Supporting Information

Bridged α-helix mimetic small molecules

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1. Materials and General Methods

All chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, Merck, TCI, Novabiochem) and used without further purification. 4-formyl-3,5-dimethoxyphenoxyethyl-functionalized polystyrene resins (PAL-PS) (0.90 mmol/g) were supplied by Midwest Bio-Tech. Normal-phase chromatography was performed on Merck silica gel 60-PF254. LC/MS (Agilent Technology) characterization was performed using a C18 reversed-phase HPLC column (2 μm, 4.6 mm × 50 mm). A gradient elution of 10% B in 2 min followed by 100% B in 13 min was used at flow rate of 0.7 mL/min (solvent A: 100% H2O, 0.1% trifluoroacetic acid (TFA); B: 100% acetonitrile, 0.1% TFA). Preparative reversed-phase HPLC purification was performed with a C18 reversed-phase column (5 μm, 25 mm × 125 mm) using a linear gradient from 10% B to 100% B by changing solvent composition over 40 min. NMR spectra were recorded on a Bruker DPX 300 (1H-NMR at 300MHz; 13C-NMR at 75 MHz) and a Bruker DPX 600
(1H-NMR at 600MHz; 13C-NMR at 150 MHz) spectrometer. The chemical shifts were reported in δ ppm and the coupling constants were reported in Hz. High Resolution Mass Spectra (HRMS) were obtained using an Agilent 7890 high-resolution mass spectrometer at the Korea Basic Science Institute (KBSI), Ochang, Korea.

2. Synthesis of mono-Alloc Protected Amine (1a-1g)

To a solution of diaminoalkanes or diaminetriethylene glycol (1 equiv.) and Et3N (1.5 equiv.) in DCM, trityl chloride (0.5 equiv.) in DCM (20 mL) was added over 10 min at 0°C. The mixture was stirred overnight at room temperature (rt). After 12 hr, the completion of the reaction was confirmed by TLC. Then, the mixture was extracted with DCM and water. The combined organic layers were dried over Na2SO4, and filtered. The filtrate was concentrated and purified by a silica gel column eluting with 10% MeOH in DCM. The resulting mono-Trityl protected amine (1 equiv.) and Et3N (3 equiv.) was dissolved in anhydrous DCM. Ally chloroformate (1.05 equiv.) was added to the solution at 0 °C. The reaction mixture was stirred at rt overnight. The completion of the reaction was confirmed by TLC, and the mixture was extracted with DCM and water. The combined organic layers were dried over Na2SO4 and filtrated. The filtrate was concentrated and purified by a silica gel column at Hexane : EA = 4 : 1. The purified Alloc and Trityl-protected amine was treated with 40% TFA in DCM at rt for 2 hr to give 1a-g. The resulting mixture was filtered and dried under air.

**Allyl (8-aminoctyl)carbamate·TFA (1a)**

1H NMR (300 MHz, CDCl3): δ 0.85-1.80(m, 12H), 2.98 (t, 2H), 3.19-3.32 (m, 2H), 3.65-3.90 (m, 2H), 5.22-5.35 (m, 4H), 5.94 (m, 1H). ESI-LC/MS : calc. mw = 228.18 found m/z = 229.2 [M+H]+.
Allyl (9-aminooctyl)carbamate·TFA (1b) $^1$H NMR (300 MHz, CDCl3): δ 1.00-1.80 (m, 14H), 2.95-3.20 (m, 2H), 3.60-3.90 (m, 2H), 4.59 (m, 2H), 4.71-4.88 (m, 2H), 5.10-5.35 (m, 2H), 5.92 (m, 1H); ESI-LC/MS : calc. mw = 242.20 found m/z = 243.2 [M+H]$^+$. 

Allyl (10-aminooctyl)carbamate·TFA (1c) $^1$H NMR (300 MHz, CDCl3): δ 1.31 (s, 10H), 1.49-1.90 (m, 6H), 3.20 (m, 2H), 4.57-4.71 (m, 4H), 5.22-5.35 (m, 2H), 5.94 (m, 1H); ESI-LC/MS : calc. mw = 256.22 found m/z = 257.2 [M+H]$^+$. 

Allyl (11-aminooctyl)carbamate·TFA (1d) $^1$H NMR (300 MHz, CDCl3): δ 0.86-1.80 (m, 18H), 2.91-3.30 (m, 2H), 4.58 (m, 2H), 4.71-4.88 (m, 2H), 5.21-5.34 (m, 2H), 5.91 (m, 1H); ESI-LC/MS : calc. mw = 270.23 found m/z = 271.2 [M+H]$^+$. 

Allyl (12-aminooctyl)carbamate·TFA (1e) $^1$H NMR (300 MHz, CDCl3): δ 1.24-1.48 (m, 20H), 3.07-3.13 (m, 2H), 4.52 (m, 2H), 5.19-5.34 (m, 2H), 5.91 (m, 1H); ESI-LC/MS : calc. mw = 284.25 found m/z = 285.2 [M+H]$^+$. 

Allyl (2-(2-(2-aminooethoxy)ethoxy)ethoxy)ethyl)carbamate · TFA (1f) $^1$H NMR (300 MHz, CDCl3): δ 2.37 (t, 2H), 3.36 (m, 2H), 3.53 (m, 6H), 3.61 (m, 6H), 4.55 (d, 2H), 5.19 (m, 2H), 5.91 (m, 1H). ESI-LC/MS : calc. mw = 276.17 found m/z = 277.2 [M+H]$^+$. 

