Supporting Information

Self-Assembling Cyclic D,L-α-Peptides as Modulators of Plasma HDL Function. A Supramolecular Approach Towards Anti-Atherosclerotic Agents

Yannan Zhao,† Luke J. Leman,† Debra J. Search,† Ricardo A. Garcia,‡ David A. Gordon,‡ Bruce E. Maryanoff,‡ M. Reza Ghadiri†*

†Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037
‡Cardiovascular Drug Discovery, Bristol-Myers Squibb Company, Pennington, NJ 08534
Peptides were synthesized by using standard Fmoc chemistry with an Advanced Chemtech Apex 396 peptide synthesizer. A typical synthesis was performed on 0.09-mmol scale using 0.6 mmol/g Rink amide MBHA resin and N-hydroxysuccinimide (NHS) to activate the amino acids. The side chain to yield a Gln residue after cleavage from the resin. Standard side chain protecting groups included Gln(Trt), Asn(Trt), Lys(Boc), Orn(Boc), diaminopropionic acid(Boc), diaminobutyric acid(Boc), His(Trt), Ser(tBu), Trp(Boc), Glu(OtBu). Chain elongations were achieved using 1,3-diisopropylcarbodiimide (DIC) and HOBt in N,N-diisopropylethylamine (DIPEA) in DMF.

Solid-phase peptide synthesis

Peptides were purified by preparative reverse-phase (RP)-HPLC on a Vydac 218TP C18 or 214TP C4 column. Purity was confirmed by analytical RP-HPLC. Purified peptides were characterized by analytical HPLC and MALDI-TOF mass spectrometry. Analytical RP-HPLC was performed using a Vydac 214TP C-4 column or a Zorbax 300SB-C18 column connected to a Hitachi D-7000 HPLC system. Binary gradients of solvent A (99% H₂O, 0.9% acetonitrile, 0.1% TFA) and solvent B (90% acetonitrile, 9.9% H₂O, 0.07% TFA) were employed for HPLC.

The N-methylated derivative, c[wL°Mo2wSeQ°Mo2sO] (83), was prepared by loading a 2-chlorotriyl chloride resin with Fmoc-Ser-OAll (All, allyloxy carbonyl) via the side chain. The peptide synthesis proceeded through the Gln residue, using Fmoc-MeLeu-OH (NovaBiochem). The amine of the Gln residue was methylated selectively on the solid support following the method of Biron et al. 51. The synthesis was continued as described above. Coupling reactions to methylated amine groups were carried out using PyBroP (5 mol-equiv) and i-Pr₂NET (12 mol-equiv) in DMF for 12 h. The wMeLwReMeQeR (27) peptide was prepared in a similar fashion, with side chain anchoring at the D-Glu residue.

