Supplementary Information

Cyclodextrin-based Peptide Self-Assemblies (Spds) that Enhance Peptide-based Fluorescence Imaging and Antimicrobial Efficacy

Jin-Biao Jiao,[a,c,‡] Guan-Zhen Wang,[b,e,‡] Xi-Le Hu,[a,‡] Yi Zang,[b] Stéphane Maisonneuve,[c] Adam C. Sedgwick,[d] Jonathan L. Sessler,[d*] Juan Xie,[c*] Jia Li,[b,e,f*] Xiao-Peng He,[a*] and He Tian[a]

[a]Key Laboratory for Advanced Materials & and Joint International Research Laboratory of Precision Chemistry and Molecular Engineering, Feringa Nobel Prize Scientist Joint Research Center, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, P. R. China

[b]National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 189 Guo Shoujing Rd., Shanghai 201203, P. R. China

[c]Université Paris-Saclay, ENS Paris-Saclay, CNRS, PPSM, 61 av President Wilson, F-94235 Cachan, France

[d]Department of Chemistry, The University of Texas at Austin, 105 East 24th Street A5300, Austin, Texas 78712-1224, United States

[e]University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing100049, P. R. China

[f]Open Studio for Druggability Research of Marine Natural Products, Pilot National Laboratory for Marine Science and Technology (Qingdao), 1 Wenhai Road, Aoshanwei, Jimo, Qingdao, 266237, China

[‡]Equal contribution

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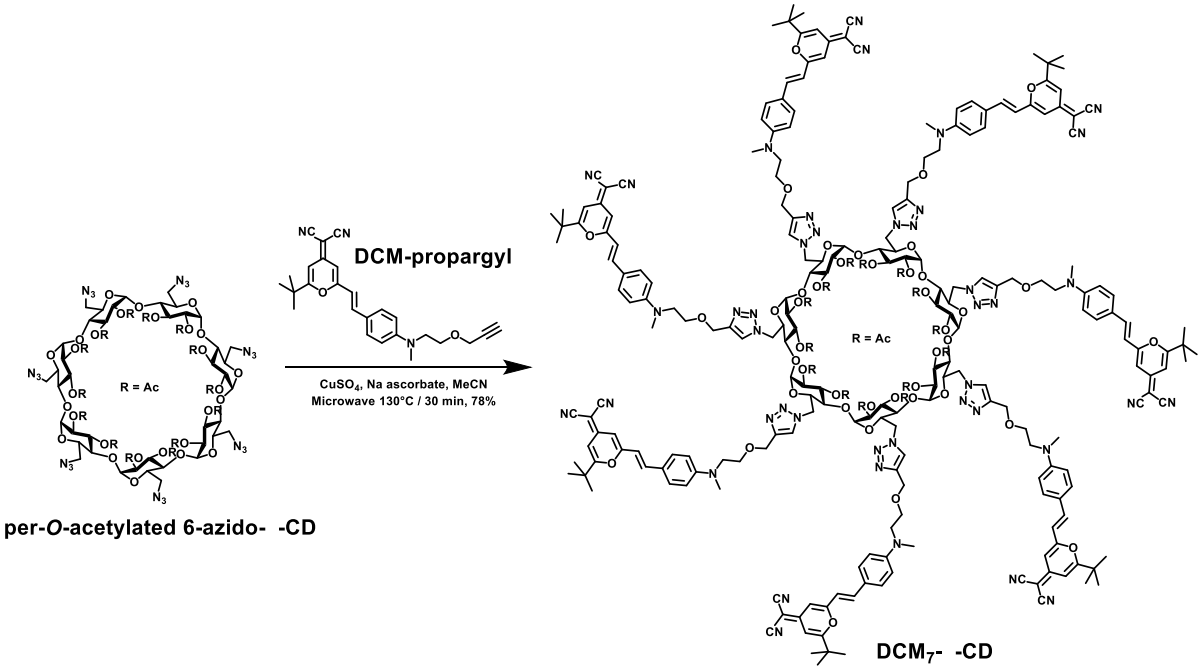
S1. Additional Schemes and Figures



