Supporting Information

Application of oxime-diversification to optimize ligand interactions within a cryptic pocket of the polo-like kinase 1 polo-box domain

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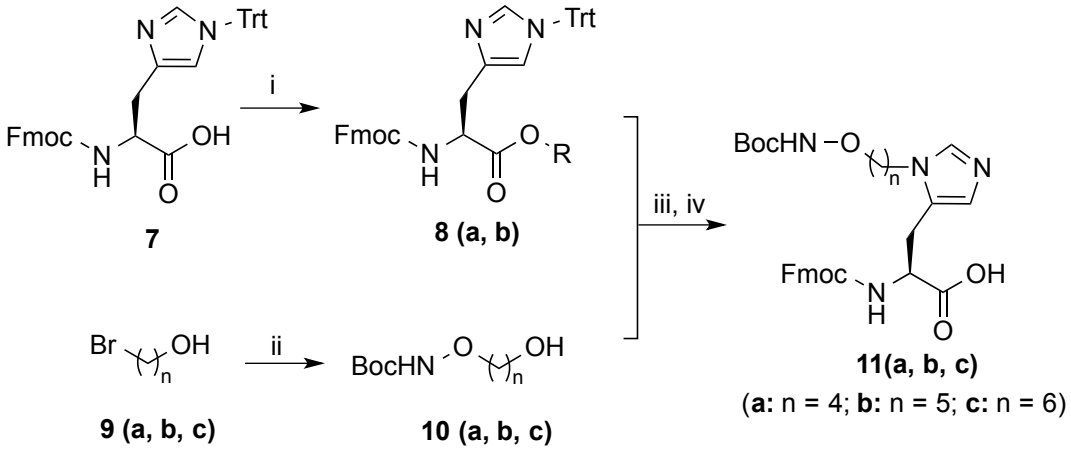
Synthesis

General synthetic. ^{1}H and ^{13}C NMR data were obtained on a Varian 400 MHz spectrometer or a Varian 500 MHz spectrometer and are reported in ppm relative to TMS and referenced to the solvent in which the spectra were collected. Solvent was removed by rotary evaporation under reduced pressure and anhydrous solvents were obtained commercially and used without further drying. Purification by silica gel chromatography was performed using Combiflash with EtOAc–hexanes or CH_2Cl_2-MeOH solvent systems. Electrospray ionization-mass spectrometra (ESI-MS) were acquired with an Agilent LC/MSD system equipped with a multimode ion source. High resolution mass spectrometric (HRMS) were acquired with a LTQ-Orbitrap-XL at 30 K resolution by LC/MS-ESI.

Synthesis of *N*-Fmoc-His-[*N(π)*–$(CH_2)_n$–O–NHBoc]-OH (11a – 11c)



Scheme S1. Synthesis of *N*-Fmoc-His-[*N(π)*–$(CH_2)_n$–O–NHBoc]-OH (**11a – 11c**). *Reagents and Conditions:* i) DIAD, PPh_3, BnOH (**a**) or 2,4-di-MeO-BnOH (**b**); ii) BocNHOH, DBU, CH_2Cl_2; iii) Tf_2O, Fmoc-His[*N(τ)*-Trt]-OBn (**8a**), DIEA, CH_2Cl_2; iv) H_2, Pd/C (10%), MeOH.

General procedure A (Mitsunobu Reaction) to prepare benzyl esters of *N*-Fmoc-His 8(a, b).[1] (*E*)-diisopropyl diazene-1,2-dicarboxylate (DIAD, 6.35 mL, 32.3 mmol) was added to a solution of PPh_3 (8.46 g, 32.3 mmol) in dry THF (100 mL) at 0° C under argon and the mixture was stirred (approximately 3 minutes) to yield a white suspension. Stirring was continued at 0° C (10 minutes) and then benzyl alcohol (32.3 mmol) was

added and the white suspension was stirred (1 h). A solution of *N*-Fmoc-His(Trt)-OH (**19**, 10 g, 16.14 mmol) in THF (100 mL) was added and the resulting clear solution was stirred (3 h). The mixture was concentrated and purified by silica gel chromatography to afford **8(a, b)** as sticky oils.

General procedure B to prepare *N*-Boc protected aminoxyl alcohols 10(a-c).[2] DBU (39 mmol) was added dropwise to a solution of *tert*-butyl hydroxycarbamate (36 mmol) and related bromides **9(a-c)** (33mmol) in CH_2Cl_2 (5 mL) at room temperature. The reaction mixture was stirred at room temperature overnight. The mixture was purified by silica gel chromatography. *N*-Boc protected aminoxyl alcohols **10(a-c)** were afforded.

General procedure C to prepare *N*(π)-alkylated His residues 11 (a-c) and 21(a-f).[3] According to our former report, a solution of alcohols **10**, **14** or **20** (2 mmol) and DIEA (2 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise to a solution of trifluoromethanesulfonic anhydride (2.0 mmol, 1.0 M in CH_2Cl_2) in CH_2Cl_2 (2.5 mL) under argon at -78° C and stirred (20 minutes). A solution of benzyl N^{α}-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^{τ}-trityl-L-histidinate **8a** (2.0 mmol for **11**) or 2,4-dimethoxybenzyl N^{α}-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^{τ}-trityl-L-histidinate **8b** (2.0 mmol for **21**) in CH_2Cl_2 (5 mL) was added at -78° C and the mixture was stirred and allowed to come to room temperature and stirred (overnight), then solvent was removed by evaporation. For **11**, a solution in MeOH was hydrogenated over Pd•C and the resultant mixture was filtered and the filtrate was concentrated. Deprotection of **21** was achieved using TFA:TIS (10:1, 10 mL). The resulting products were purified by silica gel chromatography to afford the *N*(π)-alkylated His residues **11 (a-c)** or **21(a-f)**.

Benzyl N^{α}-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^{τ}-trityl-L-histidinate (8a). Treatment of *N*-Fmoc-His(Trt)-OH (**7**) and benzyl alcohol as outlined in general procedure A provided **8a** as a viscous syrup in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 7.6 Hz, 2H), 7.66 (t, J = 6.9 Hz, 2H), 7.46 – 7.30 (m, 18H), 7.17 – 7.11 (m, 7H), 6.68 (d, J = 8.2 Hz, 1H), 6.56 (s, 1H), 5.10 (dd, J = 12.9, 22.7 Hz, 2H), 4.75 - 4.70 (m, 1H), 4.37 – 4.26 (m, 3H), 3.15 - 3.07 (m, 2H). ESI-MS m/z: 710.3 (MH^+).

2,4-Dimethoxybenzyl N^{α}-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^{τ}-trityl-L-histidinate (8b). Treatment of *N*-Fmoc-His(Trt)-OH (**7**) and benzyl alcohol as outlined in

general procedure A provided **8b** as a viscous syrup in 51% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.6 Hz, 2H), 7.64 (dd, J = 7.5, 4.4 Hz, 2H), 7.43 - 7.30 (m, 14H), 7.15-7.13 (m, 6H), 7.08 (d, J = 8.2 Hz, 1H), 6.61 (s, 1H), 6.54 (d, J = 8.3 Hz, 1H), 6.39 (d, J = 2.3 Hz, 1H), 6.33 (dd, J = 8.4, 2.4 Hz, 1H), 5.10 - 4.96 (m, 2H), 4.69 – 4.64 (m, 1H), 4.40 – 4.36 (m, 1H), 4.31 - 4.22 (m, 2H), 3.76 (s, 3H), 3.66 (s, 3H), 3.11 – 3.08 (m, 2H). ESI-MS m/z: 770.3 (MH^+).

***tert*-Butyl 4-hydroxybutoxycarbamate (10a).** Treatment of commercially available 4-bromobutan-1-ol (**9a**) as outlined in general procedure B provided compound **10a** as a colorless oil in 36% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (bs, 1H), 3.90 (t, *J* = 5.9 Hz, 2H), 3.69 (t, *J* = 5.9 Hz, 2H), 1.75 – 1.67 (m, 4H), 1.48 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 157.08, 81.76, 76.67, 62.36, 29.35, 28.21 (3C), 24.57.

