Electronic Supplementary Information

Stimuli-Responsive α-Helical Peptide Gatekeepers for Mesoporous Silica Nanocarriers

*Jeonghun Lee, Seungjong Han, Jinyoung Lee, Minhyuek Choi, Chulhee Kim**

Department of Polymer Science and Engineering, Inha University, Incheon 22212, Korea

***Corresponding Author**: chk@inha.ac.kr

Experimental Section

Materials. Cetyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane, propargyl bromide, copper (II) sulfate, trifluoroacetic acid (TFA), ninhydrin, piperidine, doxorubicin (DOX), glutathione (GSH), acetic anhydride, triethylamine, poly(ethylene glycol) methyl ether and sodium ascorbate were obtained from Aldrich and used as received. N,N'-dimethylformamide (DMF), triisopropylsilane were purchased from Acros organics. 3,6-Dioxa-1,8-octane-dithiol, N,N-diisopropylcarbodiimide (DIPC) were purchased from TCI. 1-Hydroxybenzotriazole (HOBt) and Rink Amide MBHA resin were purchased from Advanced Chem. Tech. Fmoc-L-Dap(N_3)-OH was purchased from IRIS Biotech GmbH. N-α-Fmoc protected amino acids from Novabiochem were used as received.

Synthesis of Si-NH_2. A MCM-41-type mesoporous silica nanoparticle (Si-NP) with pore size of 2.5 nm was prepared as previously described.[1] Then, 3-aminopropyltriethoxysilane (3.5 mL) was added into an ethanol solution (20 mL) of MCM-41 (50 mg), which was then allowed to react overnight at 60 °C. The resulting solid was washed thoroughly with ethanol.

Synthesis of Si-alkyne. A methanol solution (14 mL) of Si-NH_2 (100 mg) was allowed to react with propargyl bromide (1 mL) overnight at 50 °C. The resulting solid was washed thoroughly with methanol.

Peptide synthesis. All peptides were synthesized using Fmoc-chemistry in solid-phase peptide synthesis as shown in Fig. S1.[2] Fmoc-protected amino acids were assembled on Rink Amide MBHA resin. The coupling reaction for each amino acid was carried out using a 3-molar excess of the corresponding Fmoc amino acid and coupling reagents in DMF. DIPC (0.3 mmol), HOBt (0.3 mmol) and Fmoc-protected amino acid (0.3 mmol) in DMF (3 ml) were added to Rink amide resin (200 mg. 0.1 mmol), and the resulting solution was stirred for 4 h at room temperature. After filtration, the resin

was washed three times with DMF (3 ml) and methanol (3 ml), respectively. The coupling reaction was repeated until no color change was observed in the ninhydrin test. After completion of the solid-phase synthesis, the peptide was deprotected and cleaved from the resin by treatment with a mixture of TFA/triisopropylsilane/3,6-dioxa-1,8-octane-dithiol/H_2O (94:1:2.5:2.5, v/v) at room temperature for 4 h. After cleavage of the peptide from the resin, the resin was filtered and excess of TFA was removed in solution, and then the peptide was obtained by precipitation into cold diethylether at -20 ºC. The successful synthesis and high purity of the peptide were confirmed by HPLC and ESI-mass spectroscopy analyses (Fig. S5-8). N_3-^{D}CILC(LLKK)$_2$: calcd 1567.98, obsd 1567.7 $[M+H^+]^+$, 784.1 $[M+2H^+]^{2+}$, N_3-^{D}CILCLLKK: calcd 1085.62, obsd 1086.4 $[M+H^+]^+$, 543.8 $[M+2H^+]^{2+}$, 1108.5 $[M+Na^+]^+$.

Intramolecular disulfide bond formation. Air was bubbled overnight into a solution of N_3-^{D}CILCLLKK or N_3-^{D}CILC(LLKK)$_2$ in acetonitrile/10 mM ammonium bicarbonate solution (50:50, v/v). The oxidized peptides were obtained after drying in vacuo. Successful intramolecular disulfide bond formation was confirmed by mass spectrometry (Fig. S9 and S10). N_3-SS-^{D}CILC(LLKK)$_2$: calcd 1565.98, obsd 1567.1 $[M+H^+]^+$, 784.7 $[M+2H^+]^{2+}$, N_3-SS-^{D}CILCLLKK: calcd 1083.62, obsd 1084.8 $[M+H^+]^+$, 543.1 $[M+2H^+]^{2+}$.