Scheme S1. Synthesis of **DCM_7-*β*-CD**

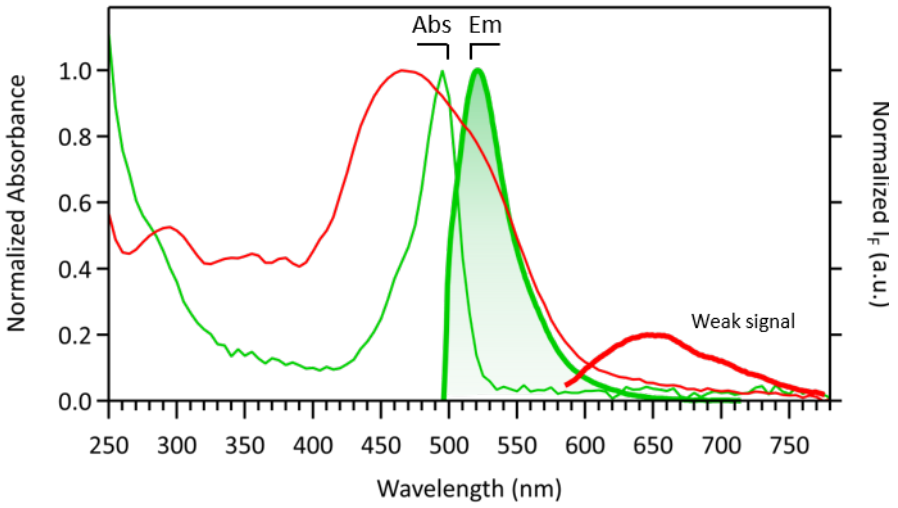


Fig. S1. Absorption and emission spectra of FITC (green) and **DCM_7-β-CD** (red) measured in phosphate buffered saline solution (0.1 M, pH 7.4). λ_{ex} = 470 nm.

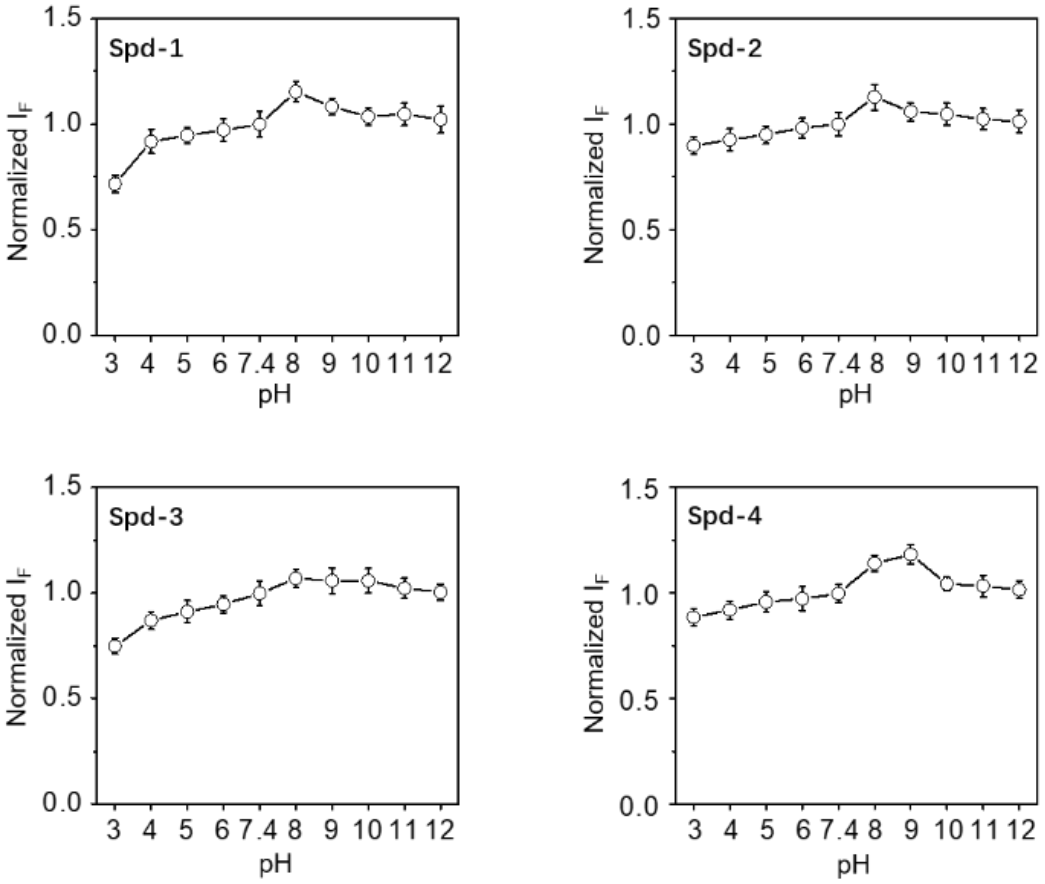


Fig. S2. Normalized fluorescence intensity of **Spds(1-4)** across a range of pH (3-12). **Spds** (**P1**/ **DCM_7-β-CD** = 0.17 μM/7.3 μM; **P2**/ **DCM_7-β-CD** = 0.25 μM/6 μM; **P3**/ **DCM_7-β-CD** = 0.25 μM/5 μM; **P4**/ **DCM_7-β-CD** = 0.25 μM/3.5 μM). All fluorescence measurements were carried out in phosphate buffered saline solution (0.1 M, pH 7.4) with an excitation wavelength of 470 nm.

