Supporting Information

Indentification of High Affinity Polo-like Kinase 1 (Plk1) Polo-box Domain Binding Peptides Using Oxime-based Diversification

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Supplementary Tables

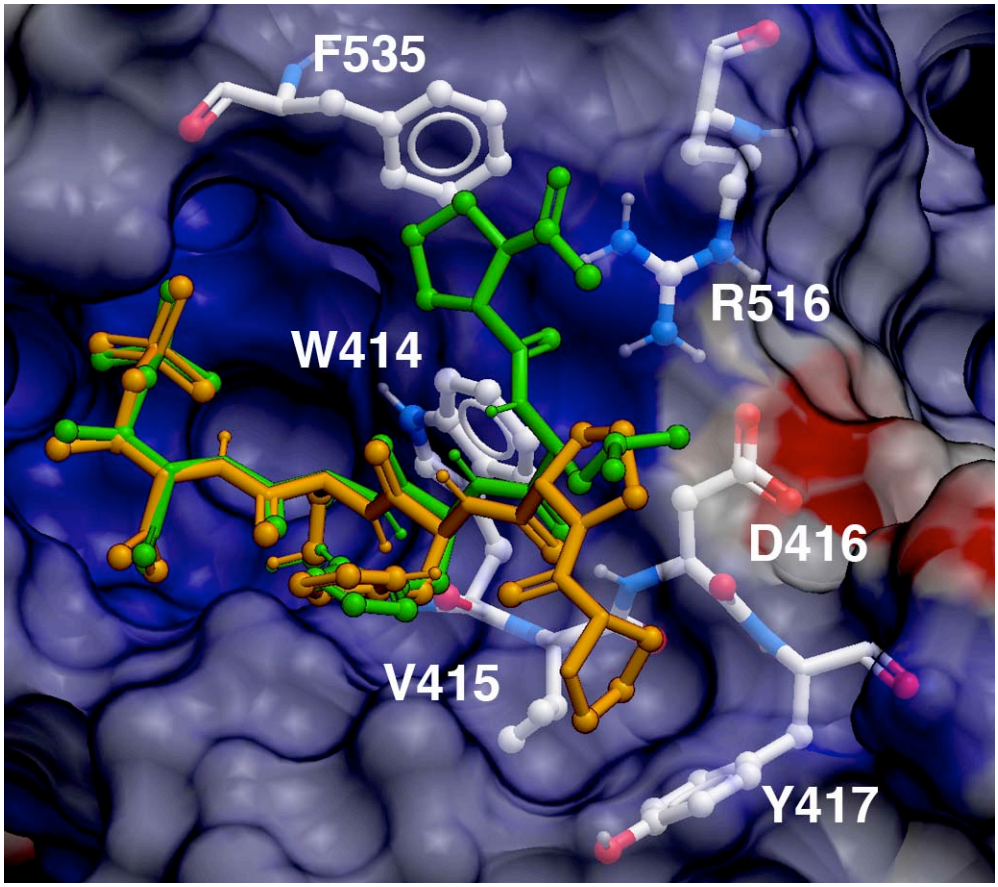


Fig. S1. Overlay of co-crystal structure data comparing the Plk-1 PBD binding of "Ac-PLHSpT" (PDB: 3HIK; shown in gold) with "PPHSpT" (PDB: 3C5L; shown in green). Protein residues interacting with *N*-terminal Prolines are shown with residue numbering. Electrostatic surface is for the 3C5L structure. The Figure was generated using ICM Chemist Pro software by Molsoft, Inc. (www.molsoft.com).

General Synthetic Procedures. All experiments involving moisture-sensitive compounds were conducted under dry conditions (positive argon pressure) using standard syringe, cannula, and septa apparatus. Solvents: All solvents were purchased anhydrous (Aldrich) and used directly. HPLC-grade hexanes, EtOAc, CH_2Cl_2, and MeOH were used in chromatography. TLC: analytical TLC was performed on Analtech pre-coated plates (Uniplate, silica gel GHLF, 250 microns) containing a fluorescence indicator; 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 TMS. Low resolution mass spectra (ESI) were measured using an Agilent 1200 LC/MSD-SL system, and high resolution mass spectra (ESI or APCI) were obtained from the UCR Mass Spectrometry Facility, Department of Chemistry, University of California, 3401 Watkins Dr., Riverside CA, 92521. Aldehydes **a** – **i** (**Fig. S2**) were purchased from Sigma-Aldrich. Aldehydes **j** – **m** were purchased from Rieke®Fine Chemicals. Aldehydes **n**[1] **o**[2] and **p**[3] were prepared according to the indicated literature procedures.

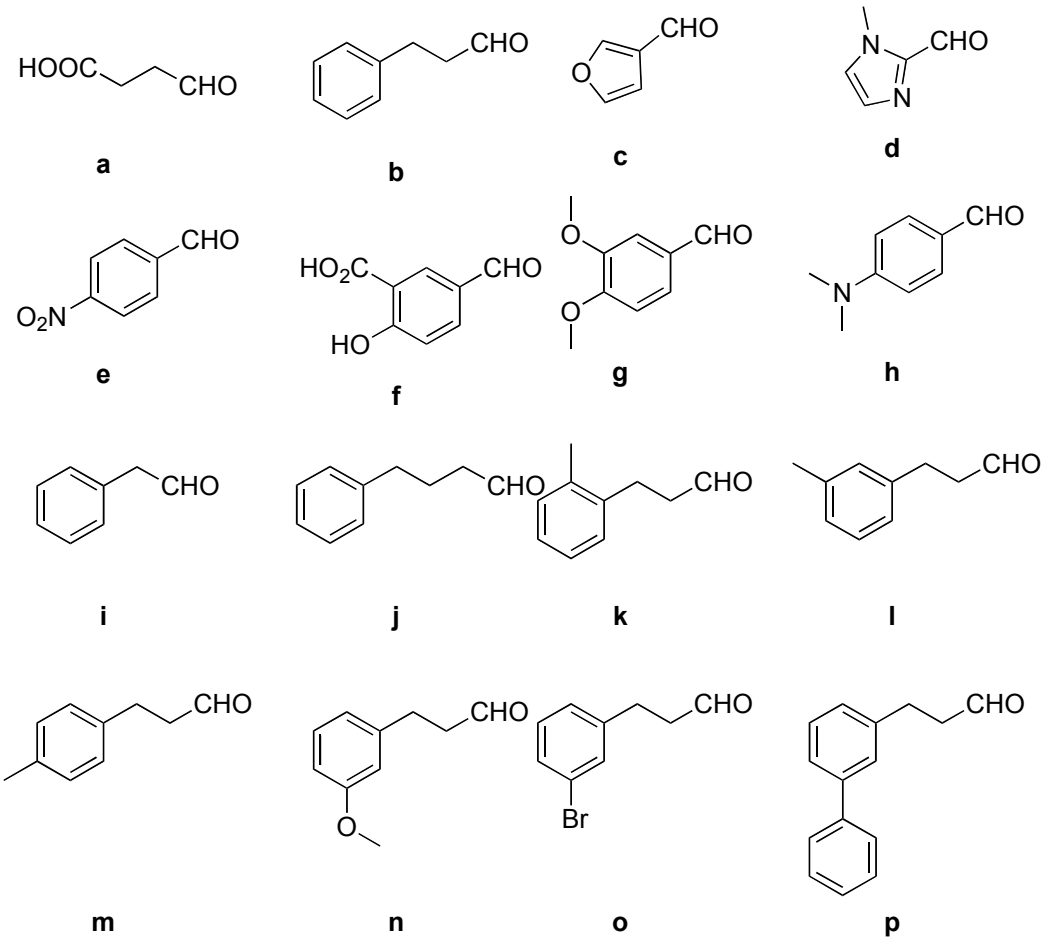
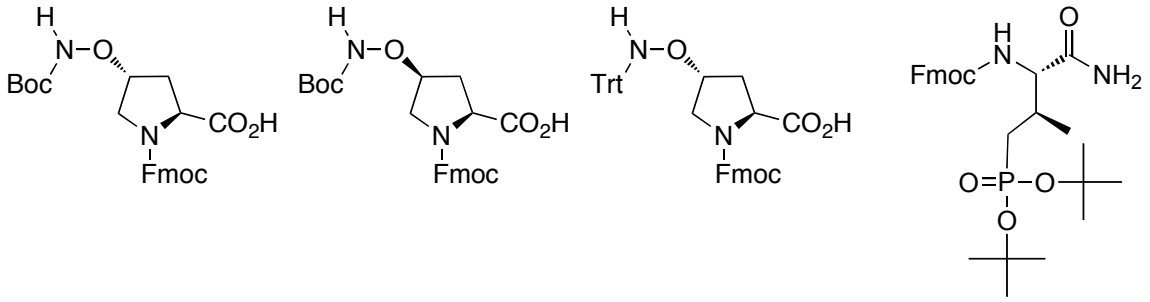


Fig. S2. Structures of aldehydes used to prepare oxime-containing peptides **4** and **5**.

General Procedures for Peptide Synthesis. Boc-protected 4-*trans*-aminooxy and 4-*cis*-aminooxy proline derivatives (**s1** and **s2**, respectively, **Fig. S3**) were prepared as previously reported[4] and used in the preparation of aminooxy-containing peptides **2** and **3**, respectively. Fmoc-Thr(PO(OBzl)OH)-OH and other Fmoc protected amino acids were purchased from Novabiochem. Peptides were synthesized on NovaSyn®TGR resin (Novabiochem, cat. no. 01-64-0060) using standard Fmoc solid-phase protocols in N-Methyl-2-pyrrolidone (NMP). 1-O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (5.0 eq.), hydroxybenzotriazole (HOBT) (5.0 eq.) and N,N-Diisopropylethylamine (DIPEA) (10.0 eq.) were used as coupling reagents. The N-terminal was acetylated by 1-acetylimidazole. The final resin was washed with N, N-dimethylforamide (DMF), methanol, dichloromethane and ether then dried under vacuum (over night). Peptide **6** was prepared using N-trityl protection of the proline aminooxy group (**s3**) (**Fig. S3)**.[4] In this latter case, the fully assembled resin (200 mg) was treated with dichloromethane (DCM) (containing 1% trifluoroacetic acid, 2.5% triisopropylsilane) 5 times, 2 minutes for each time, then treated with 3-phenylpropionic acid (5.0 eq.), HOBT (5.0 eq.) and DIC (5.0 eq) for 2 hours at room temperature. All peptides were cleaved from resin (200 mg) by treatment with 5 mL of trifluoroacetic acid : triisopropylsilane : H_2O (90 : 5 : 5) (4 h). Peptides **7** and **8** were prepared using the 4-phenylbutyl-containing proline analogues **s7** and **s10**, respectively (see below). Peptides having the pTyr residue substituted with (2*S*,3*R*)-2-amino-3-methyl-4-phosphonobutyric acid (Pmab) utilized the previously reported reagent **s4** (**Fig. S3**).[5] For peptide pull-down assays, a cysteine residue was attached to the amino-terminus by means of a 6-aminohexanoylamide spacer. Completed resins were filtered off and the filtrate was

concentrated under vacuum, then precipitated with cold ether and the precipitate washed with cold ether. The resulting solid was dissolved in 50% aqueous acetonitrile 5 mL) and purified by reverse phase preparative HPLC using a Phenomenex C_{18} column (21 mm dia x 250 mm, cat. no: 00G-4436-P0) with a linear gradient from 0% aqueous acetonitrile (0.1% trifluoroacetic acid) to 100% acetonitrile (0.1% trifluoroacetic acid) over 30 minutes at a flow rate of 10.0 mL/minute. Lyophilization gave product peptides as white powders.



s1 4-*trans*-aminooxy

s2 4-*cis*-aminooxy

s3 4-*trans*-aminooxy (Trityl-protected)

s4

Fig. S3. Structures of non-coded amino acid derivatives employed in the current work.