Synthesis of silica nanoparticles with peptide gatekeepers. Surfactant was removed from silica nanoparticles two times by stirring Si-alkyne (60 mg) in 10 mL of ethanol with 200 mg of ammonium nitrate at 80 °C for 30 min. The resulting solid was washed thoroughly with ethanol. For cargo loading, surfactant-removed Si-alkyne (20 mg) was soaked in a DMF solution (0.2 mL) of DOX (1 mg) and stirred overnight. Then, 10 mg of peptide with intramolecular disulfide bond in 1 mL of DMF, copper (II) sulfate (14.98 mg) in 100 μL of water, sodium ascorbate (11.69 mg) in 100 μL of water were added. The click reaction for conjugation of the peptides onto the surface of MSNs was performed by microwave (150 W) exposure at 90 °C with stirring for 15 min. The resulting solid was washed thoroughly with DMF and distilled water. The conjugation of each peptide onto silica nanoparticles was

confirmed by FT-IR. The amounts of peptides on the surface of MSNs were calculated using 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) titration of thiol groups. After reduction of the disulfide bond on the surface of peptide-modified MSNs using dithiothreitol in distilled water, the resulting particles were washed thoroughly using DMF and distilled water. Then the surface thiol unit was reacted with DTNB in tris buffer (pH = 8.0). The weight percentages of the peptide units on the surface of MSNs were calculated from the UV/vis absorbance at 412 nm of the resulting solution. The weight percentage of the peptide units on the surface of Si-SS-^{D}CILCLLKK and Si-SS-^{D}CILC$(LLKK)_2$ was 1.6 and 1.8 wt%, respectively.

PEGylation of MSNs. For surface functionalization of Si-SS-^{D}CILC$(LLKK)_2$ and Si-SS-^{D}CILCLLKK with PEG, a THF solution of methoxy poly(ethylene glycol) (MW 2000, 1 g, 0.5 mmol) was allowed to react with hexamethylene diisocyanate (0.45 mmol) containing dibutyltin dilaurate (23 μmol) for 1.5 h at 50 °C. The resulting mixture was added to a THF solution of MSNs with peptide gatekeepers (20 mg) and stirred for 1 d. The resulting product was washed thoroughly with THF and distilled water by centrifugation.

Liposome leakage assay.[3] Liposome was prepared by a conventional rotary evaporation method. Briefly, DPPC (36.7 mg) and cholesterol (4.83 mg) in 1 mL chloroform were placed in a 50 mL flask and were reduced into a thin film using a rotary evaporator under vacuum. The lipid was hydrated in 10 mL distilled water with 16 mM calcein. After ten freeze-thaw cycles, the liposomes were extruded through a 200 nm polycarbonate membrane under high pressure (Avanti Polar Lipids Inc., USA.) at RT. Free calcein was removed by dialysis method. MSNs or peptide were added into quartz cuvette containing 3 mL PBS buffer (10 mM, pH 7.4) and 100 μL of calcein encapsulated liposomes. Then, the PL intensity of calcein was measured (excitation wavelength = 495 nm).

Transmission electron microscopy. TEM images were obtained using a Philips CM 200 instrument operated at an acceleration voltage of 120 kV. TEM samples were prepared by placing a drop of

dispersed sample in distilled water (100 mg·L-1) onto a 300-mesh copper grid coated with carbon film. About 2 min after deposition, a filter paper was touched to the grid to remove surface water. The samples were dried before measurement.

Fluorescence measurements. All the fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorophotometer with an excitation wavelength of 485 nm (absorption maximum wavelength of DOX). Emission and excitation slit widths were set at 15 nm and 3 nm, respectively.

Fourier transform infrared spectroscopy. FT-IR spectra were obtained using VERTEX 80V vacuum spectrometer.

Zeta-potential analysis. Zeta-potential values were obtained using an OTSUKA Particle Size Analyzer ELS-Z2 using the dispersed samples in distilled water.

Circular dichroism. CD spectra were recorded on a J-815 spectropolarimeter (Jasco, Tokyo, Japan) using a quartz cell of 10 mm path length between 190 and 250 nm at room temperature. The concentration of peptide was 30 uM in 10 mM PBS buffer solution at pH 7.4 containing 50% CH_3CN. Three scans with a scan speed of 10 nm/min were averaged for each measurement. CD spectra were expressed as the mean residue ellipticity.

Dynamic light scattering. Dynamic light scattering (DLS) measurements were performed using a Brookhaven BI-200SM goniometer and BI-9000AT digital autocorrelator at room temperature. The scattered light of a He-Ne laser (Research Electro-optics, 35 mW) operated at 632.8 nm was measured at an angle of 90° and collected on an autocorrelator. The sample solutions were purified through a Millipore syringe filter (pore size = 0.2 μm). The average hydrodynamic diameters and the size distribution were calculated using CONTIN fitting procedure provided by the instrument software.