S3
3. General Synthesis of Bridged Triazine-Piperazine-Triazines

3.1 Synthesis of bridged compounds 8a-8g

PAL-PS resins (100mg, 0.90 mmol/g) were swelled in THF (4 mL). To the suspension, mono Alloc-protected amines 1a-g (5 equiv.) were added, followed by the addition of AcOH 2.5% (v/v). After shaking at rt for 1 hr, NaBH(OAc)₃ (7 equiv.) was added, and the reaction was continued at rt for 16 hr. The resins were thoroughly washed with DMF (3×), MeOH (2×), DCM (3×). Mono-substituted dichlorotriazine (5 equiv.) was loaded on the resins by treating DIPEA (10 equiv.) in THF (1 mL) at rt for 3 hr. Nosyl-protected piperazine derivative was incorporated to the resin-bound triazine derivative in the presence of DIPEA (10 equiv.) in NMP at 75 °C overnight. The beads were drained and thoroughly washed with DMF (3×), CH₂Cl₂ (3×), MeOH (3×), and DMF (3×) at the end of each reaction step. To remove the nosyl protecting group, 2-mercaptoethanol (20 equiv.) and 1,8-diazabicyclo[5.4.0]undec-7ene (DBU) (10 equiv.) were treated in 1 mL of DMF at rt for 3 hr. Next, cyanuric chloride was introduced on the resins (5a-g) by treating with cyanuric chloride (5 equiv.) and DIPEA (10 equiv.) in THF at rt for 3 hr. Then, Alloc protecting group was deprotected by treating Pd(PPh₃)₄ (0.2 equiv.) and PhSiH₃ (10 equiv.) in anhydrous DCM (1 mL) at rt under N₂ gas atmosphere overnight. On-resin cyclization was processed by treating DIPEA (10 equiv.) in NMP at 60 °C for 18 hr (7a-g). Subsequently, tyramine was introduced to the remained chloride in 7a-g by treating tyramine (20 equiv.) and DIPEA (20 equiv.). For cleavage from the resins, a cleavage cocktail solution (95% TFA, 2.5% triisopropylsilane (TIS), and 2.5% water) was added to the resins, which were shaken at rt for 2 hr. The crude products (8a-g) were analyzed by LC/MS and purified by HPLC (Fig. S1). Synthesis procedures of 8h-m was described
in Section 3.2. The purified compounds were characterized by \(^1\)H NMR, \(^{13}\)C NMR, and HRMS (Fig. S2).

3.2 Synthesis of bridged compounds 7h-m

Synthesis of 5h-m were conducted by the same procedures with Section 3.1. The Nosyl protection group was removed by treating 2-mercaptoethanol (20 equiv.) and 1,8-diazabicyclo[5.4.0]undec-7ene (DBU) (10 equiv.) in 1 mL of DMF at rt for 3 hr. Next, mono-substituted cyanuric chloride was introduced on the resin (6h-m) by treating with 5 equiv. of tyramine-substituted cyanuric chloride (9) and DIPEA (10 equiv.) in THF at rt for 3 hr. Then, various amines were introduced to the remained chloride by treating R\(_2\)-NH\(_2\) (20 equiv.) and DIPEA (20 equiv.). For cleavage from the resins, a cleavage cocktail solution (95% TFA, 2.5% triisopropylsilane (TIS), and 2.5% water) was added to the resins, which were shaken at rt for 2 hr. The crude products (7h-m) were analyzed by LC/MS and purified by HPLC. The purified compounds were under coupling reaction to obtain bridged compounds (8h-m).

3.2.1. Synthesis of compound 8h

Compound 7h, 1,4-diethynylbenzene (1 equiv.), and CuSO\(_4\)-5H\(_2\)O (1 equiv.) were dissolved in t-butanol : water = 1 : 1 solution in 1mg/ml concentration. Then, solution of tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 1equiv) and sodium ascorbate (3 equiv.) in t-butanol : D.W. = 1 : 1 were added slowly. The reaction mixture was stirred for overnight (12 hr) at rt. The crude products (8h) were analyzed by LC/MS and purified by HPLC. The purified compounds were characterized by \(^1\)H NMR, \(^{13}\)C NMR, and HRMS (Fig. S2).
3.2.2. Synthesis of compound 8i

Compound 7i and CuSO$_4$·5H$_2$O (4.4 equiv.) were dissolved in t-butanol : water = 1 : 1 solution in 1mg/ml concentration. Then, 4.4 equiv. of sodium ascorbate was added slowly and stirred for overnight (12 hr) at rt. The crude products (8i) were analyzed by LC/MS and purified by HPLC. The purified compounds were characterized by $^1$H NMR, $^{13}$C NMR, and HRMS (Fig. S2).

3.2.3. Synthesis of compound 8j

Compound 7j and DIPEA (2 equiv.) were dissolved in DMSO : NMP = 1 : 4 solution in 1mg/ml concentration. Then, 1.5 equiv. of BOP was added and stirred for overnight (15 hr) at rt. The crude products (8j) were analyzed by LC/MS and purified by HPLC. The purified compounds were characterized by $^1$H NMR, $^{13}$C NMR, and HRMS (Fig. S2).

3.2.3. Synthesis of compound 8k

Compound 7k and 1.2 equiv. of 2-methyl-6-nitrobenzoic anhydride (MNBA) were dissolved in DCM. Then, 2.4 equiv. of 4-dimethylaminopyridine (DMAP) was added and stirred for overnight (15 hr) at rt. The crude products (8k) were analyzed by LC/MS and purified by HPLC. The purified compounds were characterized by $^1$H NMR, $^{13}$C NMR, and HRMS (Fig. S2).

3.2.4. Synthesis of compound 8l and 8m

Compound 7l or 7m and 4 equiv. of DIPEA were dissolved in DMF. Then, 2 equiv. of 1,3-bis(bromomethyl)benzene or 4,4′-bis(bromomethyl)-1,1′-biphenyl was added and stirred for 4 hr at rt. The crude products (8l and 8m) were analyzed by LC/MS and purified
by HPLC. The purified compounds were characterized by $^1$H NMR, $^{13}$C NMR, and HRMS (Fig. S2).