***tert*-Butyl (5-hydroxypentyl)oxycarbamate (10b).** Treatment of commercially available 5-bromopentan-1-ol (**9b**) as outlined in general procedure B provided compound **10b** as a colorless oil in 28% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H), 3.84 (t, *J* = 6.4 Hz, 2H), 3.63 (t, *J* = 6.4 Hz, 2H), 2.15 (s, 1H), 1.68 - 1.55 (m, 4H), 1.47 (s, 9H), 1.49 - 1.44 (m, 2H).13C NMR (101 MHz, CDCl3) δ 157.00, 81.55, 76.61, 62.41, 32.30, 28.22 (3C), 27.64, 22.07.

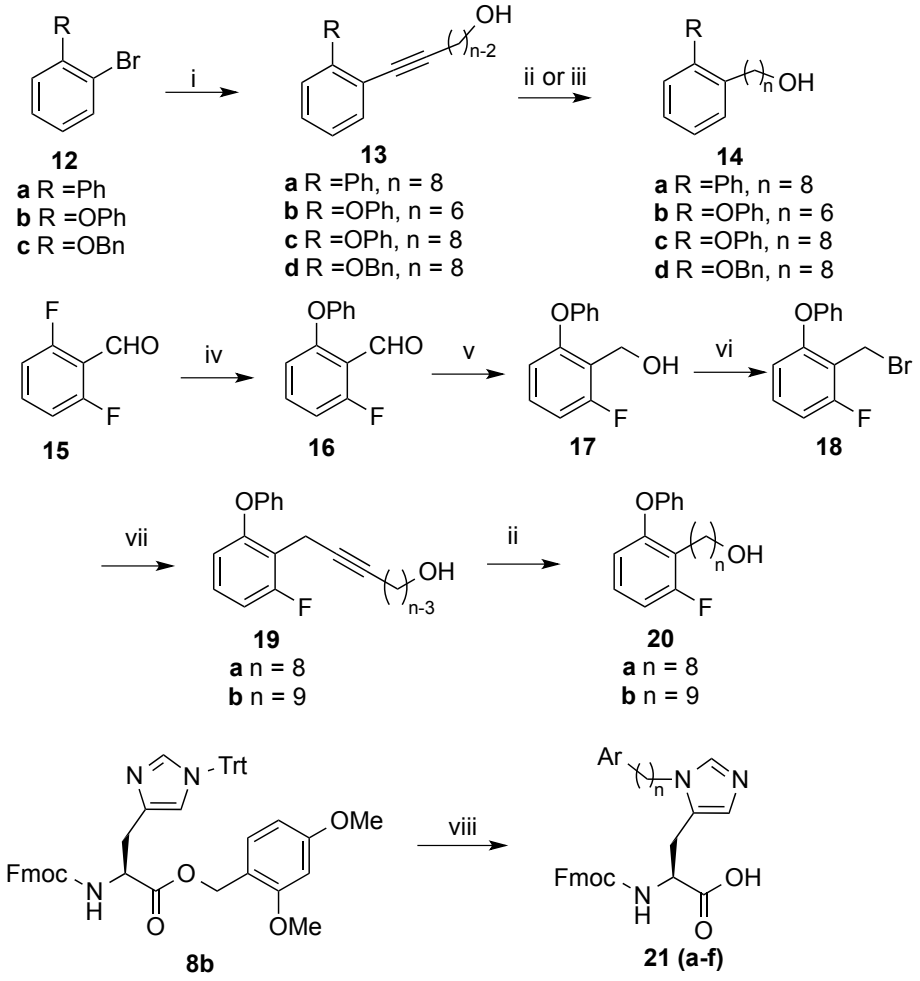
***tert*-Butyl (6-hydroxyhexyl)oxycarbamate (10c).** Treatment of commercially available 6-bromohexan-1-ol (**9c**) as outlined in general procedure B provided compound 1**0c** as a colorless oil in 56% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (s, 1H), 3.87 (t, J = 6.5 Hz, 2H), 3.66 (t, J = 6.5 Hz, 2H), 1.67-1.64 (m, 2H), 1.62 – 1.58 (m, 2H), 1.50 (s, 9H), 1.46-1.40 (m, 5H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.99, 81.45, 77.30, 62.48, 32.45, 28.20 (3C), 27.89, 25.57, 25.42. ESI-MS m/z: 234.2 (MH^+).

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(4-(((*tert*-butoxycarbonyl)amino)oxy)butyl)-L-histidine (11a).** Compound **11a** was prepared in 52% yield from **8a** and **10a** according to general procedure C. ESI-MS m/z: 565.2 (MH^+).

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(5-(((*tert*-butoxycarbonyl)amino)oxy)pentyl)-L-histidine (11b).** Compound **11b** was afforded in 19% yield from **8a** and **10b** according to general procedure C. ESI-MS m/z: 579.3 (MH^+).

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(6-(((*tert*-butoxycarbonyl)amino)oxy)hexyl)-L-histidine (11c).** Compound **11c** was afforded in 24% yield from **8a** and **10c** according to general procedure C. ESI-MS m/z: 593.3 (MH^+).

Synthesis of *N*-Fmoc-His-[*N(π)*–CH_2]$_n$–Aryl]-OH (21a – 21f)



Scheme S2. Synthesis of *N*-Fmoc-His-[N(π)–CH_2]$_n$–Aryl]-OH (**21a – 21f**). *Reagents and Conditions:* i) CH≡CH-$(CH_2)_{n-2}$OH, $Pd(PPh_3)_2Cl_2$, CuI, 70°C; ii) H_2, Pd/C; iii) H_2, $(Ph_3P)_3RhCl$; iv) PhOH, K_2CO_3, DMA, 165°C; v) $NaBH_4$, MeOH; vi) CBr_4, PPh_3, CH_3CN; vii) CuI, TBAI, Cs_2CO_3, CH≡CH-$(CH_2)_{n-3}$OH; viii) Tf_2O, Ar-$(CH_2)_n$-OH (**14a-14d, 20a, b**), DIEA, CH_2Cl_2then TFA, TIS, CH_2Cl_2.

General procedure D to prepare alcohols 13(a-d) under Sonogashira conditions.[4] To a solution of bromide **12(a-c)** (0.3 mmol) and

bis(triphenylphosphine)palladium (II) dichloride (9 μmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0.3 mmol) and copper (I) iodide (0.03 mmol) in DMF (1.5 ml) was added the appropriate alkyne related alkyne (0.45 mmol) and the vessel was flushed with argon, sealed and heated (70° C, overnight). The resulting dark mixture was cooled to room temperature and purified by silica gel chromatography to afford **13(a-d)**.

General procedure E to prepare alcohols 14(a-c) and 20(a, b) using Pd•C. To a solution of alkyne **13(a-c)** or **19(a, b)** (10 mmol) in MeOH (10 mL) was added Pd•C (30 mg, 10%) and the mixture was hydrogenated under H_2. When all starting material had been consumed (TLC), the reaction mixture collected by filtration and concentrated. The resulting residue was purified by silica gel chromatography to afford alcohols **14(a-c)** or **20(a, b)**.

General procedure F to prepare alcohols 19 (a,b) using coupling reaction.[5,6] To a solution of benzyl bromide (**18**, 10 mmol) in acetonitrile (30 mL) under argon was added copper (I) iodide (10 mmol), cesium carbonate (11 mmol), and tetrabutylammonium iodide (10 mmol). Terminal alkynes (15 mmol) were then added dropwise and the reaction mixture was heated (75° C, overnight). The mixture was cooled to room temperature and extracted (EtOAc) and the extracts washed (aqueous NH_4Cl, then brine), dried (Na_2SO_4) and concentrated. Purification (silica gel chromatography) provided alcohols **19(a, b)**.

8-([1,1'-Biphenyl]-2-yl)oct-7-yn-1-ol (13a). Treatment of **12a** and oct-7-yn-1-ol as outlined in general procedure D provided **13a** as a brown oil in 43% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62-7.60 (m, 2H), 7.53 (d, J = 7.6 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.38 - 7.33 (m, 3H), 7.28 (td, J = 7.4, 1.8 Hz, 1H), 3.64 (t, J = 6.7 Hz, 2H), 2.32 (t, J = 6.9 Hz, 2H), 1.62 - 1.32 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.61, 140.83, 132.99, 129.41, 129.29 (2C), 127.77 (2C), 127.73, 127.18, 126.92, 122.31, 93.25, 80.25, 62.93, 32.62, 28.50, 28.35, 25.29, 19.44.