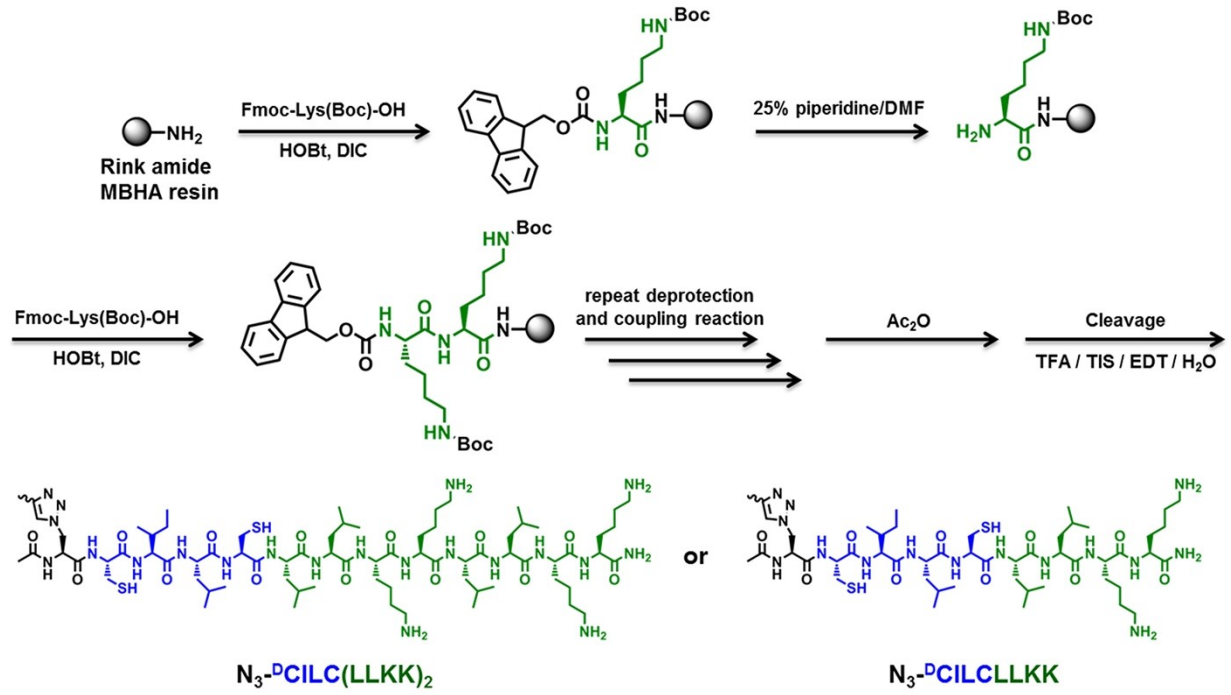

Fig. S1 Synthetic route to N_3-^{D}CILC(LLKK)$_2$ and N_3-^{D}CILCLLKK peptides.

