

# Supporting Information

## Binding Inhibitors of the Bacterial Sliding Clamp by Design

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## Peptide Synthesis

### Materials for Peptide Synthesis.

Tritylchloride polystyrene resin was purchased from Pepchem. Natural and unnatural amino acid 9-fluorenylmethoxycarbonyl- (Fmoc-) derivatives were purchased from Auspep, Fluka, Bachem, NovaBiochem, Milligen, Biosearch or Advanced Chemtech. Piperidine, diisopropylethyl amine (DIPEA), trifluoroacetic acid (TFA) and solvents were purchased from Auspep. Other reagents were purchased from Sigma, Aldrich or Fluka. A 96-well block and manifold from Porvair was used for library parallel synthesis. High performance liquid chromatography (HPLC) analyses were performed using a Zorbax SB300 C-18 column (4.6 x 50 mm) from Agilent Technologies and a gradient of two buffers: A, 0.1% TFA in water; and B, 0.1% TFA in 90% acetonitrile. Two preparative HPLC methods were performed to purify peptides: using the same buffer system to develop a linear gradient on a Zorbax SB300 C-18 column (10 x 250 mm; Agilent Technologies) or buffer A in combination with 0.1% TFA in 95% acetonitrile delivering a linear gradient to a Jupiter C-18 column (10 x 250 mm; Phenomonex). Mass spectrometry (MS) was performed on Micromass Q-TOF.

### Solid Phase Peptide Synthesis.

Each peptide has been given a 'synthesis number' (see Tables S1–S4) to simplify referral to their synthesis. The standard Fmoc/tBu protection strategy was used. Molar equivalents (eq.) relative to the resin loading are given. All peptides were prepared using a 2-chlorotrityl chloride polystyrene resin. Amide couplings were performed in dimethylformamide (DMF) for 10 min with pre-activated Fmoc-amino acid (3 eq. relative to the resin loading) using *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) (2.7 eq.) and DIPEA (4 eq.). The Fmoc-group was cleaved using 1:1 piperidine:DMF for 2 x 1 min. Acetylation of peptides **20–30** and **61–76** used acetic anhydride (3 eq) and DIPEA (4 eq.). Attachment of the first Fmoc-amino acid: The amino acid derivative (1.5 eq.) was dissolved in anhydrous dichloromethane (DCM, ca. 8 mL/g of resin) and DIPEA (1.5 eq.) was added. The solution was added to the resin and the suspension was stirred or shaken. After 5 min, additional DIPEA (3 eq.) was added. After another 45–60 min, methanol (1 mL/g of resin) was added and allowed to react for 15 min before the resin was drained, washed three times with DCM and DMF, and six times with DCM and dried *in vacuo*. In the case of peptides **17–19**, the remaining couplings were carried out and the peptides were cleaved from the support using a cleavage mixture of TFA:DCM:water:triisopropylsilane (TIPS) 70:20:5:5 (2–3 mL/100 mg resin). Peptides **17** and **18** were isolated by preparative HPLC after preparation of peptide **17** and each verified by MS. The remaining peptides were prepared using a 96-well synthesis block.

### Synthesis in the 96-Well Block.

In the case of peptides **20–30**, the first amino acid was coupled to 2-chlorotrityl chloride resin weighed out in the 96-well block. The reaction protocol was as described above for attachment of the first amino acid, except without stirring. At the end of the peptide synthesis, the Fmoc-group of the last amino acids was removed, except for peptide **56** which was left protected with Fmoc. In the case of peptides **31–76**, the resin loaded with the first amino acid attached was suspended in a "zero-gravity" mixture of chloroform and DMF (ca. 1:1). Aliquots of this

suspension were transferred to the wells while the suspension was being kept homogeneous by gentle shaking. About 30 mg (30  $\mu$ mol) of resin was loaded in each well.

For peptides **31–60**, the resin-bound peptide amine was treated with a derivatization reagent (3 eq.) and DIPEA (4 eq.) in 200  $\mu$ L DMF. The specific derivatization reagents are listed in Table S6. As derivatization reagents, carboxylic acid chlorides, sulfonyl chlorides, and chloroformates were used directly with DIPEA added to the resin prior to addition of the reagent, while carboxylic acids were used together with HBTU (2.7 eq.) and DIPEA (4 eq.) as standard couplings. Reactions were carried out overnight.

