One-Bead-One-Compound Screening Approach to the Identification of Cyclic Peptoid Inhibitors of Cyclophilin D as Neuroprotective Agents from Mitochondrial Dysfunction

Supporting Information

Soonsil Hyun,[a]† Namjoon Park,[b]† So Hee Nam,[c] Dae Hee Cheon,[c] Yan Lee,*[c] Hyun-Suk Lim*[d] and Jaehoon Yu*[b]

[a]The Institute of Molecular biology & Genetics, Seoul National University, Seoul 08826, Korea.

[b]Department of Chemistry & Education, Seoul National University, Seoul 08826, Korea. E-mail: jhoonyu@snu.ac.kr

[c]Department of Chemistry, Seoul National University, Seoul 08826, Korea. E-mail: gacn@snu.ac.kr

[d]Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang 790-784, South Korea. E-mail: hslin@postech.as.kr

† These authors equally contributed.

Table of Contents:

METHODS

All the chemicals were purchased from Sigma Aldrich Korea.

OBOC library screening.

TentaGel® B Boc/NH_2 (Rapp Polymere, #B30902.12) bifunctional resin was used to prepare the cyclic peptoid linked beads for OBOC based screening. A library was constructed using the pathways shown in Scheme 1. Two copies of 10^5 OBOC library were used for screening. OBOC library resins were swelled on dimethylformamide (DMF) for 30 min, and washed with distilled water and TBS buffer (5 mL × 5 min, each 3 times). Swelled resins were equilibrated with 5 mL of TBS buffer containing 0.05% Tween®20 (TBST) for 6 h at 4 ℃ with occasional rotation (25 rpm). Streptavidin-conjugated magnetic bead (Dynabeads™ M-270 Streptavidin, Thermo Fisher Scietific) was used to screen the bound peptoids to biotinylted CypA. Before selection, 200 μL of magnetic beads (10 mg/mL) were washed with TBS buffer (1 mL) once and incubated with startingBlock™ (Thermo Scientific) containing 2% bovine serum albumin (Aldrich, BSAV-RO, fraction V) and 0.05% Tween®20 for 1 h at RT to reduce nonspecific binding. As a prescreening step, half (500 μL) of streptavidin-conjugated magnetic beads preincubated with blocking buffer were placed in a separate Eppendorf tube and placed on a magnet to remove the supernatant.

Then, OBOC library resins, swollen in 1 mL of TBST, were added to streptavidin-conjugated magnetic beads preincubated with blocking buffer and incubated for 1 h at 4 ℃. The non-specific binder peptoid loaded resins were eliminated by using this prescreening step and the unbound resins were used for screening.

Biotinylated CypA was prepared using activated biotinylation reagent, EZ-Link™ Sulfo-NHS-LC-LC biotin (Thermo Scientific). Final 100 pmol of biotinylated CypA was loaded on the streptavidin-conjugated magnetic beads preincubated with blocking buffer above by incubating 100 nM of biotinylated CypA with Dynabeads™ in 1 mL of TBST for 1 h at 4 ℃. The supernatant was removed and a slurry of unbound resin in 1 mL of TBST was added to CypA loaded Dynabeads™. The library of resins was incubated with CypA O/N at 4 ℃ with occasional rotation, washed with TBST (1 mL × 1 min, 3 times), and finally, subjected to sequence analysis utilizing the previously reported protocol.[1]

Cyclic peptoid synthesis

Synthesis of unlabeled peptoids. Rink amide MBHA resins (100 mg, 0.051 mmol, 0.51 mmol/g) were swollen with DMF (5 mL × 5 min, RT). The resins were placed in a microwave vessel with 5 mL of 20% piperidine in DMF and

irradiated for 2 min (ramping time for 1 min) at 5 W power on an SPS microwave peptide synthesizer (Discover, CEM). The resins were washed with DMF (5 mL × 1 min, 3 times) and dichloromethane (DCM, 5 mL × 1 min, 3 times). Then, a solution of *N*-α-Fmoc-Cys(4-methoxytrityl)-OH (63 mg, 0.10 mmol), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP, 53 mg, 0.10 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 17.8 μL, 0.10 mmol) in 5 mL of DMF were added and microwave irradiated for 5 min (ramping time for 2 min) at 5 W power. The temperature was set at 35 °C for both Fmoc deprotection and coupling steps.

For peptoid coupling, the selected primary amines were first reacted with bromoacetic acid (BAA) (2 M in DMF, 1.0 mmol, 20 eq) at RT for 2 h, and the resulting glycine derivative were coupled using diisopropylcarbodiimide (DIC) (2 M in DMF, 1.0 mmol, 20 eq) with microwave irradiation at 35 °C for 2 min. This bromoacetylation-amidation sequence was repeated 5 times to complete the sequence for preparation of each penta-peptoid. Following completion of the fifth amidation step in each case, the beads were treated with chloroacetic anhydride (CAA) (2 M in DMF, 1.0 mmol, 20 eq) for promote chloroacetylation. For selective deprotection of the methoxytrityl group on the Cys residue, the resins were treated with 2 mL of a solution of 2%

trifluoroacetic acid (TFA), 5% triisopropylsilane (TIS) in DCM and shaken for 2 min. This cycle was repeated 10 times to ensure complete removal of the methoxytrityl group. After being washed, the beads were treated with 10% DIPEA in DMF for neutralization (RT, 10 min). The linear peptoid loaded resins were then treated with 2M DIPEA in DMF overnight at RT to bring about macrocyclization. Crude cyclic peptoids were recovered from the resin by incubation with a cleavage cocktail containing 95% TFA, 2.5% TIS and 2.5% water for 2 h at RT. All peptoids were then purified by HPLC using Zorbax C_{18} (3 μm, 4.6 x 150 mm) column as the stationary phase. For the mobile phase, buffer A (water with 0.1% v/v TFA) and buffer B (acetonitrile with 0.1% v/v TFA) were used as a gradient.

Synthesis of TAMRA-labeled peptoids. TAMRA-labeled peptoids were synthesized with the same procedure with the synthesis of unlabeled cyclic peptoids. Instead of Fmoc-Cys(4-methoxytrityl)-OH, Fmoc-Lys(4-methoxytrityl)-OH (100 mg, 0.16 mmol) solution was added with PyBOP (92.1 mg, 0.18 mmol) and DIPEA (22. 9 μL, 0.13 mmol) in 5 mL of DMF and irradiated for 5 min using microwave (ramping time for 2 min) at 5 W power. Amine of this Lys will be conjugated with 5(6)-TAMRA (5-(and 6)-Carboxytetramethylrhodamine) at the end of the synthesis. After washing step, Fmoc protection group was removed by 5 mL of 20% piperidine in DMF by irradiating for 2min using

microwave. After washing, the solution of Fmoc-NH-PEG-COOH (9 atoms) (60 mg, 0.16 mmol), PyBOP (92.1 mg, 0.18 mmol), DIPEA (22. 9 μL, 0.13 mmol) were added and irradiated for 5 min. After washing, Fmoc deprotection and another washing step, Fmoc-Cys(tert-butylthio)-OH (67.3 mg, 0.16 mmol), PyBOP (92.1 mg, 0.18 mmol), DIPEA (22. 9 μL, 0.13 mmol) were added to the resins and irradiated with microwave for 5 min. After Fmoc deprotection, coupling of peptoid monomers was performed using the same procedure used in the synthesis of unlabeled peptoids. After CAA coupling and washing step, tert-butylthio protection group of Cys was deprotected by modified Beekman protocol.[2] In brief, the resins were moved to 5 mL round bottom flask and tributylphosphine (32.5 μL, 0.13 mmol) in NMP (N-methyl pyrolidone) : water = 9:1 (1620 uL: 180 uL) was added and stirred for 1 h in N2. After washing step, same procedure was repeated with 10 equivalents (65 μL, 0.26 mmol) of tributylphosphine instead of 5 equivalents. Cyclization and Mmt deprotection were performed with the same procedure used in the synthesis of unlabeled peptoids. The solution of 5(6)–TAMRA (44.8 mg, 0.1 mmol), N-Hydroxybenzotriazole (HOBt, 14.1 mg, 0.1 mmol), 2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU, 43 mg, 0.1 mmol), DIPEA (30.5 μL, 0.17 mmol) was added to the resins for the

TAMRA coupling (2 h, RT). All TAMRA-labeled peptoids were then purified with the same HPLC methods used in the purification of unlabeled peptoids.