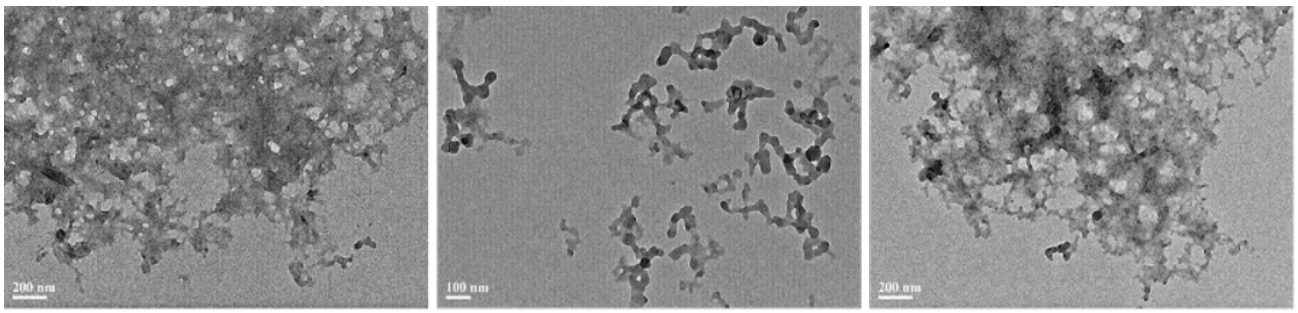


Fig. S3. (a) High-resolution transmission electron microscopic images of **DCM_7-*β*-CD** (4 μM), **Spd-1** (**P1/DCM_7-*β*-CD** = 4 μM/4 μM), and **Spd-1** (**P1/DCM_7-*β*-CD** = 4 μM/4 μM) with Caspase-3 (400 nM) recorded on a JEM-2100 TEM system.