After the last solid phase reaction, the resins were washed six times with DMF, ten times with DCM and dried by a stream of air (suction). The resins were then treated with the cleavage cocktail (1 mL/30 mg resin). After cleavage for a period of 2 h, the suspensions were filtered and the resins were washed twice with the cleavage cocktail. The resulting filtrates were analysed by MS and HPLC and the samples were concentrated to dryness *in vacuo* using a Genevac freeze dryer. The crude peptides thus obtained in a solid form were washed three times with diethyl ether and then purified by HPLC, if needed. All peptides were more than 95% pure by HPLC and MS analyses were consistent with the calculated values.

## Small Molecule Synthesis

### Materials and Instrumentation.

Melting points were determined on a Bausch & Lomb hot-stage melting point apparatus and are uncorrected.  $^1\text{H}$  and  $^{13}\text{C}$  spectra were recorded on a Bruker AV400 NMR spectrometer. Chemical shifts were measured in ppm relative to  $\text{CDCl}_3$  then related to tetramethylsilane. High resolution electron impact mass spectra (HR-EIMS) were recorded on a Thermoquest MAT 95XL, using an ionization energy of 70 eV. Accurate mass measurements were obtained with a resolution of 5000–10000 using perfluorokerosene as the reference compound. Radial chromatography was conducted on Merck silica gel 60PF<sub>254</sub> on a chromatotron (Harrison). Analytical HPLC was conducted on a Waters Breeze system, Alltima C18 5 $\mu$  column (4.6 x 150 mm) with detection at 230 nm, flow rate 1.0 mL/min, solvent 90–100% 0.1% TFA in methanol/0.1% TFA in water. Petroleum spirit refers to the fraction boiling from 40–60°C.

### Synthesis of 5-Butyryl-2,2-dimethyl-4,6-dioxocyclohexane-1-carbonitrile.

Butyric anhydride (7.1 g, 45 mmol) was added to a stirred, chilled (0–5°C) solution of 2,2-dimethyl-4,6-dioxocyclohexane carbonitrile (4.95 g, 30 mmol) in DCM (10 mL). 4-Dimethylaminopyridine (200 mg, 1.64 mmol) and triethylamine (7.6 g, 0.75 mol) were then added dropwise over 10 min and the resulting solution stirred at room temperature for 16 h. Water (200 mL) was then added, the pH adjusted to 3 and the mixture stirred for 30 min then extracted with diethyl ether (2 x 200 mL) and benzene (200 mL). The combined organic phases were washed with water (2 x 200 mL) and saturated NaCl solution (20 mL), dried ( $\text{MgSO}_4$ ), filtered and concentrated under reduced pressure to give a cream solid (6.8 g, 96%). A sample purified by radial chromatography (eluent 1:1 DCM:petroleum spirit) gave 5-butyl-2,2-dimethyl-4,6-dioxocyclohexanecarbonitrile as a colourless solid, mp 106–108°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CHCl}_3$ - $d_3$ )  $\delta$  ppm 0.92 (t,  $J$  = 7.3 Hz, 3 H), 1.14 (s, 3H), 1.25 (s, 3H), 1.52–1.66 (m, 2H), 2.62 (d,  $J$  = 18.3 Hz, 1H), 2.69 (d,  $J$  = 18.3 Hz, 1H), 2.84–3.01 (m, 2H), 3.54 (s, 1H).  $^{13}\text{C}$  NMR

(100 MHz,  $\text{CHCl}_3\text{-}d_3$ )  $\delta$  ppm 13.7, 17.8, 23.2, 28.3, 33.8, 42.1, 45.4, 53.3, 110.5, 115.3, 185.0, 196.1, 206.0. HR-EIMS for  $\text{C}_{13}\text{H}_{17}\text{NO}_3$  required, 235.1203; found, 235.1201.