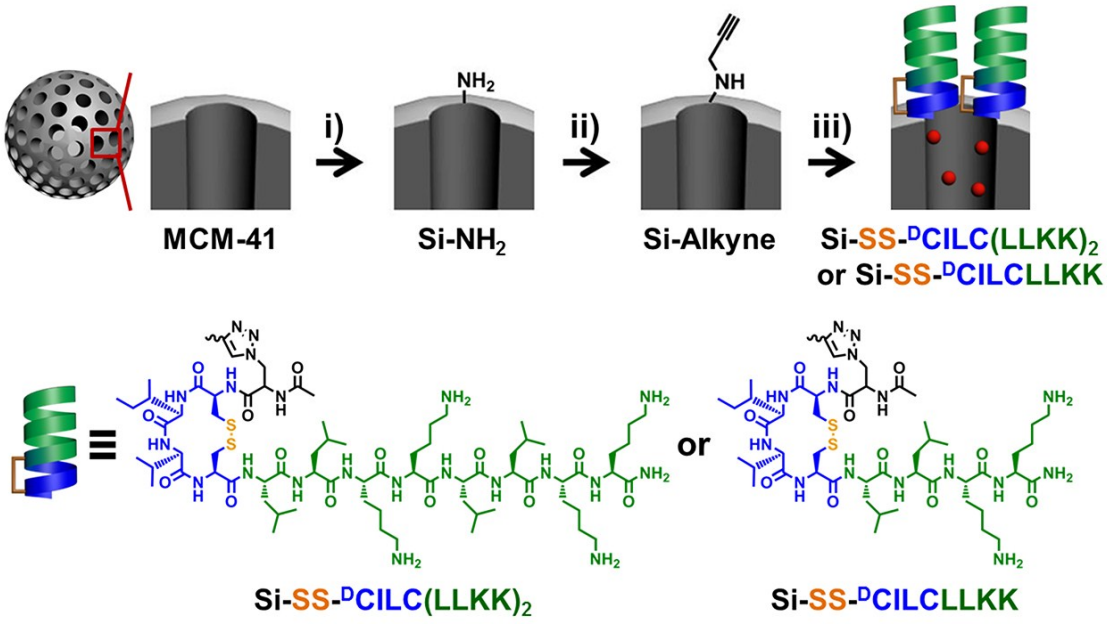

Fig. S2 Synthetic route to Si-SS-^{D}CILC(LLKK)$_2$ and Si-SS-^{D}CILCLLKK. Conditions: i) APTES; ii) propargyl bromide; iii) CTAB removal, DOX loading, copper(II) sulfate, sodium ascorbate, and azido-conjugated peptides (SS-N_3-^{D}CILCLLKK or SS-N_3-^{D}CILC(LLKK)$_2$).

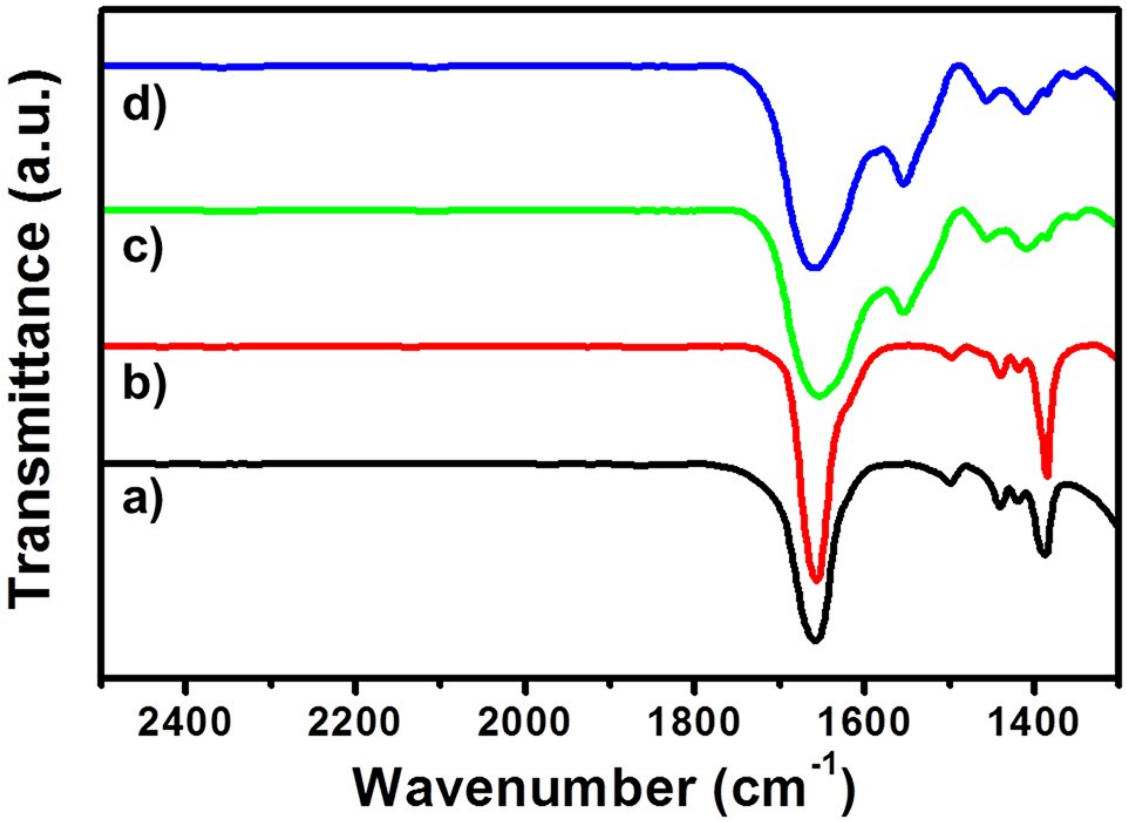

Fig. S3 FT-IR spectra of Si-NH_2 (a), Si-Alkyne (b), Si-SS-^{D}CILC(LLKK)$_2$ (c) and Si-SS-^{D}CILCLLKK (d).