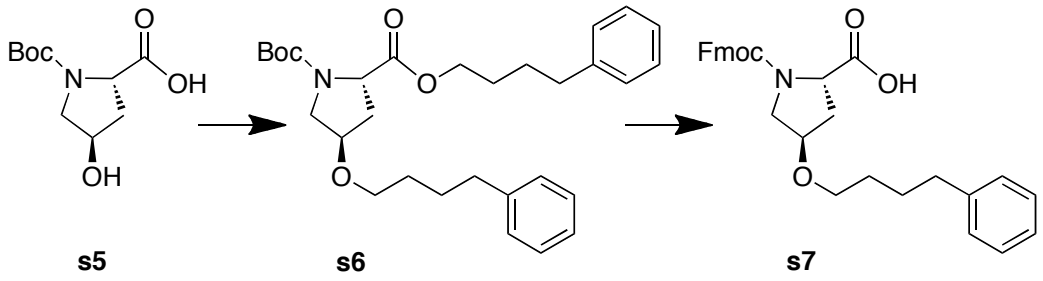


Fig. S4. Preparation of reagent **s7**.

Preparation of Proline Analogue s6. To a suspension of sodium hydride (60% in mineral oil, 1.90 g, 47.5 mmol) in DMF (30 mL) at 0°C, was added a solution of Boc-L-hydroxyproline (**s5**) (5.0 g, 21.6 mmol) in DMF (30 mL) dropwise over 5 minutes. The mixture was kept at 0 °C (15 minutes) then 4-phenyl-1-iodobutane (prepared from 4-phenyl-1-butanol[6]) (16.9 g, 64.8 mmol) was added and the mixture was allowed to come to room temperature and stirred (over night). The reaction was quenched by the addition of saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (300 mL). The organic layer was washed, dried and purified by silica gel column chromatography (hexanes : EtOAc) to yield **s6** (**Fig. S4**) as a colorless oil (5.1 g, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.23 (m, 5 H), 7.16 – 7.13 (m, 5 H), 4.34 (dd, *J* = 8.0, 6.4 Hz, 0.3 H), 4.27 (t, *J* = 7.6 Hz, 0.7 H), 4.14 – 4.08 (m, 2 H), 3.98 (m, 1 H), 3.60 – 3.55 (m, 2 H), 3.42 – 3.33 (m, 2 H), 2.65 – 2.58 (m, 4 H), 2.25 (m, 1 H), 1.98 (m, 1 H), 1.70 – 1.60 (m, 8 H), 1.41 (s, 3.5 H), 1.36 (s, 5.5 H). ESI (+VE) *m/z*: 518.4 $(M + Na)^+$. HR-ESI/APCI MS cacld for $C_{30}H_{42}NO_5$ $(M + H)^+$: 496.3058, Found: 496.3072.

Preparation of Proline Analogue s7. A mixture of **s6** (5.00 g, 10.1 mmol) and LiOH monohydrate (848 mg, 20.2 mmol) in a solution of THF (30 mL), MeOH (10 mL) and H_2O (15 mL) was stirred at room temperature (3 h). Organic solvent was removed by

rotary evaporation and the resulting aqueous phase was washed with ether (50 mL x 2), acidified to pH 3–4 using 1N aqueous HCl and extracted with EtOAc (150 mL). The EtOAc extract was washed, dried ($NaSO_4$) and evaporated to a colorless oil, which was treated with a mixture of TFA (30 mL) and dichloromethane (30 mL) at room temperature (2 h). Solvent was removed and the resulting residue was dried under igh vacuum (2 h). The residue was then dissolved in dioxane (30 mL) and to this was added H_2O (30 mL), followed by solid $NaHCO_3$ (4.20 g, 50.0 mmol) and FmocOSu (3.71 g, 11.0 mmol), and and them mixture was stirred at room temperature (overnight). Dioxane was removed by rotary evaporation the remaining aqueous phase was washed with ether (50 mL x 20), acidfifed to pH 3–4 using 1N aqueous HCl and extracted with EtOAc (200 mL). The EtOAc layer was washed (brine), dried (NaSO4) and evaporated to provide analytically pure **s7** as a thick oil (5.1g, 100% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (brs, 1 H), 7.74 (d, *J* = 7.6 Hz, 1 H), 7.68 (d, *J* = 7.6 Hz, 1 H), 7.57 – 7.46 (m, 2 H), 7.40 – 7.22 (m, 7 H), 7.18 – 7.12 (m, 2 H), 4.49 (t, *J* = 7.6 Hz, 0.5 H), 4.45 – 4.32 (m, 2.5 H), 4.24 (t, *J* = 7.2 Hz, 0.5 Hz), 4.15 – 4.00 (m, 1.5 H), 3.70 (m, 0.40 H), 3.60 – 3.55 (m, 1.6 H), 3.44 – 3.34 (m, 2 H), 2.64 – 2.57 (m, 2 H), 2.35 (m, 1 H), 0.86 (m, 1 H), 1.70 – 1.55 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7, 176.5, 171.8, 159.1, 158.6, 155.9, 154.9, 143.7, 142.2, 141.3, 128.4, 128.3, 127.1, 119.9, 76.8, 76.2, 69.2, 68.0, 60.7, 58.1, 57.5, 52.0, 51.7, 47.1, 36.8, 35.6, 35.0, 29.3, 27.9, 21.0, 14.1. ESI (+VE) *m/z*: 508.3 $(M + Na)^+$. HR-ESI/APCI MS cacld for $C_{30}H_{32}NO_5$ $(M + H)^+$: 486.2275, Found: 486.2278.

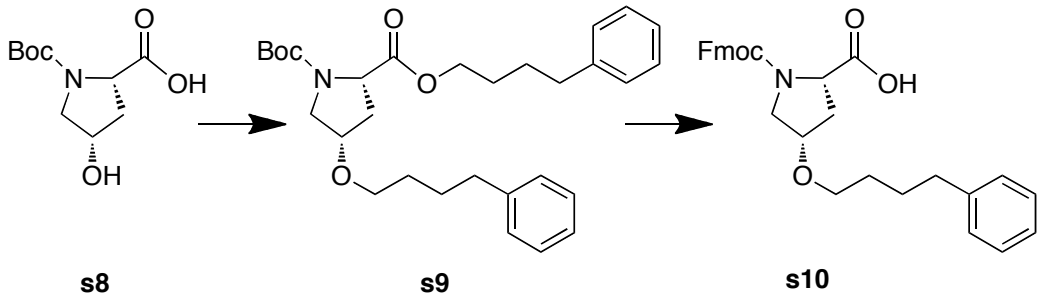


Fig. S5. Preparation of reagent **s10**.

Preparation of Proline Analogue s9. Proline analogue **s9** was prepared from N-Boc-*cis*-4-hydroxy-L-proline (**s8**) in 29% yield by the same method described above for the preparation of **s6**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.22 (m, 5 H), 7.18 – 7.12 (m, 5 H), 4.39 (dd, *J* = 8.4, 3.6 Hz, 0.4 H), 4.27 (dd, *J* = 8.4, 4.0 Hz, 0.6 H), 4.15 – 3.98 (m, 2 H), 3.93 (m, 1 H), 3.63 (m, 0.60 H), 3.55 (dd, *J* = 11.2, 5.2 Hz, 0.4 H), 3.43 (ddd, *J* = 18.4, 11.6, 3.2 Hz, 1 H), 3.36 – 3.28 (m, 2 H), 2.65 – 2.55 (m, 4 H), 2.30 – 2.15 (m, 2 H), 1.70 – 1.55 (m, 6 H), 1.55 – 1.46 (m, 2 H), 1.45 (s, 3.5 H), 1.40 (s, 5.5 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 172.0, 154.2, 153.8, 142.3, 142.1, 141.9, 128.3, 125.8, 79.9, 79.8, 77.4, 76.3, 68.9, 64.8, 57.8, 57.4, 52.0, 51.4, 36.2, 35.7, 35.4, 35.0, 29.4, 28.4, 28.3, 28.1, 27.9, 27.6. ESI (+VE) *m/z*: 518.4 $(M + Na)^+$. HR-ESI/APCI MS cacld for $C_{30}H_{42}NO_5$ $(M + H)^+$: 496.3058, Found: 496.3068.

Preparation of Proline Analogue s10. Proline analogue **s10** was prepared from **s9** in 30% yield using the same methodology described above for the preparation of **s7**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (brs, 1 H), 7.73 (d, *J* = 7.6 Hz, 1 H), 7.69 (d, *J* = 7.2 Hz, 1 H), 7.60 – 7.50 (m, 2 H), 7.40 – 7.25 (m, 7 H), 7.17 – 7.10 (m, 2 H), 4.50 -4.43 (m, 1.6 H), 4.40 – 4.30 (m, 1.4 H), 4.23 (m, 0.60 H), 4.17 (m, 0.40 H), 4.00 (m, 1 H), 3.63 – 3.53 (m, 2 H), 3.45 (m, 0.5 H), 3.40 – 3.30 (m, 1.5 H), 2.60 – 2.53 (m, 2 H), 2.42 (m, 0.5 H), 2.30 (m, 1 H), 2.20 (m, 0.5 H), 1.70-1.50 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8, 174.5, 155.7, 143.9, 143.6, 142.3, 141.3, 128.3, 127.8, 127.0, 125.7, 125.0, 120.0, 76.3, 68.8, 67.8, 60.4, 58.1, 57.6, 52.2, 47.1, 36.0, 35.5, 34.1, 31.6, 29.1, 27.8, 25.4, 22.6, 21.0, 14.4. ESI (+VE) *m/z*: 508.3 $(M + Na)^+$. HR-ESI/APCI MS cacld for $C_{30}H_{32}NO_5$ $(M + H)^+$: 486.2275, Found: 486.2299.

Analytical HPLC Conditions. The purities of peptides were determined by either using a Waters XBridge^{TM} BEH 130 column (C_{18} 5 μm 4.6 mm dia x 250 mm) with a linear gradient from 0% aqueous acetonitrile (0.1% trifluoroacetic acid) to 100% acetonitrile (0.1% trifluoroacetic acid) over 30 minutes at a flow rate of 1.0 mL/minute, detection at 220 nm or a Phenomenex column Gemini column (C_{18} 5 μm, 110 Å, 4.60 mm dia x 250 mm) with a linear gradient from 5% aqueous acetonitrile (0.1% trifluoroacetic acid) to 100% acetonitrile (0.1% trifluoroacetic acid) over 40 minutes at a flow rate of 1.0 mL/minute, detection at 220 nm.