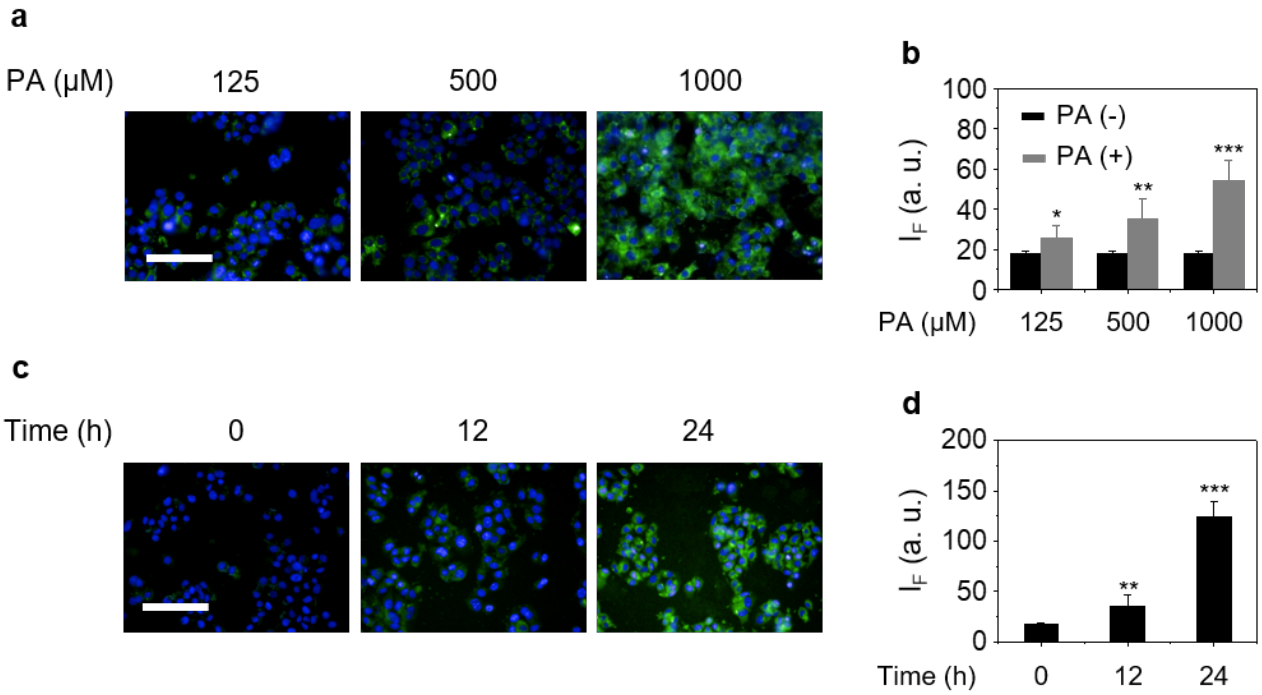


Fig. S4. Images showing that the fluorescence enhancement of **Spd-1** in apoptotic Hep-G2 cells is dependent on both the concentration of PA (palmitic acid) and the time of incubation. Fluorescence imaging (a) and quantification (b) of Hep-G2 cells treated with PA for 12 h at 0.125, 0.5 and 1 mM, followed by incubation with **Spd-1** (**P1**/**DCM$_7$-*β*-CD** = 1 μM/8 μM) for 30 min (*P < 0.05 with respect to PA(-); **P < 0.01 with respect to PA(-); ***P < 0.001 with respect to PA(-)). Fluorescence imaging (a) and quantification (b) of Hep-G2 cells treated with 0.5 mM PA for 0, 12 and 24 h, followed by incubation with **Spd-1** (**P1**/**DCM$_7$-*β*-CD** = 1 μM/8 μM) for 30 min. Scale bar = 100 μm. Excitation and emission channels for FITC are 460-490 nm and 500-550 nm, respectively. Cell nuclei were stained by Hoechst (excitation and emission channels are 360-400 nm and 410-480 nm, respectively). Statistical analysis was carried out by Students' t-test. All experiments were run in triplet. Scale bars are S. D. (n = 3).