Synthesis of Cy5.5-labeled peptoid. Synthesis of Cy5.5-labeled peptoid I11 was performed following the same procedure with the synthesis of TAMRA-labeled peptoids. At the final step, instead of 5(6)-TAMRA and other reagents, the solution of Cyanine 5.5 NHS ester (11.7 mg, 0.015 mmol), DIPEA (3.1 μL, 0.02 mmol) in 1 mL of DMF was added to the ca. 20 mg resins (O/N, RT).

Anti-inflammatory activity

Jurkat cells were maintained in RPMI medium supplemented with 10% fetal bovine serum, penicillin and streptomycin at 37 °C in a 5% CO_2 incubator. To monitor the expressed mRNA levels of the proinflammatory cytokine interleukin 2 (IL-2), Jurkat cells (4×10^5 cells) were treated with 10 ng/mL of Phorbol 12-myristate 13-acetate (PMA) and 1 μg/mL of ionomycin in the presence of 40 μM of cyclic peptoids for 6 h at 37 °C in a 5% CO_2 incubator. As a positive control, 10 nM of CsA was used. The cells were then centrifuged at 14,000 rpm for 2 min at 4 ℃ and the supernatants were removed. The RNA was isolated using TRIzol™ Reagent (Invitrogen™) following the manufacturer's instructions. The isolated RNA was quantified with multimode microplate reader (Spark; TECAN) and 1 ug of each was used to prepare the cDNA using

TOPscript™ RT DryMix (Enzynomics). The cDNA was incubated with RNase H for 20 min at 37℃. The expressed IL-2 level of each sample was examined using TOPreal™ qPCR 2 × PreMIX (Enzynomics, #RT500M) following a modification of the manufacturer's instructions. The housekeeping gene GAPDH was used as a reference for qPCR. The qPCR was performed with real-time PCR cycler (Rotor-Gene Q, QIAGEN).

Binding affinity measurement

Surface Plasmon Resonance (SPR)

Binding affinity experiments were performed at BIACORE 3000 (GE Healthcare). Phosphate buffered saline (PBS) was used as a running buffer for the entire SPR assay. Carboxylic acids on the surface of CM5 sensor chip were activated by a mixture of equivalent volume of EDC (0.4 M) / NHS (0.1 M). A solution of streptavidin (1 mg/mL) was 1,000-fold diluted in sodium acetate (pH 5.5) and immobilized on both analyzing channel and the reference channel by using the amine coupling method. Streptavidin (*c.a.* 5,000 RU) was immobilized at each flow channel. For further immobilization of biotinylated CypA and biotin on each channel, CypA and biotin were diluted in PBS before injection. CypA were immobilized at 3232.6 RU and 2600 RU for two independent experiments. Then, 10 mM of cyclic peptoid in DMSO was diluted to 20 μM in PBS and analyzed for SPR screening. As a negative control, 0.2% DMSO in

PBS was used. The maximum response units from the association phases were used to determine the relative binding efficiencies of the peptoids.

Fluorescence anisotropy assay

Binding constants of the peptoid to Cyps were determined by using fluorescence anisotropy titration. To monitor fluorescence anisotropy changes of 5-TAMRA-labeled peptoids upon addition of CypA (Sino Biological, #10436-H08E) or CypD (LifeSpan Biosciences, LS-G3522),[34] 20 or 40 nM 5-TAMRA-peptoids in 35 mM HEPES buffer (10 mM NaCl, pH = 7.8) were used as the fluorescence probes. The probe solution was excited at 550 nm and monitored at 580 nm (each band path 10 nm) at 25 °C in a final volume of 500 μL using a LS55 fluorescence spectrometer (Perkin Elmer) equipped with a thermo-controlled water circulator. The integration time was 10 s. A solution of CypA (or CypD) was added and the anisotropy was monitored at each concentration of CypA (or CypD). The average results of 5 measurements were used and binding affinities, K_d, were calculated using Prism 8 (GraphPad Software Inc.) by an equation (eq. 1),

$$A = A_0 + \Delta A\frac{([Cyp] + [Peptoid]_0 + K_d) - \sqrt{([Cyp] + [Peptoid]_0 + K_d}}{2[Peptoid]_0}$$

(eq. 1)

where A and A_0 are the fluorescence anisotropy of labeled peptoids in the presence and absence of Cyps, respectively, ΔA is the difference of fluorescence anisotropy between in the presence and in the absence of Cyp. [*Cyp*] and $[Peptoid]_0$ are the concentrations of Cyp and initial peptoid, respectively.

Mitochondria potential measurement

JC-1 assay. A JC-1 kit (Sigma CS0390A) was used to measure the difference of mitochondrial membrane potential ($\Delta\psi_m$) in SH-SY5Y cells according to the manufacturer's protocol. Briefly, a solution (3 mL) of JC-1 dye in Staining Solution (Sigma CS0390) was added to a suspension of SH-SY5Y cells (3×10^6 cells in 3 mL in DMEM complete media) and incubated with final 2 μM of JC-1 dye (Sigma T4069) for 20 min at 37 °C. Mitochondrial membrane potential, JC-1 ratio, was determined by measuring the fluorescence intensity at emission 590 nm with excitation at 485 nm for red fluorescence (J-aggregate) and at 526 nm with excitation at 485 nm for green fluorescence (JC-1 monomer) using a microplate reader (Tecan Spark®) set at 37 °C. Ratio red/green fluorescence 15 min after adding antimycin A was calculated for each condition and levels were relative to untreated cells. A total of three independent experiments were performed and each experiment was duplicated.

Confocal fluorescence microscopy.

SH-SY5Y cells (5 x 10^4 cells/well) were seeded on a 35 mm x 10 mm confocal microscope dish (SPL, 200350) and incubated 24 h. Cells were stained with JC-1 (2 μM) at 37 °C for 15 min and washed with complete DMEM media. Antimycin A (20 μM), CsA (1 μM) and I11 (1 μM) were added and the samples were incubated for 15 min. After PBS washing, cells were observed using confocal laser scanning microscope (Zeiss, LSM 880) with a 40x lens. Representative fluorescence images at 1 μM of CsA and I11 are shown (Fig. 4b). For the measurement of JC-1 monomer intensity, 488 nm laser was used with the filter range of 493-544 nm. For the J-aggregate, 561 nm laser was used with the filter range of 577-631 nm.

Cell Cytotoxicity (WST-1 assay)

SH-SY5Y cells (1.0 × 10^4 cells/well) were seeded on a 96-well plate and incubated 24 h. Cells were incubated with final 10 μM of each peptoid in 5% DMSO in DMEM media. After incubation at 37 °C for 24 h, cell viability was analyzed using a WST-1 assay (DoGen, cat. EZ-3000). Absorbance of each well was measured at 450 nm using a multimode microplate reader (TECAN, Spark). Viability was calculated as the ratio of the absorbance of chemical treated cells to that of not treated cells.