Human plasma remodeling

Quantitation by densitometry of Western blots. Plasma remodeling studies were carried out to measure the effectiveness of the cyclic D,L-α-peptides in promoting the formation of pre-β HDL from mature human plasma HDLs. Procedures were modified from reported protocols. 52. Blood samples from normal healthy human donors were obtained from TSRI Normal Blood Donor Program. EDTA-anticoagulated whole blood was centrifuged at 1,500 x g for 15 min within 1 h of being drawn from the donor. The plasma layer was aliquoted and used immediately or frozen at -80 °C for future use. Peptide stock solutions were prepared at ~15 mM peptide in 50% DMSO for initial screenings, or at ~2 mM peptide in 10% sucrose for remodeling assays at lower concentrations and cholesterol efflux assays. Plasma samples (30 µL) were treated with peptide stocks, and the samples were incubated at 37 °C for 0.5–1 h, and then quenched by adding 270 µL of 50% sucrose (1:9 dilution). Samples were mixed with 6x native loading buffer (125 mM tris, pH 6.8, 0.02% bromophenol, 20% glycerol), of which 6 µL was loaded onto a 4–20% polyacrylamide gel. The molecular weight ladder used was High Molecular Weight Calibration Kit (Amersham Biosciences, #17-0445-01). Gels were run at constant 80 V at 4 °C for 14 h using a Laemli buffer system (25 mM tris, 192 mM glycine, pH 8.3). Gels were then blotted onto nitrocellulose membranes (0.45 µm, Bio-Rad) at constant 30 V at 4 °C for 2 h using the Laemli buffer (no methanol). The membrane was blocked with 3% bovine serum albumin (BSA) in TBS (20 mM tris, 150 mM NaCl, pH 7.6) for 1 h at 23 °C, and then washed once for 10 min with TBST (20 mM tris, 150 mM NaCl, pH 7.6, 0.1% tween-20). The membrane was next incubated with 3% BSA in TBST containing a ~1.8000 dilution of HRP-conjugated goat anti-human apoA-I antibody (Academy Bio-Medical, #11H-G1a) for 1 h at 23 °C. The membrane was extensively washed with TBST (at least 6 x 10 min) and once with TBS. The membrane was incubated with chemiluminescence reagent (Thermo SuperSignal West Pico) for 5 min, and then exposed to photographic film (Kodak BioMax Light). The films were scanned and quantified by densitometry using the program LabWorks (v. 4.0.0.8). The data from individual blots were normalized by scaling the densitometry values using a "no-treatment" sample and a 4F (43 µM) sample from each individual blot as the low and high signals, respectively.
Quantitation by ELISA assay. Pre-β1-HDL levels were also assayed by ELISA (Daiichi, cat # 289194). 10 µL of peptide stock (0.9 mM in 50% DMSO or 10% sucrose) were mixed with 40 µL of freshly thawed frozen human plasma and incubated for 1 h at 37°C. Remodeling was stopped by 21-fold dilution in stabilization buffer (provided by the kit). Samples were further diluted to final 101-fold in dilution buffer (provided by the kit) and either assayed immediately or stored at -80°C until time of assay. All kit components, stored at 4°C, were warmed to room temperature prior to use. Buffers, substrate and calibrator were prepared according to manufacturer instructions. 50 µL of either sample or calibrator were added to each test well in duplicate to a 96-well flat-bottom plate. The plate was covered and incubated at room temperature for 1 h, protected from light. The liquid was then aspirated and the wells were washed 4 times according to manufacturer instructions. 50 µL of enzyme-labeled anti-pre-β HDL MAb, provided by the kit, were added to each test well. The plate was incubated at room temperature for 1 h, protected from light. The liquid was then aspirated and the wells were washed 4 times according to manufacturer instructions. 50 µL of substrate solution were added to each test well. The plate was incubated at room temperature for 10 min, protected from light, after which the reaction was stopped by addition of 50 µL of stop reagent (provided by the kit) to each test well. Absorbance was measured at wavelength 495 nm and using 660 nm for reference. A standard curve was calculated by using a quadratic fit. The data were normalized across multiple ELISA assays by including the 4F peptide as a high signal and 10% sucrose as a low signal in every assay.

Cholesterol efflux
Preparation of labeling medium. These procedures were modified from a reported protocol. The labeling medium was prepared by complexing the unlabeled cholesterol and BODIPY-cholesterol (20% of the total cholesterol) with methyl-β-cyclodextrin (CD) at a molar ratio of 1:40 (total cholesterol/CD). Unlabeled cholesterol and BODIPY-cholesterol were dissolved in chloroform and dried in a round bottom flask by rotor-evaporation in the dark to form a thin film. The cholesterol mixture was solubilized by adding 20 mM CD in MEM-HEPES buffer. The suspension was sonicated in a water bath (37°C) for 1 h, stirred at 37°C for 3 h, filtered using a 0.45-µm syringe filter, and diluted with an equal volume of MEM-HEPES buffer containing 4 µg/mL Sandoz ACAT (acyl-CoA:cholesterol acyltransferase) inhibitor (Sigma). The final concentrations of BODIPY-cholesterol, unlabeled cholesterol, CD, and ACAT inhibitor in the labeling medium were 0.025 mM, 0.1 mM, 10 mM, and 2 mg/mL, respectively.