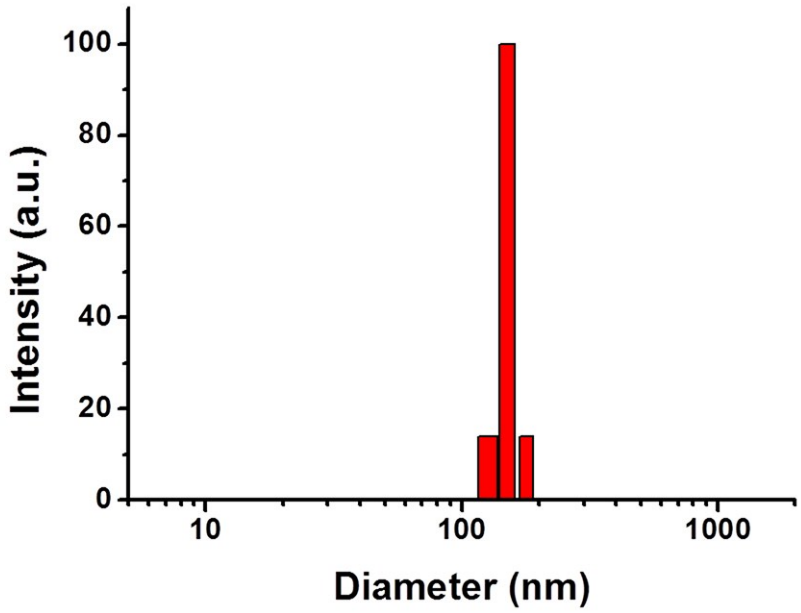

Fig. S4 Hydrodynamic diameter of Si-SS-^{D}CILC(LLKK)$_2$-PEG obtained from dynamic light scattering.

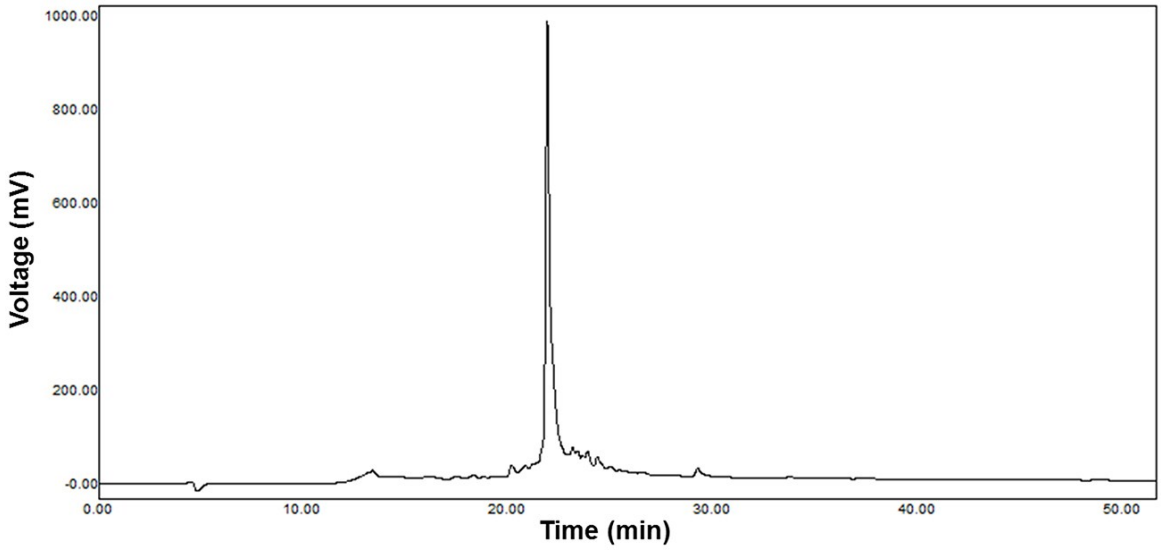

Fig. S5 HPLC chromatogram of N_3-^{D}ClLC(LLKK)$_2$.