6-(2-Phenoxyphenyl)hex-5-yn-1-ol (13b). Treatment of **12b** and hex-5-yn-1-ol as outlined in general procedure D provided **13b** as a brown oil in 66% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.35 - 7.31 (m, 2H), 7.26 - 7.24 (m, 1H), 7.11 - 7.07 (m, 2H), 6.99 - 6.95 (m, 3H), 3.59 (t, *J* = 6.1 Hz, 2H), 2.40 (t, *J* = 6.4 Hz, 2H), 1.61 - 1.54 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.70, 156.94, 133.62, 129.57 (2C), 129.02, 123.74,

122.66, 119.81, 117.81 (2C), 116.85, 95.18, 76.53, 62.39, 31.68, 24.68, 19.32. DUIS-MS m/z: 267 (MH^+).

8-(2-Phenoxyphenyl)oct-7-yn-1-ol (13c). Treatment of **12b** and oct-7-yn-1-ol as outlined in general procedure D provided **13c** as a yellow oil in 34% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, J = 7.6, 1.7 Hz, 1H), 7.24 (dd, J = 8.6, 7.3 Hz, 2H), 7.19 - 7.13 (m, 1H), 6.99 (t, J = 7.5 Hz, 2H), 6.89 (d, J = 7.5 Hz, 2H), 6.85 (d, J = 8.2 Hz, 1H), 3.53 (t, J = 6.6 Hz, 2H), 2.26 (t, J = 6.8 Hz, 2H), 1.48 - 1.36 (m, 4H), 1.33 - 1.21 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.65, 157.13, 133.64, 129.53 (2C), 128.87, 123.58, 122.74, 119.60, 118.02 (2C), 116.85, 95.47, 76.23, 62.95, 32.60, 28.43, 28.41, 25.21, 19.50. ESI-MS m/z: 295.2 (MH^+).

8-(2-(Benzyloxy)phenyl)oct-7-yn-1-ol (13d). Treatment of **12c** and oct-7-yn-1-ol as outlined in general procedure D provided **13d** as a yellow oil in 16% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.1 Hz, 2H), 7.42 - 7.37 (m, 3H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.21 (td, *J* = 8.3, 1.7 Hz, 1H), 6.93 - 6.90 (m, 2H), 5.17 (s, 2H), 3.63 (t, *J* = 6.6 Hz, 2H), 2.49 (t, *J* = 7.0 Hz, 2H), 1.68 - 1.62 (m 2H), 1.59 - 1.48 (m, 4H), 1.43 - 1.36 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.13, 137.17, 133.43, 128.75, 128.41 (2C), 127.68, 126.94 (2C), 120.84, 114.08, 112.84, 94.64, 76.83, 70.41, 62.91, 32.64, 28.73, 28.62, 25.29, 19.69. ESI-MS m/z: 309.2 (MH^+).

8-([1,1'-Biphenyl]-2-yl)octan-1-ol (14a). Treatment of **13a** as outlined in general procedure E provided **14a** as slightly a yellow oil in 97% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 - 7.30 (m, 2H), 7.27 - 7.23 (m, 1H), 7.24 - 7.19 (m, 4H), 7.16 - 7.09 (m, 2H), 3.52 (t, J = 6.7 Hz, 2H), 2.50 - 2.47 (m, 2H), 1.46 - 1.41 (m, 2H), 1.39 - 1.34 (m, 2H), 1.21 - 1.15 (m, 2H), 1.14 - 1.08 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 142.04, 141.82, 140.31, 130.02, 129.25 (2C), 129.21, 127.98 (2C), 127.31, 126.70, 125.54, 63.06, 32.98, 32.76, 31.29, 29.32, 29.22 (2C), 25.68.

6-(2-Phenoxyphenyl)hexan-1-ol (14b). Treatment of **13b** as outlined in general procedure E provided **14b** as a colorless oil in 65% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 - 7.31 (m, 2H), 7.28 - 7.27 (m, 1H), 7.20 - 7.16 (m, 1H), 7.12 - 7.06 (m, 2H), 6.95 - 6.90 (m, 3H), 3.62 (t, J = 6.6 Hz, 2H), 2.66 - 2.63 (m, 2H), 1.67 - 1.61 (m, 2H), 1.58 - 1.52 (m, 2H), 1.39 - 1.36 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.12, 154.32, 134.38, 130.58, 129.63 (2C), 127.11, 123.90, 122.40, 119.75, 117.56 (2C), 63.00, 32.67, 30.07, 29.99, 29.14, 25.47.

8-(2-Phenoxyphenyl)octan-1-ol (14c). Treatment of **13c** as outlined in general procedure E provided **14c** as a colorless oil in 79% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (t, J = 8.0 Hz, 2H), 7.29 - 7.27 (m, 1H), 7.18 (td, J = 7.6, 1.7 Hz, 1H), 7.09 (dt, J = 14.8, 7.4 Hz, 2H), 6.95 (d, J = 7.8 Hz, 2H), 6.91 (d, J = 8.0 Hz, 1H), 3.64 (t, J = 6.7 Hz, 2H), 2.64 (dd, J = 8.9, 6.7 Hz, 2H), 1.64 - 1.60 (m, 2H), 1.59 - 1.53 (m, 2H), 1.37 - 1.29 (m, 8H).^{13}C NMR (126 MHz, $CDCl_3$) δ 158.14, 154.34, 134.52, 130.58 (2C), 129.62, 127.05, 123.87, 122.38, 119.73, 117.57 (2C), 63.07, 32.78, 30.09 (2C), 29.35(2C), 29.29, 25.70. ESI-MS m/z: 299.2 (MH^+).

8-(2-(Benzyloxy)phenyl)octan-1-ol (14d).[7] Wilkinson's catalyst tris(triphenylphosphine)rhodium(I) chloride (0.16 g, 0.17 mmol) was added to a solution of 8-(2-(benzyloxy)phenyl)oct-7-yn-1-ol (**11d,** 0.52 g, 1.69 mmol) in tBuOH:THF (1:1, 10 mL). The solution was stirred under H_2 at room temperature (overnight) and the resulting mixture was concentrated and the residue was purified by silica gel chromatography to afford **14d** as a colorless oil (410 mg, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, J = 7.6 Hz, 2H), 7.44 (t, J = 7.5 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.20 (t, J = 8.4 Hz, 2H), 6.95 (t, J = 7.3 Hz, 2H), 5.13 (s, 2H), 3.66 (t, J = 6.7 Hz, 2H), 2.77 - 2.68 (m, 2H), 1.75 (s, 1H), 1.71 - 1.65 (m, 2H), 1.60 - 1.56 (m, 2H), 1.42 - 1.34 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.58, 137.63, 131.73, 129.97, 128.51(2C), 127.70, 127.04 (2C), 126.80, 120.67, 111.67, 69.81, 63.08, 32.79, 30.37, 29.97, 29.56, 29.51, 29.41, 25.77. APCI-MS m/z: 313.2 (MH^+), 345.3 (MNa^+).

2-Fluoro-6-phenoxybenzaldehyde (16). To a solution of 2,6-difluorobenzaldehyde (**15,** 11.2 mL 102 mmol) and phenol (9.6 g, 102 mmol) in dimethylacetamide (DMA) (50 mL) was added potassium carbonate (14.1 g, 102 mmol) and the mixture was stirred at reflux (2 h). The reaction mixture was brought to room temperature and partitioned between H_2O (100 ml) and CH_2Cl_2, dried (Na_2SO_4), concentrated and purified by silica gel chromatography to provide **16** as a colorless oil (14.1 g, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (s, 1H), 7.47 - 7.40 (m, 3H), 7.23 (t, J = 7.4 Hz, 1H), 7.09 (dd, J = 8.6, 1.2 Hz, 2H), 6.89 - 6.85 (m, 1H), 6.66 (d, J = 8.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.79 (1C, d, J = 2.3 Hz), 162.90 (1C, d, J = 263.4 Hz), 160.50 (1C, d, J = 5.2 Hz), 155.63, 135.73 (1C, d, J = 11.6 Hz), 130.19 (2C), 124.90, 119.85 (2C), 116.03 (1C, d, J = 9.5 Hz), 113.48 (1C, d, J = 3.7 Hz), 110.81 (d, J = 21.2 Hz). ESI-MS m/z: 239.0 ($M+Na^+$).