Preparation of efflux medium. Just before the initiation of the efflux assays, the human plasma frozen at -80°C was thawed, and 80 µL of plasma was incubated with 20 µL of cyclic peptide stocks (peptide stocks were 1-3 mg/mL in 10% sucrose) or the vehicle (10% sucrose) at 37°C for 30 min. The treated plasma samples (100 µL) were then incubated with 25% PEG 8000 solution in 200 mM glycine buffer, pH 8.5 (40 µL) to precipitate apoB-containing lipoproteins. After a 15-min incubation, the precipitate was removed by high-speed centrifugation (13,000 rpm, 10 min, 4°C), and the supernatant (60 µL) was immediately diluted with MEM-HEPES buffer (1.5 mL) to give ~2% whole plasma. This solution, containing the peptide remodeled-HDL lipoprotein fraction as cholesterol acceptors, was used as the efflux medium.

Cholesterol efflux from mouse macrophage cells. Mouse macrophage J774A.1 cells (TIB-67; American Type Culture Collection, Manassas, VA) were seeded in 12-well culture plate at 3×10⁵ cells/mL and cultured to 80–90% confluency in DMEM with 10% FBS at 5% CO₂. Cells were rinsed twice with MEM-HEPES, incubated for 1 h with 0.25 mL of labeling medium containing CD/BODIPY-cholesterol/unlabeled cholesterol and 2 µg/mL Sandoz ACAT inhibitor, followed by washing twice with MEM-HEPES. The cholesterol-laden cells were equilibrated for 18 h in DMEM containing 5% LPDS, 2 µg/mL Sandoz ACAT inhibitor, and 0.3 mM Cpt-cAMP. After equilibration, the cells were washed twice with MEM-HEPES and incubated with 0.25 mL of efflux media for 4 h. At the end of the incubation time, the efflux media was removed and centrifuged (1200 rpm, 10 min) to remove floating cells, and the fluorescence intensity of 100 µL cell-free media was recorded using a Tecan GENios plate reader (excitation 485 nm, emission 535 nm). The obtained signals were background subtracted using the signal from unlabeled cells. The cell monolayers were rinsed twice with ice-cold MEM-HEPES and solubilized with 0.25 mL 1% cholic acid by shaking on a plate shaker for at least 4 h at room temperature; then the fluorescence intensity of 100 µL cell lysate was recorded and background subtracted using the signal from unlabeled cell lysate. Fractional efflux of BODIPY-cholesterol was calculated based on the fluorescence intensity of the media divided by the total fluorescence values (medium + lysate). BODIPY-cholesterol efflux to the media containing vehicle (10% sucrose)-treated plasma was 29 ± 0.7%, and was subtracted from cholesterol efflux values of all acceptors. Measurements were made in quadruplicate.

Cellular cytotoxicity and hemolysis
The assays were performed as described in ref. S5.

