Supporting Information for

A New Class of Peptidomimetics Targeting the Polo-Box Domain of Polo-Like Kinase 1

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Figure S1. Schematics of synthesis of **22** and **24**

Reagents and conditions: (a) acid (5 eq), HBTU (5 eq), HOBT (5 eq), DIEA (10 eq), DMF, rt 1 h; (b) 20% piperidine in DMF, 1x10 min, 2x3 min; (c) TFA (2 mL), 5% TIS, 5% H₂O, 2 h.

Figure S2. Schematics of synthesis of **22-Cys-PEG** and **24-Cys-PEG**

Figure S3. Schematics of synthesis of **22-Cys-PEG-FITC** and **24-Cys-PEG-FITC**

Reagents and conditions: (a) i. Fmoc-PEG-OH (3 eq), HBTU (3 eq), HOBT (3 eq), DIEA (6 eq), DMF, rt 2 h; ii. 20% piperidine in DMF, 1x10 min, 2x3 min; (b) FITC (1.5 eq), DIEA (3 eq), DMF, 2 h; (c) TFA (2 mL), 5% TIS, 5% H₂O, 2 h.

Table S1. Data collection and refinement statistics

	7	8	22	2
Data collection				
Beamline	PLSII-7A	PLSII-7A	PLSII-7A	PF BL-1A
Wavelength (Å)	1.0000	1.0000	0.97934	0.9640
Space group	<i>P</i> 1	<i>P</i> 4 ₃ 2 ₁ 2	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁
a, b, c (Å)	37.592, 39.651, 39.942	57.760, 57.760, 137.568	57.927, 58.300, 66.753	39.390, 61.270, 51.260
α, β, γ (°)	93.725, 97.085, 102.915	90, 90, 90	90, 90, 90	90, 103.2, 90
Resolution range (Å)	50.0 - 1.8 (1.83 - 1.80)	50.0 - 2.7 (2.75 - 2.70)	50.0 - 1.9 (1.93 - 1.90)	50.0 - 1.6 (1.66 - 1.60)
Reflections	20,085/47,511	6,904/112,963	18,067/208,591	30,636/99,423
(unique/total)				
Completeness (%)	97.1 (96.0)	99.9 (100)	97.6 (100)	98.4 (85.6)
Redundancy	2.4 (2.4)	16.4 (17.0)	11.5 (11.7)	3.2 (2.7)
<i>R</i> -merge (%)*	0.035 (0.129)	0.126 (0.793)	0.069 (0.434)	0.071 (0.365)
<i>I</i> /σ(<i>I</i>)	20.6	8.8	15.1	19.5
Refinement				
Resolution range (Å)	40.0 - 1.80	53.26 - 2.70	43.91 - 1.90	50.0 - 1.60
<i>R</i> _{work} **	0.159	0.182	0.191	0.189
<i>R</i> _{free}	0.196	0.245	0.236	0.204
Number of atoms				
Protein	1,775	1,725	1,699	1,809
Ligand	56	56	57	85
Salt			10 (SO ₄)	
Water	100	1	47	163
<i>B</i> factor (Å ²)				
Protein	20.706	48.91	33.728	18.5
Ligand	16.349	40.801	28.262	13.8
Salt			52.145 (SO ₄)	
Water	26.704	34.79	34.582	29.0
Rmsd				
Bond length (Å)	0.0204	0.0126	0.0185	0.0046
Bond angles (°)	1.9777	1.6275	1.9921	1.23
Ramachandran plot				
Favored (%)	91.0	84.8	90.6	97.3

Allowed (%)

9.0

15.2

9.4

2.7

Values in parentheses correspond to the highest-resolution shell.

$$* R\text{-merge} = \frac{\sum \sum |I_i - \langle I \rangle|}{\sum \sum I_i}$$

$$** R\text{-factor} = \frac{\sum |F_{\text{obs}}(hkl) - F_{\text{calc}}(hkl)|}{\sum |F_{\text{obs}}(hkl)|}$$