$8a$-TFA $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 0.96-1.12 (m, 4H), 1.13-1.49 (m, 8H), 2.84 (m, 3H), 3.18 (m, 4H), 3.35 (m, 1H), 3.56-3.90 (m, 6H), 4.20 (m, 1H), 4.45 (m, 1H), 4.80 (br s, 1H), 4.90-4.93 (m, 1H), 6.68 (m, 2H), 6.83 (m, 1H), 6.86-7.07 (m, 4H), 7.70-7.27 (m, 4H), 7.32-7.61 (m, 5H), 7.64 (br s, 1H), 7.77 (m, 1H); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 25.9, 26.4, 28.6, 28.9, 29.5, 29.7, 34.6, 36.3, 39.1, 41.0, 42.2, 44.2, 44.6, 53.5, 115.4, 115.4, 115.6, 117.3, 118.6, 118.8, 119.3, 123.2, 123.5, 127.1, 127.2, 128.7, 128.8, 129.0, 129.4, 129.9, 130.1, 131.5, 136.5, 155.9, 156.2, 157.1, 162.0, 162.4, 163.8; HRMS (ESI) calculated for C$_{45}$H$_{52}$N$_{12}$O$_2$ [M + H]$^+$: 793.4411; Found: 793.4414.

$8b$-TFA $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 1.14-1.72 (m, 14H), 2.85 (m, 3H), 2.95-3.10 (m, 1H), 3.06-3.42 (m, 4H), 3.43-3.63 (m, 2H), 3.69 (m, 3H), 3.82 (m, 1H), 3.95 (m, 1H), 4.20 (m, 1H), 4.43 (m, 1H), 4.81 (br s, 1H), 4.91 (m, 1H), 6.68 (m, 2H), 6.84 (m, 1H), 6.93-7.10 (m, 5H), 7.10-7.15 (m, 3H), 7.20 (m, 1H), 7.30-7.50 (m, 4H), 7.54-7.70 (m, 1H), 7.72-7.90 (m, 1H); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 26.0, 26.3, 26.5, 28.6, 28.9, 29.2, 29.7, 34.6, 37.4, 37.7, 40.1, 40.5, 42.5, 43.7, 44.8, 54.9, 115.4, 115.6, 117.3, 118.6, 118.8, 119.3, 119.4, 122.7, 123.2, 123.3, 123.5, 127.2, 128.8, 128.9, 129.2, 129.8, 129.9, 131.7, 136.0, 153.8, 154.1, 154.5, 156.2, 157.3, 162.9, 163.0, 163.6, 163.8; HRMS (ESI) calculated for C$_{46}$H$_{54}$N$_{12}$O$_2$ [M + H]$^+$: 807.4569; Found: 807.4571.

$8c$-TFA $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 0.98-1.10 (m, 3H), 1.26-1.44 (m, 10H), 1.50-1.72 (m, 3H), 2.72-2.99 (m, 4H), 3.15 (m, 3H), 3.50 (m, 3H), 3.54-3.76 (m, 3H), 3.85 (m, 1H),
4.60-4.85 (m, 3H), 5.08 (br s, 1H), 5.17 (m, 1H), 6.74 (m, 2H), 6.87 (m, 1H), 7.05 (m, 5H), 7.10 (m, 2H), 7.12 (m, 1H), 7.24 (m, 1H), 7.39 (m, 3H), 7.58 (m, 1H), 7.62-7.80 (m, 2H); $^1$H-NMR (150 MHz, CDCl$_3$) δ 25.9, 26.4, 28.5, 28.6, 28.9, 29.5, 29.7, 34.6, 35.9, 36.4, 39.1, 41.3, 42.5, 44.2, 44.6, 53.5, 115.4, 115.5, 117.4, 118.8, 118.9, 119.2, 119.3, 123.2, 123.5, 123.8, 127.1, 127.2, 128.7, 128.8, 129.0, 129.4, 129.9, 130.1, 131.5, 136.5, 154.3, 154.4, 155.8, 156.2, 157.1, 162.4, 163.8, 164.0; HRMS (ESI) calculated for C$_{47}$H$_{56}$N$_{12}$O$_{2}$ [M + H]$^+$: 821.4727; Found: 821.4731.

8d·TFA $^1$H-NMR (600 MHz, CDCl$_3$) δ 1.11-1.55 (m, 18H), 2.86 (m, 4H), 2.96-3.31 (m, 4H), 3.40 (m, 2H), 3.49 (m, 1H), 3.65 (m, 2H), 3.80 (m, 1H), 4.55-4.90 (m, 3H), 5.13 (br s, 1H), 5.23 (br s, 1H), 6.74 (m, 2H), 6.90 (m, 1H), 7.05(m, 6H), 7.11 (m, 2H), 7.18 (m, 1H), 7.27 (m, 1H), 7.35 (m, 1H), 7.41 (m, 3H), 7.59 (m, 1H); $^1$H-NMR (150 MHz, CDCl$_3$) δ 25.8, 26.2, 28.4, 29.0, 29.3, 29.6, 29.7, 30.7, 30.9, 34.5, 36.1, 39.0, 40.2, 40.4, 42.6, 43.6, 44.2, 53.2, 115.5, 117.4, 118.7, 118.8, 119.1, 119.4, 123.3, 123.5, 123.8, 127.0, 127.2, 128.7, 128.8, 129.1, 129.5, 129.9, 130.1, 136.7, 137.1, 154.5, 156.0, 156.2, 156.3, 157.1, 162.1, 162.4, 163.8, 164.1; HRMS (ESI) calculated for C$_{48}$H$_{58}$N$_{12}$O$_{2}$ [M + H]$^+$: 835.4884; Found: 835.4482.

