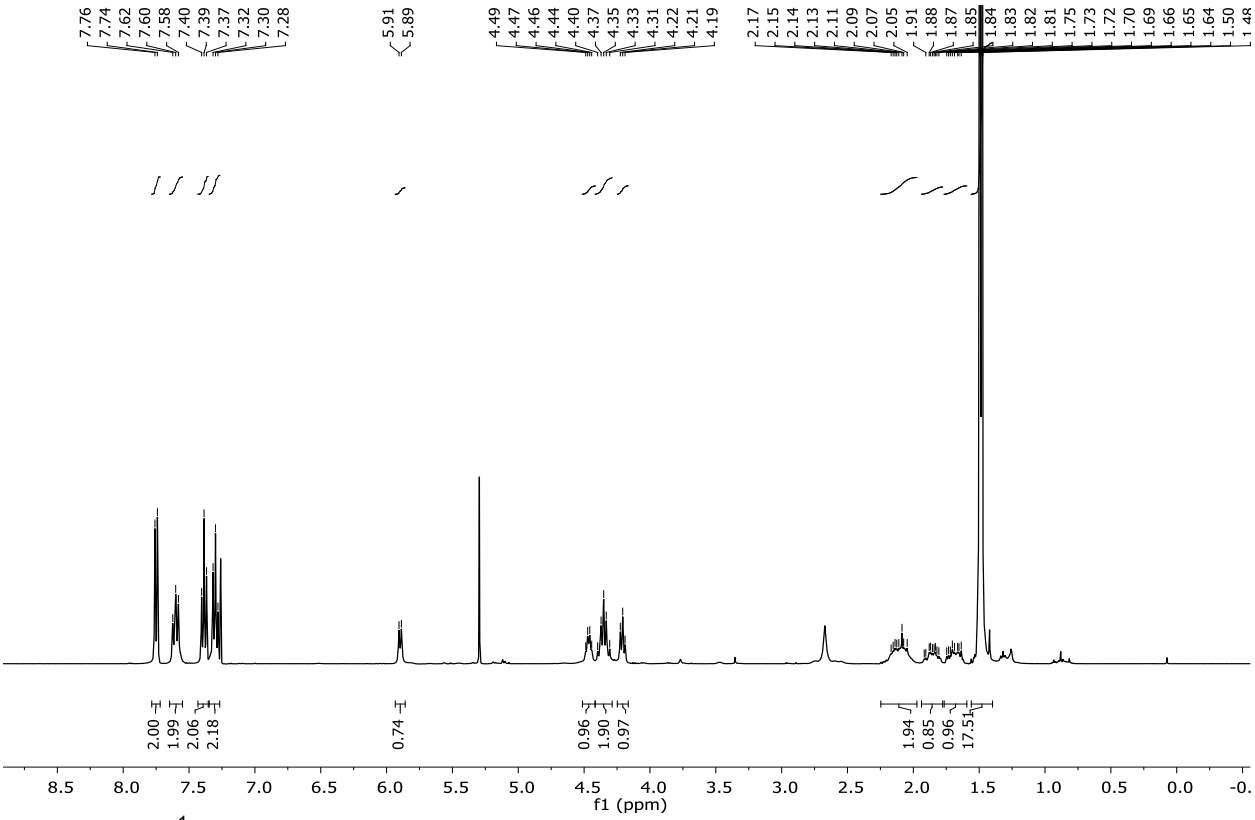

Figure S48. ^{1}H NMR spectra (400 MHz) of **29** in $CDCl_3$.