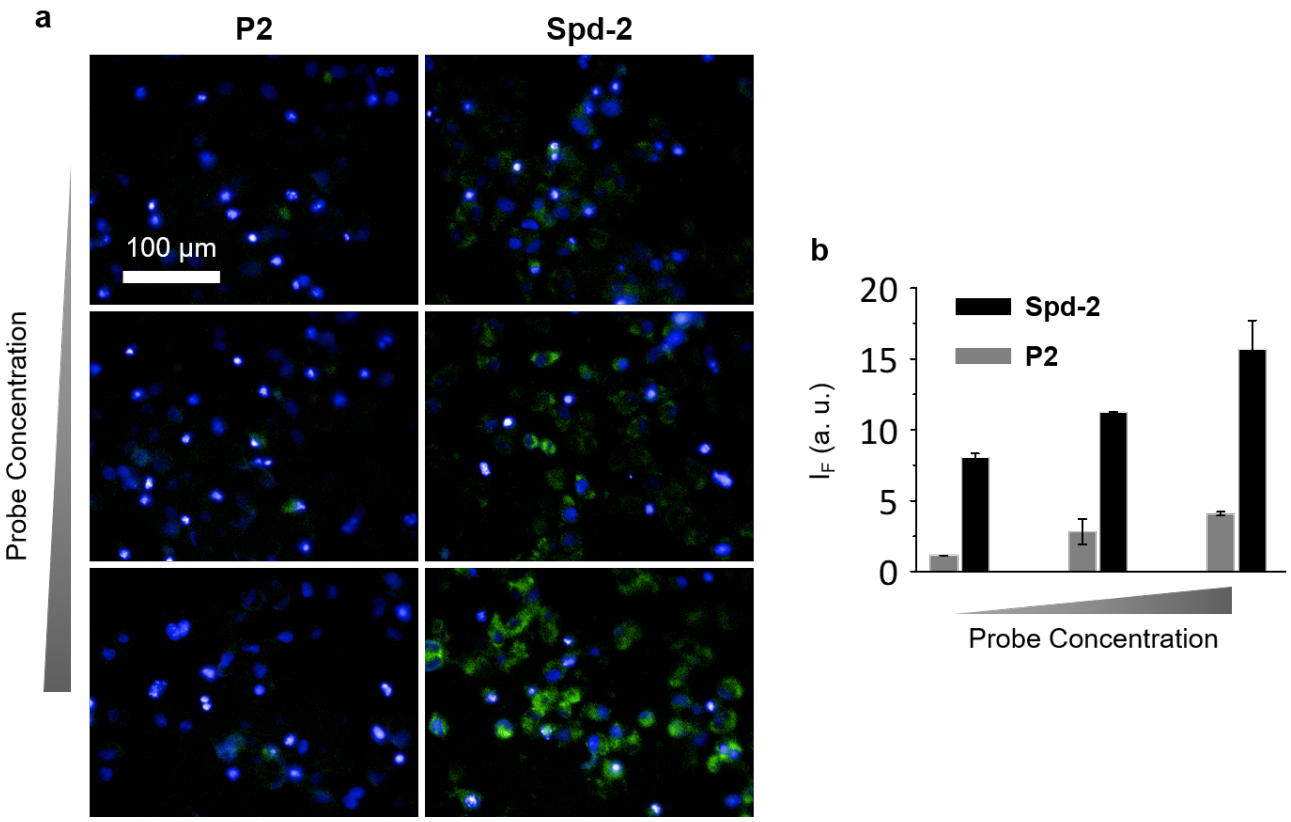


Fig. S5. Dose-dependent fluorescence imaging (a) and quantification (b) of HeLa cells (pre-incubated with 500 μM of palmitic acid (PA)) treated with **P2** (0.25 μM, 0.5 μM, 1 μM) or **Spd-2** (**P2**/**DCM$_7$-*β*-CD** = 0.25/4 μM, 0.5/5 μM, 1/8 μM). Excitation and emission channels for FITC are 460-490 nm and 500-550 nm, respectively. Cell nuclei were stained by Hoechst (excitation and emission channels are 360-400 nm and 410-480 nm, respectively). Scale bar = 100 μm (applicable to all images in Fig. S5a).