8e·TFA $^1$H-NMR (600 MHz, CDCl$_3$) δ 1.10-1.75 (m, 20H), 2.86 (m, 4H), 3.10-3.30 (m, 4H), 3.35-3.58 (m, 3H), 3.66 (m, 2H), 3.80 (m, 1H), 4.56-4.89 (m, 3H), 5.09 (br s, 1H), 5.21 (m, 1H), 6.77 (m, 2H), 6.90 (m, 1H), 7.02-7.20 (m, 8H), 7.24 (m, 1H), 7.31 (m, 1H), 7.39 (m, 3H), 7.59 (m, 1H), 7.60-7.80 (m, 1H); $^1$H-NMR (150 MHz, CDCl$_3$) δ 25.7, 28.1, 28.2, 28.3, 28.5, 28.6, 29.3, 29.4, 34.5, 36.2, 39.1, 40.1, 40.2, 40.4, 42.5, 43.5, 44.5, 44.6, 53.4, 115.4, 115.6, 117.3, 118.8, 118.9, 119.1, 119.2, 123.4, 123.5, 123.8, 127.0, 128.7, S8
129.0, 129.3, 129.8, 130.1, 131.4, 131.6, 136.6, 137.0, 154.3, 154.6, 154.7, 156.2, 157.1, 162.5, 162.6, 163.6, 163.8; HRMS (ESI) calculated for $C_{49}H_{60}N_{12}O_2 \ [M + H]^+$: 849.5040; Found: 849.5041.

8f·TFA $^1$H-NMR (600 MHz, MeOD) $\delta$ 2.81 (m, 3H), 2.99 (m, 1H), 3.13 (m, 1H), 3.25 (m, 1H), 3.36-3.71 (m, 20H), 3.71-4.08 (m, 3H), 3.41-3.80 (m, 1H), 4.19 (m, 2H), 4.51 (m, 1H), 4.97 (br s, 1H), 6.70 (m, 2H), 6.87 (m, 1H), 7.04 (m, 6H), 7.20 (m, 3H), 7.28-7.45 (m, 5H), 7.56 (br s, 1H); $^{13}$C-NMR (150 MHz, MeOD) $\delta$ 34.0, 34.1, 36.0, 38.4, 39.7, 39.8, 42.1, 42.3, 44.0, 44.1, 54.4, 69.2, 69.9, 70.0, 113.2, 113.7, 115.0, 115.6, 117.5, 118.6, 119.5, 123.4, 126.5, 128.3, 128.9, 129.0, 129.2, 129.4, 129.5, 129.6, 137.1, 137.3, 155.1, 155.4, 155.8, 157.1, 161.5, 161.7, 161.9, 162.2, 162.7; HRMS (ESI) calculated for $C_{45}H_{52}N_{12}O_5 \ [M + H]^+$: 841.4262; Found: 841.4258.

8g·TFA $^1$H-NMR (600 MHz, MeOD) $\delta$ 2.85 (m, 5H), 3.36-3.71 (m, 26H), 4.43-4.63 (m, 1H), 4.65-4.76 (m, 1H), 6.70-6.75 (m, 2H), 6.92 (br s, 1H), 7.05 (m, 6H), 7.20 (m, 3H), 7.28-7.45 (m, 5H), 7.56 (br s, 1H); $^{13}$C-NMR (150 MHz, MeOD) $\delta$ 29.0, 29.3, 31.6, 34.0, 34.1, 35.5, 38.9, 39.5, 39.6, 42.2, 42.3, 43.4, 44.0, 46.6, 48.2, 54.0, 68.5, 68.8, 69.8, 70.1, 70.2, 70.4, 115.0, 117.7, 118.3, 118.4, 118.7, 123.1, 123.2, 126.4, 128.2, 129.0, 129.2, 129.4, 129.5, 129.6, 155.2, 155.8, 157.3, 157.4, 161.6, 161.8, 161.9; HRMS (ESI) calculated for $C_{47}H_{57}N_{12}O_6 \ [M + H]^+$: 885.4524; Found: 885.4524.

8h·TFA $^1$H-NMR (600 MHz, MeOD) $\delta$ 1.29-1.45 (m, 3H), 1.97-2.15 (m, 2H), 2.32-2.57 (m, 2H), 2.68-2.93 (m, 6H), 3.48 (m, 3H), 3.62 (m, 3H), 3.88 (m, 1H), 4.07-4.27 (m, 2H), 4.35 (m, 1H), 4.42 (m, 1H), 6.80 (m, 2H), 6.89-6.99 (m, 2H), 7.01-7.22 (m, 11H), 7.34-
7.49 (m, 4H), 7.30-7.44 (m, 4H), 7.57 (m, 1H), 7.69-7.87 (m, 4H); \(^1^3\)C-NMR (150 MHz, MeOD) \(\delta\) 17.2, 24.1, 27.0, 26.8, 29.3, 31.9, 33.9, 37.4, 40.4, 42.2, 42.3, 113.6, 115.0, 115.1, 115.2, 117.8, 118.4, 118.6, 118.8, 119.1, 122.5, 123.1, 123.4, 123.6, 124.6, 125.4, 126.0, 126.1, 126.3, 126.4, 128.3, 129.0, 129.4, 129.5, 130.4, 142.4, 147.2, 147.3, 154.8, 155.8, 157.5, 161.5, 161.8; HRMS (ESI) calculated for C\(_{51}\)H\(_{51}\)N\(_{18}\)O\(_2\) [M + H]^+: 947.4442; Found: 947.4436.

8i-TFA \(^1\)H-NMR (600 MHz, MeOD) \(\delta\) 1.57-1.71 (m, 4H), 1.73-1.86 (m, 4H), 2.00 (m, 8H), 2.28 (m, 2H), 2.86 (m, 3H), 3.15-3.33 (m, 3H), 3.41 (m, 4H), 3.52-3.73 (m, 3H), 4.55-4.84 (m, 3H), 5.05-5.23 (m, 1H), 6.69-6.85 (m, 2H), 6.89 (m, 1H), 6.94-7.29 (m, 9H), 7.40 (m, 3H), 7.51-7.71 (m, 2H), 7.71-7.91 (m, 1H); \(^1^3\)C-NMR (150 MHz, MeOD) \(\delta\) 18.1, 25.6, 26.1, 28.1, 34.6, 35.9, 36.2, 39.1, 40.3, 42.5, 43.6, 44.5, 50.9, 53.3, 69.0, 115.4, 115.5, 115.6, 118.8, 118.9, 119.1, 119.2, 119.3, 123.3, 123.6, 123.8, 124.0, 127.1, 128.6, 128.7, 129.0, 129.2, 129.9, 131.3, 136.6, 137.0, 154.1, 154.5, 156.0, 156.2, 157.0, 162.5, 163.3; HRMS (ESI) calculated for C\(_{47}\)H\(_{54}\)N\(_{15}\)O\(_2\) [M + H]^+: 860.4585; Found: 860.4586.