Mouse pharmacokinetics
Male mice (20 g) of a BALB/cByJ background were obtained from the Rodent Breeding Colony of the Department of Animal Resources at TSRI, and were maintained on a chow diet. The cyclic peptide c[wLwReQeR], was dissolved in PBS containing 1% sucrose, and sterile filtered through a 0.22-µm syringe filter before injection. Mice were fasted beginning 12 h before dosing, and continued the fast until after the 8-h time point blood draw was completed. Groups of three mice received a 16-mg/kg dose of the cyclic peptide via intraperitoneal injection (0.3 mL). Blood was drawn (30–60 µL) from the retro-orbital sinus into a heparinized capillary tube before dosing (0 min) and at different intervals from 30 min to 8 h after dosing. Plasma was isolated immediately from the whole blood by centrifugation at 5000 rpm for 10 min at 4°C. Immediately after the plasma was isolated, 20 µL of plasma was acidified with 20 µL of 5% TFA to break peptide-protein interactions, and 40 µL of acetonitrile was then added. After vortexing for 30 s, the mixture was centrifuged at 13000 rpm for 10 min at 4°C. The resultant supernatant was analyzed by using LC-MS SIM as described below.
Cyclic peptide concentrations were quantified by using reverse-phase HPLC coupled with mass spectrometry. The electrospray ionization mass spectrometry measurements were carried out in the positive ionization mode using a single quadrupole mass spectrometer (Hewlett Packard HP 1100 MSD series). 10 or 20 µL of sample were injected through a C8 reverse-phase column (Zorbax 300-SB, 4.6 mm×150 mm, 5 µm) using a flow rate of 1.5 mL/min and binary gradients of solvent A (99% H2O, 0.1% formic acid, 0.01% TFA) and solvent B (99% acetonitrile, 0.1% formic acid, 0.01% TFA). Mass detection was carried out in the selected ion monitoring (SIM) mode for the positive molecular ion, with the optimized fragmentor and capillary voltages of 180 V and 5 kV, respectively. The selected monitoring mass for cyclic peptide c[wLwReQeR], 21, was 592.8 ([M+2H]⁺). For quantitative calibration, standard curves were established using mouse EDTA-anticoagulated plasma spiked with various concentrations of the peptide. The calibration curve was established by using linear fitting of the data, with correlation coefficient ≥ 0.98. The limit of detection was 1 µM.

In vivo plasma remodeling
Cyclic peptide c[wLwReQeR], 21, was dissolved in 10% sucrose, and sterile filtered through a 0.22-µm syringe filter before injection. Mice were fasted beginning 12 h before dosing, and continued the fast until after the 8-h time point blood draw was completed. Groups of three mice received a 20-mg/kg dose of the cyclic peptide via intraperitoneal injection (0.3 mL). Blood was drawn (30–60 µL) from the retro-orbital sinus into a heparinized capillary tube before dosing (0 min) and at different intervals from 30 min to 8 h after dosing. Plasma was isolated immediately from the whole blood by centrifugation at 5000 rpm for 10 min at 4°C, after which 10 µL of plasma were mixed with 90 µL of 50% sucrose, vortexed for 30 s, and stored at 4°C until all time points had been collected (samples were stored from 0–8 h). Samples were then mixed with 20 µL of 6x native loading buffer (125 mM tris, pH 6.8, 0.02% bromophenol, 20% glycerol). 6 µL of these samples were loaded onto a 4–20% polyacrylamide gel. Gels were run at constant 80 V at 4°C for 14 h using a Laemli buffer system (25 mM tris, 192 mM glycine, pH 8.3). Gels were then blotted onto nitrocellulose membranes (0.45 mm, Bio-Rad) at constant 30 V at 4°C for 2 h using the Laemli buffer (no methanol). The membrane was blocked with 5% non-fat dry milk (NFDM) in TBST (20 mM tris, 150 mM NaCl, pH 7.6, 0.1% Tween-20) for 1 h at 23°C, and then washed once for 10 min with TBST. The membrane was next incubated with 1% NFDM in TBST containing a ~1:5,000 dilution of rabbit anti-mouse apoA-I antibody (Meridian Life Science) as primary antibody for 1 h at 23°C. The membrane was extensively washed with TBST (at least 6 x 10 min), and incubated with 1% NFDM containing a ~1:50,000 dilution of HRP-conjugated anti-rabbit IgG (Thermo) as secondary antibody for 1 h at 23°C. The membrane was extensively washed with TBST (at least 6 x 10 min), and once with TBS. The membrane was incubated with ECL reagent (Thermo SuperSignal West Pico) for 5 min, and then imaged using photographic film (Kodak BioMax Light).