Table S1. ESI-Mass Spectral Data and HPLC Purity of Synthetic Peptides.[a]

No	Expected $(M + H)^+$	Observed $(M + H)^+$	Expected $(M - H)^-$	Observed $(M - H)^-$	HPLC Purity
2	706.3	706.6			95.8%
3	706.3	706.6			98.0%
4b	822.3	822.3			100% (two oxime isomer ratio: ~1/4)
5b	822.3	822.3			100% (two oxime isomer ratio: ~1/4)
6	838.3	838.3			79.9%
7	823.4	823.3			100%
7(S4A)	807.4	807.4	805.4	805.3	80.2%
7(pT5T)	743.4	743.2			100%
7*	821.4	821.4	819.4	819.3	100%
7*(S4A)	805.4	805.3	803.4	803.4	77%
8	823.4	823.5	821.4	821.4	100%
8(S4A)	807.4	807.4	805.4	805.4	82.2%
7	997.5	997.3	995.5	994.6	92.6%
*7**	995.4	995.2	993.5	992.7	90.6%

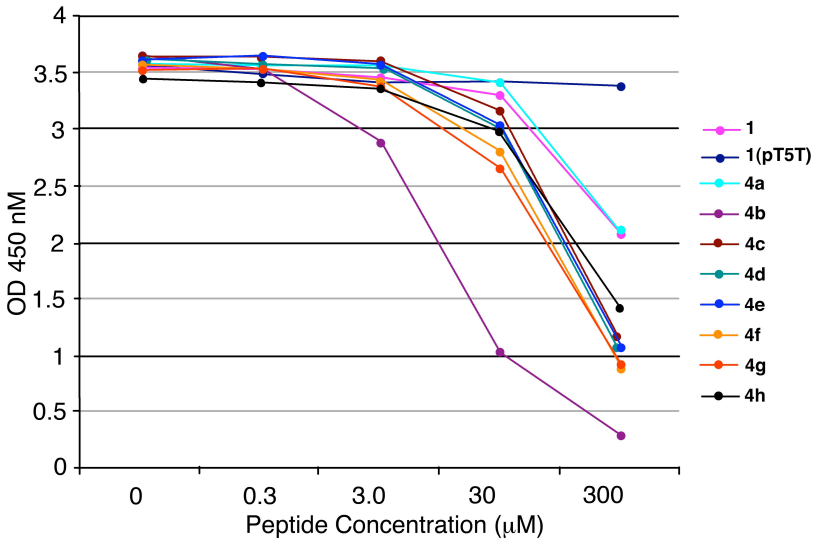
7*(S5A)	979.5	979.3	977.5	976.6	89.0%
7(pT5T)	917.5	917.4			92.8%

[a]Compound numbers in red italics indicate that the peptides are *N*-terminally modified to contain Cys-(6-aminohexanoylamide) functionality for use in peptide pull-down assays.

Table S2. HRMS of Selected Peptides.

No	Expected $(M + H)^+$	Observed $(M + H)^+$	Expected $(M - H)^-$	Observed $(M - H)^-$
2			704.2774	704.2745
3			704.2774	704.2787
4b	822.3546	822.3551		
5b			820.3400	820.3357
7			821.3604	821.3590
8			821.3604	821.3585
7*	821.3957	821.3977		
7*(S4A)	805.4008	805.4033		

a



b

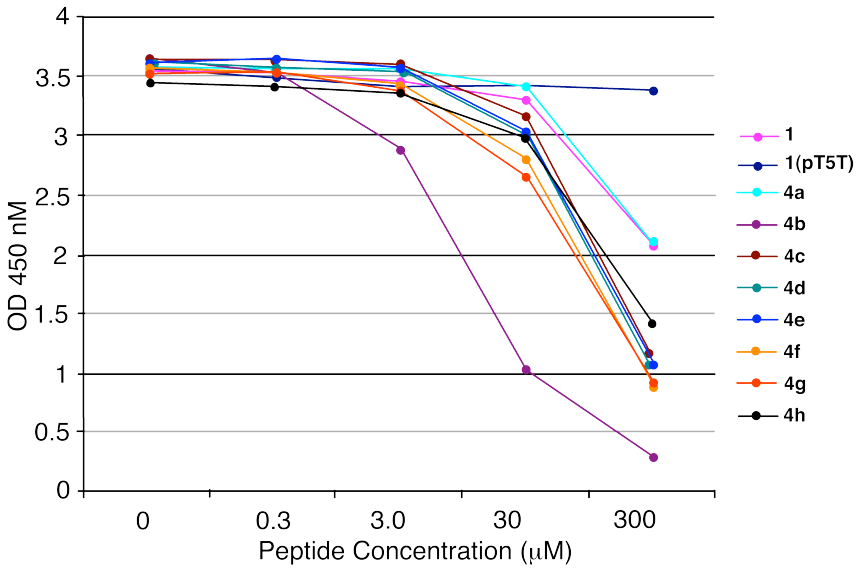


Fig. S6. Plk1 PBD binding curves obtained from ELISA-based PBD inhibition assays as described in the Experimental Methods. Peptide structures are shown in Figure 2 of the published text: (a) Data obtained with oxime library **4**; (b) Data obtained with oxime library **5**. Representative graphs from three independent experiments are shown.

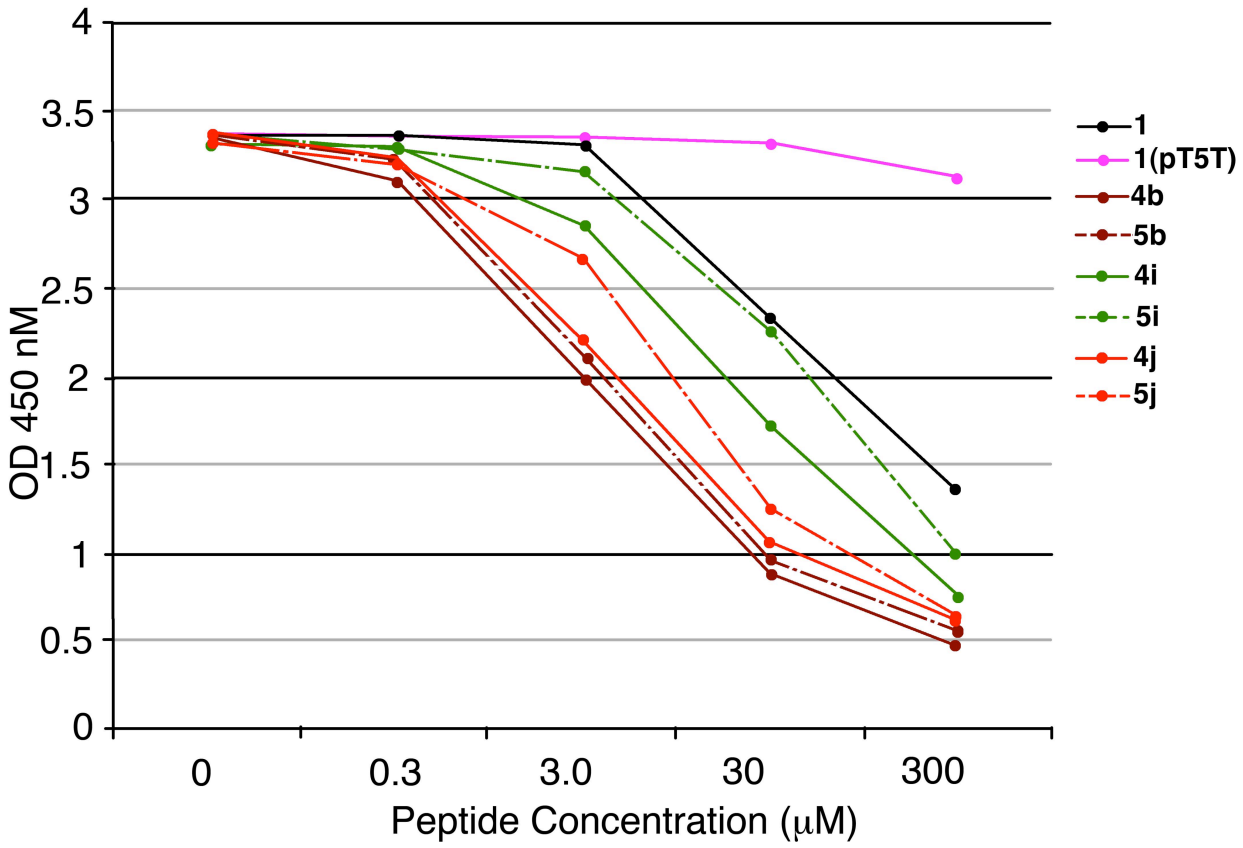


Fig S7. Plk1 PBD binding curves obtained from ELISA-based PBD inhibition assays as described in the above. A representative graph from three independent experiments is shown.

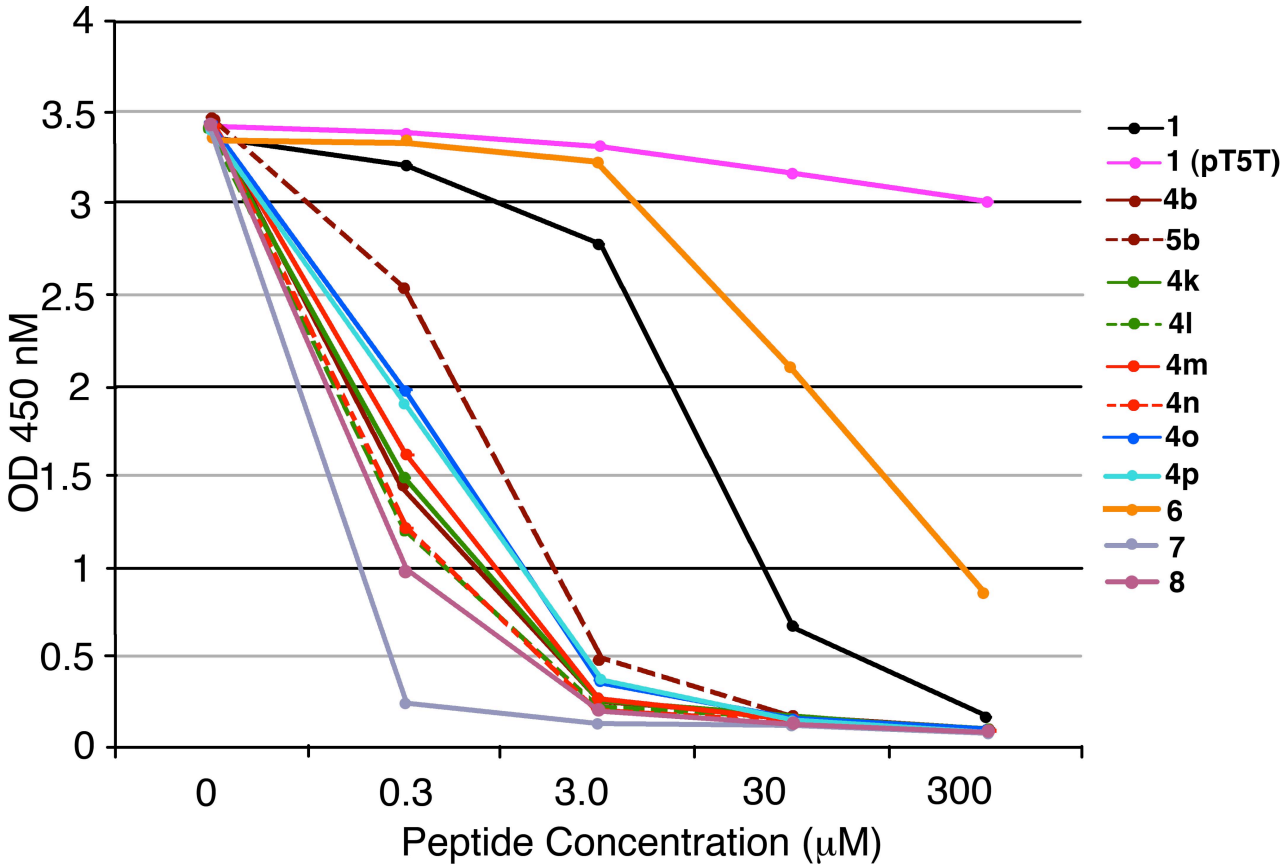


Fig S8. Plk1 PBD binding curves obtained from ELISA-based, Plk1 PBD inhibition assays as described in the Experimental Methods. Representative graphs are shown from three independent experiments.