8j-TFA \(^1\)H-NMR (600 MHz, CDCl\(_3\)) \(\delta\) 1.25-1.51 (m, 4H), 1.51-1.89 (m, 7H), 1.91-2.27 (m, 3H), 2.67-3.06 (m, 5H), 3.08-3.44 (m, 6H), 3.55 (m, 2H), 3.65 (m, 2H), 3.35-3.57 (m, 1H), 4.57-4.94 (m, 2H), 5.00 (br s, 1H), 6.66-6.81 (m, 2H), 6.88 (m, 1H), 6.94-7.13 (m, 6H), 7.13-7.29 (m, 2H), 7.30-7.44 (m, 4H), 7.58 (m, 1H), 7.70-7.88 (m, 1H); \(^1^3\)C-NMR (150 MHz, CDCl\(_3\)) \(\delta\) 24.7, 25.6, 26.8, 27.7, 28.8, 34.6, 35.8, 36.3, 38.8, 39.3, 40.8, 42.3, 43.4, 44.4, 52.5, 53.3, 62.0, 115.4, 117.3, 118.8, 119.1, 119.3, 123.2, 123.5, 123.7, 126.9, 127.0, 128.6, 129.0, 129.4, 129.8, 131.5, 131.6, 136.7, 137.2, 154.0, 154.2, 154.4, 154.8, 155.8, 157.5, 161.5, 161.8; HRMS (ESI) calculated for C\(_{47}\)H\(_{54}\)N\(_{15}\)O\(_2\) [M + H]^+: 860.4585; Found: 860.4586.
156.0, 157.1, 162.4, 163.4, 163.6, 163.8; HRMS (ESI) calculated for C_{47}H_{56}N_{13}O_{3} [M + H]^+: 850.4629; Found: 850.4631.

**8k·TFA** ^1^H-NMR (600 MHz, CDCl\textsubscript{3}) δ 1.42 (m, 2H), 1.57 (m, 2H), 1.62-1.86 (m, 6H), 1.91 (m, 2H), 2.50-2.92 (m, 6H), 2.97-3.35 (m, 4H), 3.45 (m, 2H), 3.62 (m, 2H), 3.68-3.96 (m, 4H), 4.43-4.73 (m, 3H), 5.00 (br s, 1H), 6.62 (m, 1H), 6.86-6.99 (m, 2H), 7.01-7.13 (m, 5H), 7.13-7.29 (m, 4H), 7.34-7.43 (m, 3H), 7.57 (m, 1H), 8.14 (m, 1H), 8.18-8.32 (m, 1H); ^1^C-NMR (150 MHz, CDCl\textsubscript{3}) δ 25.6, 26.2, 29.8, 34.1, 35.9, 36.2, 36.9, 38.7, 40.8, 42.3, 43.4, 44.4, 52.5, 53.3, 62.0, 115.4, 117.4, 118.8, 119.2, 121.3, 121.5, 123.2, 123.4, 123.5, 123.7, 126.9, 127.2, 128.5, 128.6, 129.0, 129.6, 129.8, 131.5, 137.0, 138.4, 149.2, 154.4, 155.3, 157.1, 162.2, 163.3, 163.8, 164.0; HRMS (ESI) calculated for C_{47}H_{55}N_{12}O_{4} [M + H]^+: 851.4469; Found: 851.4465.

**8l·TFA** ^1^H-NMR (600 MHz, CDCl\textsubscript{3}) δ 2.78-2.87 (m, 6H), 3.00 (m, 1H), 3.13-3.28 (m, 2H), 3.16 (m, 2H), 3.62-3.71 (m, 4H), 3.71-3.99 (m, 4H), 4.45-4.70 (m, 5H), 5.02 (br s, 1H), 6.75 (m, 2H), 6.89 (m, 1H), 7.03 (m, 6H), 7.14-7.26 (m, 3H), 7.32-7.50 (m, 4H), 7.56 (m, 1H), 7.68 (br s, 1H), 7.78 (m, 1H), 8.19 (br s, 1H), 8.27 (br s, 1H); ^1^C-NMR (150 MHz, CDCl\textsubscript{3}) δ 32.0, 34.5, 35.8, 36.2, 37.0, 38.8, 39.0, 40.2, 42.6, 43.7, 44.2, 53.4, 115.2, 115.6, 117.2, 118.8, 119.1, 119.3, 123.4, 123.6, 123.9, 126.9, 127.2, 127.4, 127.5, 129.0, 129.9, 136.4, 136.9, 138.1, 138.3, 138.5, 138.7, 154.1, 154.3, 154.6, 154.7, 155.8, 155.9, 156.1, 156.2, 157.0; HRMS (ESI) calculated for C_{40}H_{53}N_{12}O_{2}S_{2} [M + H]^+: 905.3856; Found: 905.3859.