LPS acute inflammation assay
This experiment was carried out in collaboration with Bristol Myers Squibb (Hopewell, NJ, USA). Peptide 21 (7.5 mg/kg) or vehicle (phosphate-buffered saline, pH 7.4) was administered to BALB-C mice (n=5 per group) via i.p. injection 4 h prior to LPS challenge. In vivo challenge with LPS from Salmonella enterica serotype Typhimurium was administered at 0.03 mg/kg via i.p. injection. A third group of animals did not receive any treatment or LPS challenge to provide baseline cytokine levels. Retro-orbital plasma samples were taken serially post challenge to measure the plasma profiles of key cytokines using custom-designed multiplex luminex panels.

Statistical analysis
Data are expressed as the mean ± S.D. Statistical significance was determined by Student’s t-test, as determined by using GraphPad Prism software (version 5.0d). p values <0.05 were considered as statistically significant.

SUPPORTING REFERENCES
(S1) Biron, E.; Chatterjee, J.; Kessler, H. Optimized selective N-methylation of peptides on solid support. J. Pept. Sci. 2006, 12, 213-219.
(S2) Troutt, J. S.; Alborn, W. E.; Mosior, M. K.; Dai, J.; Murphy, A. T.; Beyer, T. P.; Zhang, Y.; Cao, G.; Konrad, R. J. An apolipoprotein A-I mimetic dose-dependently increases the formation of pre-beta1 HDL in human plasma. J. Lipid Res. 2008, 49, 581-587.
(S3) Sankaranarayanan, S.; Kellner-Weibel, G.; de la Llera-Moya, M.; Phillips, M. C.; Asztalos, B. F.; Bittman, R.; Rothblat, G. H. A sensitive assay for ABCA1-mediated cholesterol efflux using BODIPY-cholesterol. J. Lipid Res. 2011, 52, 2332-2340.
(S4) Asztalos, B. F., de la Llera-Moya, M., Dallal, G. E., Horvath, K. V., Schaefer, E. J., and Rothblat, G. H. Differential effects of HDL subpopulations on cellular ABCA1- and SR-BI-mediated cholesterol efflux. J. Lipid Res. 2005, 46, 2246-2253.
(S5) Dartois, V.; Sanchez-Quesada, J.; Cabezas, E.; Chi, E.; Dubbelde, C.; Dunn, C.; Granja, J.; Gritzen, C.; Weinberger, D.; Ghadiri, M. R.; Parr, T. R., Jr. Systemic antibacterial activity of novel synthetic cyclic peptides. Antimicrob. Agents Chemother. 2005, 49, 3302-3310.
Table S1. Sequences and rank order effectiveness of peptides screened for plasma HDL remodeling activity.