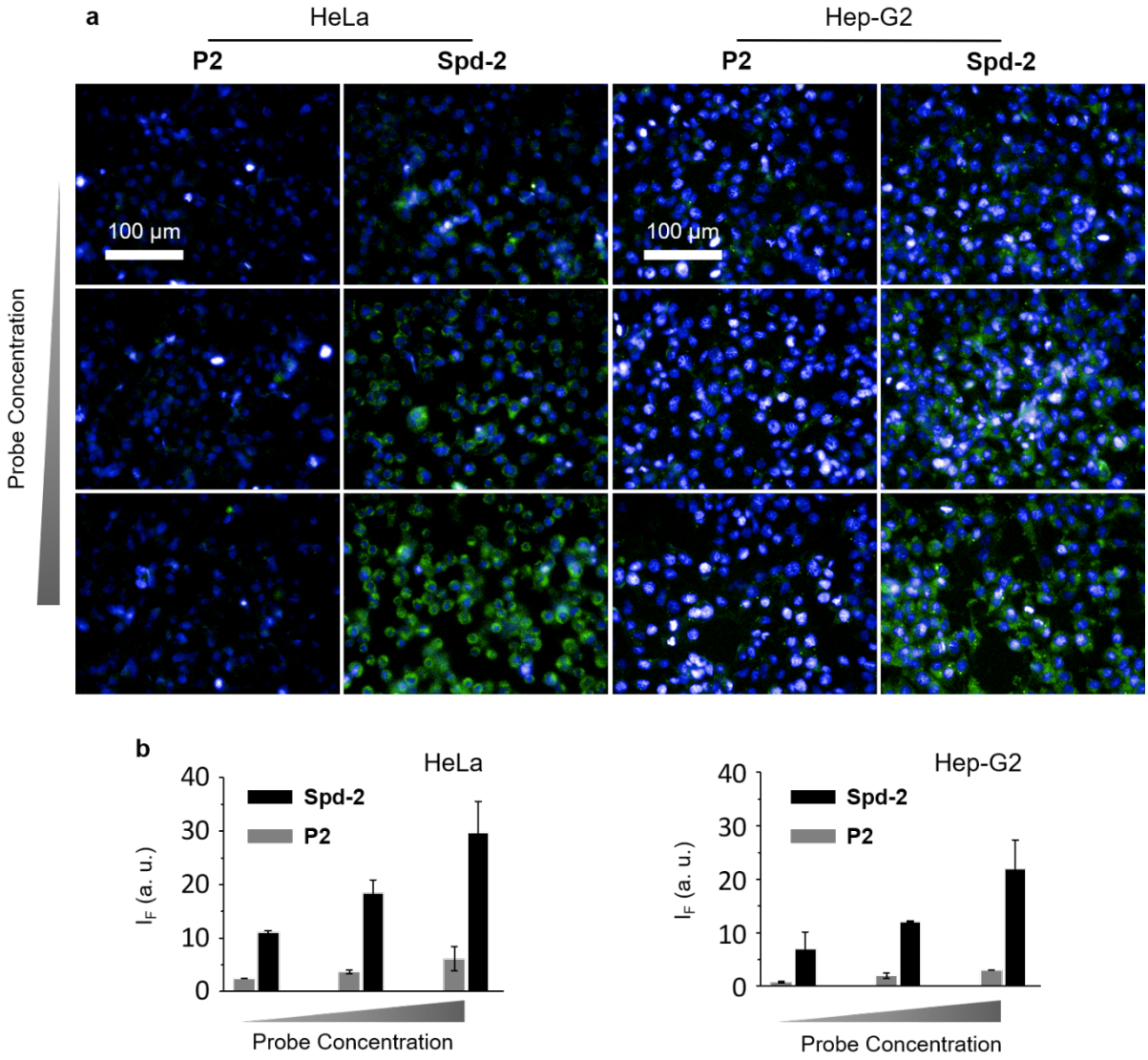


Fig. S6. Dose-dependent fluorescence imaging (a) and quantification (b) of HeLa and Hep-G2 cells (pre-incubated with 1 μM of cisplatin) treated with **P2** (0.25 μM, 0.5 μM, 1 μM) or **Spd-2** (**P2**/**DCM$_7$-*β*-CD** = 0.25/4 μM, 0.5/5 μM, 1/8 μM). Excitation and emission channels for FITC are 460-490 nm and 500-550 nm, respectively. Cell nuclei were stained by Hoechst (excitation and emission channels are 360-400 nm and 410-480 nm, respectively). Scale bar = 100 μm (applicable to all images in Fig. S6a).

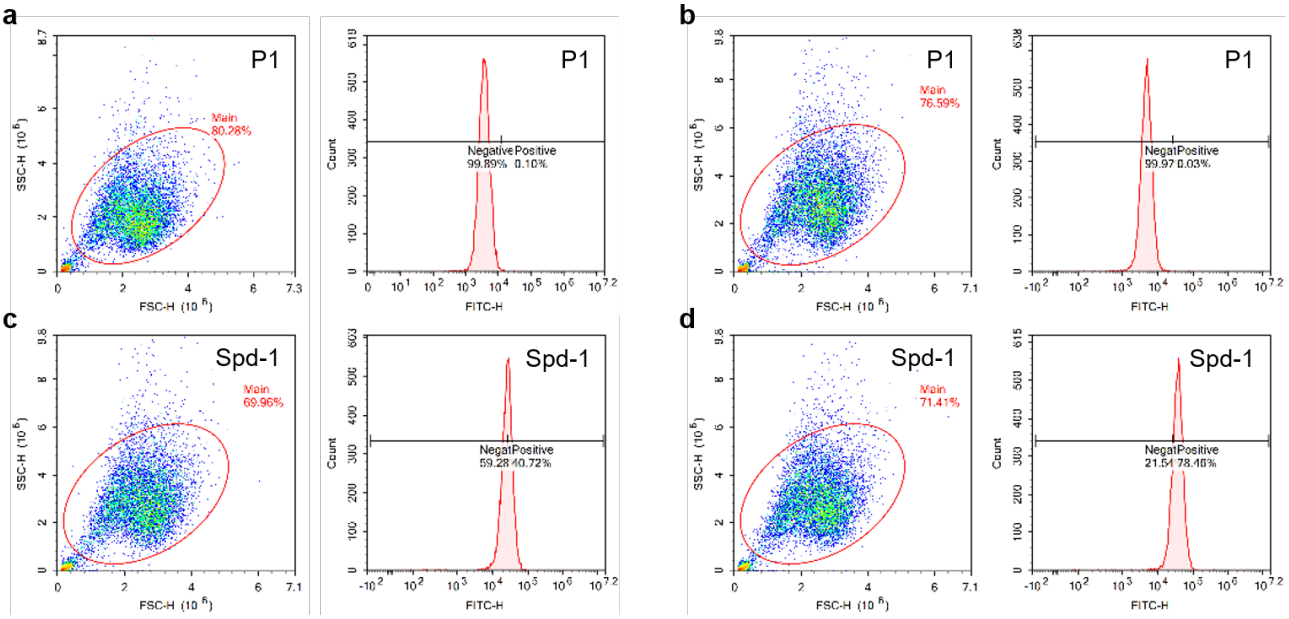


Fig. S7. Analysis of cell internalization of **P1** and **Spd-1** using flow cytometry. PA-induced apoptotic cells were incubated with **P1** at (a) 0.2 μM and (b) 0.5 μM, and **Spd-1** at (b) **P1**/ **DCM$_7$-*β*-CD** = 0.2 μM/4 μM and (d) **P1**/**DCM$_7$-*β*-CD** = 0.5 μM/5 μM for 30 min. The FITC channel was chosen to specify apoptosis positive cells. All experiments were run in triplet.