**8m·TFA** ^1^H-NMR (600 MHz, CDCl\textsubscript{3}) δ 2.62 (m, 2H), 2.73 (m, 1H), 2.83 (m, 2H), 2.94-
2.97 (m, 4H), 3.08-3.43 (m, 4H), 3.52 (m, 1H), 3.66-3.96 (m, 8H), 4.13 (m, 1H), 4.47-4.79 (m, 2H), 6.77-6.87 (m, 2H), 6.88-7.00 (m, 1H), 7.01-7.15 (m, 7H), 7.16-7.27 (m, 6H), 7.33-7.46 (m, 6H), 7.50-7.64 (m, 2H), 7.67 (m, 1H), 8.00-8.15 (m, 1H); $^{13}$C-NMR (150 MHz, CDCl$_3$) δ 27.0, 27.7, 30.0, 34.8, 35.9, 36.5, 38.6, 39.9, 41.8, 42.7, 42.9, 53.4, 53.8, 76.8, 77.0, 77.2, 115.7, 117.3, 118.8, 119.1, 119.2, 119.4, 123.1, 123.2, 123.4, 123.5, 123.6, 124.0, 125.3, 126.0, 126.2, 126.3, 127.0, 127.1, 127.4, 127.9, 128.2, 128.5, 128.7, 128.8, 129.0, 129.1, 129.3, 129.9, 136.7, 137.2, 137.4, 137.9, 138.1, 154.0, 154.6, 155.7, 157.1, 161.7, 161.9, 163.5, 163.8; HRMS (ESI) calculated for C$_{55}$H$_{57}$N$_{12}$O$_2$S$_2$ [M + H]$^+$: 981.4169; Found: 981.4166.

4. Protein Purification.

The expression plasmid encoding BH3-binding domain of human Mcl-1 (amino acids 172–320) tagged with GST were transformed into BL21 (DE3) E. coli cells. Expression of Mcl-1 protein was induced by treating 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 20 °C overnight. Resuspended cell pellets in lysis buffer (20 mM Tris, pH 7.2, 250 mM NaCl, complete protease tablet (Roche)) were lysed by sonication. After centrifugation, cell lysates were applied to a 20mL GSTrap HP column (GE Life Sciences) according to the manufacturer’s instructions. Purified GST-tagged Mcl-1 were under cleavage reaction by thrombin protease to cleave the GST-tag. Mcl-1 was obtained after another round of loading to GSTrap HP column to remove GST and passing through the size exclusion chromatography.

5. Competitive Fluorescence Polarization (FP) Assay

BH3-binding domain of Mcl-1 protein (0.8 μM) was incubated with the fluorescently
labeled BH3 peptide (KALETLRRVGDGVQRNHETAF) (50 nM) in binding buffer (50 mM Tris, pH 8.0, 100 mM NaCl, and 0.01% Tween20) in a 384-well plate at rt for 30 min. The concentration of Mcl-1 protein for competitive FP assay was determined by the binding curve of fluorescently-labeled BH3 to Mcl-1 protein (Fig. S3). Next, varying concentrations of bridged triazine-piperazine-triazines (8a-8m) or BH3 peptide were added. After incubation at rt for 1 hr, fluorescence anisotropy values were measured by a Tecan F200 Microplate Reader (λ<sub>ex</sub>: 485 nm; λ<sub>em</sub>: 535 nm). Using following equations Y = Bottom + (Top – Bottom)/(1 + 10<sup>-logIC<sub>50</sub></sup>), IC<sub>50</sub> values were determined with nonlinear regression and fitted by GraphPad Prism® 4 software. Maximum anisotropy value means the anisotropy value when 0.8 μM Mcl-1 protein is incubated with the fluorescently-labeled BH3 peptide. Minimum anisotropy values were determined when the fluorescently-labeled BH3 peptide was incubated without Mcl-1 protein. Ki values were calculated by the following equation \( K_i = [I]_{50}/([L]_{50}/K_D + [P]_0/K_D + 1) \) as previously reported.<sup>1</sup>

For binding assay with PAS-B domain of NCOA1, the fluorescently-labeled 21-mer STAT6 peptide (LLPPTEQDLTKLLLLEGQGESG) (1 μM) was incubated with NCOA1 (5 μM) in binding buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, and 0.01% Tween20) for 30 min at rt. Next, varying concentrations of 8f, 8g or yl-2<sup>2</sup>, known to bind NCOA1, were treated. After incubation for 1 hr, binding activity was determined as described above. For binding assay with BCL-XLΔC, 20 nM of the TAMRA-labeled BAK-BH3 peptide (GQVGRQALIIGDDINR)<sup>3</sup>, known to bind BCL-XLΔC protein, was incubated with the BCL-XLΔC in binding buffer (PBS buffer, pH 7.4, 150 mM NaCl, and 0.05% Tween20) in a 384-well plate for 30 min. Then, varying concentrations of 8f, 8g or a BAK peptide were added. After incubation for 1 hr, fluorescence anisotropy values were
measured by a Tecan F200 Microplate Reader (\(\lambda_{\text{ex}}\): 535 nm; \(\lambda_{\text{em}}\): 595 nm). Binding activity was determined as described above.

6. Quantum Mechanical (QM) Calculations
The geometry optimization of each of the 9c, 8f and 8d compounds was performed by the Gaussian 09 program.\(^4\) The calculation was conducted by using the \textit{ab initio} theory at the HF/6-31G* level\(^5\) in the gas phase. The partial atomic charges of these compounds were calculated with the restrained electrostatic potential (RESP).\(^6\)

7. Molecular Docking Search and Molecular Dynamics (MD) Simulations
The flexible molecular docking search of each of the 9c, 8f and 8d compounds to the Mcl-1 surface was performed by using AutoDock Vina software package.\(^7\) This was done with a box centered on Mcl-1 and employing 40 \times 40 \times 46 grid points. We used the X-ray determined structure (PDB ID: 3MK8\(^8\)) of the Mcl-1 protein as an initial conformation. For the 9c, 8f, and 8d compounds, we used their geometry optimized structures as initial conformations for the flexible docking search. The docked complex conformations of the best scoring were used as initial structures for running molecular dynamics (MD) simulations.