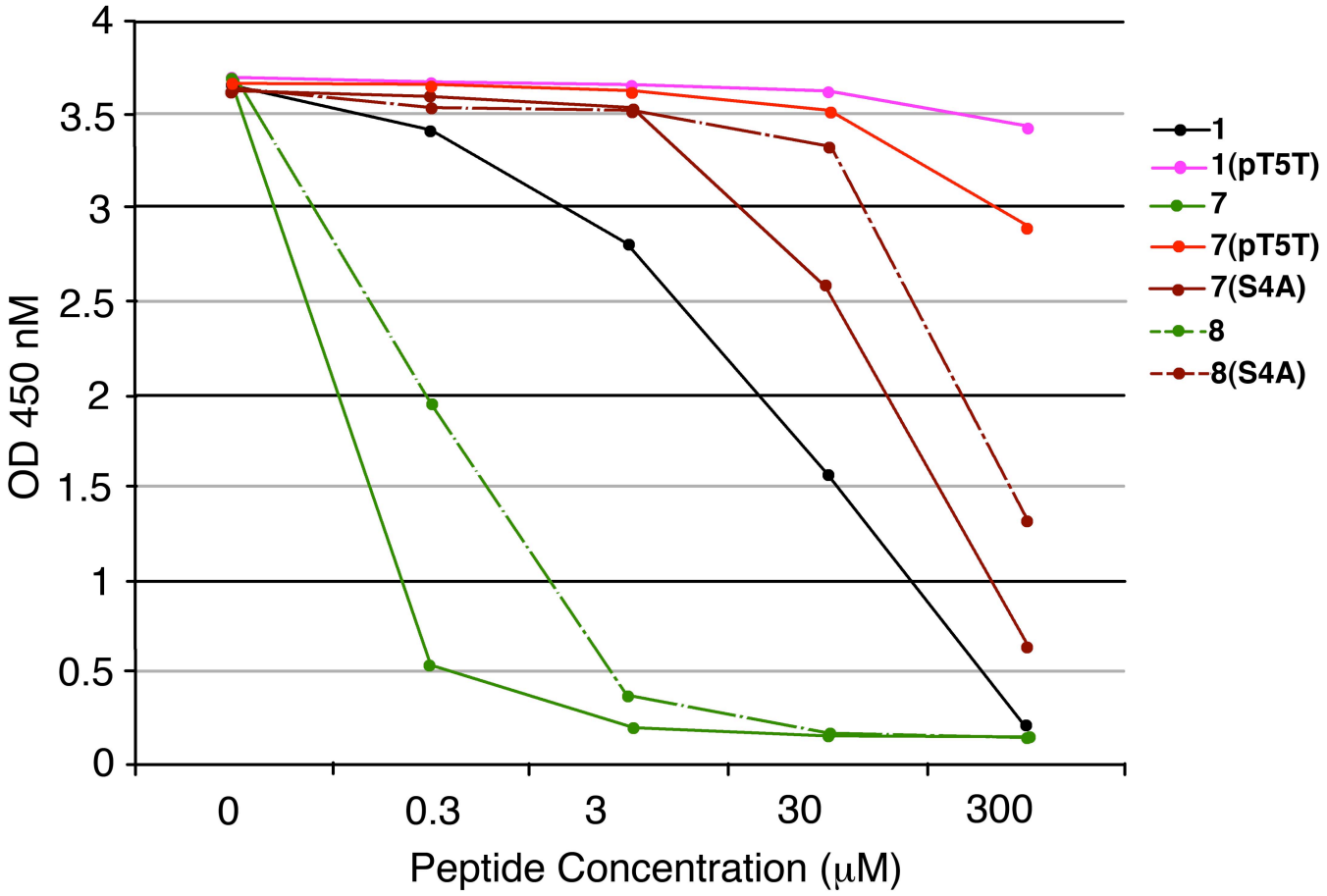


Fig S9. Plk1 PBD binding curves obtained from ELISA-based, Plk1 PBD inhibition assays as described in the Experimental Methods. Representative graphs are shown from three independent experiments.

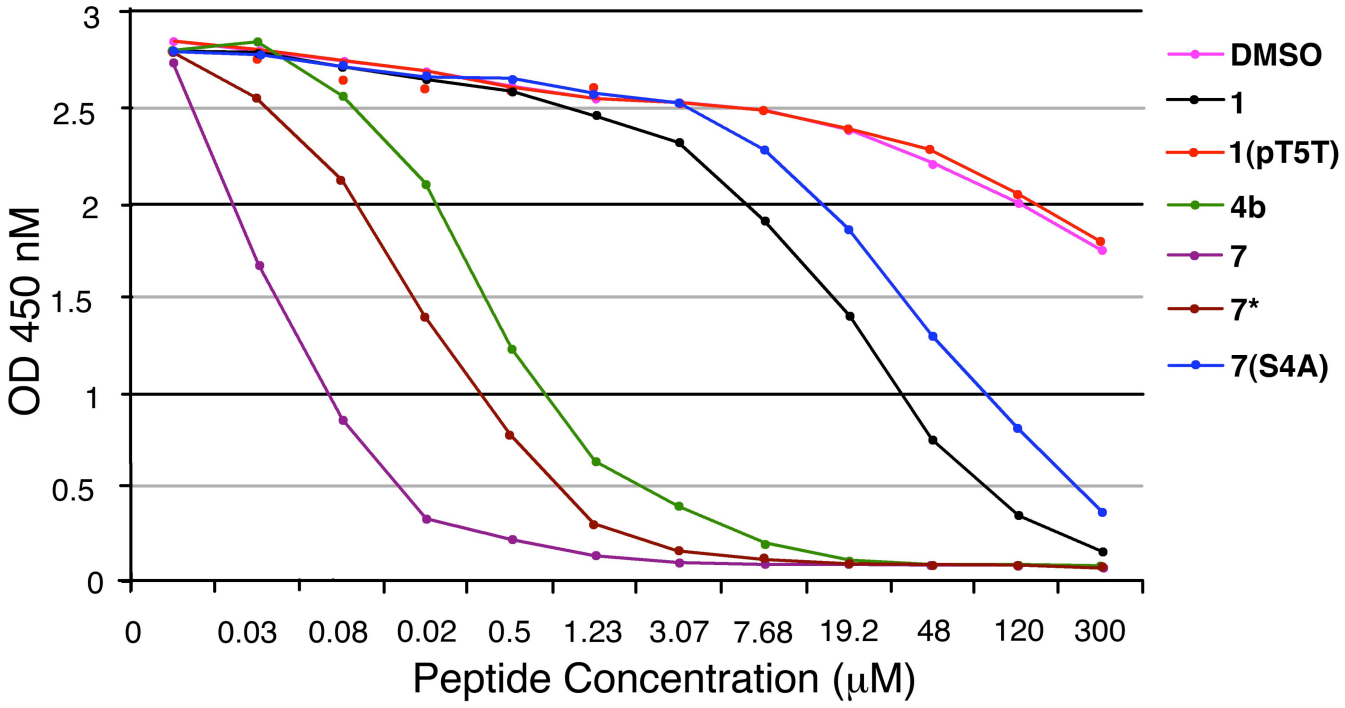


Fig. S10. Plk1 PBD binding curves obtained from ELISA-based PBD inhibition assays as described in the Experimental Methods using an expanded concentration range. A representative graph from three independent experiments is shown.

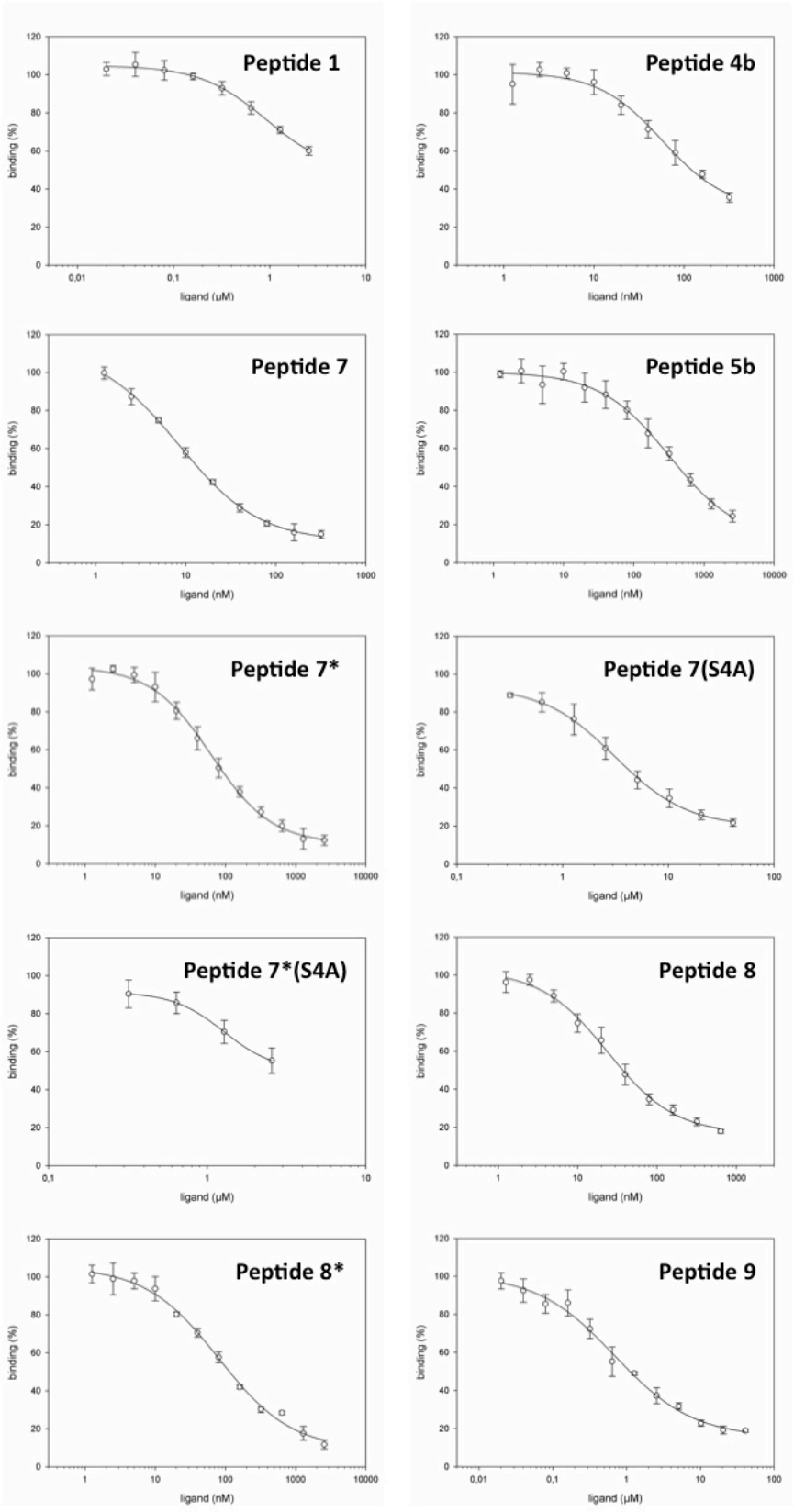


Fig. S11. Results of fluorescence polarization assays showing inhibition of binding of 5-carboxyfluorescein-GPMQSpTPLNG-OH (5-CF-**9**) and the Plk1 PBD as described in the Experimental Procedures of the published text.