(2-Fluoro-6-phenoxyphenyl)methanol (17). To a solution of **16** (8.1 g, 37.6 mmol) in MeOH (100 mL) was added portion-wise $NaBH_4$ (1.4 g, 37.6 mmol) at 0° C and the resulting mixture was stirred at 0 °C (30 minutes). The mixture was concentrated, partitioned between EtOAc and brine, dried (Na_2SO_4), concentrated and the resulting residue purified by silica gel chromatography to yield **17** as a colorless oil (7.8 g, 95% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 - 7.37 (m, 2H), 7.23 - 7.16 (m, 2H), 7.06 - 7.04 (m, 2H), 6.86 (t, J = 8.9 Hz, 1H), 6.66 (d, J = 8.3 Hz, 1H), 4.85 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.70 (1C, d, *J* = 247.3 Hz), 156.95 (1C, d, *J* = 7.4 Hz), 156.62, 129.98 (2C), 129.57 (1C, d, *J* = 10.5 Hz), 124.02, 119.41 (1C, d, *J* = 18.0 Hz), 119.04 (2C), 113.77 (1C, d, *J* = 3.3 Hz), 110.48 (1C, d, *J* = 22.6 Hz), 54.02 (1C, d, *J* = 5.1 Hz).

2-(Bromomethyl)-1-fluoro-3-phenoxybenzene (18). To a solution of **17** (9.1 g, 41.7 mmol) in acetonitrile (100 mL) was added triphenylphosphine (16.4 g, 62.5 mmol). The resulting suspension was cooled to 0° C and CBr_4 (20.7 g, 62.5 mmol) was added and the miture was stirred at room temperature (30 minutes). The mixture was diluted with EtOAc and then concentrated and purified by silica gel chromatography to provide **18** as a colorless oil (5.89 g, 50% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43-7.40 (m, 2H), 7.23 - 7.19 (m, 2H), 7.12 - 7.09 (m, 2H), 6.86 (t, J = 9.2 Hz, 1H), 6.63 (d, J = 8.4 Hz, 1H), 4.70 (d, J = 1.3 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.56 (1C, d, *J* = 250.5 Hz), 156.91 (1C, d, *J* = 6.4 Hz), 156.34, 130.16 (1C, d, *J* = 10.5 Hz), 129.98 (2C), 124.30, 119.61 (2C), 117.10 (1C, d, *J* = 17.2 Hz), 113.42 (1C, d, *J* = 3.2 Hz), 110.11 (1C, d, *J* = 21.6 Hz), 20.10 (1C, d, *J* = 5.4 Hz).

8-(2-Fluoro-6-phenoxyphenyl)oct-6-yn-1-ol (19a). Treatment of **18** and hept-6-yn-1-ol as outlined in general procedure F provided **19a** as a colorless oil in 46% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 - 7.33 (m, 2H), 7.19 - 7.12 (m, 2H), 7.03 - 7.00 (m, 2H), 6.89 - 6.84 (m, 1H), 6.68 (d, J = 8.3 Hz, 1H), 3.62 - 3.59 (m, 2H), 2.11 - 2.07 (m, 2H), 1.63 - 1.35 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.48 (d, J = 246.9 Hz), 157.31, 155.96 (d, J = 7.4 Hz), 129.67 (2C), 128.02 (d, J = 10.3 Hz), 123.31, 118.58 (2C), 117.64 (d, J = 18.1 Hz), 114.61 (d, J = 3.3 Hz), 110.57 (d, J = 22.3 Hz), 79.68, 76.94, 62.88, 32.26, 28.51, 24.90, 18.69, 13.01. ESI-MS m/z: 313.2 (MH^+).

9-(2-Fluoro-6-phenoxyphenyl)non-7-yn-1-ol (19b). Treatment of **18** and oct-7-yn-1-ol as outlined in general procedure F provided **19b** as a colorless oil in 38% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 - 7.28 (m, 2H), 7.17 - 7.08 (m, 2H), 7.01 - 6.98 (m, 2H), 6.87 -

6.82 (m, 1H), 6.66 (d, J = 8.3 Hz, 1H), 3.59 - 3.57 (m, 2H), 2.05 (tt, J = 7.0, 2.4 Hz, 2H), 1.62 - 1.26 (m, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.46 (d, *J* = 246.9 Hz), 155.94 (d, *J* = 7.3 Hz), 155.91, 129.65 (2C), 127.99 (d, *J* = 10.3 Hz), 123.31, 118.57 (2C), 117.64 (d, *J* = 18.1 Hz), 114.57 (d, *J* = 3.2 Hz), 110.54 (d, *J* = 22.3 Hz), 79.84, 76.79, 62.82, 32.58, 28.68, 28.49, 25.21, 18.64, 12.94. ESI-MS m/z: 327.2 (MH^+).

8-(2-Fluoro-6-phenoxyphenyl)octan-1-ol (20a). Treatment of **19a** as outlined in general procedure E provided **20a** as a colorless oil in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (dd, J = 8.6, 7.3 Hz, 2H), 7.13 - 7.08 (m, 2H), 6.99 (d, J = 8.0 Hz, 2H), 6.85 (t, J = 8.8 Hz, 1H), 6.68 (d, J = 8.2 Hz, 1H), 3.64 (t, J = 6.7 Hz, 2H), 2.73 - 2.69 (m, 2H), 1.69 (bs, 1H), 1.63 - 1.53 (m, 4H), 1.37 - 1.32 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.08 (d, *J* = 244.6 Hz), 157.59, 155.99 (d, *J* = 8.3 Hz), 129.75, 126.90 (d, *J* = 10.3 Hz), 123.06 (2C), 122.19 (d, *J* = 18.8 Hz), 118.16 (2C), 114.62 (d, *J* = 3.1 Hz), 110.47 (d, *J* = 23.1 Hz), 62.99, 32.77, 29.47, 29.37, 29.30, 29.28, 25.72, 23.05 (d, *J* = 2.7 Hz). ESI-MS m/z: 317.2 (MH^+).

9-(2-Fluoro-6-phenoxyphenyl)nonan-1-ol (20b). Treatment of **19b** as outlined in general procedure E provided **20b** as a colorless oil in 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 - 7.33 (m, 2H), 7.13 - 7.07 (m, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.84 (t, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 8.2 Hz, 1H), 3.64 (t, *J* = 6.6 Hz, 2H), 2.71 (t, *J* = 7.6 Hz, 2H), 1.89 (bs, 1H), 1.59 (dp, *J* = 13.6, 7.1 Hz, 4H), 1.35 - 1.29 (m, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.08 (d, *J* = 244.6 Hz), 157.58, 155.99 (d, *J* = 8.4 Hz), 129.74(2C), 126.88 (d, *J* = 10.4 Hz), 123.05, 122.20 (d, *J* = 18.8 Hz), 118.16 (2C), 114.60 (d, *J* = 3.1 Hz), 110.45 (d, *J* = 23.1 Hz), 62.98, 32.78, 29.49, 29.48, 29.43, 29.41, 29.26, 25.74, 23.05 (d, *J* = 2.7 Hz).

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(8-(2-phenylphenyl)octyl)-L-histidine (21a).** Treatment of **8b** and **14a** as outlined in general procedure C provided **21a** as a colorless oil in 94% yield. ESI-MS m/z: 642.3 (MH^+). HRMS cacld. for $C_{41}H_{44}N_3O_4$: 642.3326 (MH^+); found 642.3321.