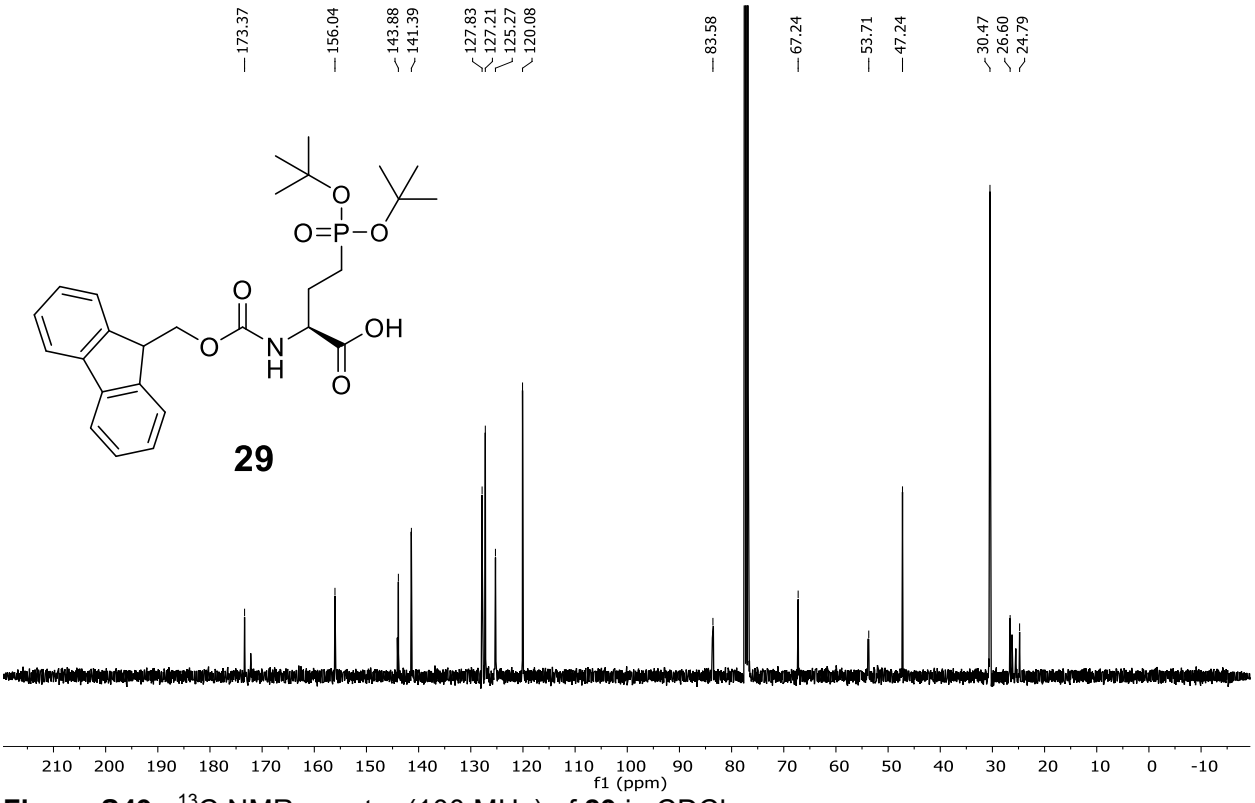

Figure S49. ^{13}C NMR spectra (100 MHz) of **29** in $CDCl_3$.

Analytical Traces for New Peptides

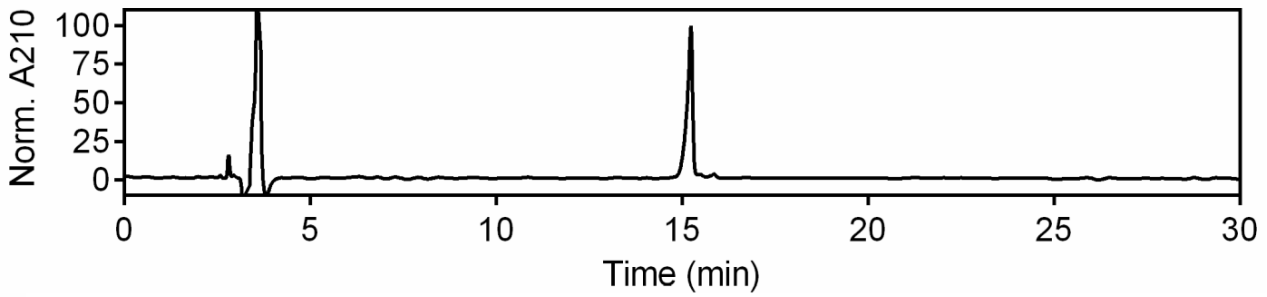

Figure S50. Analytical HPLC trace of **7**.