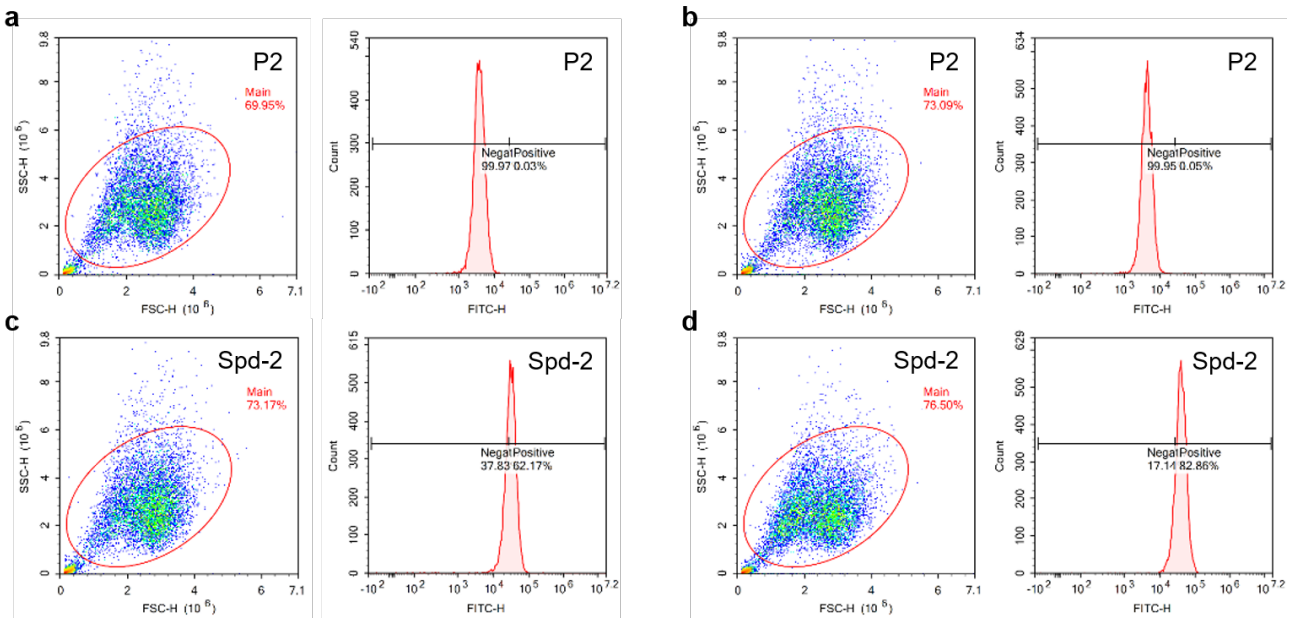


Fig. S8. Analysis of cell internalization of **P2** and **Spd-2** with flow cytometry. PA induced apoptotic cells were incubated with **P2** at (a) 0.25 μM and (b) 0.5 μM, and **Spd-2** at (b) **P2**/ **DCM$_7$-*β*-CD** = 0.25 μM/5 μM and (d) **P2**/**DCM$_7$-*β*-CD** = 0.5 μM/5 μM for 30 min. The FITC channel was chosen to specify apoptosis positive cells. All experiments were run in triplet.