**Synthesis of (Z)-5-(1-((4'-Fluorobiphenyl-4-yl)methoxyimino)butyl)-2,2-dimethyl-4,6-dioxocyclohexanecarbonitrile (3).**

*N,N*-Diethylethylenediamine (0.35 g, 3.0 mmol) was added to a stirred suspension of 2-[(4'-fluorobiphenyl-4-yl)methoxy]isoindoline-1,3-dione (0.70 g, 2.0 mmol) in ethanol (10 mL). The resulting suspension was stirred at room temperature for 2 h then the pH adjusted to 3.5 by addition of glacial acetic acid. 5-Butyryl-2,2-dimethyl-4,6-dioxocyclohexanecarbonitrile (0.47 g, 2.0 mmol) was then added and the mixture stirred for 16 h at room temperature. It was then diluted with water (20 mL), the pH adjusted to 3.5 and extracted with diethyl ether (2 x 30 mL) and benzene (30 mL). The combined organic phases were washed with water and saturated NaCl solution, dried ( $\text{MgSO}_4$ ), filtered and concentrated under reduced pressure. The crude material was subjected to radial chromatography (1:3 ethyl acetate:petroleum spirit elution) then recrystallized from 2:1 diethyl ether:petroleum spirit to give (Z)-5-(1-((4'-fluorobiphenyl-4-yl)methoxyimino)butyl)-2,2-dimethyl-4,6-dioxocyclohexanecarbonitrile (**3**) (340 mg, 39%) as a cream solid, m.p. 96–98°C. The purity of this compound was greater than 95% by HPLC.  $^1\text{H}$  NMR (400 MHz,  $\text{CHCl}_3\text{-}d_3$ )  $\delta$  ppm 0.99 (t, 3H), 1.19 (s, 3H), 1.24 (s, 3H), 1.49–1.63 (m, 2H), 2.46–2.62 (m, 2H), 2.87–3.04 (m, 2H), 3.39 (bs, 1H), 5.09 (s, 2H), 7.09–7.16 (m, 2H), 7.44 (d, 2H), 7.52–7.60 (m, 4H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CHCl}_3\text{-}d_3$ )  $\delta$  ppm 14.3, 20.4, 23.5, 28.3, 29.0, 34.0, 44.9, 53.5, 76.5, 105.1, 115.6, 115.8, 115.9, 127.3, 128.6, 128.7, 129.1, 134.7, 136.5, 136.6, 140.7, 161.4, 163.8, 165.8, 184.0, 185.4. HR-EIMS for  $\text{C}_{26}\text{H}_{27}\text{FN}_2\text{O}_3$  required, 434.2000; found, 434.1984.

**Synthesis of 6-(5,5-Dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione.**

Sodium (0.70 g, 30 mmol) was added to ethanol (10 mL) and the suspension stirred until all the sodium had reacted. Ethyl acetoacetate (3.8 mL, 30 mmol) and 1-(5,5-dimethyl-1,3-dioxan-2-yl)ethanone (4.74 g, 30 mmol) were then added and the mixture heated to reflux for 6 h. The ethanol was removed under reduced pressure then ice-water (25 mL) was added and the mixture washed with diethyl ether (3 x 25 mL). The pH of the aqueous phase was adjusted to 2 with 6 M hydrochloric acid, and the mixture extracted with diethyl ether (3 x 25 mL). The combined latter organic phases were washed with saturated NaCl solution (25 mL), dried ( $\text{MgSO}_4$ ), filtered and concentrated under reduced pressure to give 6-(5,5-dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione (2.78 g, 38%) as a cream solid. mp 108–111°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CHCl}_3\text{-}d_3$ )  $\delta$  ppm 0.56 (s, 3H), 0.91 (s, 3H), 1.32 (s, 3H), 2.31 (d,  $J = 16.3$  Hz, 1H), 2.83 (dd,  $J = 1.4, 16.5$  Hz, 1H), 3.06 (d,  $J = 20.8$  Hz, 1H), 3.31 (dd,  $J = 1.4, 20.8$  Hz, 1H), 3.23 (d,  $J = 11.0$  Hz, 1H), 3.28 (d,  $J = 11.0$  Hz, 1H), 3.42 (dd,  $J = 2.6, 11.1$  Hz, 1H), 3.46 (dd,  $J = 2.6, 11.1$  Hz, 1H), 4.25 (s, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CHCl}_3\text{-}d_3$ )  $\delta$  ppm 21.3, 22.5, 23.8, 30.0, 43.1, 44.3, 76.5, 76.8, 80.8, 102.3, 168.2, 199.4. EIMS  $m/z$  157 ( $[\text{M}-\text{C}_5\text{H}_9\text{O}]^+$ , 2%), 115 ( $[\text{C}_6\text{H}_{11}\text{O}_2]^+$ ).