X-ray Crystallography

Protein purification and crystallization. Plk1 PBD protein (residues 371-603) was purified as previously described.[7] Crystals were grown using the hanging drop vapor diffusion method. PBD protein at 12 mg/mL in 10 mM Tris pH 8, 0.5 M NaCl, 10 mM DTT, 2% DMSO and 2 mM peptide **7** was mixed with an equal volume of reservoir solution consisting of 31% (w/v) PEG 3350, 0.1 M bis-Tris pH 5.5 and 240 mM $MgCl_2$. Crystals began appearing overnight and reached maximum size over several days.

Data collection, and structure determination and refinement. Crystals were flash cooled by direct transfer from the mother liquor into a nitrogen cold stream. Data were collected at 100 K on a Mar345 image plate detector with a Rigaku RU-300 home X-ray source. The data were processed with the HKL[8] and CCP4[9] software suites. The structure was solved by molecular replacement using AMoRe[10] using chain A of structure 3FVH (RCSB accession code)[7] as a search model, and refined using PHENIX[11] with manual fitting in XtalView[12] **Fig. S12** was created using Xtalview[12] and PyMOL (http://www.pymol.org/).

Table S3. Data Collection and Refinement Statistics

PBD ID	4DFW
Space group	P1
a (Å)	35.7
b (Å)	34.8
c (Å)	47.1
α (°)	76.4
β (°)	87.0
γ (°)	69.1
Resolution range (Å)	15-1.55
Average redundancy	3.1
Completeness[1]	91.0% (82.4%)
R_{sym}[1]	0.042 (0.100)
Average I/σ[1]	21.5 (5.0)
R/R_{free} (%)	15.5 / 19.6

[1]Values for the highest resolution shell are shown in parentheses.

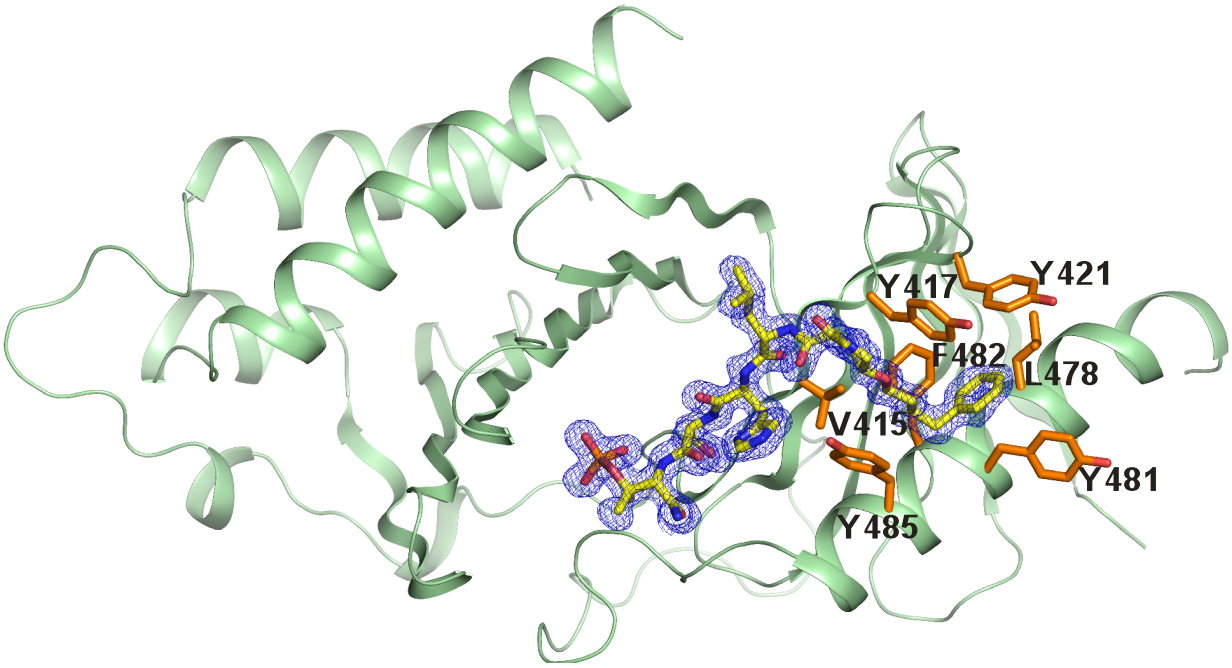


Fig. S12. SigmaA weighted 2Fo-Fc electron density map contoured at 1 σ and 1.55 Å resolution around peptide **7** ligand (stick rendering with yellow carbons). The PBD protein is shown in green cartoon rendering with hydrophobic side chains around the phenyl-alkyl substituent on peptide **7** shown in stick rendering with orange carbons.

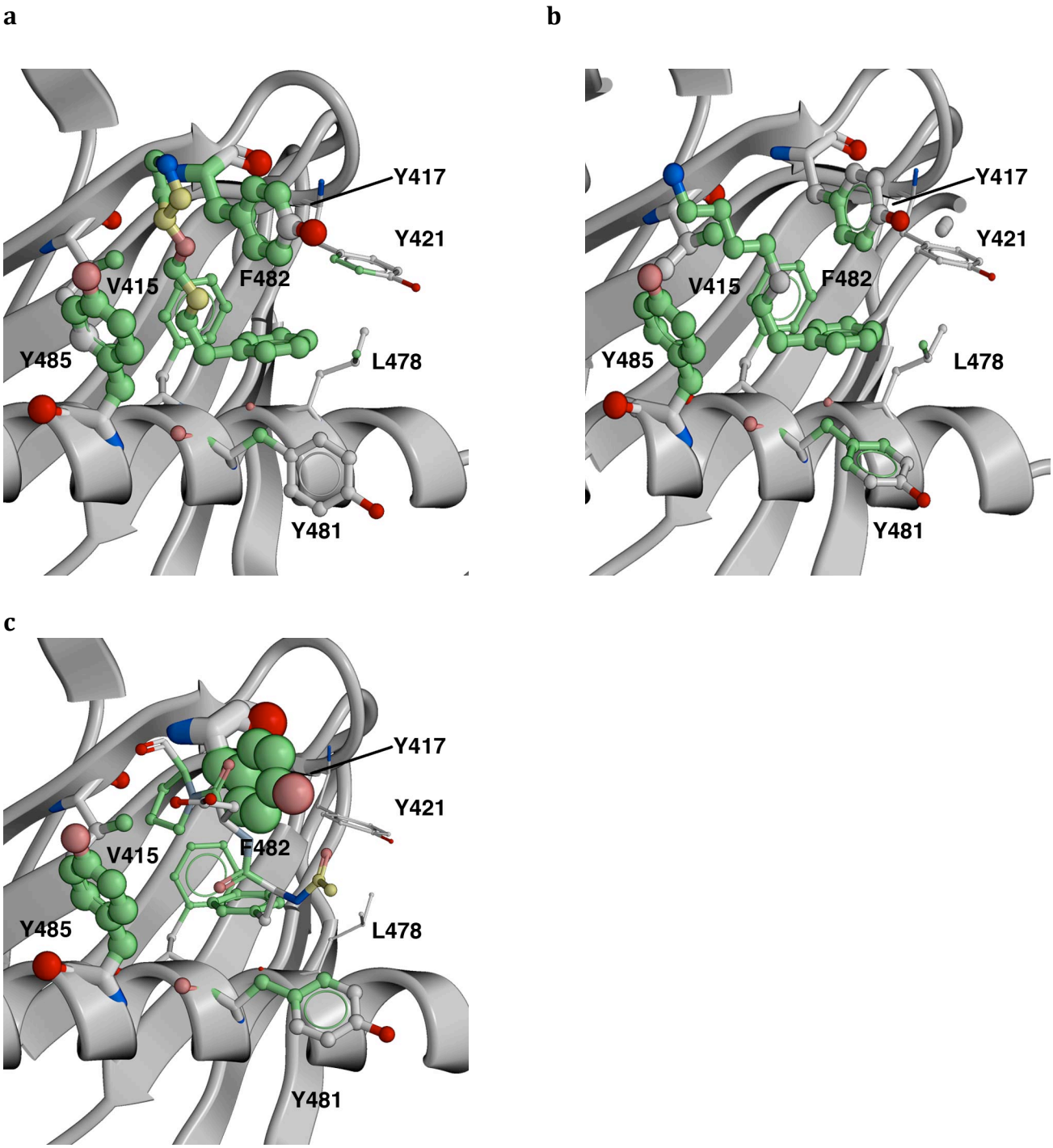


Fig. S13. Depiction of Plk1 PBD-bound ligands (a) **7**; (b) **10** PDB: 3RQ7) and (c) **11** (PDB: 3P37) showing contact regions for selected regions. Green = hydrophobic contact, with ball and stick size relative to degree of interaction. Graphics and numberical data (provided in Table S2) were produced using the Contact Residues option within ICM Chemist Pro by Molsoft, Inc. (www.molsoft.com). Final graphics were assembled using Adobe Photoshop CS4.

Table S4. Ligand – residue Contact Areas for Plk1 PBD-bound Peptides Shown in Fig. S13.[a]

Peptide	V415			Y417			Y421			Y481		
	CA	**EA**	**%**	**CA**	**EA**	**%**	**CA**	**EA**	**%**	**CA**	**EA**	**%**
7	34	78	44	48	171	28	10	189	5	28	186	15
10	40	86	46	36	146	24	8	181	4	26	199	13
11	32	82	40	**74**	**110**	**67**	8	125	6	30	191	15

Peptide	F482			Y485			L478		
	CA	**EA**	**%**	**CA**	**EA**	**%**	**CA**	**EA**	**%**
7	23	129	18	50	139	36	**14**	**146**	**10**
10	22	141	16	46	204	23	**17**	**147**	**12**
11	17	133	13	52	158	33	5	141	4

[a]Column Heading: CA (contact area); EA (exposed area); % (ratio of CA to EA). Data was generated using the Contact Residues option of ICM Chemist Pro by Molsoft, Inc. (www.molsoft.com).