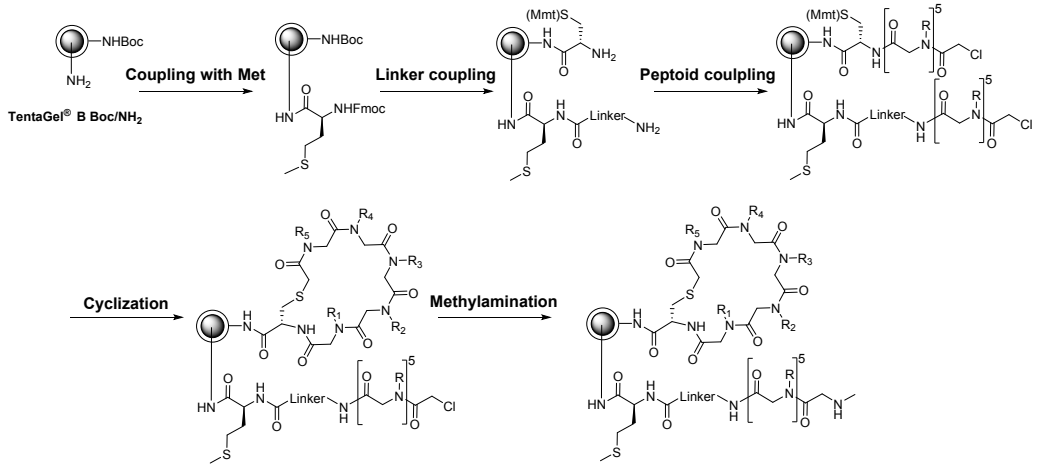
Blood-brain barrier (BBB) permeability using a PAMPA model based on porcine brain lipid extract

BBB permeability of the peptoids and CsA was analyzed using parallel an artificial membrane permeability assay (Corning, cat. 353015) as described previously.[5] The experiment was carried out in triplicate. Five μL of 2% (w/v) brain polar lipid extract (Avanti, cat. 141101P) in dodecane was loaded on the porous PVDF membrane carefully before use. Two hundred μL of 100 μM peptoids and CsA in 5% DMSO in PBS were loaded in each donor well. In each acceptor well, 300 μL of PBS with 5% DMSO was loaded. After 16 h at room temperature, the concentrations of peptoids and CsA in each well were determined using a HPLC (Agilent 1100 series) and calibration curves obtained from serial diluted solutions of each compound. A reverse phase C_{18} (3 μm, 4.6 x 150 mm) column was used as the stationary phase. For the mobile phase, buffer A (water with 0.1% v/v TFA) and buffer B (acetonitrile with 0.1% v/v TFA) were used as a gradient. The gradient conditions are as follows: linear gradient 40-60% B over 10 min. In case of CsA, linear gradient of buffer C (Water:ACN:MeOH = 64:34:3) was used at 65 °C.

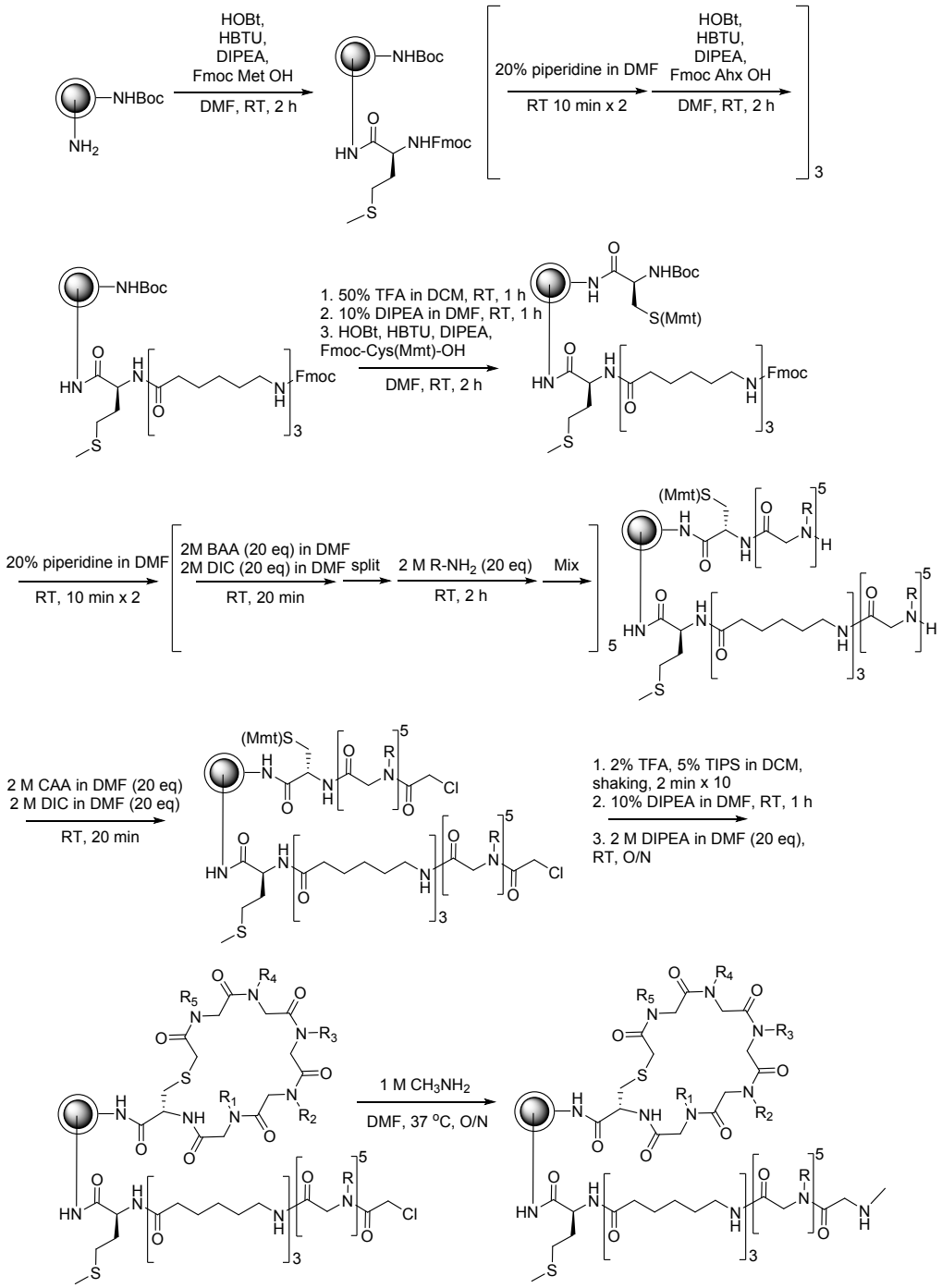
***In vivo* biodistribution of Cy5.5-labelled I11**

Female Balb/c Nu mice were purchased from Raonbio Ltd (Korea) at an age of 6 week. All animal studies were performed in accordance with the Seoul National University Institutional Animal Care and Use Committee (SNU-180116-2-6). Four hundred μM of Cy5.5-I11 was prepared in a vehicle solution (saline:Tween-20:DMSO=83.3:10:6.7). One hundred twenty five μL (50 nmoles, 86.9 μg) was injected intravenously to each mice via tail (n=3). While the mice being anesthetized with isoflurane in oxygen gas, *in vivo* imaging was performed at 30, 60, 90, 230 min post-injection with IVIS Spectrum CT (Perkin Elmer, excitation/emission: 675/720 nm). Mice were sacrificed 230 min post-injection, and then brain, kidney, heart, spleen, lung and liver were collected from each mouse without delay. Fluorescence intensity of Cy5.5-I11 in each organ was measured and its biodistribution (%) was analyzed. Each distribution (%) was calculated by the ratio of total radient efficiency [(p/sec/cm^2/sr)/(μW/cm^2)] for each organ to the total radient efficiency sum of analyzed six organs.

Scheme S1. Preparation of cyclic peptoids library.



Scheme S2. Detailed synthesis of peptoid library.



Scheme S3. Screening of OBOC cyclic peptoid library.