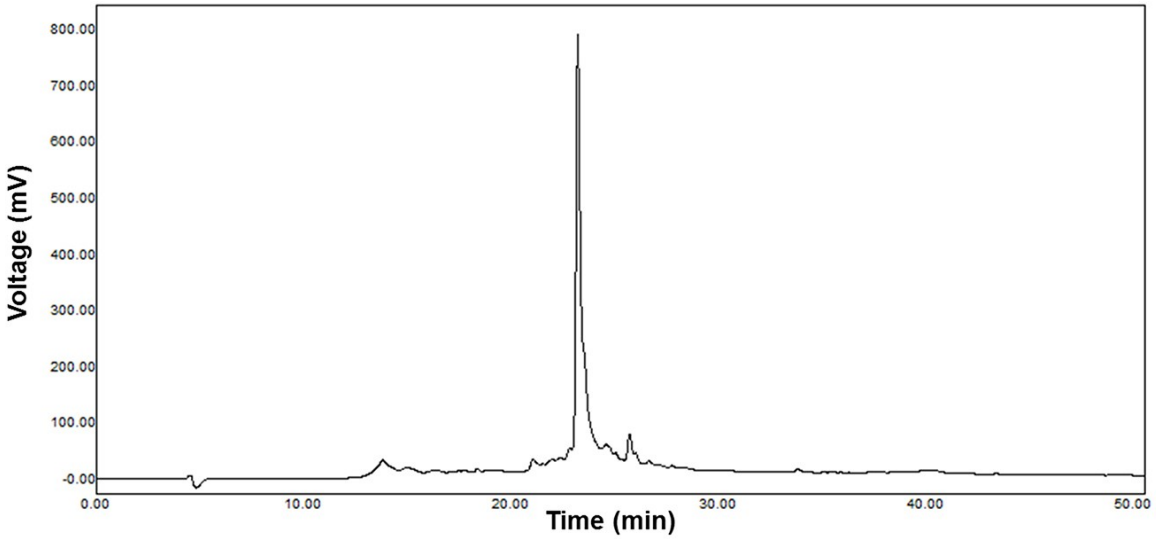

Fig. S6 HPLC chromatogram of N_3-^{D}ClLCLLKK.

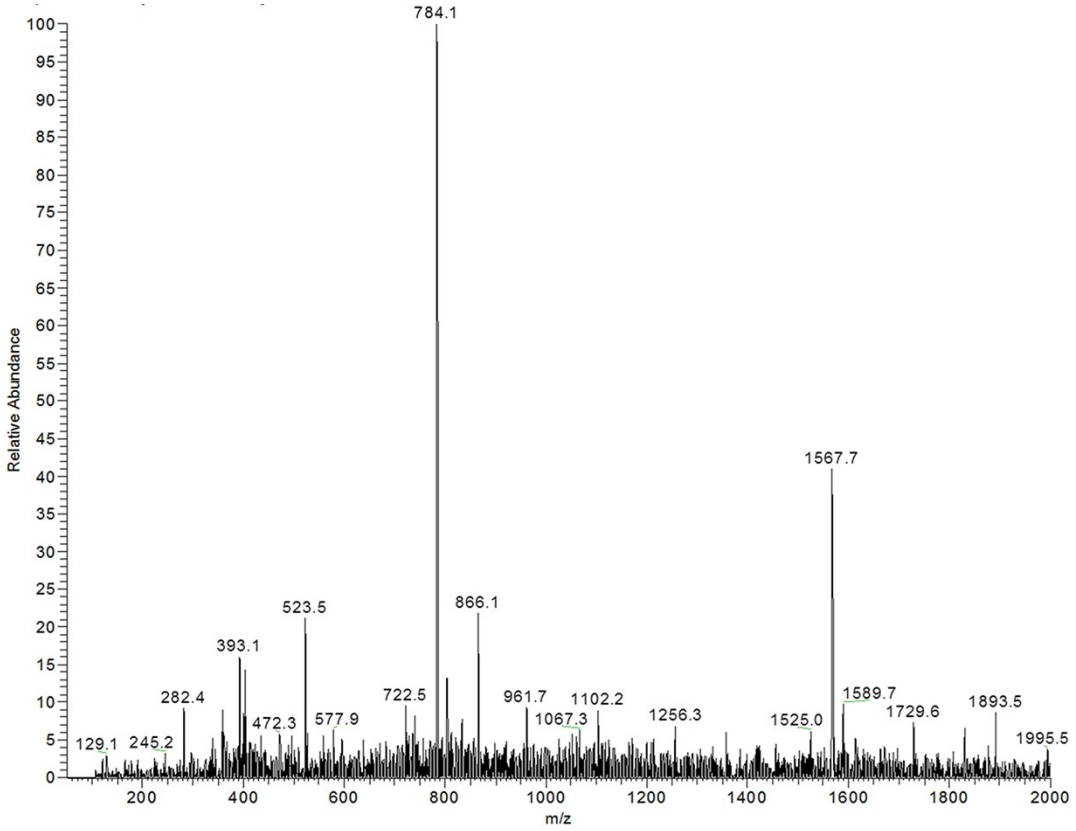

Fig. S7 LC-MS spectrum of N_3-^{D}CILC(LLKK)$_2$.