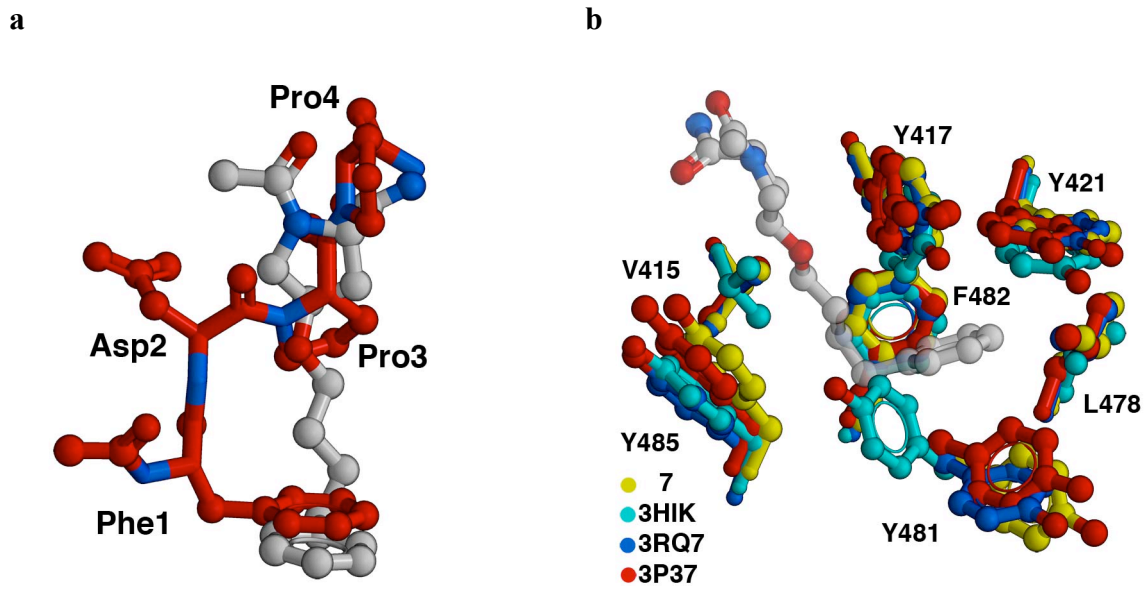
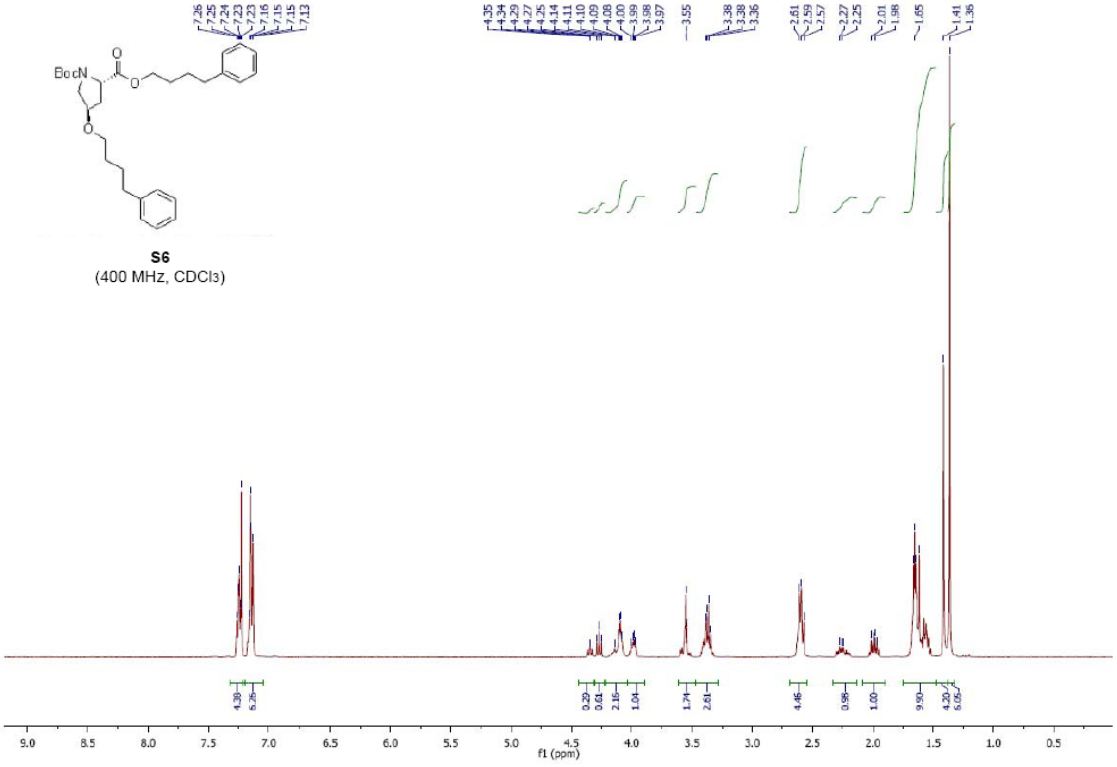


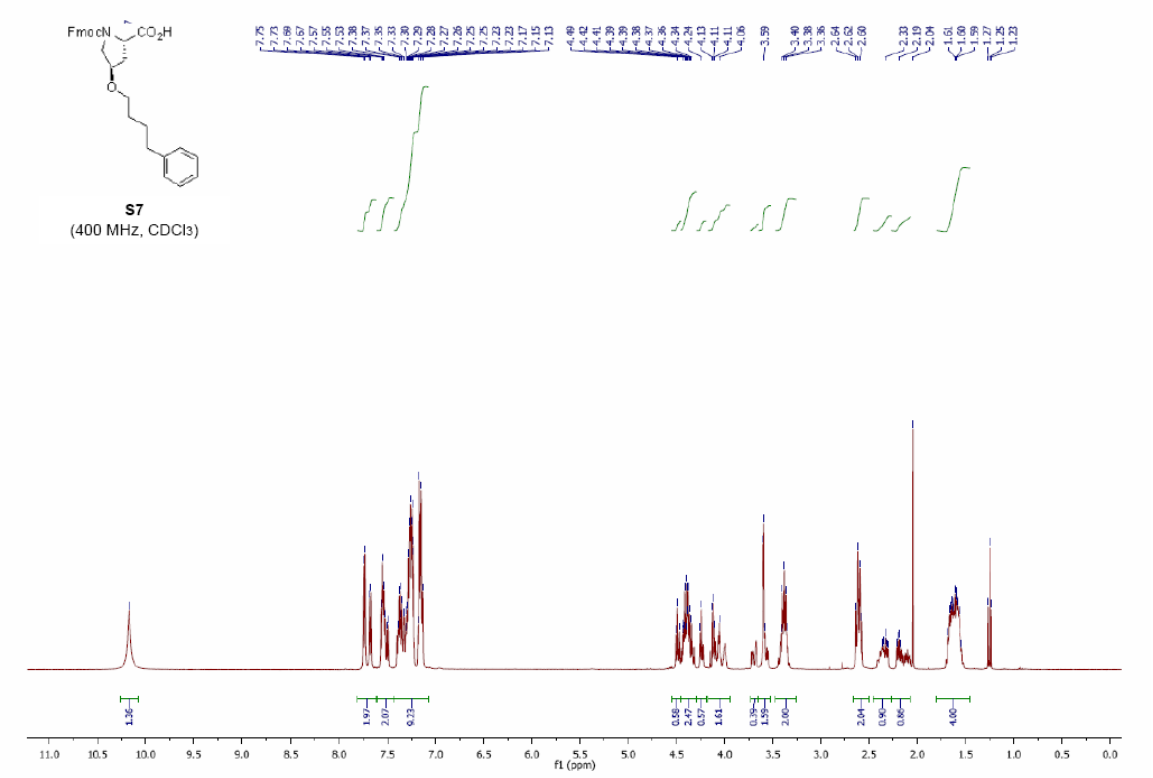
Fig. S14. Plk1 PBD interactions. (a) Overlay of the Ph-$(CH_2)_4$-O-Pro portion of peptide **7** (shown carbon = grey) with the *N*-terminal residues Phe-Asp-Pro-Pro of peptide **11** (PBD: 3P37, shown carbon = red). (b) Comparison of key side chain residues observed with the Plk1 PBD binding of **7** (yellow); **1** (PBD: 3HIK, cyan); **10** (PBD: 3RQ7, blue) and **11** (PBD: 3P37, red). The Ph-$(CH_2)_4$-O-Pro portion of peptide **7** is shown carbon with carbon = grey, nitrogen = blue and oxygen = red. Superposition was generated using ICM Chemist Pro by Molsoft, Inc. (www.molsoft.com). Final graphics were assembled using Adobe Photoshop CS4.

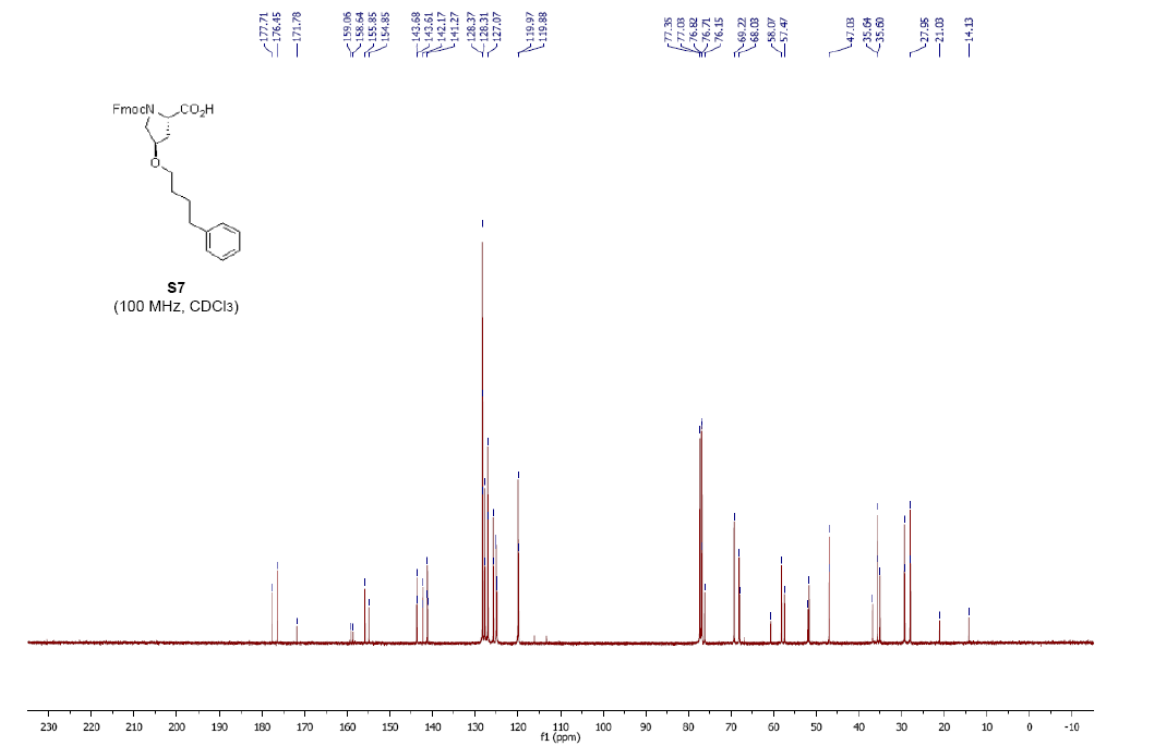
^{1}H and ^{13}C NMR spectra of s6, s7, s9, s10 and Selected HPLC Traces of Peptides 2, 3, 4b, 5b, 7 & 8.