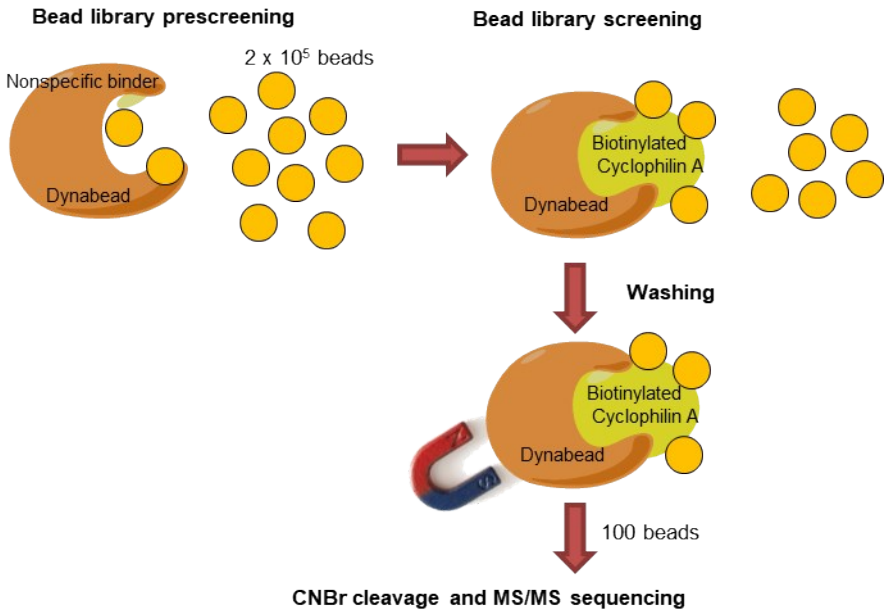


Figure S1. A plot of lipophilicity (cLogP) vs frequency of monomer. Values of cLogP were calculated by using ChemAxon and plotted against the frequency in the screened library (Spearman's r = -0.651, P = 0.047).

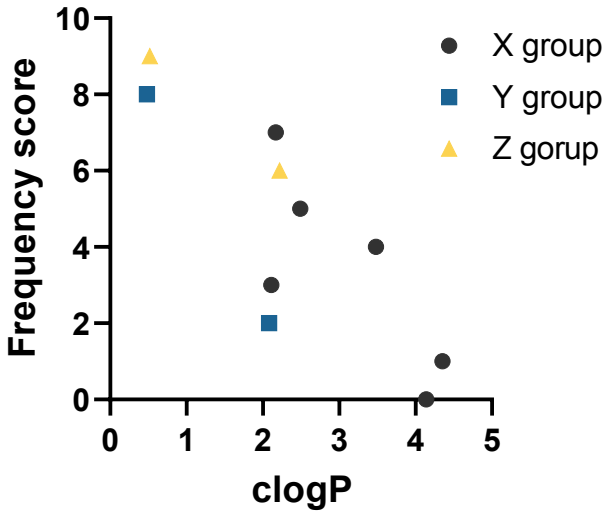
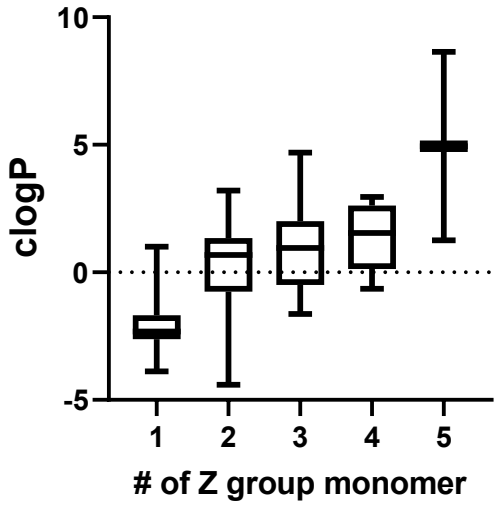


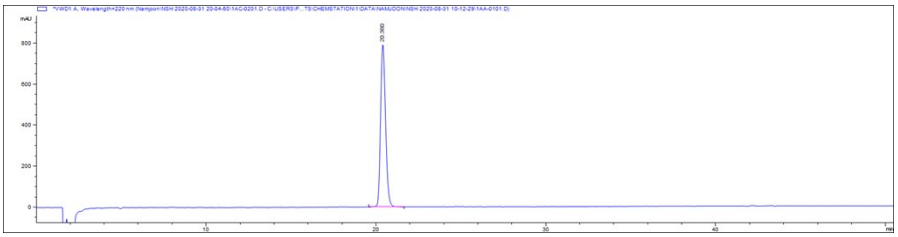
Figure S2. A plot of number of Z monomer vs clogP. (Spearman's r = 0.393, P < 0.0001).



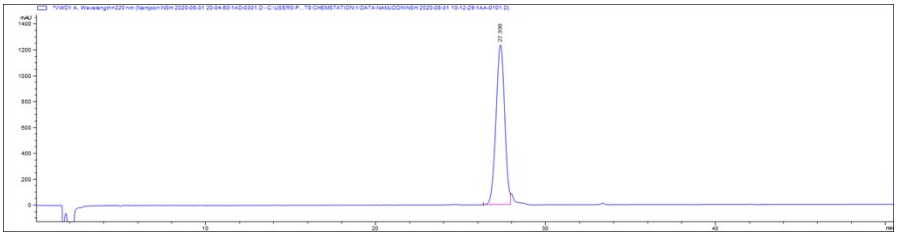
# of Z group monomer	1	2	3	4	5
Ave	-1.99	0.18	0.88	1.35	4.94
s.d.	1.41	1.70	1.52	1.35	5.23

Figure S3. Chromatograms of the peptoids. A Phenomenex C_{18} (3 μm, 4.6 x 150 mm) column was used as the stationary phase. For the mobile phase, buffer A (water with 0.1% v/v TFA) and buffer B (acetonitrile with 0.1% v/v TFA) were used as a gradient. The gradient conditions are as follows: 5 min, 5% B followed by linear gradient 5-70% B over 25 min. Each peptoid was labeled on a respective chromatogram.

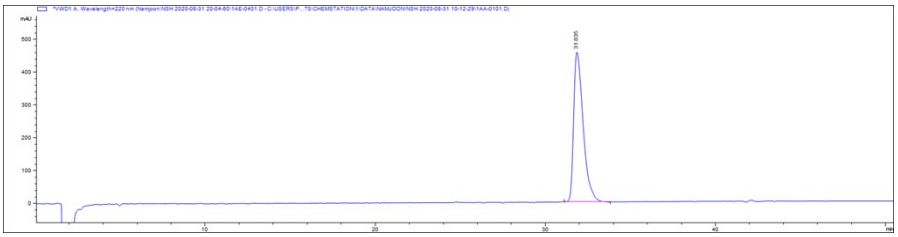
H3 was obtained as a white powder. The HPLC chromatogram of H3 is shown below (>99% purity).



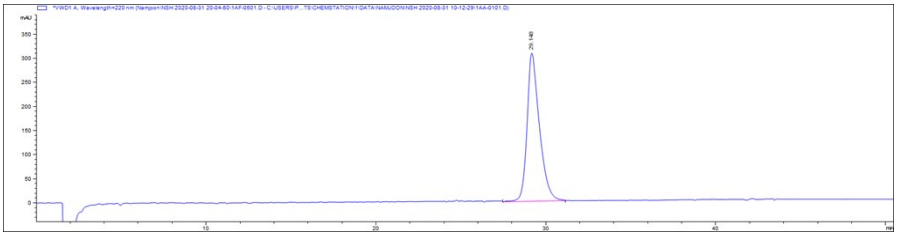
H9 was obtained as a white powder. The HPLC chromatogram of H9 is shown below (96% purity).



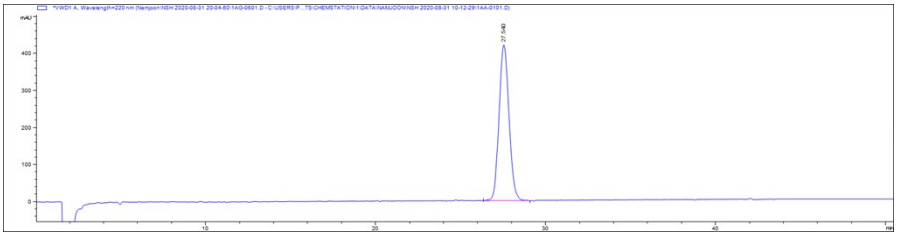
H11 was obtained as a white powder. The HPLC chromatogram of H11 is shown below (>99% purity).