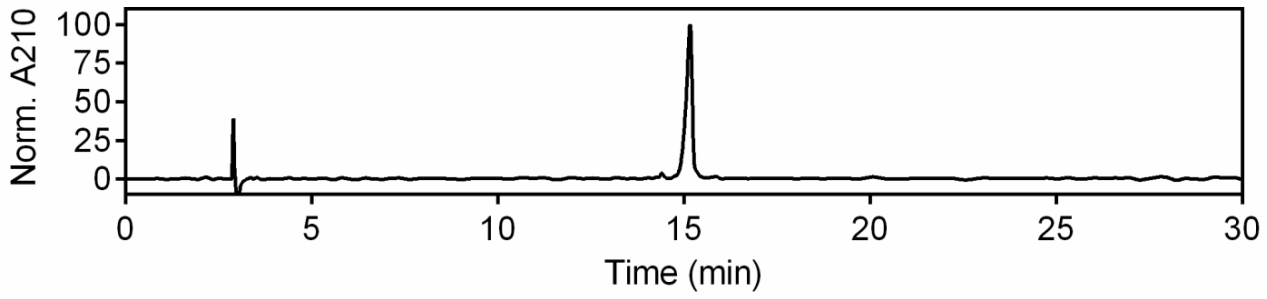

Figure S51. Analytical HPLC trace of **19**.

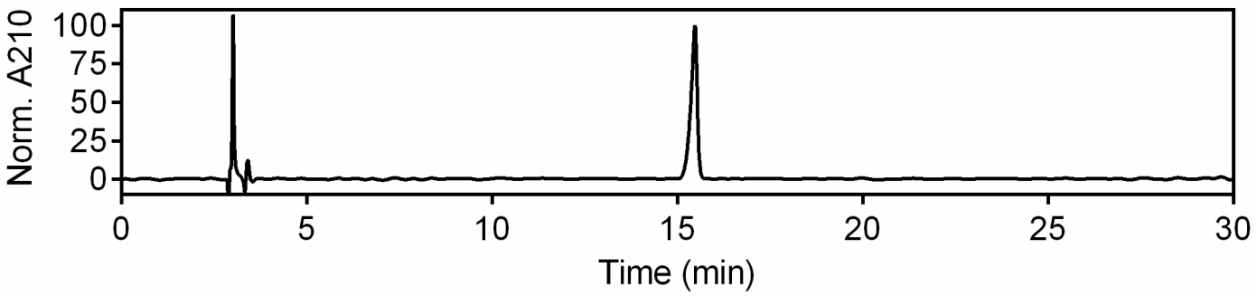

Figure S52. Analytical HPLC trace of **20**.

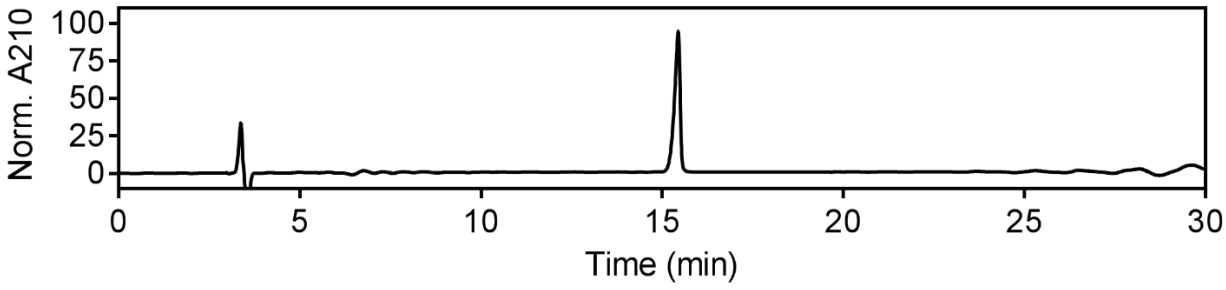

Figure S53. Analytical HPLC trace of **21**.

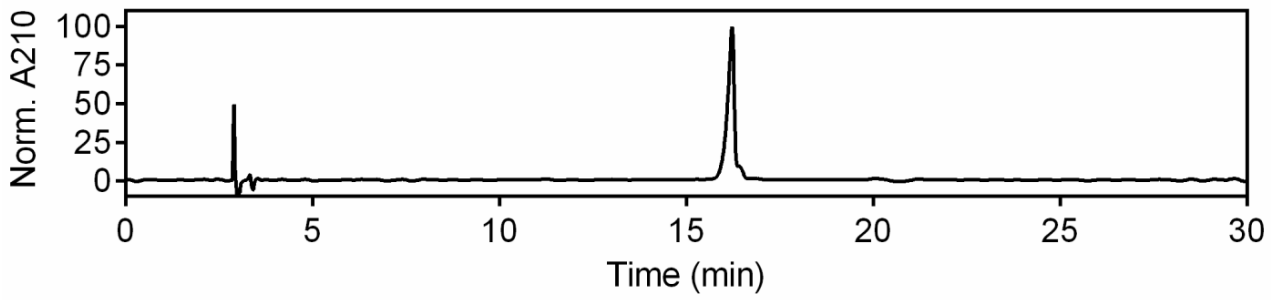

Figure S54. Analytical HPLC trace of **22**.