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(6-(2-phenoxyphenyl)hexyl)-L-histidine (21b).** Treatment of **8b** and **14b** as outlined in general procedure C provided **21b** as a colorless oil in 97% yield. ESI-MS m/z: 630.3 (MH^+). HRMS cacld. for $C_{39}H_{40}N_3O_5$: 630.2962 (MH^+); found 630.2962.

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(6-(2-phenoxyphenyl)octyl)-L-histidine (21c).**Treatment of **8b** and **14c** as outlined in general procedure C provided **21c** as a colorless oil in 92% yield. ESI-MS m/z: 658.3 (MH^+). HRMS cacld. for $C_{41}H_{44}N_3O_5$ (MH^+): 658.3275; found 658.3272.

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(8-(2-benzoxyphenyl)octyl)-L-histidine (21d).** Treatment of **8b** and **14d** as outlined in general procedure C provided **21d** as a colorless oil in 79% yield. ESI-MS m/z: 672.3 (MH^+).

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(8-(2-fluoro-6-phenoxyphenyl)octyl)-L-histidine (21e).**Treatment of **8b** and **20a** as outlined in general procedure C provided **21e** as a colorless oil in 54% yield. ESI-MS m/z: 676.3 (MH+). HRMS Calcd. for $C_{41}H_{43}FN_3O_5$(MH^+): 676.3181; found 676.3166.

***N*$^{\alpha}$-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*N*$^{\pi}$-(9-(2-fluoro-6-phenoxyphenyl)nonyl)-L-histidine (21f).** Treatment of **8b** and **20b** as outlined in general procedure C provided **21f** as a colorless oil in 50% yield. ESI-MS m/z: 690.3 (MH^+). HRMS calcd. for $C_{42}H_{45}FN_3O_5$ (MH^+): 690.3338; found 690.3318.

General Solid-Phase Peptide Synthesis. *N(π)*-alkylated His residues were prepared using previously reported methodology.[3] Protected amino acids used were Fmoc-Thr(PO(OBzl)OH)-OH, Fmoc-Ser(O^{t}Bu)-OH, N(π)-alkylated Fmoc-His-OH (**11a-c** and **21a-f**), Fmoc-Leu-OH, and Fmoc-Pro-OH (purchased from Novabiochem). Peptides were synthesized on a NovaSyn® TGR resin (Novabiochem Cat#. 855009) using standard Fmoc solid-phase protocols in *N*-methyl-2-pyrrolidone (NMP). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (5.0 equivalents), and *N*,*N*-diisopropylethylamine (DIPEA) (10.0 equivalents) were used as coupling reagents. Unnatural amino acid residues were coupled using 2.5 equivalents. Deprotection was performed using 20% piperidine in DMF (15 minutes, twice). Amino-terminal acetylation was performed using 1-acetylimidazole. Finished resins were washed with NMP, MeOH, CH_2Cl_2, and Et_2O, dried under vacuum, and then cleaved by treatment with a solution of TFA:H_2O:TIS (95:2.5:2.5) (5 h). The resin was removed by filtration, and the filtrate was concentrated under vacuum and the resulting residue dissolved in 50% aqueous acetonitrile (5 mL) and purified by reverse-phase

preparative high pressure liquid chromatography (HPLC) using a Waters 2535 system having photodiode array detection and Phenomenex C18 columns (Cat. No. 00G-4436-P0-AX, 250 mm × 21.2 mm 10 μm particle size, 110 Å pore) at a flow rate of 10 mL/minute. Binary solvent systems consisting of A = 0.1% aqueous TFA and B = 0.1% TFA in acetonitrile were employed with gradients as indicated. Products were obtained as amorphous solids following lyophilization.

Peptide 4a. Peptide **4a** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 100% over 30 minutes, retention time = 12.47 minutes). ESI-MS *m/z*: 762.3 (MH^+). HRMS calcd. for $C_{30}H_{53}N_9O_{12}P$ (MH^+): 762.3546; found: 762.3575.

Peptide 4b. Peptide **4b** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 100% over 30 minutes, retention time = 12.38 minutes). ESI-MS *m/z*: 776.4 (MH^+). HRMS calcd. for $C_{31}H_{55}N_9O_{12}P$ (MH^+): 776.3702; found: 776.3689.

Peptide 4c. Peptide **4c** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 100% over 30 minutes, retention time = 12.65 minutes). ESI-MS *m/z*: 790.3 (MH^+). HRMS calcd. for $C_{32}H_{57}N_9O_{12}P$(MH^+): 790.3859; found 790.3880.

Peptide 6e'-8. Peptide **6e'-8** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 80% over 30 minutes, retention time = 20.52 minutes). ESI-MS *m/z*: 939.4 (MH^+). HRMS calcd. for $C_{46}H_{68}N_8O_{11}P$: 939.4740; Found 939.4748.

Peptide 6f'-8. Peptide **6f'-8** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 80% over 30 minutes, retention time = 20.20 minutes). ESI-MS *m/z*: 969.4 (MH^+). HRMS calcd. for $C_{47}H_{70}N_8O_{12}P$: 969.4845; Found 969.4862.

Peptide 6g'-6. Peptide **6g'-6** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 80% over 30 minutes, retention time = 19.57 minutes). ESI-MS *m/z*: 927.4 (MH^+). HRMS calcd. for $C_{44}H_{64}N_8O_{12}P$: 927.4376; Found 927.4384.

Peptide 6g-8. Peptide **6g-8** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 80% over 30 minutes, retention time = 20.79 minutes). ESI-MS *m/z*: 973.4 (MH^+). HRMS calcd. for $C_{46}H_{67}FN_8O_{12}P(MH^+)$: 973.4595; found 973.4563.

Peptide 6g'-8. Peptide **6g'-8** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 80% over 30 minutes, retention time = 20.66 minutes). ESI-MS *m/z*: 955.4 (MH^+). HRMS calcd. for $C_{46}H_{68}N_8O_{12}P$: 955.4689; Found 955.4699.

Peptide 6g-9. Peptide **6g-9** was purified by reverse phase preparative HPLC using a linear gradient (solvent B from 0 to 80% over 30 minutes, retention time = 21.25 minutes). ESI-MS *m/z*: 987.4 (MH^+). HRMS calcd. for $C_{47}H_{69}FN_8O_{12}P(MH^+)$: 987.4751; found 987.4719.

Plk1 PBD Competition ELISA Assays

Transfection and Protein Lysate Production. Plasmids encoding full length Plk1 or isolated Plk1 PBD linked to a 3x myc tagged were purchased from Addgene (deposited by Prof. Erich Nigg). HEK293T cells were plated on 10 cm culture dishes at 4M cells per plate. Following 24 h, the cells were transfected with 10 μg of plasmid DNA using 20 μL of TurboFect transfection reagent (Pierce Biotechnology) according to the manufacturer's instructions. Following either 24 h or 48 h, the cells were harvested using trypsin, washed with PBS 7.4 buffer, and lysed in lysis buffer [PBS 7.4 + 0.5% NP-40 + protease/phosphatase inhibitor cocktail (Pierce Biotechnology)] using 3 freeze/thaw cycles. The lysed suspension was centrifuged at 10,000 xG for 10 minutes to pellet membrane proteins and nuclei. The supernatant was removed to provide a crude cytosolic lysate containing expressed myc-tagged Plk1 or Plk1 PBD. The total protein concentration was determined using a BCA assay kit (Pierce Biotechnology).

ELISA Inhibition Assay. All assay steps were performed at room temperature with gentle shaking. A biotinylated phosphopeptide (sequence: Biotin-Ahx-PMQS(pT)PLN-NH_2) was diluted into PBS 7.4 to 1 μM (from a 10 mM DMSO stock solution) and loaded onto the wells of a 96-well Neutravidin-coated plate (Pierce Biotechnology) at 100 μL per well (1 h).

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 (1 h). The cytosolic lysate containing myc-tagged 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 was washed 2x with PBST (150 µL) and the pre-incubated lysates were added to the plate and incubated (1 h). The wells were washed [4x with PBST (150 µL)] and then probed with anti-myc primary antibody (1:1,500 dilution, mouse monoclonal, Pierce Biotechnology) (1 h). The wells were washed [4x with PBST (150 µL)] and incubated with rabbit anti-mouse HRP conjugate (1:3,000 dilution, Pierce Biotechnology) (1 h). The wells were 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 aq. 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 using GraphPad Prism 6 software (model: log(inhibitor) vs. response -- variable slope (four parameters)) to provide IC_{50} values. The IC_{50} values from multiple independent experiments were normalized and averaged to provide values ± standard error of the mean (SEM). Values are shown in Table 1 of the text and Figures S2 and S3.