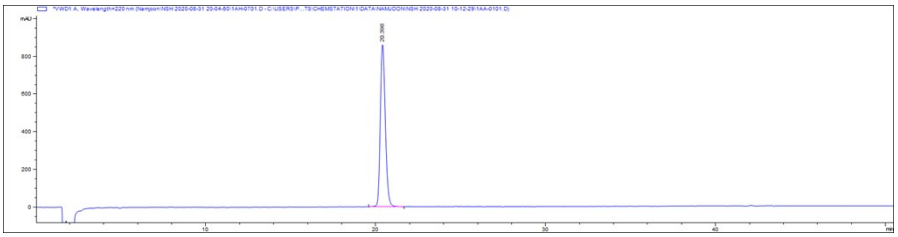
H17 was obtained as a white powder. The HPLC chromatogram of H17 is shown below (>99% purity).



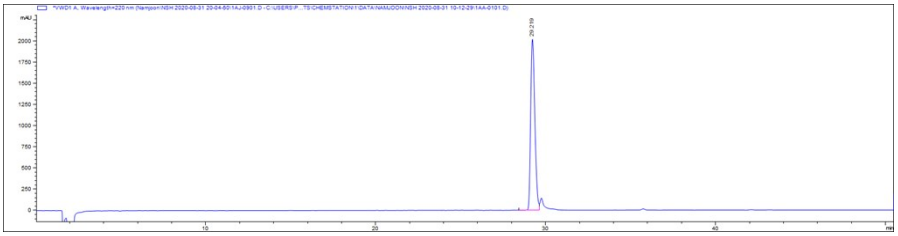
H23 was obtained as a white powder. The HPLC chromatogram of H23 is shown below (>99% purity).



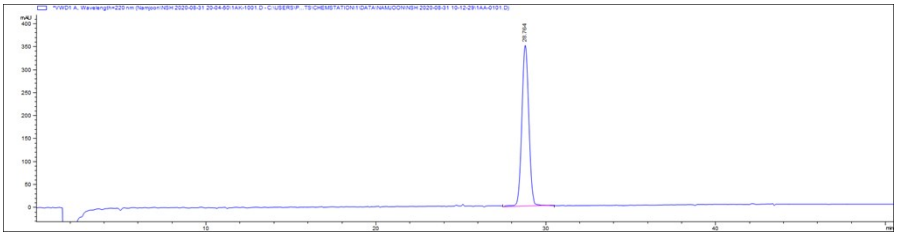
I4 was obtained as a white powder. The HPLC chromatogram of I4 is shown below (>99% purity).



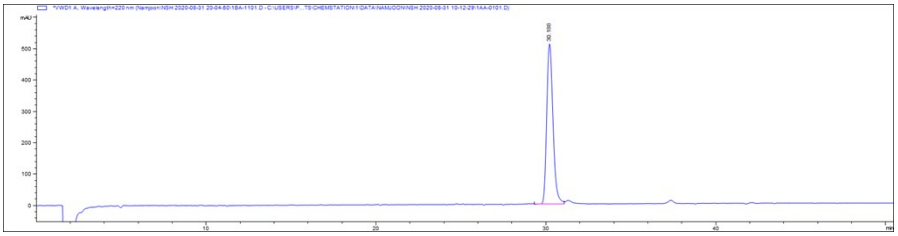
I11 was obtained as a white powder. The HPLC chromatogram of I11 is shown below (93% purity).



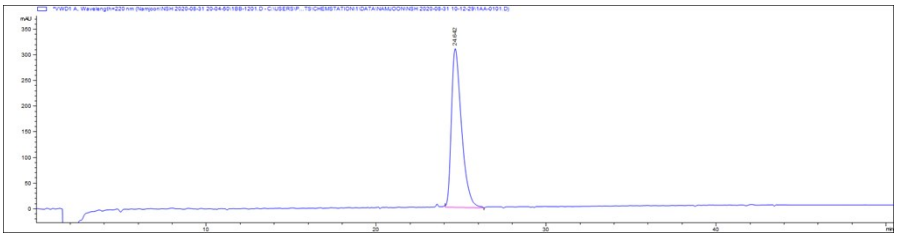
I14 was obtained as a white powder. The HPLC chromatogram of I14 is shown below (>99% purity).



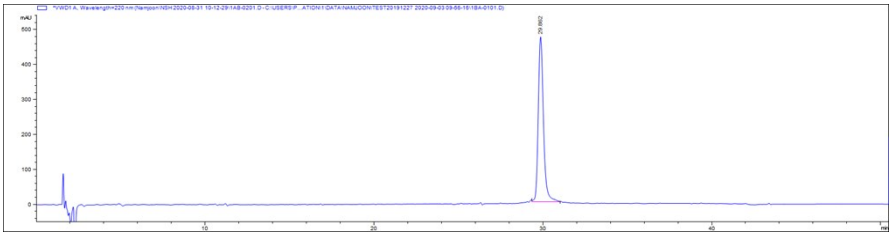
I15 was obtained as a white powder. The HPLC chromatogram of I15 is shown below (96% purity).



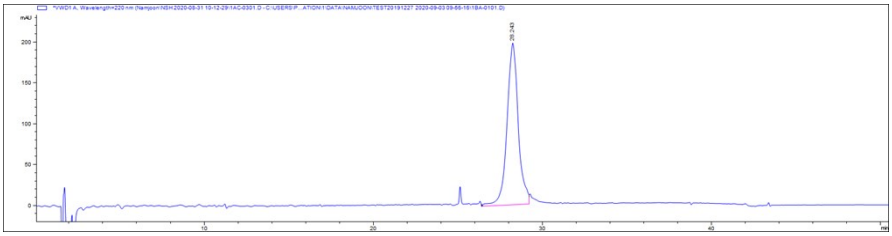
J1 was obtained as a white powder. The HPLC chromatogram of J1 is shown below (>99% purity).



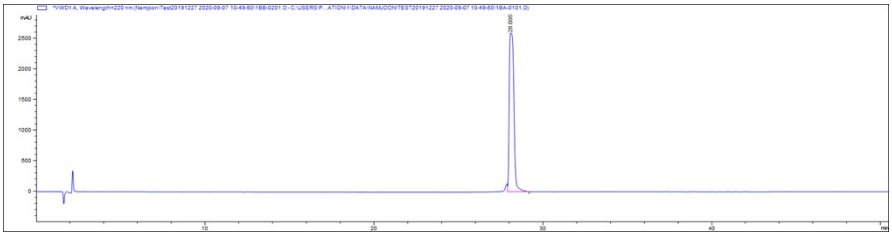
TAMRA-H11 was obtained as a red powder. The HPLC chromatogram of TAMRA-H11 is shown below (>99% purity).



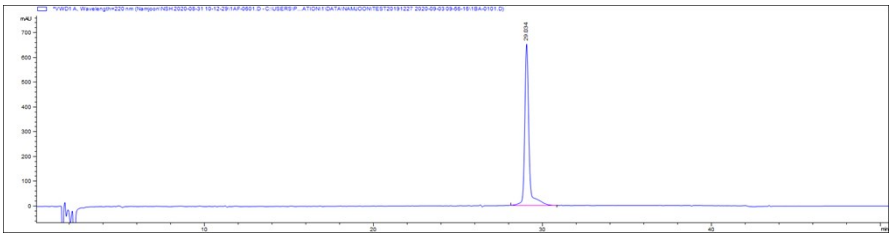
TAMRA-H17 was obtained as a red powder. The HPLC chromatogram of TAMRA-H17 is shown below (98% purity).



TAMRA-I11 was obtained as a red powder. The HPLC chromatogram of TAMRA-I11 is shown below (98% purity)



TAMRA-I15 was obtained as a red powder. The HPLC chromatogram of TAMRA-I15 is shown below (>99% purity).