^{1}H Spectrum of s6

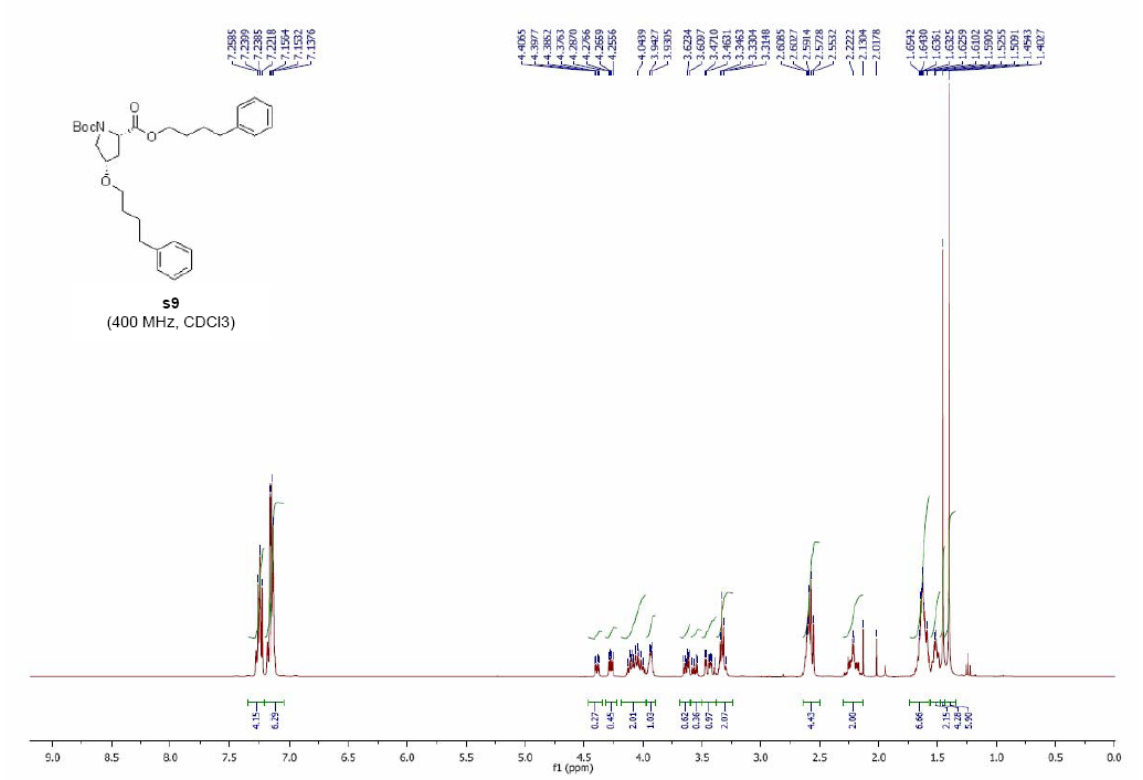


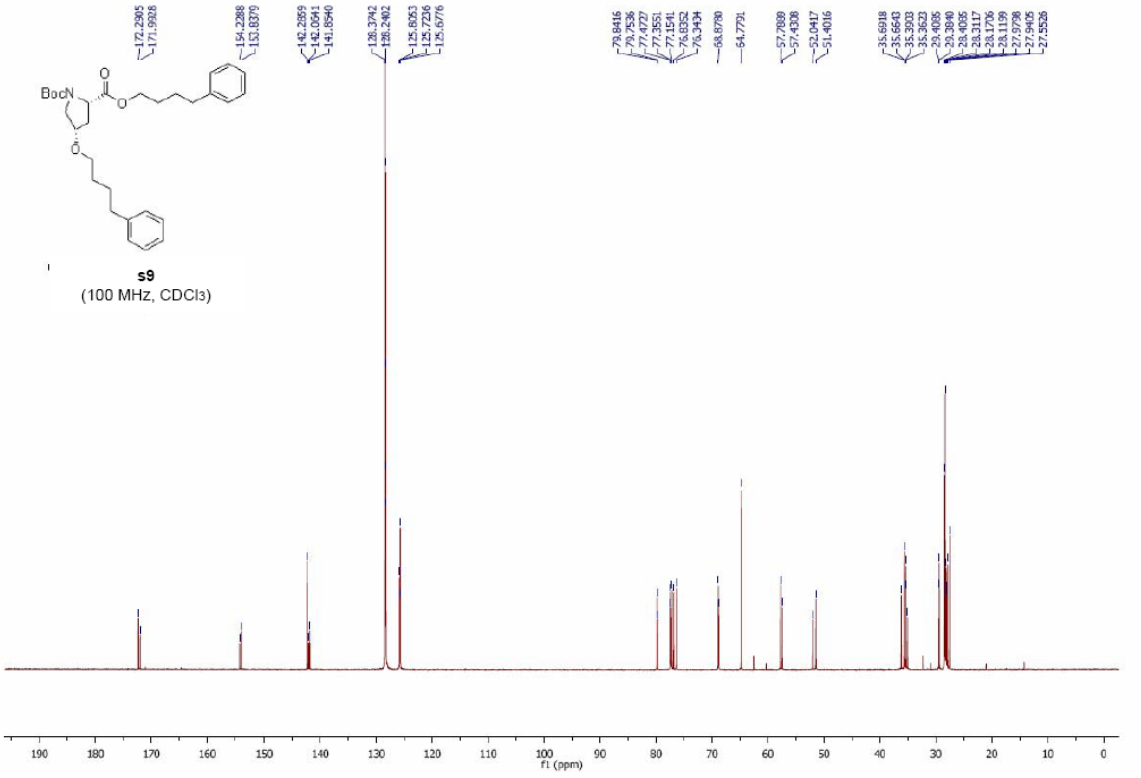
^{1}H and ^{13}C NMR Spectra of s7



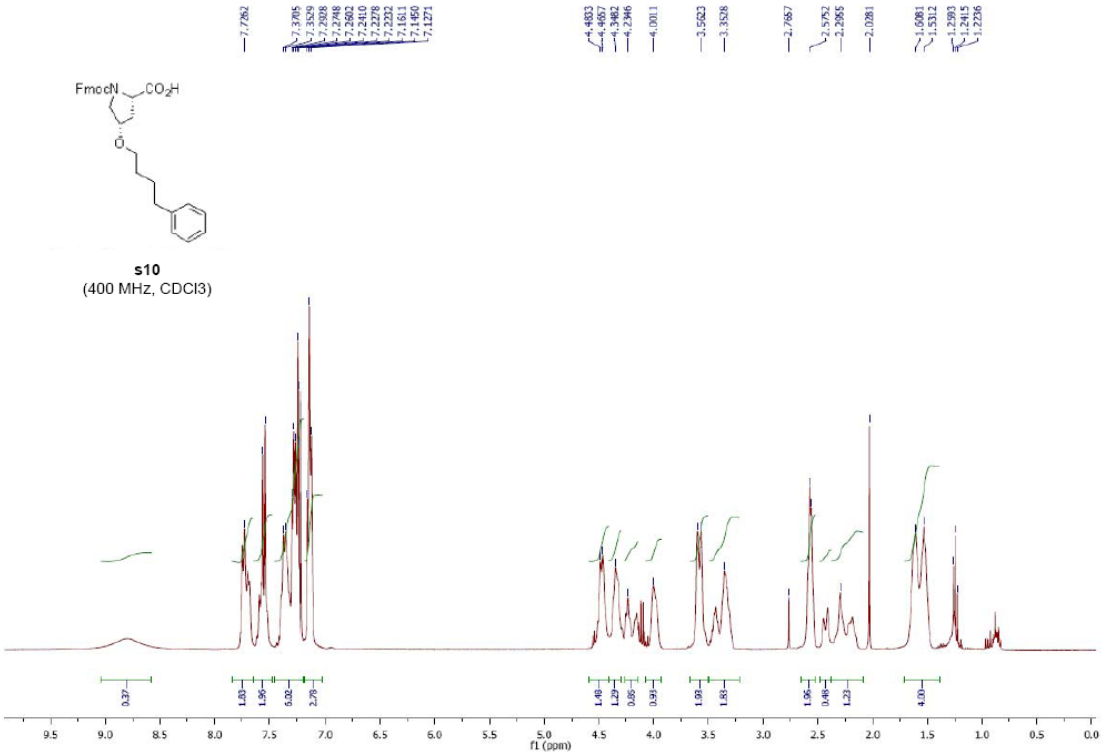


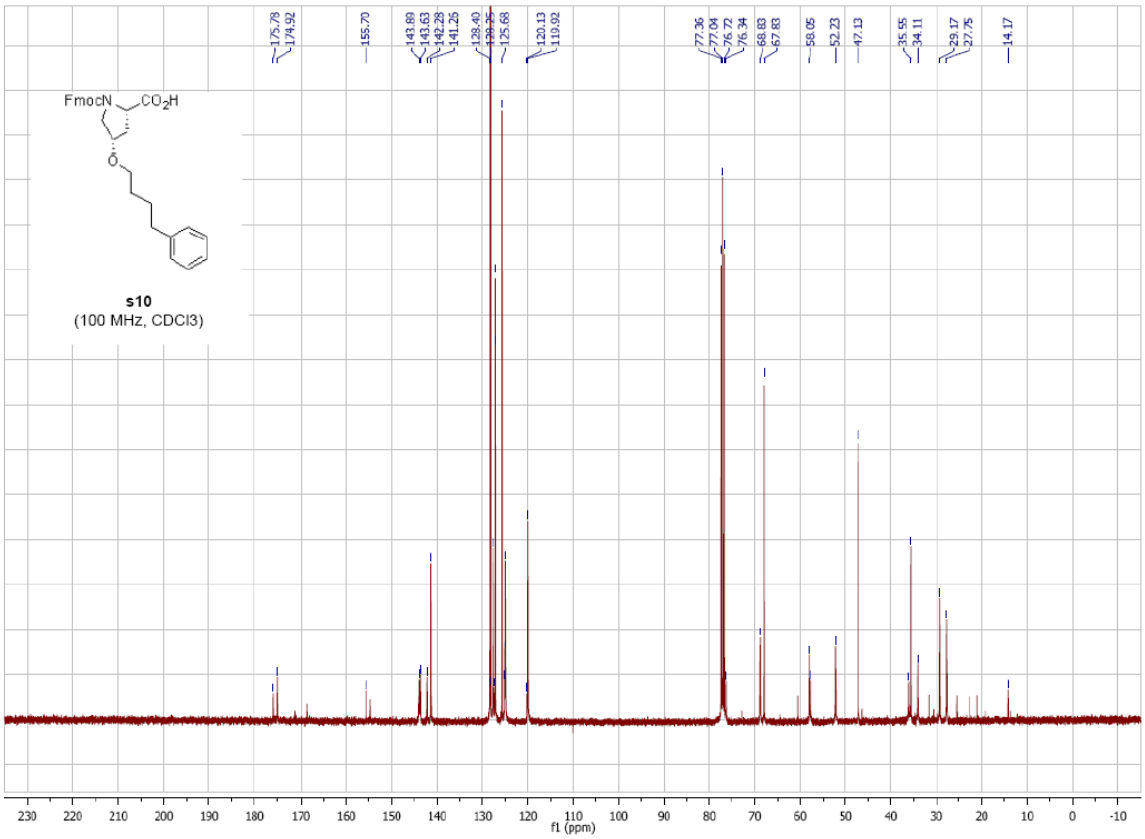
^{1}H and ^{13}C NMR Spectra of s9





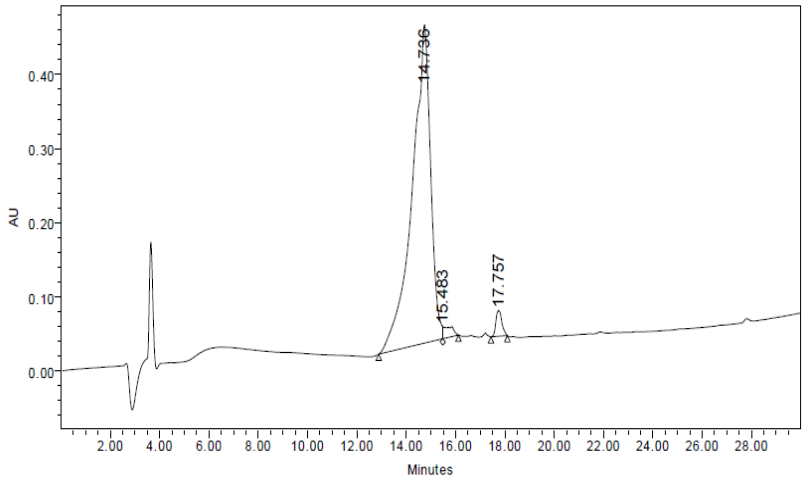
^{1}H and ^{13}C NMR Spectra of s10





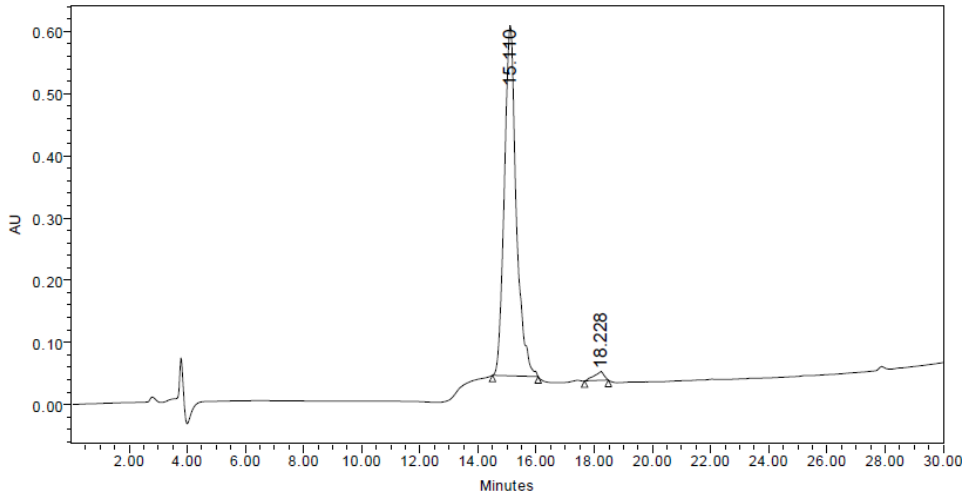
Selected analytical HPLC Traces for Peptides 2, 3, 4b, 5b, 7 & 8.

Peptide 2



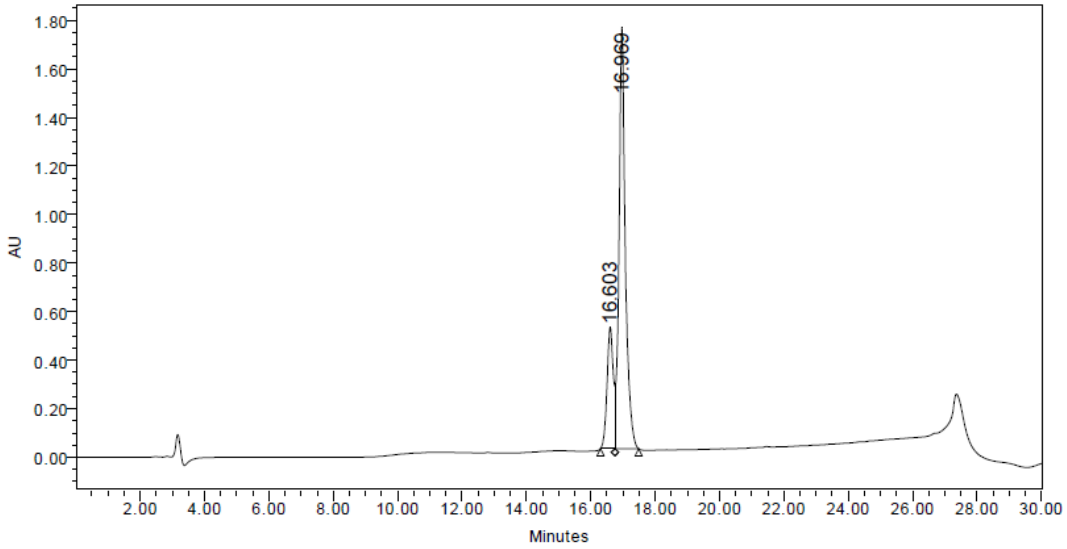
	RT	Area	% Area	Height
1	14.736	22258539	95.80	429580
2	15.483	392369	1.69	16134
3	17.757	582561	2.51	34151

Peptide 3



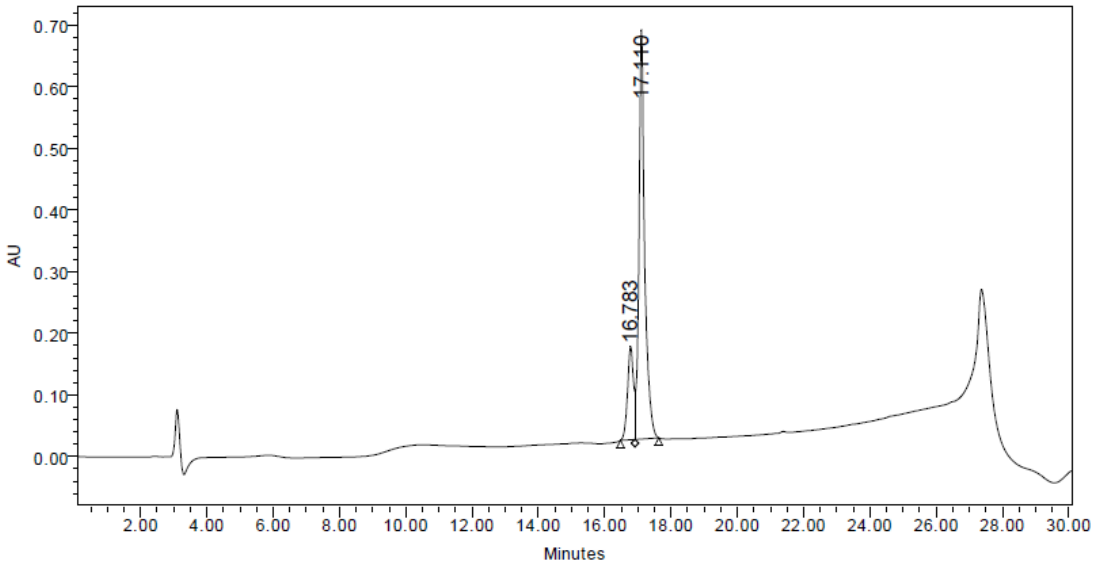