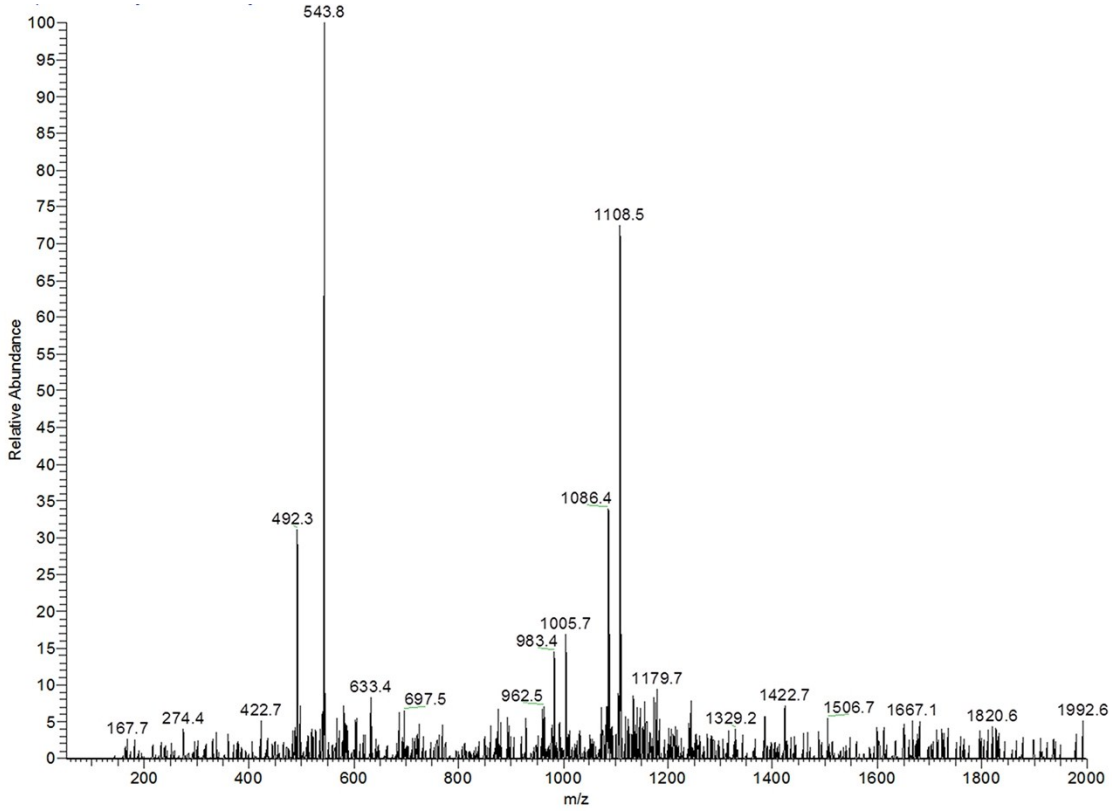

Fig. S8 LC-MS spectrum of N_3-^{D}CILCLLKK.