| peptide rank | sequence (#) | score | peptide rank | sequence | score | peptide rank | sequence | score |
|--------------|--------------|-------|--------------|----------|-------|--------------|----------|-------|
| 1            | WlWrEqEr (22)| 62.8±16.3 | 28         | yAyLkHkK (53) | 9.1±1.0 | 55         | IWkHsHkK (68) | -0.9±0.9 |
| 2            | wLwReQeR (21)| 61.3±10.5 | 29         | wAOwSeQsO (32) | 8.5±1.7 | 56         | LwLHkKeH (39) | -1.1±6.0 |
| 3            | wLwKsHkK (40)| 48.4±8.3 | 30         | wLwReQeR (27) | 8.0±0.7 | 57         | FYkFkK (69) | -1.2±3.5 |
| 4            | wFyYhGrS (51)| 42.7±10.5 | 31         | wLwKdQdK (3) | 7.8±0.9 | 58         | IWkHkKhK (41) | -1.8±1.7 |
| 5            | wLwSeQsO (31)| 37.6±12.3 | 32         | yYvYhH (62) | 7.4±2.3 | 59         | WEIYsHo (70) | -2.1±0.2 |
| 6            | wLwRdNdK (1) | 37.6±10.8 | 33         | wFwYrSsH (48) | 6.8±1.1 | 60         | yLyReNeK (20) | -2.1±0.8 |
| 7            | YlWyKhAe (55)| 32.2±2.4 | 34         | yYvYhKk (63) | 6.4±1.4 | 62         | LwKkKkK (71) | -2.7±5.2 |
| 8            | wFyYhHsH (50)| 31.2±9.8 | 35         | wLwReQeR (23) | 5.4±1.4 | 63         | LkSkSkSs (72) | -2.8±0.5 |
| 9            | wLwOeQeO (15)| 29.2±2.7 | 36         | wLwKdQdK (39) | 5.0±0.8 | 64         | LwXeQeE (12) | -3.4±0.3 |
| 10           | wLwSeQhK (34)| 25.7±6.9 | 38         | yDyWvHaK (65) | 5.3±1.1 | 65         | LwLhRkKk (42) | -4.1±0.9 |
| 11           | wLwKdDk (56)| 23.3±1.2 | 39         | wLwHeNeK (8) | 5.0±0.8 | 66         | LwSeWk (73) | -4.6±0.1 |
| 12           | LwLwSeEK (57)| 23±0.2  | 40         | wLwEeKkN (7) | 4.9±1.6 | 67         | LwOeQeK (13) | -4.8±0.3 |
| 13           | LwLwOeNeO (16)| 22.9±3.3 | 41         | wLwSeQsK (33) | 4.1±0.1 | 68         | LwXeNeK (6) | -5.9±2.6 |
| 14           | LwLwLrKe (58)| 22.8±6.1 | 42         | yGwWnNkN (45) | 3.8±0.5 | 69         | LwRdQeK (19) | -7.1±5.8 |
| 15           | wNAwReQeR (26)| 22.6±2.6 | 43         | wLwHeQeK (9) | 3.7±0.8 | 70         | LwLwKrK (74) | -7.1±2.0 |
| 16           | wLwKsK (59)| 19.3±4.0 | 44         | wBYkNnk (66) | 3.2±0.7 | 71         | WkBwKsQsK (36) | -7.1±2.3 |
| 17           | wLwKdNdK (2) | 17.0±1.1 | 45         | wLwKdQeK (4) | 3.1±2.4 | 72         | LwXeQeK (14) | -8.5±1.5 |
| 18           | wLwSeKeK (5)| 15.3±3.4 | 46         | wLwSeQeK (11) | 2.7±1.4 | 73         | IWkhHeK (75) | -8.9±1.5 |
| 19           | pyLwKsKsK (37)| 14.6±2.2 | 47         | wLwReQeO (18) | 1.9±1.7 | 75         | IWThKkK (43) | -9.8±2.1 |
| 20           | wLwRwReQeR (28)| 13.9±9.2 | 48         | wWfYkHsS (47) | 1.0±3.0 | 76         | LwLkKkRh (44) | -10.1±1.0 |
| 21           | yWyKsHaE (60)| 12.5±3.6 | 49         | yWyKsHaH (38) | 0.9±1.6 | 77         | WybNoHkKt (77) | -10.9±0.3 |
| 22           | wLwSeQsSs (46)| 12.0±0.5 | 50         | yLwSeQsSs (35) | 0.7±0.3 | 78         | yLyReQeO (78) | -12.8±0.5 |
| 23           | wLwREQeR (29)| 11.9±12.6 | 51         | WIWkHyK (67) | 0.7±1.9 | 79         | LwLhKkKk (79) | -14.6±1.1 |
| 24           | wLwReNeO (17)| 10.6±2.7 | 52         | WwWgWkSo (54) | 0.5±0.7 | 80         | YfGqKk (80) | -15.5±0.3 |
| 25           | wNBkSkSkS (61)| 9.7±0.9  | 53         | WwWwRgQeR (25) | 0.0±0.5 | 81         | WwWwReQeR (25) | -15.5±0.3 |