**Synthesis of 3-Butyryl-6-(5,5-dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione.**

Butyric anhydride (5.9 g, 38 mmol) was added to a stirred, chilled (0–5°C) solution of 6-(5,5-dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione (6.05 g, 25 mmol) in DCM (25 mL). A solution of 4-dimethylaminopyridine (165 mg, 1.35 mmol) and triethylamine (6.3 g, 63 mmol) in DCM (5 mL) was then added dropwise over 2 min at 0–5°C, and the resulting solution stirred at room temperature for 16 h. Ice (25 g) was then added, the pH adjusted to 2.5 with 6 M hydrochloric acid and the mixture stirred for 2 min. The organic layer was removed and the aqueous layer extracted with diethyl ether (50 mL) and benzene (25 mL). The combined organic phases were washed with saturated NaCl solution (2 x 10 mL), dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure to give *3-butyryl-6-(5,5-dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione* (7.7 g, 99%) as a pale yellow solid. A sample recrystallized from DCM/petroleum spirit gave mp 67–73°C. <sup>1</sup>H NMR (400 MHz, CHCl<sub>3</sub>-d<sub>3</sub>) δ ppm 0.67 (s, 3H), 0.92 (t, *J* = 7.4 Hz, 3H), 1.07 (s, 3H), 1.38 (s, 3H), 1.56–1.69 (m, 2H), 2.54 (d, *J* = 17.5 Hz, 1H), 2.83–3.06 (m, 2H), 3.09 (d, *J* = 17.5 Hz, 1H), 3.34–3.40 (m, 2H), 3.52–3.60 (m, 2H), 4.39 (s, 1H). <sup>13</sup>C NMR (100 MHz, CHCl<sub>3</sub>-d<sub>3</sub>) δ ppm 13.7, 18.5, 21.5, 22.4, 22.7, 30.1, 37.3, 40.0, 76.8, 77.0, 78.5, 102.3, 103.1, 163.1, 195.4, 202.6. HR-EIMS for C<sub>16</sub>H<sub>24</sub>O<sub>6</sub> required, 312.1567; found, 312.1563.

**Synthesis of (Z)-3-(1-(Biphenyl-4-ylmethoxyimino)butyl)-6-(5,5-dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione.**

*N,N*-Diethylethylenediamine (0.35 g, 3.0 mmol) was added to a stirred suspension of 2-(biphenyl-4-ylmethoxy)isoindoline-1,3-dione (0.66 g, 2.0 mmol) in ethanol (10 mL). The resulting suspension was stirred at room temperature for 2 h then the pH adjusted to 3.5 by addition of glacial acetic acid. 3-Butyryl-6-(5,5-dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione (0.62 g, 2.0 mmol) was then added and the mixture stirred for 16 h at room temperature. It was then diluted with water (20 mL), the pH adjusted to 3.5 and extracted with diethyl ether (2 x 30 mL) and benzene (30 mL). The combined organic phases were washed with water and saturated NaCl solution, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The crude material was subjected to radial chromatography (1:2 ethyl acetate:petroleum spirit elution) to give *(Z)-3-(1-(biphenyl-4-ylmethoxyimino)butyl)-6-(5,5-dimethyl-1,3-dioxan-2-yl)-6-methyldihydro-2H-pyran-2,4(3H)-dione* (410 mg, 42%) as a white solid. mp 75–80°C. <sup>1</sup>H NMR (400 MHz, CHCl<sub>3</sub>-d<sub>3</sub>) δ ppm 0.71 (s, 3H), 0.97 (t, *J* = 7.7 Hz, 3H), 1.14 (s, 3H), 1.42 (s, 3H), 1.52–1.64 (m, 2H), 2.45 (d, *J* = 17.7 Hz, 1H), 2.94–3.07 (m, 2H), 3.06 (d, *J* = 17.7 Hz, 1H), 3.42 (d, *J* = 11.5 Hz, 2H), 3.59–3.66 (m, 2H), 4.45 (s, 1H), 1.76 (s, 2H), 7.35 (t, *J* = 7.4 Hz, 1H), 7.41–7.48 (m, 4H), 7.58–7.65 (m, 4H). <sup>13</sup>C NMR (100 MHz, CHCl<sub>3</sub>-d<sub>3</sub>) δ ppm 14.2, 20.5, 21.0, 21.6, 22.8, 28.7, 30.2, 35.5, 76.6, 76.9, 77.0, 78.3, 96.4, 102.3, 127.1, 127.4, 127.5, 128.8, 129.0, 134.8, 140.5, 141.5, 163.7, 166.2, 179.6. HR-EIMS for C<sub>29</sub>H<sub>35</sub>NO<sub>6</sub> required, 493.2459; found, 493.2455.