The all-atom, explicit-water MD simulations were performed at 300 K, 1 bar, and neutral pH using the pmemd module of the AMBER 16 package.\(^9\) We employed the ff99SB-ILDN force field\(^10\) for protein and TIP3P model\(^11\) for water. The water molecules and 2 Cl\(^-\) counter ions were added in a cubic periodic simulation box. The particle mesh Ewald method\(^12\) was applied for treating long-distance electrostatic interactions, while a 12 Å cutoff was used for short-distance interactions. The SHAKE algorithm\(^13\) was employed
for bonds including hydrogen atoms. The initial conformation was subjected to 1,500 steps of steepest descent minimization followed by 1,500 steps of conjugate gradient minimization with 500 kcal mol\(^{-1}\)Å\(^{-2}\) harmonic restraints. Then, the second minimization process, comprising 3,000 steps of steepest descent minimizations and 3,000 steps of conjugate gradient minimization, was performed without harmonic restraints. We then equilibrated the system during 20 ps in which the temperature was gradually increased from 1 to 300 K with a constant-volume (NVT) simulation. This is followed by a 200 ps constant-pressure (NPT) simulation. Finally, we performed 100 ns NPT production run for each complex. The representative complex structure for each compound was obtained from the clustering analysis.

8. Thermodynamics Calculations
The effective binding free energy (\(\Delta f\)) was computed from \(\Delta f = f_{\text{complex}} - (f_{\text{Mcl-1}} + f_{\text{ligand}})\) using the complex, Mcl-1 and ligand conformations taken from the simulation. Here, \(f = E_u + G_{\text{solv}}\) is a sum of the internal potential energy (\(E_u\)) and the solvation free energy (\(G_{\text{solv}}\)). We calculated \(E_u\) directly from the force field used in the simulations. For \(G_{\text{solv}}\), we used the three-dimensional reference interaction site model (3D-RISM) theory.\(^{14}\) We then applied a site-directed decomposition analysis,\(^{15, 16}\) which provides the residue-specific contributions to thermodynamic functions. Fig. S5 shows the contribution to the effective binding free energy (\(\Delta f\)) from each residue. The effective binding free energy (\(\Delta f\)) for 8f is computed to be \(-12.2\) kcal/mol (\(\Delta E_u = -100.6\) kcal/mol; \(\Delta G_{\text{solv}} = 88.4\) kcal/mol), which is about 1 kcal/mol more favorable than the one for 9c (\(\Delta f = -11.2\) kcal/mol; \(\Delta E_u = -71.3\) kcal/mol; \(\Delta G_{\text{solv}} = 60.1\) kcal/mol). This is in good agreement with the experimental result. The computed binding free energy difference between 8f and 9c
stems from the additional contacts present in 8f between the PEG bridge and the Mcl-1 surface (Fig. S5 showing the residue-decomposed result for $\Delta f$). However, the gain in the effective free energy is only mild (1 kcal/mol) since the significant energy gain ($\Delta E_u = -100.6$ kcal/mol for 8f and $-71.3$ kcal/mol for 9c) is largely compensated by the desolvation penalty ($\Delta G_{\text{solv}} = 88.4$ kcal/mol for 8f and 60.1 kcal/mol for 9c). On the other hand, the effective binding free energy for 8d is found to be significantly worse ($\Delta f = -6.2$ kcal/mol; $\Delta E_u = -65.2$ kcal/mol; $\Delta G_{\text{solv}} = 59.0$ kcal/mol) than that of 8f or 9c. This mainly reflects the unfavorable interaction ($\Delta E_u$) between the hydrocarbon bridge of 8d and the hydrophilic Mcl-1 surface ($\Delta E_u = -65.2$ kcal/mol for 8d and $-71.3$ kcal/mol for 9c; whereas $\Delta G_{\text{solv}}$ values for these compounds are similar). Overall, the binding affinity of 8f is the higher than that of the parent compound 9c or the hydrocarbon bridged 8d due to the additional electrostatic interactions between the bridge part of 8f and the hydrophilic Mcl-1 surface. However, due to the desolvation penalty of the hydrophilic bridge of 8f to be able to bind to Mcl-1, the overall binding preference of 8f is only $\sim 1$ kcal/mol.


Jurkat T lymphocyte cells were cultured in medium consisting of Roswell Park Memorial Institute 1640 medium (RPMI) with 10% fetal bovine serum (FBS) and penicillin-streptomycin at 37 °C with 5% CO$_2$.

10. Co-immunoprecipitation (Co-IP)

Jurkat T cells ($1 \times 10^7$ cells) were treated with 8f, 8g or DMSO in Opti-MEM medium for 2 hr at 37 °C with 5% CO$_2$. After incubation, cells were harvested with centrifugation and
washed with cold DPBS twice. Washed cells were lysed with lysis buffer (50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 0.5% NP-40, 1 mM DTT, 1 mM EDTA, and 1×protease inhibitor) on the ice. Pre-cleared cell lysate were incubated with anti-Mcl-1 antibody (S-19, Santa Cruz) for overnight at 4 °C. After incubation, protein A/G agarose (Santa Cruz) was treated to the cell lysates for 1 hr at 4 °C. The beads were precipitated and washed with lysis buffer three times. Proteins bound to the beads were denatured and subjected to Western blotting using anti-BAK (Cell Signaling) and anti-Mcl-1 antibodies (Cell Signaling).

11. Caspase 3/7 Assay
Jurkat T cells were seeded at 3×10^4 cells per well in white 96-well plates in Opti-MEM medium. Cells were treated with varying concentrations of 8f, 8g or 9c or DMSO for 16 hr. The caspase 3/7 activity was measured using Caspase-Glo 3/7 Assay kit (Promega) according to the manufacturer’s instructions.