General Protocol for Oxime-based Diversification

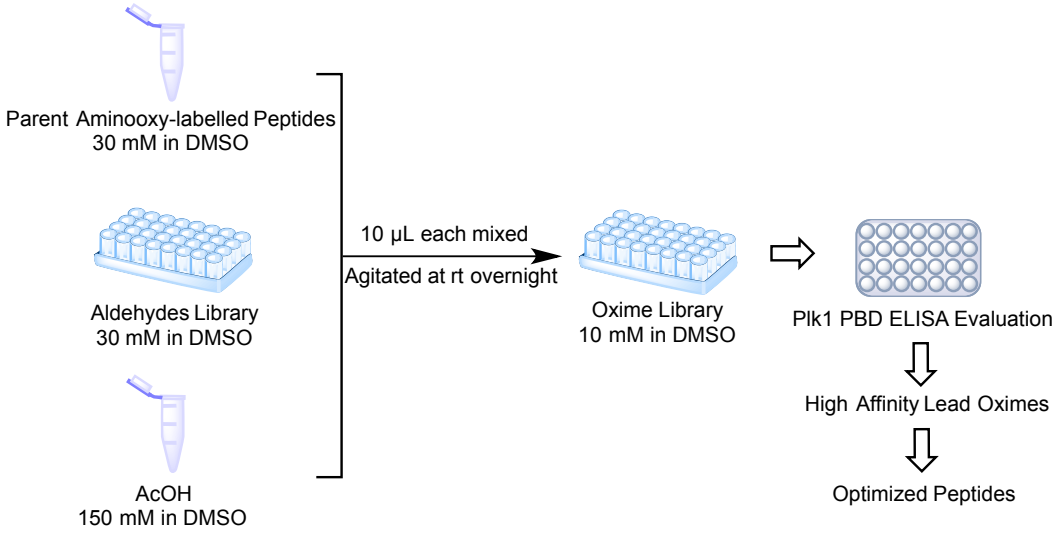
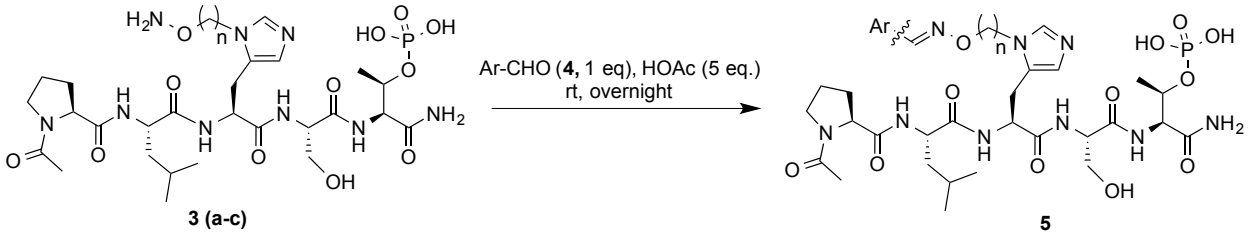
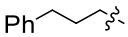
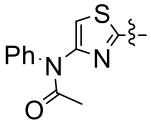
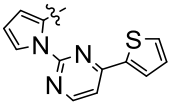
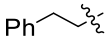
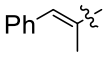
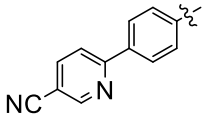
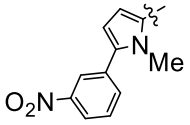
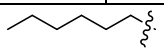
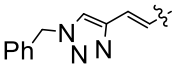
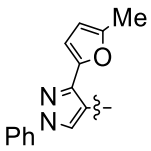


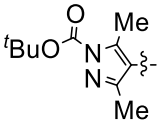
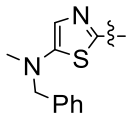
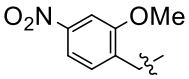
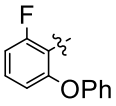
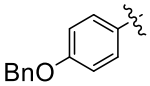
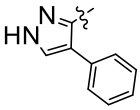
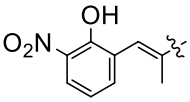
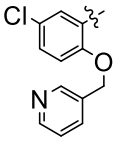
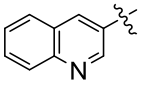
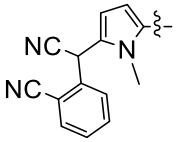
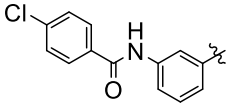
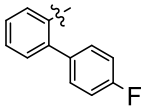
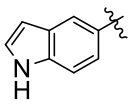
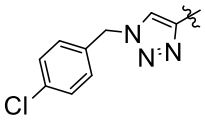
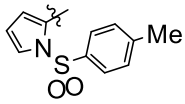
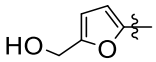
Figure S1. Schematic outline of oxime-ligation approach to peptide optimization.

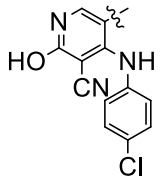
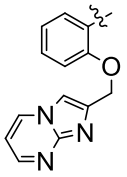
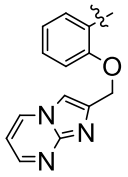
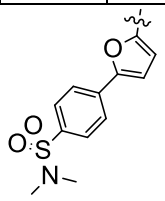
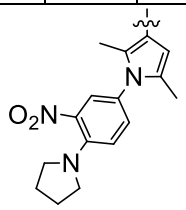
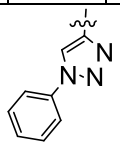
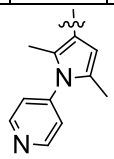
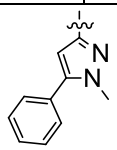
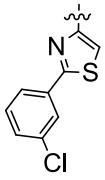
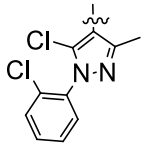
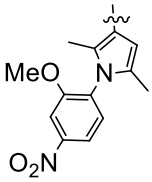
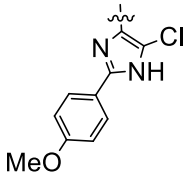
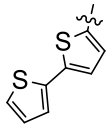
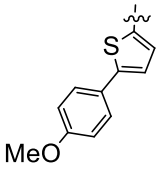
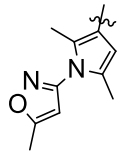
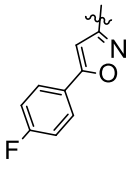
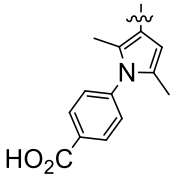
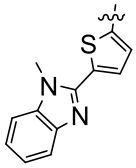
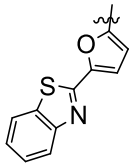
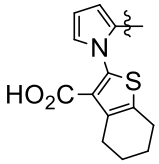
Post solid-phase diversification: Preparation of oxime-containing peptides (10 mM in DMSO).[8] A mixture of HPLC-purified aminooxy-His containing peptide (30 mM in DMSO, 10 µL), aldehyde (30 mM in DMSO, 10 µL) and acetic acid (150 mM in DMSO, 10 µL) was agitated at room temperature (overnight). Crude reaction mixtures were used directly for Plk1 PBD binding ELISA assays. Structures of oxime-containing peptides are shown in Table S1.