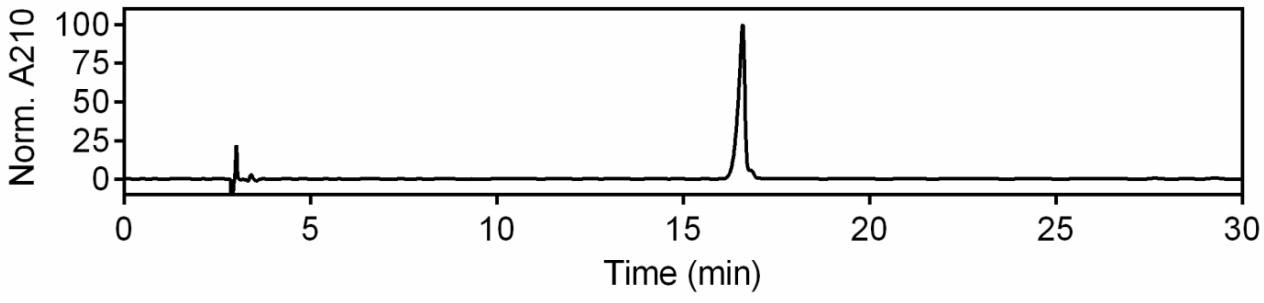

Figure S55. Analytical HPLC trace of **23**.

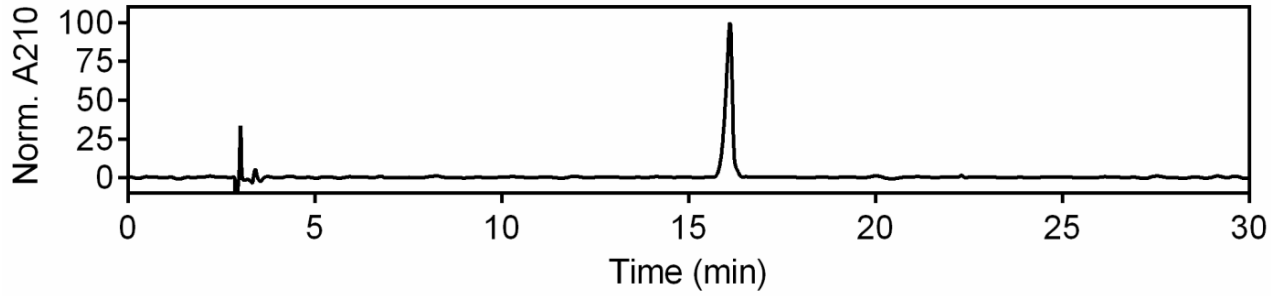

Figure S56. Analytical HPLC trace of **24**.

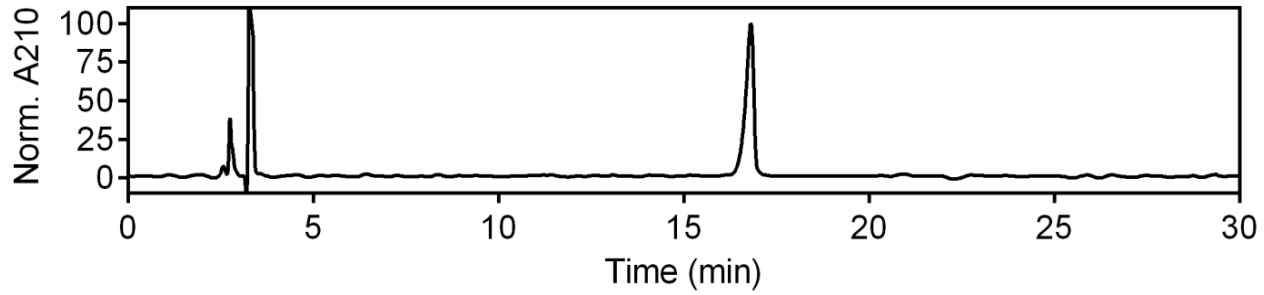

Figure S57. Analytical HPLC trace of **25**.