## Tables

**Table S1. Inhibition of  $\alpha$ - $\beta_2$  and  $\beta_2$ -consensus peptide assays by peptide variants.**  
**Series 1<sup>a</sup>**

Synthesis number	Peptide name	Sequence <sup>b</sup>	$\alpha$ - $\beta_2$ binding IC <sub>50</sub> (nM) <sup>b</sup>	SPR IC <sub>50</sub> (nM) <sup>b</sup>
1	consensus-2*	IG QLSLF GV	2,300 $\pm$ 1,100	550
2	pepDnaE-n	IG QADMF GV	96	3,000*
3	pepDnaE-13	IG QADCF GV	>1,000,000	nd
4	pepDnaE-14	IG QADMC GV	>1,000,000	nd
5	pepDnaE-15	IG QADLF GV	56,000	nd
6	pepDnaE-16	IG QADIF GV	>500,000	nd
7	pepDnaE-17	IG QADV F GV	315,000	nd
8	pepDnaE-18	IG QADFF GV	174,000	nd
9	pepDnaE-19	IG QADWF GV	345,000	nd
10	pepDnaE-20	IG QADYF GV	>500,000	nd
11	pepDnaE-21	IG QADML GV	>500,000	nd
12	pepDnaE-22	IG QADMI GV	>500,000	nd
13	pepDnaE-23	IG QADMV GV	>500,000	nd
14	pepDnaE-24	IG QADMM GV	>500,000	nd
15	pepDnaE-25	IG QADMW GV	282,000	nd
16	pepDnaE-26	IG QADML GV	>500,000	nd

<sup>a</sup> The assays were conducted as previously described<sup>19</sup>.

<sup>b</sup> The IC<sub>50</sub> values represent the molar concentration of peptide required to inhibit the interactions by 50%. Errors are standard errors in the mean of at least two independent measurements.

\* as previously reported<sup>19</sup>; nd, not done;

**Table S2. Inhibition of  $\alpha$ - $\beta_2$  and  $\beta_2$ -consensus peptide assays by peptide variants.**  
**Series 2<sup>a</sup>**

Synthesis number	Peptide name	Sequence <sup>b</sup>	$\alpha$ - $\beta_2$ binding IC <sub>50</sub> (nM) <sup>b</sup>	SPR IC <sub>50</sub> (nM) <sup>b</sup>
17	Pentamer-1	QLDLF	2,600 $\pm$ 1,400	620
18	pyroPentamer-1	pyroQLDLF	4,500 $\pm$ 700	nd
19	acPentamer-1	Ac-QLDLF	70 $\pm$ 20	400
20	acPentamer-2	Ac-QLDL[1Nal]	192 $\pm$ 11	64
21	acPentamer-3	Ac-QLDL[2Nal]	109 $\pm$ 81	162
22	acPentamer-4	Ac-QLDL[2ClF]	34 $\pm$ 8	117 $\pm$ 49
23	acPentamer-5	Ac-QLDL[3ClF]	11 $\pm$ 1	49 $\pm$ 8
24	acPentamer-6	Ac-QLDL[4ClF]	27 $\pm$ 18	20 $\pm$ 5
25	acPentamer-7	Ac-QLDL[3,4diClF]	21 $\pm$ 6	23 $\pm$ 11
26	acPentamer-8	Ac-QLDL[benzyl]	205 $\pm$ 23	124
27	acPentamer-9	Ac-QLDL4[MeF]	77 $\pm$ 11	76
28	acPentamer-10	Ac-QLDL[biP]	>3,000	nd
29	acPentamer-11	Ac-QLDL[3Pal]	2,300 $\pm$ 148	nd
30	acPentamer-12	Ac-QLDL[4Pal]	>3,000	nd

<sup>a</sup> The assays were conducted as previously described<sup>19</sup>.