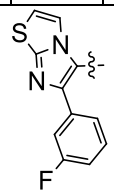
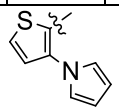
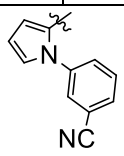
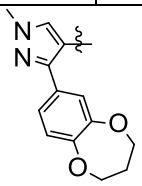
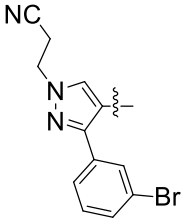
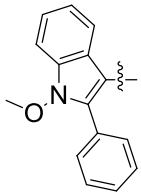
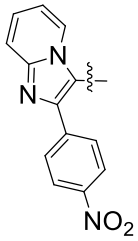
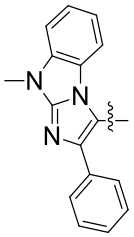
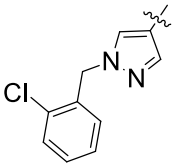
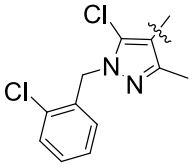
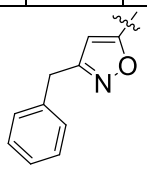
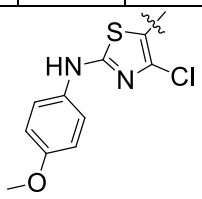
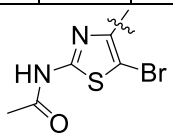
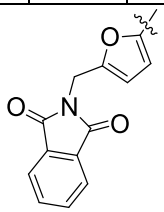
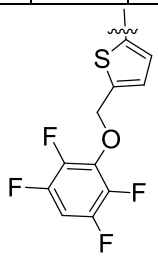
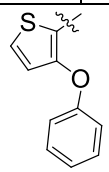
Table S1. Structure of peptide oxime libraries and their percent inhibition values as determined in Plk1 PBD binding ELISA assays.[a]

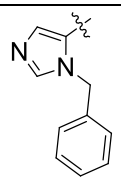
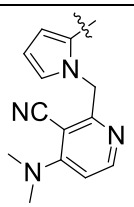
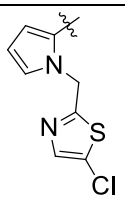
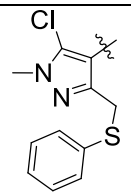
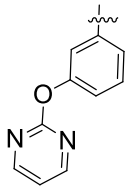
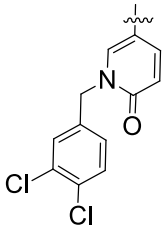
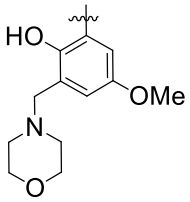
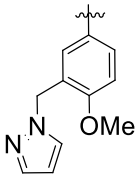
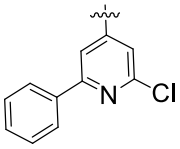
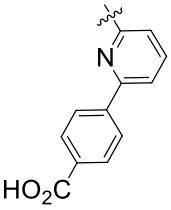
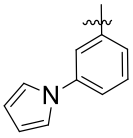
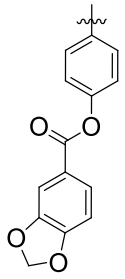
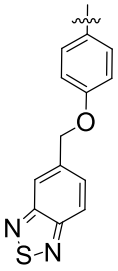
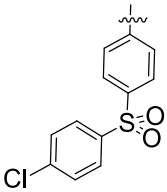
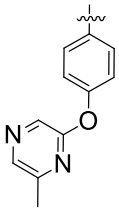
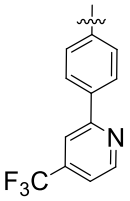


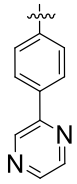
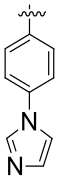
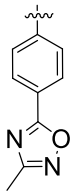
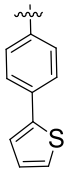
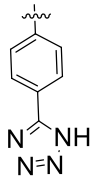
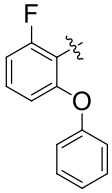
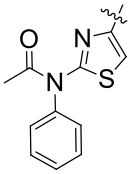
		Plk1 PBD Inhibition (%)[b]											
No.	**Conc.[c]**	**1**			**2**			**3**			**4**		
	n	*4*	*5*	*6*	*4*	*5*	*6*	*4*	*5*	*6*	*4*	*5*	*6*
A	Ar												
	100 nM	6.0	0.0	11.8	10.1	26.7	27.8	16.9	16.2	30.1	18.3	20.2	24.7
	300 nM	15.8	23.0	32.7	19.4	**82.1**	**74.4**	40.5	47.8	**84.1**	46.2	**67.2**	**70.9**
B	Ar												
	100 nM	7.3	12.2	14.5	13.5	16.5	21.1	18.9	28.5	25.3	16.8	13.6	19.7
	300 nM	5.3	23.9	42.8	22.1	53.0	**62.9**	**61.1**	**84.2**	**83.0**	31.8	44.1	**61.8**
C	Ar												
	100 nM	11.0	16.3	14.4	14.0	13.4	20.6	21.8	22.9	23.3	14.1	13.6	11.1
	300	10.1	22.4	33.9	18.2	29.3	50.9	50.1	**66.8**	**73.2**	11.3	13.8	27.5

	nM												
D	Ar												
	100 nM	7.3	12.7	15.2	11.5	14.0	18.1	13.6	7.5	11.8	15.9	23.6	15.0
	300 nM	11.2	46.1	46.8	19.5	44.6	55.5	24.0	43.0	54.4	48.0	**73.3**	**72.0**
E	Ar												
	100 nM	11.3	12.9	16.8	21.9	21.5	34.0	13.2	22.5	23.4	16.2	13.5	18.9
	300 nM	31.2	47.2	50.3	**61.9**	**71.9**	**88.5**	41.9	**67.6**	**67.6**	28.7	37.9	45.8
F	Ar												
	100 nM	12.8	9.7	20.4	17.2	12.7	30.4	11.8	13.5	17.6	15.2	18.6	16.4
	300 nM	53.6	42.7	**70.2**	51.9	**66.2**	**86.5**	19.6	39.2	55.3	25.5	56.1	48.8
G	Ar										H		
	100 nM	2.3	19.2	2.8	9.3	14.9	23.4	3.4	9.1	17.0	11.6	9.1	8.8
	300 nM	17.5	59.6	46.8	51.3	**68.2**	**82.3**	10.2	26.5	42.9	1.5	1.9	5.9
H	Ar										**1**	**2**	DMSO

	100 nM	0.0	9.1	11.0	12.3	7.9	19.0	8.0	7.5	8.1	9.1	**93.8**	0.0
	300 nM	6.5	25.9	36.7	27.5	46.1	**63.0**	0.0	0.0	26.1	0.0	**98.9**	0.0
I	Ar												
	200 nM		7.6	15.8		16.1	20.9		16.3	23.4		37.2	42.2
J	Ar												
	200 nM		56.9	38.0		9.5	7.9		14.7	15.7		10.9	12.5
K	Ar												
	200 nM		52.5	38.0		51.3	42.4		2.8	6.2		4.3	19.9
L	Ar												
	200 nM		39.7	**68.9**		34.2	52.9		10.1	11.5		30.4	46.5
M	Ar												

	200 nM		28.3	38.0		29.9	**74.5**		37.4	44.9		33.4	48.2
N	Ar												
	200 nM		34.8	52		**66.6**	**58.5**		6.0	44.3		0.0	18.7
O	Ar												
	200 nM		35.6	**74.5**		26.2	53.4		0	12.2		0	10.7
P	Ar												
	200 nM		14.3	36.6		50.6	**60.4**		3.5	34.4		24.4	**60.6**
Q	Ar												
	200 nM			**84.7**			36.5			25.0			**67**

R	Ar												
	200 nM			49.5			13.2			28.3			**65.1**
S	Ar												
	200 nM			16.8			58.3			22.9			30.3
T	Ar												
	200 nM			44.0			45.0			33.4			18.1
U	Ar												
	200 nM			41.7			36.5			22			51.6

V	Ar												
	200 nM			37.7			38.1			56.9			31.0
W	Ar										**1**	**2**	DMSO
	200 nM			28.9			**69.6**		51.3	49.9	2.7	**96.6**	0.0

[a]Inhibition values more than 60% are highlighted; [b]ELISA-based competitive inhibition of isolated PBD following incubation in crude cell lysates; [c]Oxime concentration in DMSO using 100 nM, 200 nM or 300 nM concentration as shown individually.