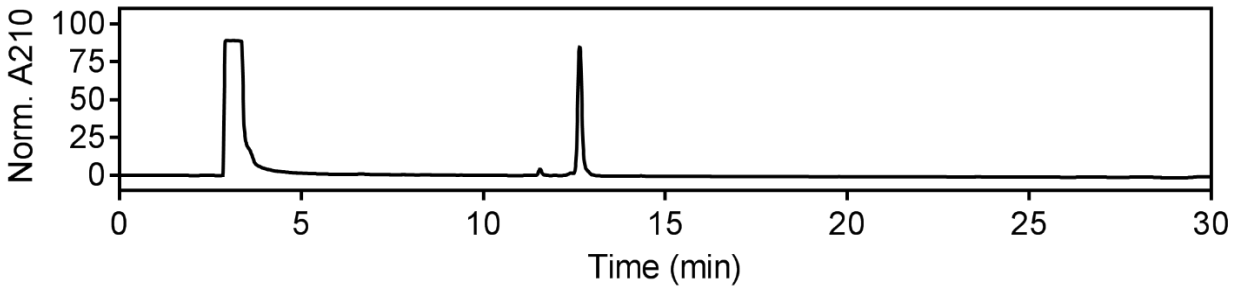

Figure S58. Analytical HPLC trace of **Plk1 PBD FP Probe** (5CF-GPMQSpTPLN-NH_2).

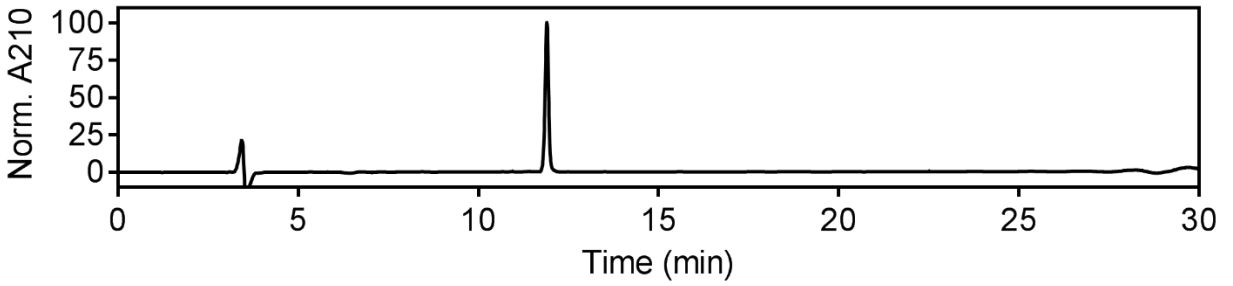

Figure S59. Analytical HPLC trace of **Plk2 PBD FP Probe** (5CF-GPMQTSpTPKN-NH_2).

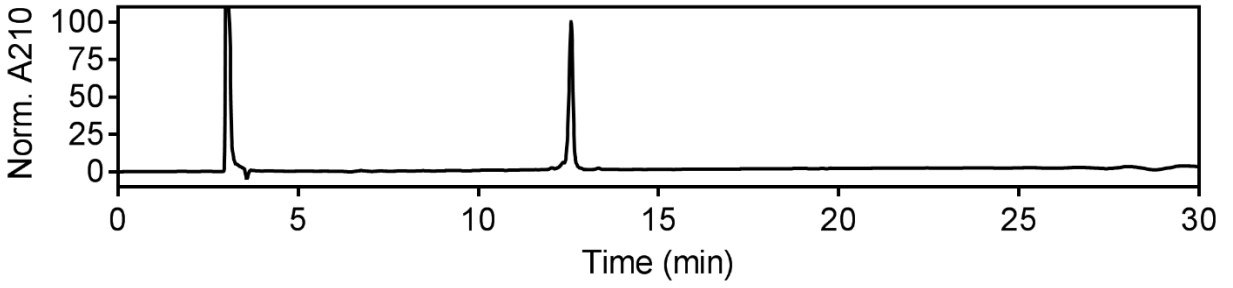

Figure S60. Analytical HPLC trace of **Plk3 PBD FP Probe** (5CF-GPLATSpTPKN-NH_2).

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