	RT	Area	% Area	Height
1	15.110	16047639	97.99	563486
2	18.228	328993	2.01	13746

Peptide 4b (2 peaks are oxime bond isomers)



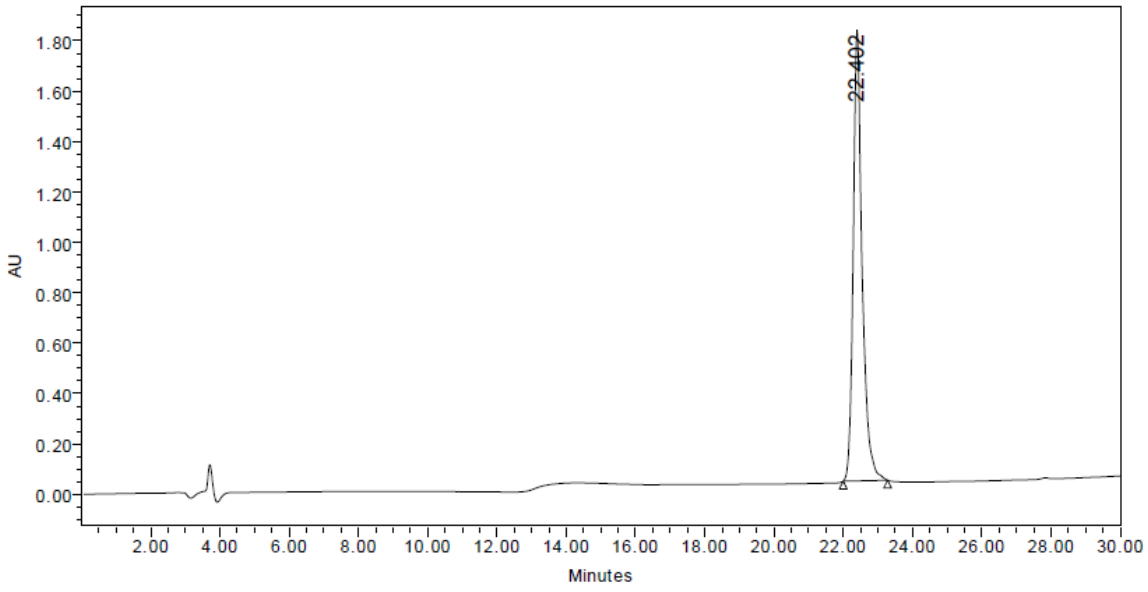
	RT	Area	% Area	Height
1	16.603	6677133	21.91	500786
2	16.969	23803849	78.09	1734952

Peptide 5b (2 peaks are oxime bond isomers)



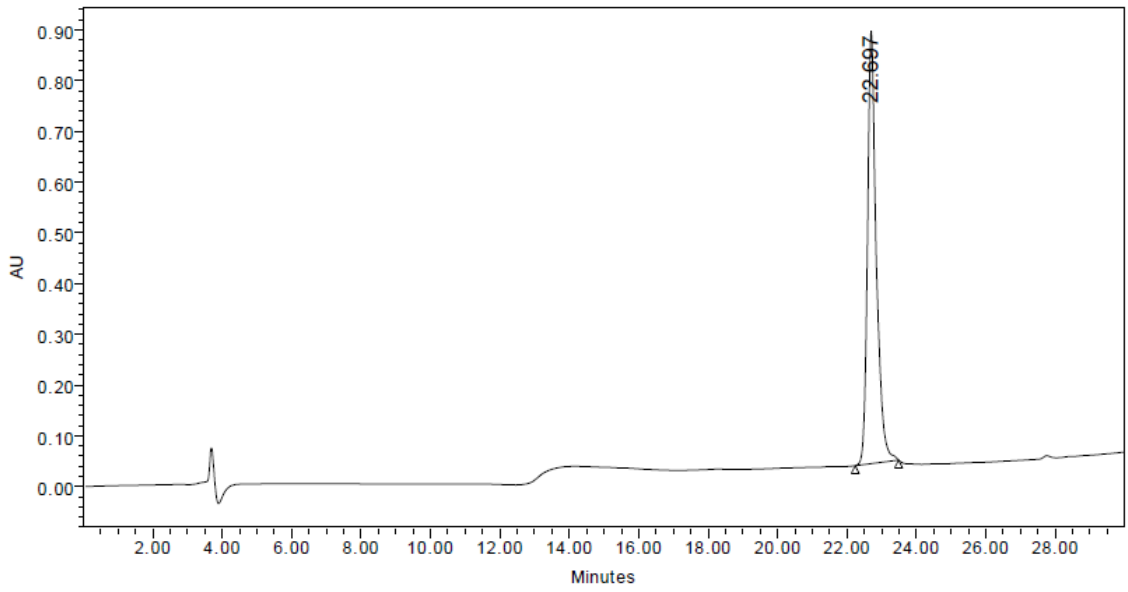
	RT	Area	% Area	Height
1	16.783	1852006	18.80	151572
2	17.110	8001419	81.20	663343

Peptide 7



	RT	Area	% Area	Height
1	22.402	32062388	100.00	1788908

Peptide 8



	RT	Area	% Area	Height
1	22.697	15023199	100.00	852355

Supplementary References

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