“Score” refers to the normalized level (µg/mL) of pre-beta HDL generated by incubation of human plasma with the peptide (180 µM), as determined by the pre-beta ELISA assay. The data were normalized across multiple ELISA assays by using the 4F peptide as a positive control and 10% sucrose as a negative control. Capital letters represent L-amino acids; small letters represent D-amino acids. Abbreviations: Z, 2,3-diaminopropionic acid; X, 2,4-diaminobutyric acid; O, ornithine; HL, homoleucine; AO, 2-amino octanoic acid; NA, 2-naphthylalanine; KB, Ne-benzyl lysine; FY, 3-pyridylalanine; mε, N-methylated amino acid; BY, benzyl tyrosine.
Figure S1. Comparison of apoA-I mimetic agents based on a cyclic D,L-α-peptide (left) and a linear α-helical peptide (right).
(a) Chemical structure of the cyclic peptide and helical wheel diagram for the helical peptide, showing the location of charged residues. Blue, cationic; red, anionic. (b) The likely mechanism of action of the cyclic D,L-α-peptides involves membrane insertion and peptide self-assembly into nanotubular complexes, whereas conventional apoA-I mimetic peptides fold into an α-helix to function within the membrane. (c) The side chains along a face of a cyclic D,L-α-peptide nanotube and a folded α-helix are positioned similarly due to similar distances between individual cyclic D,L-α-peptide subunits and turns of the helix, respectively. (d) A nanotube composed of a stack of four cyclic peptides and a helix are shown as cylinders cut down the long axis of the hydrophobic face and flattened. Dotted lines mark the hydrophobic/hydrophilic interface. Red, cationic residues; blue, anionic residues; capital letter, L-amino acid; lower case letter, D-amino acid. Note that cyclic D,L-α-peptides assemble in an antiparallel fashion in the cylindrical structure.
Figure S2. Western blots from 10% sucrose screens of cyclic D,L-α-peptides (360 µM). Peptides were added to human plasma from 10% sucrose stock solutions. The samples were incubated for 1 h at 37 °C, and then western blotted for human apoA-I to visualize HDL remodeling. The data from individual blots were normalized by scaling the densitometry values using the 10% sucrose sample and the 4F (43 µM) sample from each individual blot as the low and high signals, respectively. O, ornithine; X, diaminobutyric acid; Z, diaminopropionic acid.

Figure S3. Correlation between amount of pre-beta HDL generated by cyclic D,L-α-peptides as determined by either Western blot densitometry or ELISA.
Figure S4. Backbone N-methylation blocks the self-assembly of cyclic D,L-α-peptides. a) In the absence of backbone N-methylation, the flat ring conformation leaves the backbone carbonyl and NH atoms free to hydrogen bond with other like molecules, giving rise to elongated tubular complexes. b) The introduction of backbone N-methyl substituents (magenta balls) at every other or every fourth residue renders one face of the peptide ring incapable of intersubunit hydrogen bonding, thereby limiting the self-association to dimeric complexes. For further details, see ref S5.

Figure S5. Pharmacokinetics of cyclic D,L-α-peptide c[wLwReQeR], 1, in mice, as determined by using HPLC/MS SIM. (a) Pharmacokinetic profile for the peptide in male BALB/c mice (n = 3) after an i.p. dose of 16 mg/kg. Data are given as mean ± SD. (b) Representative MS selected ion trace for ion 592.8 ([M2H]2+) at cyclic peptide concentrations of 0 µM, 1 µM (lower limit of detection), and 6 µM in mouse plasma. The peak at 4.4 min corresponds to the cyclic peptide. (c) Representative calibration curve of cyclic peptide in mouse plasma.

y = 36230x
R² = 0.9978