Cy5.5-I11 was obtained as a blue powder. The HPLC chromatogram of Cy5.5-I11 is shown below (95% purity).

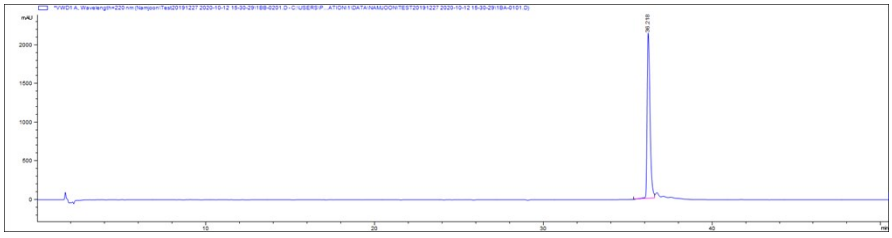


Figure S4. Chemical structures of synthesized cyclic peptoids.

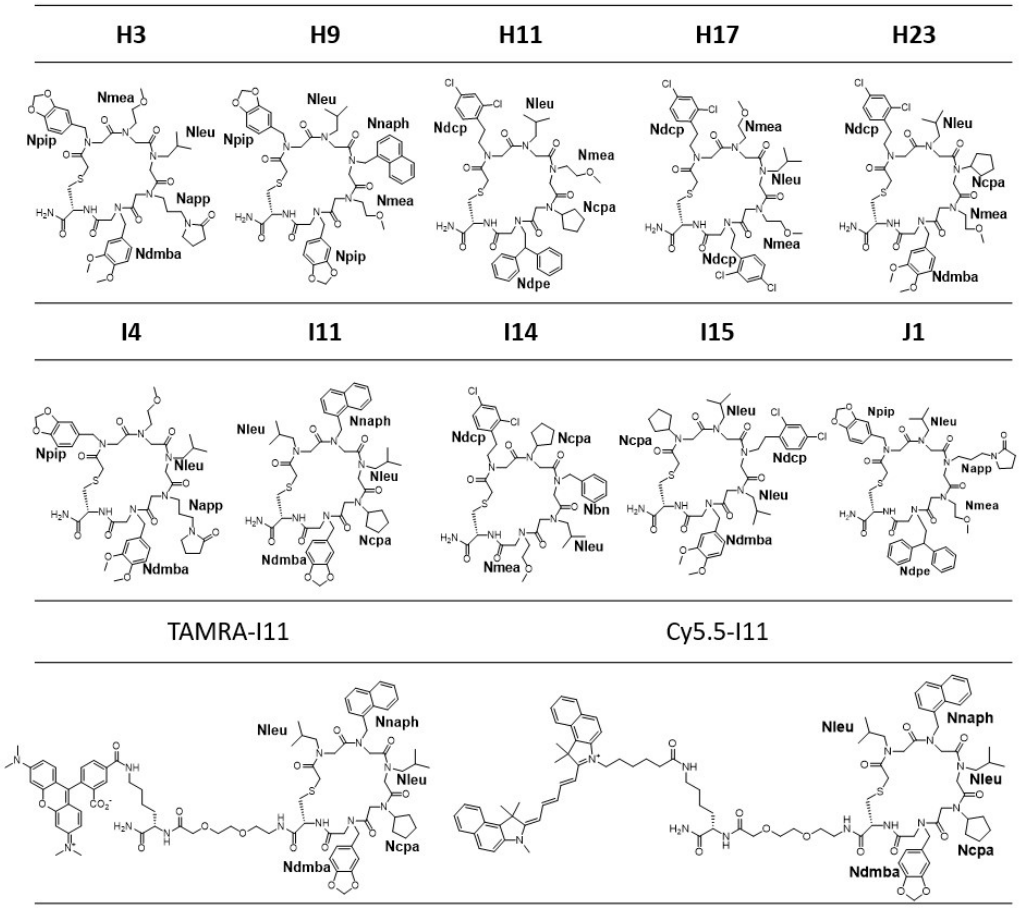


Figure S5. Fluorescence anisotropy binding curves of CsA and I11 interactions with CypA and CypD. (a) The binding of CsA to CypA (top) and CypD (bottom). (b) The binding of I11 to CypA (top) and CypD (bottom). The data points represent mean ± SD of three independent experiments.

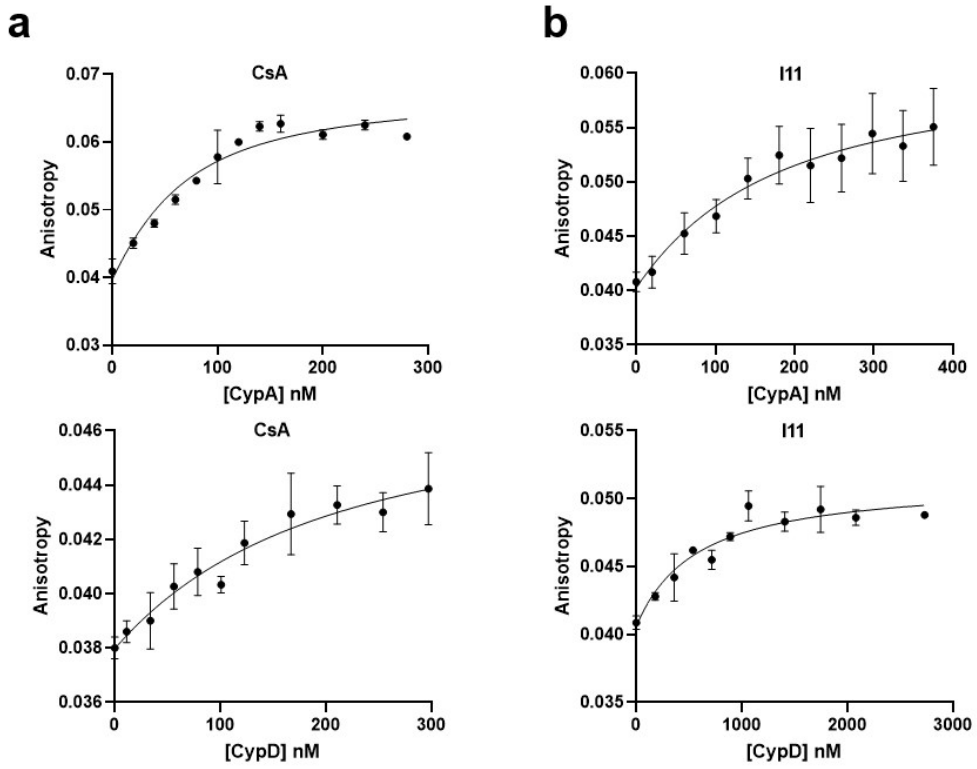


Figure S6. WST-1 cell cytotoxicity assay. SH-SY5Y cells were treated with various concentrations of macrocyclic peptoids for 24 h.

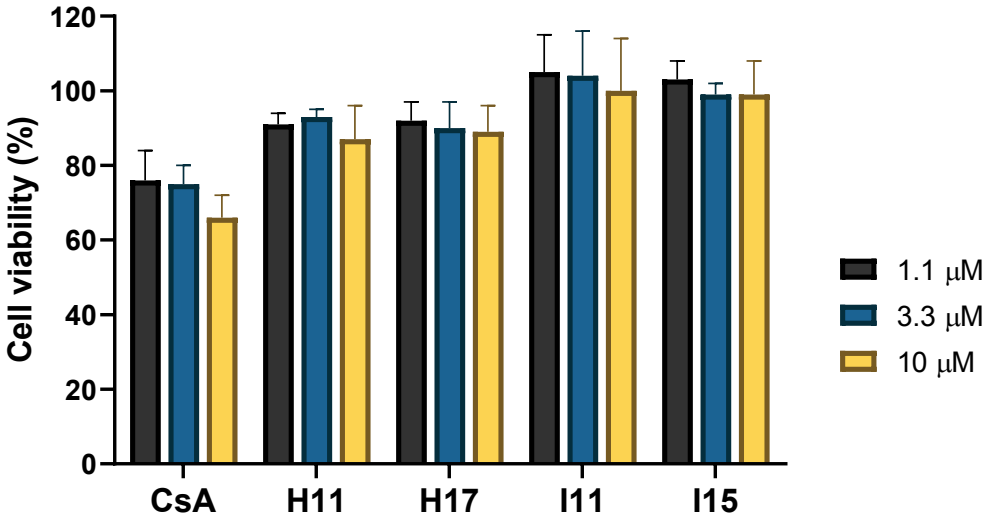


Figure S7. Biodistribution of I11. a) *In vivo* fluorescence images of mouse after intravenous injection of Cy5.5-labelled I11. Dye-labeled I11 was administered at a dose of 50 nmol/mouse via a lateral tail vein. All fluorescence images were acquired at 30, 60, 90, and 230 min. b-d) *Ex vivo* fluorescent images of major organs excised (b) and blood (c) at the end point. d) Relative distribution (%) was calculated by the ratio of total radient efficiency for each organ to the total radient efficiency sum of analyzed six organs.