<sup>b</sup> The IC<sub>50</sub> values represent the molar concentration of peptide required to inhibit the interactions by 50%. Errors are standard errors in the mean of at least two independent measurements. nd, not done; Nal, naphthylalanine; ClF, chlorophenylalanine; MeF, methylphenylalanine; biP, 4-phenylphenylalanine; Pal, pyridylalanine.

**Table S3. Substitutions at position 4 of Ac-QLDL[3,4CIF].<sup>a</sup>**

Synthesis number	Substitution at position 4	$\alpha$ - $\beta_2$ binding IC <sub>50</sub> (nM) <sup>b</sup>	SPR IC <sub>50</sub> (nM) <sup>b</sup>
61	1-naphthylalanine	>1,000	>3,000
62	2-naphthylalanine	>1,000	>3,000
63	2-chloro-phenylalanine	508 $\pm$ 110	237 $\pm$ 62
64	3-chloro-phenylalanine	577 $\pm$ 151	397 $\pm$ 1
65	4-chloro-phenylalanine	>1,000	946 $\pm$ 84
66	3,4-dichloro-phenylalanine	>1,000	>3,000
67	benzylalanine	>1,000	>3,000
68	4-methylphenylalanine	>1,000	>3,000
69	biphenylalanine	>1,000	>3,000
70	4-pyridylalanine,	$\geq$ 1,000	411 $\pm$ 18
71	3-pyridylalanine,	>1,000	986 $\pm$ 101
72	3-allylglycine	433 $\pm$ 55	76 $\pm$ 5
73	cyclohexylalanine	200 $\pm$ 35	145 $\pm$ 18
74	3-methylalanine	>1000	890 $\pm$ 60
75	methyleucine	121 $\pm$ 22	nd
76	methionine	37 $\pm$ 1	33 $\pm$ 1

<sup>a</sup> The assays were conducted as previously described<sup>19</sup>.

<sup>b</sup> The IC<sub>50</sub> values represent the molar concentration of peptide required to inhibit the interactions by 50%. Errors are standard errors in the mean of at least two independent measurements. nd, not done.



**Table S4. Amino-terminal extensions to the QLDL[3,4ClF] peptide.<sup>a</sup>**

Synthesis	Amino terminal group	$\alpha$ - $\beta_2$ binding	SPR
31	Propionyl	63 $\pm$ 11	61 $\pm$ 17
32	Pivaloyl	62 $\pm$ 19	69 $\pm$ 23
33	Cyclohexylcarboxyl	111 $\pm$ 15	59 $\pm$ 5
34	Phenoxyacetyl	195 $\pm$ 13	132 $\pm$ 20
35	Butyryl/2-Naphthoyl	75 $\pm$ 30	571 $\pm$ 41
36	Benzyloxyacetyl	67 $\pm$ 17	337 $\pm$ 18
37	3-Indoleacetyl	66 $\pm$ 7	537 $\pm$ 76
38	4-Phenylbutyryl	179 $\pm$ 6	731 $\pm$ 2
39	Phenylacetyl	236 $\pm$ 58	nd
40	1-Naphthylacetyl	361 $\pm$ 28	1,207
41	2-Naphthylacetyl	526 $\pm$ 52	422 $\pm$ 18
42	3-Indolepropionyl	375 $\pm$ 302	1861 $\pm$ 31
43	2,4-dimethoxybenzoyl	42 $\pm$ 15	27 $\pm$ 2
44	Benzoyl	69 $\pm$ 24	456 $\pm$ 80
45	2-Nitrobenzoyl	177 $\pm$ 49	462 $\pm$ 23
46	4-Nitrobenzoyl	217 $\pm$ 11	440 $\pm$ 29
47	3,5-dinitrobenzoyl	319 $\pm$ 87	1,550 $\pm$ 46
48	<i>p</i> -Anisoyl	137 $\pm$ 28	nd
49	1-Naphthoyl	220 $\pm$ 138	nd
50	Isobutyloxycarbonyl	20 $\pm$ 15	41 $\pm$ 21
51	Methyloxycarbonyl	86 $\pm$ 0.5	89 $\pm$ 10
52	Ethyloxycarbonyl	116 $\pm$ 28	327 $\pm$ 29
53	Allyloxycarbonyl	118 $\pm$ 54	398 $\pm$ 18
54	Benzyloxycarbonyl	245 $\pm$ 42	737 $\pm$ 61
55	Phenyloxycarbonyl	237 $\pm$ 59	1,069 $\pm$ 18
56	9-Fluorenyloxycarbonyl	$\geq$ 1,000	849 $\pm$ 74
57	Benzenesulfonyl	871 $\pm$ 24	nd
58	<i>p</i> -Toluenesulfonyl	309 $\pm$ 52	>3,000
59	3-Nitrobenzenesulfonyl	>1,000	nd
60	1-Naphthalenesulfonyl	>1,000	nd