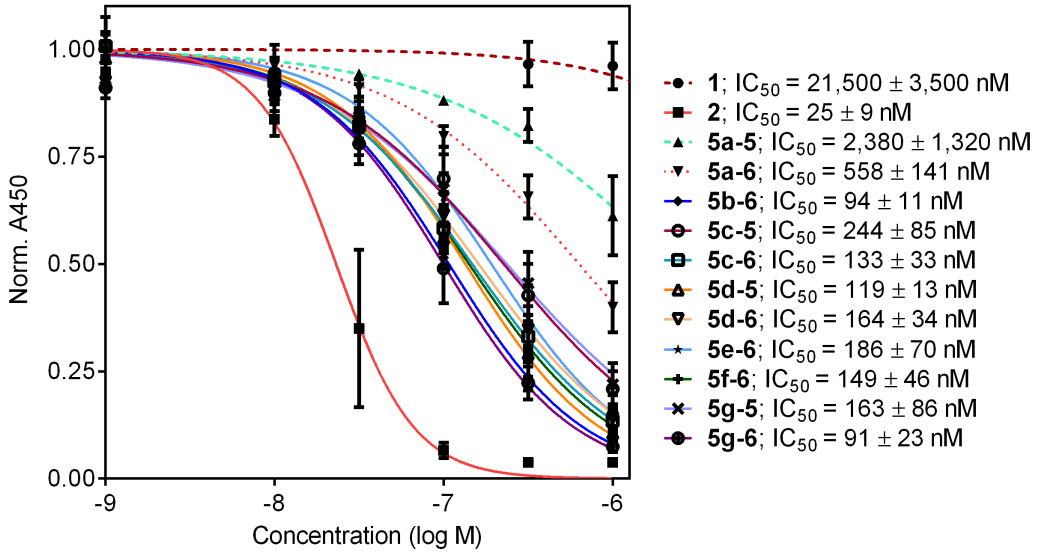


Figure S2. Inhibitory potencies of peptides obtained using ELISA-based Plk1 PBD assays determined as outlined above.

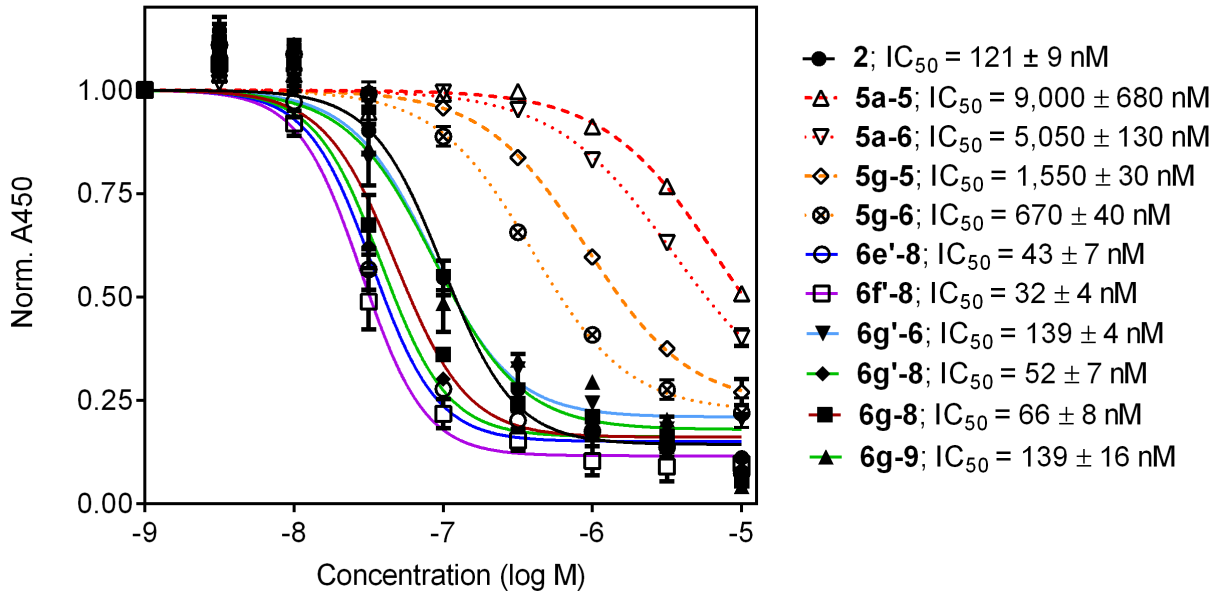


Figure S3. Inhibitory potencies of peptides obtained using ELISA-based full-length Plk1 binding assays determined as outlined above (IC_{50} values are given in Table 1 of the text).

In Silico Studies. Docking of ligands was performed with Molsoft ICM Pro® software (version 3.8-4a) using a MacIntosh computer (OSX v10.10.5 running on a 3.4 GHz Intel Core i7 processor with a NVIDIA GeForce GTX 680MX graphics processor having 2048 Mb video Ram) using default parameters and procedures.[9,10] The initial receptor was derived from the crystal structure of parent **2** bound to Plk1 PBD protein (PDB accession code 3rq7) using the standard protocol. The initial ligand was defined based on **2** using its initial PBD coordinates with positional restraint placed the pThr phosphoryl oxygens side (assigned as O9 – O11) and the sidechain oxygen atoms of the pT-1 Ser residue (assigned as O5). A rigid receptor map was constructed from protein residues contained within a bounding box surrounding the ligand and including thightly bound waters (designated as wa, wa2, wa14, wb, wb2, wb3, wb4, wb14, wb15 and wa16). Flexible ligand docking was performed using the software's internal coordinate mechanics (ICM) protocol. Subsequently, the molecular structures of **6e'-8**, **6f'-8** and **6g'-8** were generated by editing the ligand *in situ* and redocking in similar fashion. The best binding poses were selected based on calculated VlsScore values (Table S2) and shown in Figure 2 of the text.

Table S2. MolSoft ICM Docking Results.[a]

2

	ConfNum	L	Score	VlsScore	Strain	RecConf	Steric	Torsion	Electro	Hbond	Hydroph	Surface
1	1		-96.32	-108.1	11.78	3rq7_1_rec1:1	57.18	28	-13.02	-32.17	-9.466	38.14
2	2		-92.9	-108.1	15.24	3rq7_1_rec1:1	58.14	28	-12.43	-31.93	-9.333	37.94
3	3		-88.18	-104	15.8	3rq7_1_rec1:1	55.83	28	-9.294	-31.88	-9.252	37.39

6e'-8

	ConfNum	L	Score	VlsScore	Strain	RecConf	Steric	Torsion	Electro	Hbond	Hydroph	Surface
1	2		-97.17	-111.9	14.75	3rq7_1_rec1:1	60.45	29	-12.91	-32.36	-10.31	37.92
2	5		-95.32	-110.1	14.78	3rq7_1_rec1:1	59.51	29	-12.71	-32.18	-10.23	38.24
3	1		-94.18	-108	13.79	3rq7_1_rec1:1	59.35	29	-9.294	-32.32	-10.14	38.24

6f'-8

	ConfNum	L	Score	VlsScore	Strain	RecConf	Steric	Torsion	Electro	Hbond	Hydroph	Surface
1	1		-98.84	-113.9	15.1	3rq7_1_rec1:1	63.45	30	-12.08	-32.48	-10.76	38.49
2	4		-98.63	-114.1	15.51	3rq7_1_rec1:1	62.92	30	-13.17	-32.31	-10.69	37.82
3	3		-98.26	-112.4	14.16	3rq7_1_rec1:1	63.09	30	-11.76	-32.5	-10.24	39.37

6g'-8

	ConfNum	L	Score	VlsScore	Strain	RecConf	Steric	Torsion	Electro	Hbond	Hydroph	Surface
1	1		-98.36	-112.7	14.37	3rq7_1_rec1:1	62.44	29	-12	-32.49	-10.13	38.95
2	5		-97.69	-112.8	15.1	3rq7_1_rec1:1	62.05	29	-12.22	-32.5	-10.44	38.87
3	3		-94.27	-110.9	16.63	3rq7_1_rec1:1	61.42	29	-11.68	-32.54	-10.4	40.14

[a]Docking was performed as indicated. Ligand poses were selected based on the best VlsScore values (highlighted in yellow).

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