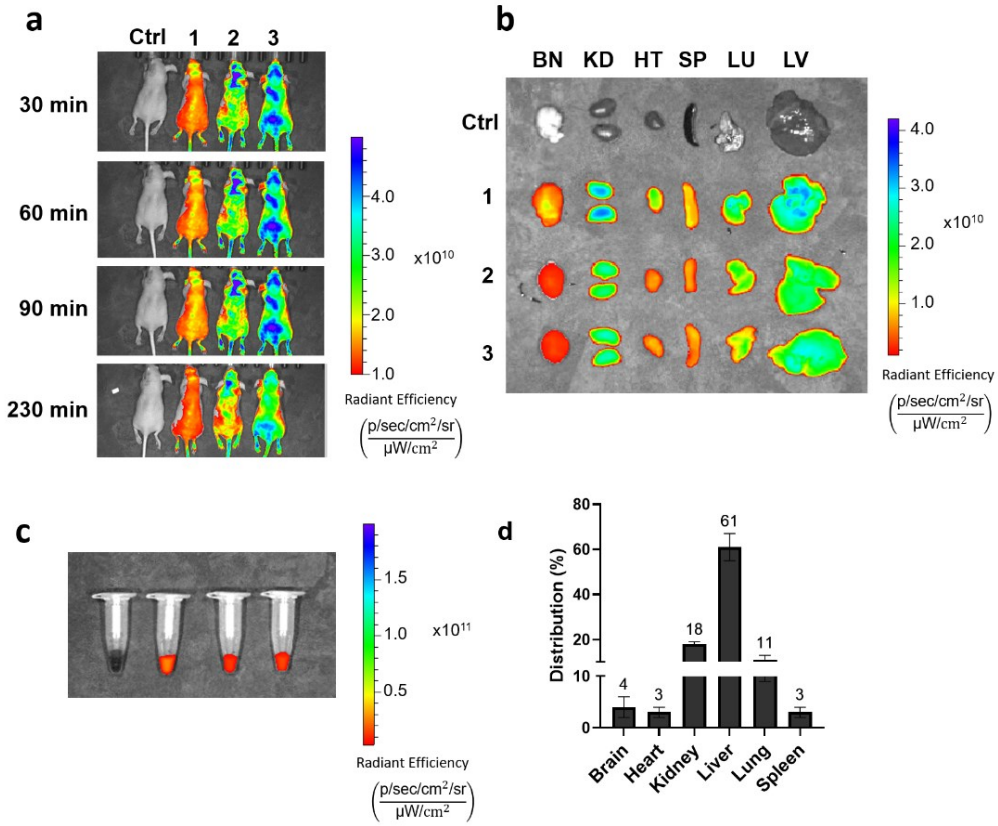


Table S1. Sequence analysis of cyclic peptoids by using MS/MS.[a]

#	Name of Sequence	Position of monomer[b] 1	2	3	4	5	#	Name of Sequence	Position of monomer[b] 1	2	3	4	5
1	F1	Ncpa	Ncpa	Ndmba	Ndmba	Nbn	**51**	H3	Ndmba	Napp	Nleu	Nmea	Npip
2	F2	Ncpa	Npip	Napp	Npip	Ndpe	**52**	H4	Ncpa	Ndmba	Ncpa	Nmea	Nnaph
3	F3	Nnaph	Nbn	Nmea	Nbn	Ndmba	**53**	H5	Ndmba	Npip	Ndmba	Ncpa	Ndpe
4	F4	Ncpa	Ndcp	Napp	Nbn	Nmea	**54**	H6	Nbn	Npip	Ndmba	Nmea	Ndmba
5	F5	Napp	Ncpa	Ncpa	Nbn	Nmea	**55**	H7	Nmea	Nnaph	Nbn	Nleu	Nmea
6	F6	Nbn	Nmea	Napp	Npip	Nnaph	**56**	H8	Ndmba	Nbn	Napp	Nleu	Nbn
7	F7	Npip	Npip	Nnaph	Ndmba	Napp	**57**	H9	Npip	Nmea	Nnaph	Nleu	Npip
8	F8	Nmea	Ncpa	Nmea	Npip	Ndcp	**58**	H10	Nmea	Nbn	Nbn	Ndmba	Nbn
9	F9	Npip	Ndmba	Ncpa	Nbn	Napp	**59**	H11	Ndpe	Ncpa	Nmea	Nleu	Ndcp
10	F10	Npip	Ndpe	Napp	Nmea	Npip	**60**	H12	Ndmba	Ncpa	Npip	Napp	Nnaph
11	F11	Nnaph	Ncpa	Napp	Ndpe	Nmea	**61**	H13	Nmea	Nbn	Ndmba	Nleu	Ndcp
12	F12	Nmea	Nleu	Napp	Ndcp	Ndpe	**62**	H14	Ncpa	Ndmba	Napp	Nmea	Nleu
13	F13	Nbn	Napp	Nnaph	Ndmba	Ndmba	**63**	H15	Nmea	Ncpa	Nbn	Ndpe	Nnaph
14	F14	Ndmba	Nmea	Nnaph	Napp	Nnaph	**64**	H16	Nbn	Ndcp	Ncpa	Napp	Ncpa
15	F15	Ndpe	Nmea	Nleu	Nleu	Nleu	**65**	H17	Ndcp	Nmea	Nleu	Nmea	Ndcp
16	F16	Ndpe	Nnaph	Nleu	Nmea	Nbn	**66**	H18	Ncpa	Nbn	Nnapa	Ncpa	Nmea
17	F17	Nleu	Nbn	Nnaph	Nbn	Ndmba	**67**	H19	Napp	Ndcp	Napp	Nleu	Nnaph
18	F18	Nbn	Ncpa	Ndpe	Nmea	Ncpa	**68**	H20	Ndpe	Nmea	Ndmba	Ndmba	Napp
19	F19	Ndmba	Ncpa	Ncpa	Nnaph	Nbn	**69**	H21	Npip	Ndmba	Nmea	Napp	Nnaph
20	F20	Ndpe	Ncpa	Npip	Npip	Ndmba	**70**	H22	Nmea	Nleu	Nnaph	Nnaph	Nnaph
21	F21	Ndmba	Nbn	Ncpa	Nleu	Ndmba	**71**	H23	Ndmba	Nmea	Ncpa	Nleu	Ndcp
22	F22	Nmea	Ncpa	Nmea	Npip	Ncpa	**72**	H24	Ncpa	Napp	Nleu	Napp	Nnaph
23	F23	Ndpe	Ndmba	Nmea	Ndcp	Napp	**73**	I1	Nbn	Ncpa	Ncpa	Nnaph	Ndpe
24	F24	Ndmba	Nbn	Ncpa	Nleu	Ndmba	**74**	I2	Napp	Ndmba	Nmea	Ndcp	Ncpa
25	G1	Napp	Ncpa	Nleu	Ndmba	Nnaph	**75**	I3	Ndpe	Nnaph	Nmea	Napp	Ncpa
26	G2	Napp	Nnaph	Nbn	Nleu	Ncpa	**76**	I4	Ndmba	Napp	Nleu	Nmea	Npip
27	G3	Napp	Ndmba	Ncpa	Ndpe	Ndmba	**77**	I5	Ncpa	Ndpe	Nleu	Npip	Npip
28	G4	Napp	Nnaph	Ncpa	Nmea	Ndpe	**78**	I6	Napp	Nleu	Nnaph	Napp	Nnaph
29	G5	Nnaph	Nmea	Ndpe	Ndmba	Npip	**79**	I7	Nnaph	Nleu	Nbn	Nbn	Nmea
30	G6	Napp	Nnaph	Ndcp	Nnaph	Nleu	**80**	I8	Ndmba	Nnaph	Ndmba	Nleu	Ncpa
31	G7	Npip	Nleu	Ncpa	Nbn	Ncpa	**81**	I9	Napp	Nnaph	Ncpa	Ndmba	Ndmba
32	G8	Napp	Nnaph	Ndpe	Nmea	Npip	**82**	I10	Nbn	Ndmba	Ncpa	Napp	Ndcp
33	G9	Napp	Napp	Nbn	Ndpe	Ndmba	**83**	I11	Npip	Ncpa	Nleu	Nnaph	Nleu
34	G10	Npip	Npip	Nbn	Nbn	Npip	**84**	I12	Nmea	Nbn	Nbn	Nmea	Nbn
35	G11			n.d.			**85**	I13	Ndpe	Ndmba	Ndpe	Ndpe	Nnaph
36	G12	Ncpa	Ndcp	Nmea	Ndmba	Nnaph	**86**	I14	Nmea	Nleu	Nbn	Ncpa	Ndcp
37	G13	Ndmba	Ncpa	Nnaph	Ndmba	Ndmba	**87**	I15	Ndmba	Nleu	Ndcp	Nleu	Ncpa
38	G14	Nmea	Ndcp	Napp	Nleu	Napp	**88**	I16	Nbn	Napp	Ndcp	Ndmba	Ncpa
39	G15	Nmea	Nbn	Ndcp	Ndmba	Napp	**89**	I17	Ndmba	Nnaph	Nbn	Nmea	Nmea
40	G16	Ndmba	Ncpa	Ncpa	Ndmba	Napp	**90**	I18	Napp	Ndmba	Nbn	Ndmba	Npip
41	G17	Ndmba	Napp	Ndcp	Ndcp	Napp	**91**	I19	Ndmba	Npip	Npip	Ncpa	Napp
42	G18	Ndmba	Npip	Ndpe	Nmea	Napp	**92**	I20	Ndmba	Ncpa	Nbn	Nmea	Napp