12. Flow Cytometry
Jurkat T cells were plated at 2×10^5 cells per well in 24-well plates in Opti-MEM medium. Cells were treated with varying concentration of 8f, 8g, 9c or DMSO for 24 hr at 37 °C with 5% CO₂. After incubation, cells were collected with centrifugation and washed with cold DPBS twice. Washed cells were resuspended in cold DPBS and incubated with DPBS containing 2.5 μg/mL propidium iodide (PI). Flow cytometry was conducted using 10,000 live cells by BD FACS Calibur flow cytometer.
Scheme S1. Synthesis of bridged α-helix mimetic small molecules
**Table S1.** Chemical structures and analytic data of 8h-m

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Chemical structure of bridge</th>
<th>% Purity</th>
<th>Reaction condition for stapling</th>
</tr>
</thead>
<tbody>
<tr>
<td>8h</td>
<td><img src="image" alt="Chemical structure of 8h" /></td>
<td>96</td>
<td>1 equiv. CuSO₄·5H₂O, 1 equiv. tris(3-hydroxypropyltriazolyl)methylamine (THPTA) and 3 equiv. of sodium ascorbate in t-butanol : D.W. = 1 : 1 for overnight</td>
</tr>
<tr>
<td>8i</td>
<td><img src="image" alt="Chemical structure of 8i" /></td>
<td>93</td>
<td>4.4 equiv. CuSO₄·5H₂O and 4.4 equiv. sodium ascorbate in t-butanol : D.W. = 1:1 at r.t. for overnight</td>
</tr>
<tr>
<td>8j</td>
<td><img src="image" alt="Chemical structure of 8j" /></td>
<td>71</td>
<td>1.5 equiv. BOP and 2 equiv. DIPEA in DMF/NMP (1:4) at r.t. for overnight</td>
</tr>
<tr>
<td>8k</td>
<td><img src="image" alt="Chemical structure of 8k" /></td>
<td>69</td>
<td>1.2 equiv. MNBA (2-Methyl-6-nitrobenzoic anhydride) and 2.4 equiv. DMAP (4-Dimethylaminopyridine) in DCM at r.t. for 15 hr</td>
</tr>
<tr>
<td>8l</td>
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<td>84</td>
<td>2 equiv. DIPEA in DMF at r.t. for 4 hr</td>
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<tr>
<td>8m</td>
<td><img src="image" alt="Chemical structure of 8m" /></td>
<td>73</td>
<td>2 equiv. DIPEA in DMF at r.t. for 4 hr</td>
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</table>

* Determined by analytical reversed-phase HPLC of crude products.
**Table S2.** The cLogP (calculated log Poctanol/water) values of 8a-8m and 9c

<table>
<thead>
<tr>
<th>Compound</th>
<th>cLogP</th>
<th>Compound</th>
<th>cLogP</th>
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<tr>
<td>8a</td>
<td>5.89</td>
<td>8h</td>
<td>3.94</td>
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<tr>
<td>8b</td>
<td>6.45</td>
<td>8i</td>
<td>4.53</td>
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<tr>
<td>8c</td>
<td>7.02</td>
<td>8j</td>
<td>3.48</td>
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<td>8d</td>
<td>7.58</td>
<td>8k</td>
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</tr>
<tr>
<td>8e</td>
<td>8.15</td>
<td>8l</td>
<td>6.69</td>
</tr>
<tr>
<td>8f</td>
<td>2.38</td>
<td>8m</td>
<td>8.78</td>
</tr>
<tr>
<td>8g</td>
<td>2.05</td>
<td>9c</td>
<td>3.46</td>
</tr>
</tbody>
</table>
Figure S1. LC/MS data for 8a-m.

8a **Calculated mass**: 792.43

8b **Calculated mass**: 806.45

8c **Calculated mass**: 820.46
Figure S1. (Cont’d)

8d  Calculated mass: 834.48

8e  Calculated mass: 848.50

8f  Calculated mass: 840.42
Figure S1. (Cont’d)
Figure S1. (Cont’d)
Figure S1.
Figure S2. $^1$H and $^{13}$C NMR spectra for 8a-m.

8a
Figure S2. (Cont’d)

8b
Figure S2. (Cont’d)

8c
Figure S2. (Cont’d)

8d
Figure S2. (Cont’d)

8e
Figure S2. (Cont’d)

8f
Figure S2. (Cont’d)

8g
Figure S2. (Cont’d)

8h
Figure S2. (Cont’d)

8i
Figure S2. (Cont’d)

8j
Figure S2. (Cont’d)

8k
Figure S2. (Cont’d)
Figure S2.

8m
Fig. S3 Binding curve of fluorescently-labeled BH3 peptide binding to Mcl-1

$K_D = 0.37 \, \mu\text{M}$
**Fig. S4** Comparison of the Mcl-1 binding interfaces between BH3, 9c, 8f, and 8d. (A) BH3/Mcl-1 complex, (B) parent compound 9c/Mcl-1 complex, (C) PEG bridged compound 8f/Mcl-1 complex, (D) hydrocarbon bridged compound 8d/Mcl-1 complex.
**Fig. S5** Residual decomposition of the effective binding free energy ($\Delta f$) of (A) parent compound 9c/Mcl-1 complex, (B) PEG bridged compound 8f/Mcl-1 complex, and (C) hydrocarbon bridged compound 8d/Mcl-1 complex.
**Fig. S6** Co-immunoprecipitation assay. Jurkat T lymphocyte cells were treated with 8g for 2.5 hr. The effect of 8g on the Mcl-1/BAK interaction was analyzed by Mcl-1 immunoprecipitation and BAK Western analysis. The results are representative of three independent experiments.

<table>
<thead>
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<th>8g (μM)</th>
<th>Input</th>
<th>IP : Mcl-1</th>
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</thead>
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<td></td>
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<td>2</td>
</tr>
<tr>
<td>Mcl-1</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>BAK</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>
**Fig. S7** Cellular activities of 9c, 8g, and 8f. Flow cytometry analysis for monitoring apoptotic cells.
Fig. S8 (A) Inhibition curves of 8f, 8g, and yl-2 (a known inhibitor of the NCOA1/STAT6 interaction)² peptide for fluorescein-labeled STAT6 peptide binding to NCOA1 PAS-B domain, (B) Inhibition curves of 8f, 8g, and a BAK peptide (a known inhibitor)³ for fluorescently-labeled BH3 peptide binding to BCL-XLΔC. Error bars represent standard deviation from three independent experiments.
References