<sup>a</sup> The assays were conducted as previously described<sup>19</sup>.

<sup>b</sup> The IC<sub>50</sub> values represent the molar concentration of peptide required to inhibit the interactions by 50%. Errors are standard errors in the mean of at least two independent measurements.  
nd, not done.

**Table S5. Crystallographic data**

<i>X-ray data</i>	
Symmetry	P2 <sub>1</sub>
Unit Cell	a=79.2 b=66.5 c=83.1 β=115.86
Resolution range (Å)	25-1.90 (1.97–1.90)
<i>R</i> -merge <sup>†</sup> (%)	8.2 (43.7)
< <i>I</i> /σ <i>I</i> >	13.8 (2.7)
Observations	57969 ( 5356)
Completeness	94.8 (87.9)
Multiplicity	3.7 (3.0)
<i>Structure</i>	
<i>R</i> , <i>R</i> -free <sup>‡</sup>	24.3, 31.7
RMS Deviations from ideal geometry:	
Bond lengths (Å)	0.022
Bond angles (°)	2.205
Torsion angles, period 1 (°)	9.034
chiral-volumes (Å <sup>3</sup> )	0.175
planar groups(Å)	0.010
Ramachandran statistics:	1.57%
Residues in preferred region	94%
Residues in allowed region	4.1%
Outliers	1.6%

Numbers in parentheses correspond to the highest resolution bin.

<sup>†</sup>  $R\text{-merge} = \frac{\sum_{hkl} \sum_i |I(hkl)_i - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I(hkl)_i}$

<sup>‡</sup>  $R = \frac{\sum_{hkl} |F_o(hkl) - F_c(hkl)|}{\sum_{hkl} |F_o(hkl)|}$ , where  $F_o$  and  $F_c$  are the observed and calculated structure factors, respectively.

**Table S6. Derivatization reagents used in generating variants of the CBM pentapeptide**

Synthesis	Derivatization reagent
31	Propionic anhydride
32	Pivaloyl chloride
33	Cyclohexylcarboxylic acid
34	Phenoxyacetyl chloride
35	Butyric acid/2-naphthoic acid
36	Benzyloxyacetyl chloride
37	3-Indolyl acetic acid
38	4-Phenylbutyric acid
39	Phenylacetyl chloride
40	1-Naphthyl acetic acid
41	2-Naphthyl acetic acid
42	3-Indolyl propionic acid
43	2,4-dimethoxybenzoic acid
44	Benzoyl chloride
45	2-Nitrobenzoyl chloride
46	4-Nitrobenzoic acid
47	3,5-dinitrobenzoyl chloride
48	p-Anisoyl chloride
49	1-Naphthoic acid
50	Isobutylchloroformate
51	Methylchloroformate
52	Ethylchloroformate
53	Allylchloroformate
54	Benzylchloroformate
55	Phenylchloroformate
56	—
57	Benzenesulfonyl chloride
58	p-Toluenesulfonyl chloride
59	3-Nitrobenzenesulfonyl chloride
60	1-Naphthalenesulfonyl chloride