43	G19	Ndmba	Npip	Npip	Ncpa	Nnaph	**93**	I21	Nmea	Npip	Ndpe	Npip	Napp
44	G20	Napp	Nbn	Nbn	Nnaph	Napp	**94**	I22			n.d.		
45	G21	Napp	Nbn	Napp	Nnaph	Nbn	**95**	I23	Nmea	Ncpa	Npip	Napp	Ndmba
46	G22			n.d.			**96**	I24	Nmea	Npip	Nmea	Nmea	Npip
47	G23	Nnaph	Napp	Nbn	Nleu	Napp	**97**	J1	Ndpe	Nmea	Napp	Nleu	Npip
48	G24	Ndmba	Nbn	Nmea	Npip	Nleu	**98**	J2	Ndcp	Napp	Nmea	Ncpa	Napp
49	H1	Napp	Ndcp	Napp	Napp	Nmea	**99**	J3	Nmea	Ncpa	Napp	Ndcp	Ndpe
50	H2	Nmea	Nnaph	Nnaph	Ndcp	Nmea	**100**	J4	Nnaph	Ncpa	Npip	Ncpa	Nnaph

[a]Nleu; Isobutylamine, Nmea; 2-Methoxyethylamine, Ncpa; Cyclopentylamine, Napp; 1-(3-Aminopropyl)-2-pyrrolidinone, Ndmba; 3,4-Dimethoxybenzylamine, Nbn; Benzylamine, Npip; Piperonylamine, Nnaph; 1-Naphthylmethylamine, Ndcp; 2,4-Dichlorophenethylamine, Ndpe; 2,2-Diphenylethylamine. [b]n.d., not determined due to the sample quality.

Table S2. Monomer frequency and score.

Monomer	Frequency	Frequency score[a]
Nleu	37	2
Nmea	63	8
Ncpa	61	6
Napp	64	9
Ndmba	63	7
Nbn	50	5
Npip	43	3
Nnaph	48	4
Ndcp	26	0
Ndpe	28	1

[a] The frequency score is shown as 0-9.

Table S3. Sequence and MS data of 10 selected cyclic peptoids.[a]

Peptoid	Sequence	MS $[M+H]^+$	
		calcd	obsd
H3	cyclo[Cys-Ndmba-Napp-Nleu-Nmea-Npip]	969.43	969.63
H9	cyclo[Cys-Npip-Nmea-Nnaph-Nleu-Npip]	968.38	968.60
H11	cyclo[Cys-Ndpe-Ncpa-Nmea-Nleu-Ndcp]	980.38	980.61
H17	cyclo[Cys-Ndcp-Nmea-Nleu-Nmea-Ndcp]	962.25	964.41
H23	cyclo[Cys-Ndmba-Nmea-Ncpa-Nleu-Ndcp]	950.36	950.49
I4	cyclo[Cys-Ndmba-Napp-Nleu-Nmea-Npip]	969.43	969.60
I11	cyclo[Cys-Npip-Ncpa-Nleu-Nnaph-Nleu]	900.43	900.53
I14	cyclo[Cys-Nmea-Nleu-Nbn-Ncpa-Ndcp]	890.34	890.36
I15	cyclo[Cys-Ndmba-Nleu-Ndcp-Nleu-Ncpa]	948.38	948.51
J1	cyclo[Cys-Ndpe-Nmea-Napp-Nleu-Npip]	999.46	999.52
TAMRA-H11	Rho-Lys-peg-cyclo[Cys-Ndpe-Ncpa-Nmea-Nleu-Ndcp]	1665.69	1665.16
TAMRA-H17	Rho-Lys-peg-cyclo[Cys-Ndcp-Nmea-Nleu-Nmea-Ndcp]	1647.56	1649.03
TAMRA-I7	Rho-Lys-peg-cyclo[Cys-Nnaph-Nleu-Nbn-Nbn-Nmea]	1565.71	1565.32
TAMRA-I11	Rho-Lys-peg-cyclo[Cys-Npip-Ncpa-Nleu-Nnaph-Nleu]	1585.74	1586.15
TAMRA-I15	Rho-Lys-peg-cyclo[Cys-Ndmba-Nleu-Ndcp-Nleu-Ncpa]	1633.69	1633.96
Cy5.5-I11	Cy5.5-Lys-peg-cyclo[Cys-Npip-Ncpa-Nleu-Nnaph-Nleu]	1738.92	1738.76

[a] All mass spectrometric data of peptoids were from analysis of $[M+H]^+$peaks.

1. K. J. Lee and H.-S. Lim, *Org. Lett.*, 2014, **16**, 5710-5713.
2. N. J. Beekman, W. M. Schaaper, G. I. Tesser, K. Dalsgaard, S. Kamstrup, J. P. Langeveld, R. S. Boshuizen and R. H. Meloen, *J. Pept. Res.*, 1997, **50**, 357-364.
3. K. Kajitani, M. Fujihashi, Y. Kobayashi, S. Shimizu, Y. Tsujimoto and K. Miki, *Proteins*, 2008, **70**, 1635-1639.
4. In the OBOC screening step, we used CypA instead of CypD due to the solubility concern of the commercially available wild type enzyme.
5. O. Tsinman, K. Tsinman, N. Sun and A. Avdeef, *Pharm. Res.*, 2011, **28**, 337-363.