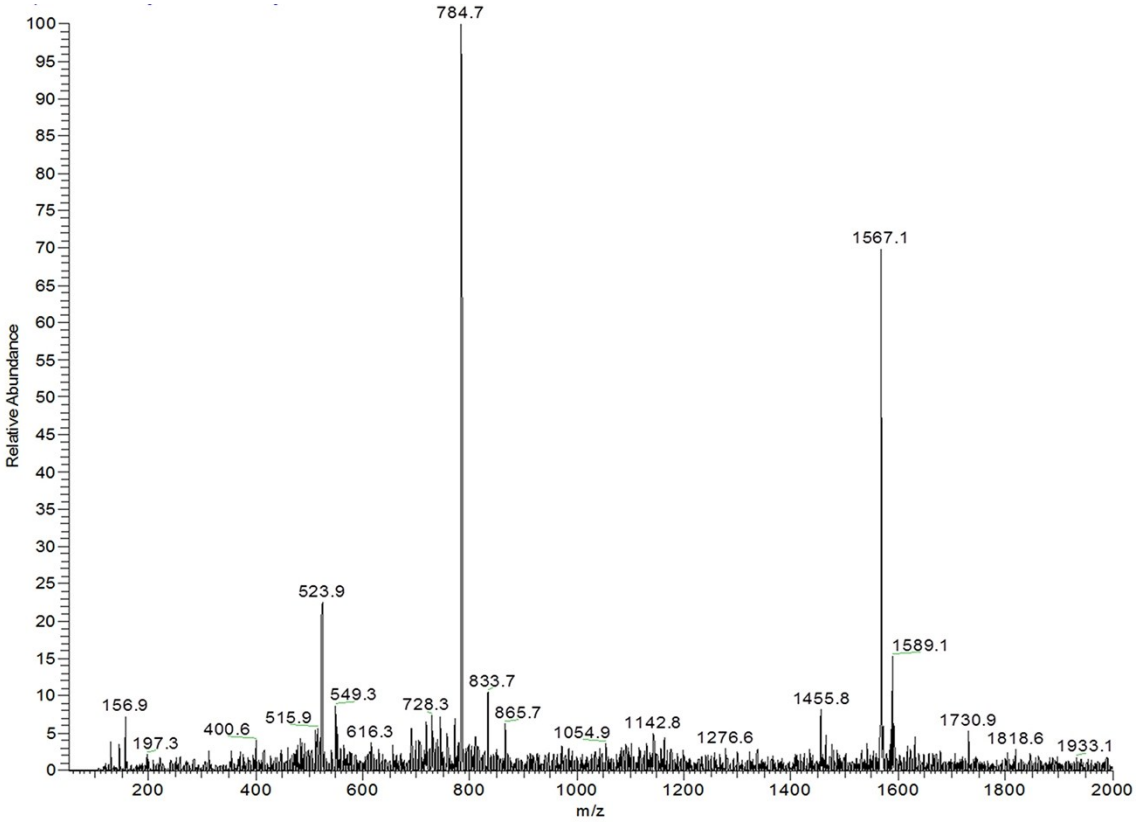

Fig. S9 LC-MS spectrum of N_3-SS-^{D}CILC(LLKK)$_2$.

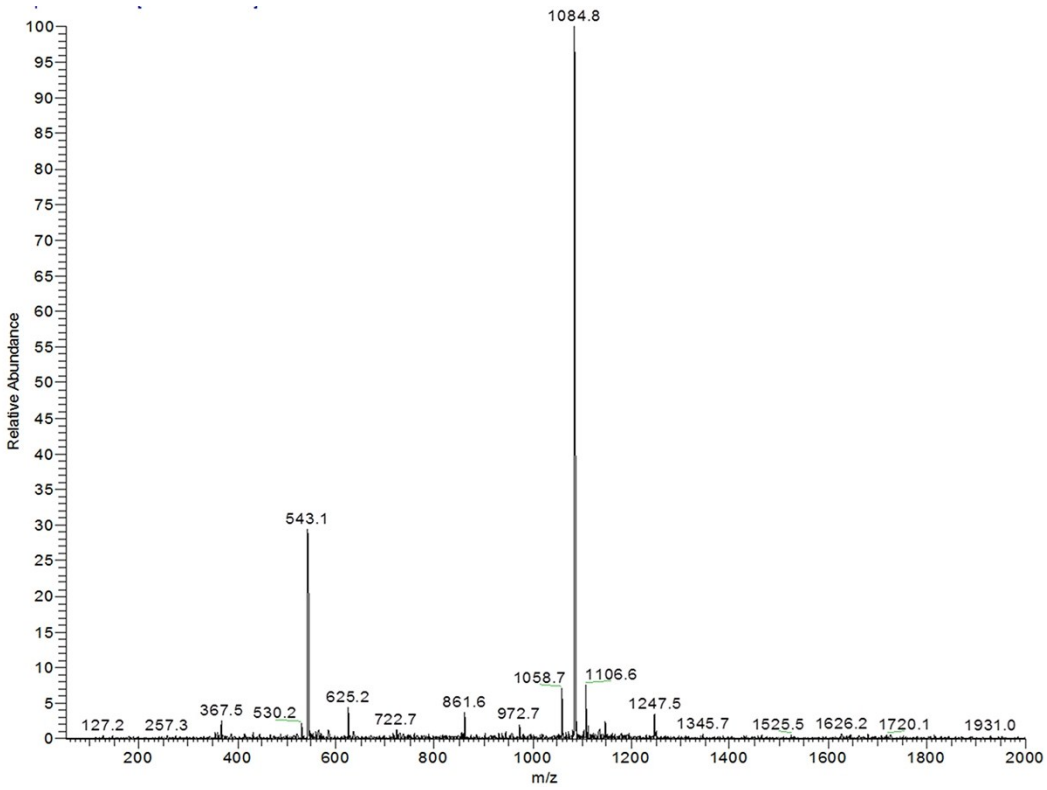

Fig. S10 LC-MS spectrum of N_3-SS-^{D}CILCLLKK.

References

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3 L. Luan, Q. Meng, L. Xu, Z. Meng, H. Yan and K. Liu, *J. Mater. Chem. B*, 2015, **3**, 1068-1078.