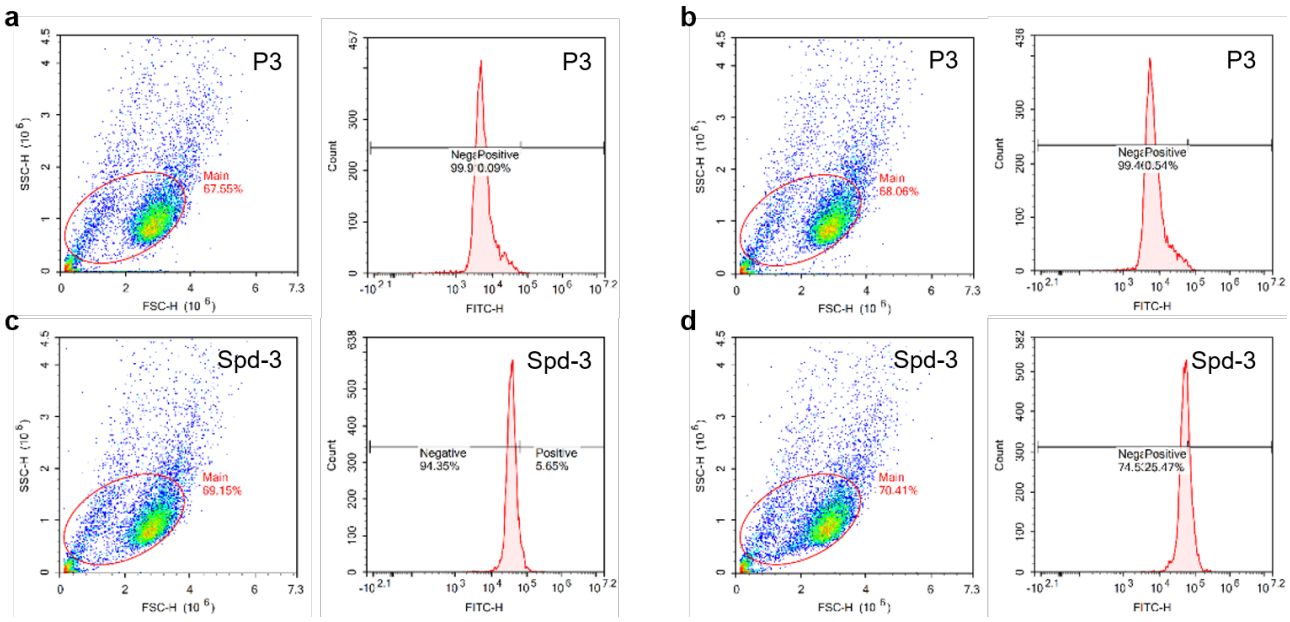


Fig. S9. Detection of cell internalization of **P3** and **Spd-3** with flow cytometry. HeLa cells stably expressing mCherry-α tubulin were incubated with **P3** of (a) 0.25 μM, (b) 0.5 μM and **Spd-3** of (c) **P3**/**DCM$_7$-*β*-CD** = 0.25 μM/5 μM and (d) **P3**/**DCM$_7$-*β*-CD** = 0.5 μM/5 μM for 30 min. The FITC channel was chosen to specify apoptosis positive cells. All experiments were run in triplet.

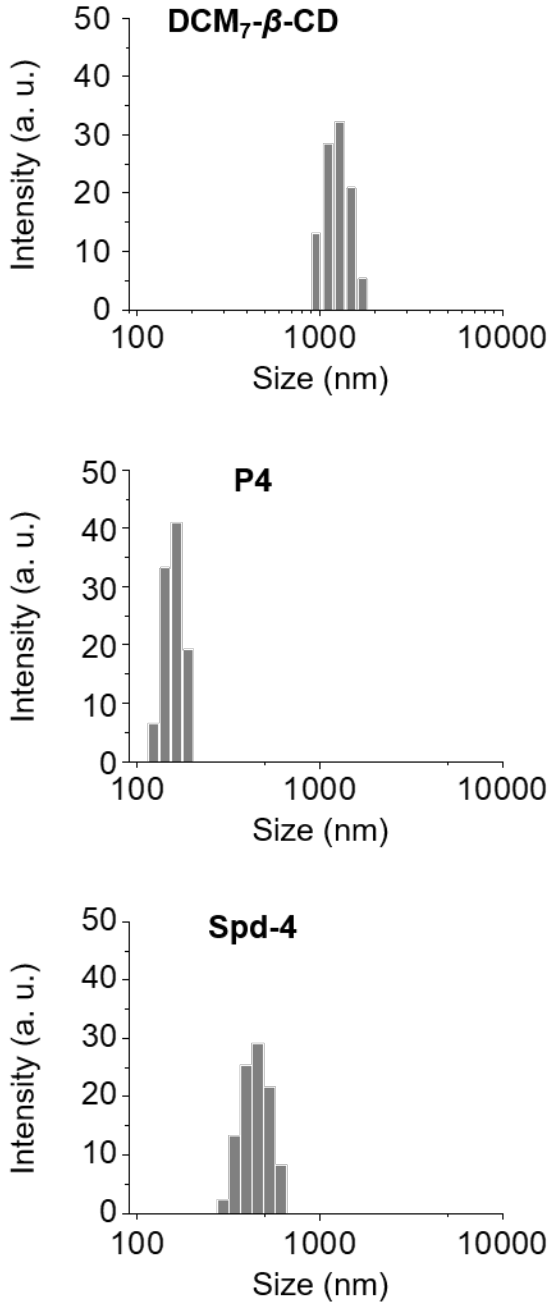


Fig. S10. Dynamic light scattering analyses of **DCM$_7$-*β*-CD** (4 μM), **P4** (4 μM) and **Spd-4** (**P4**/**DCM$_7$-*β*-CD** = 4 μM/4 μM).

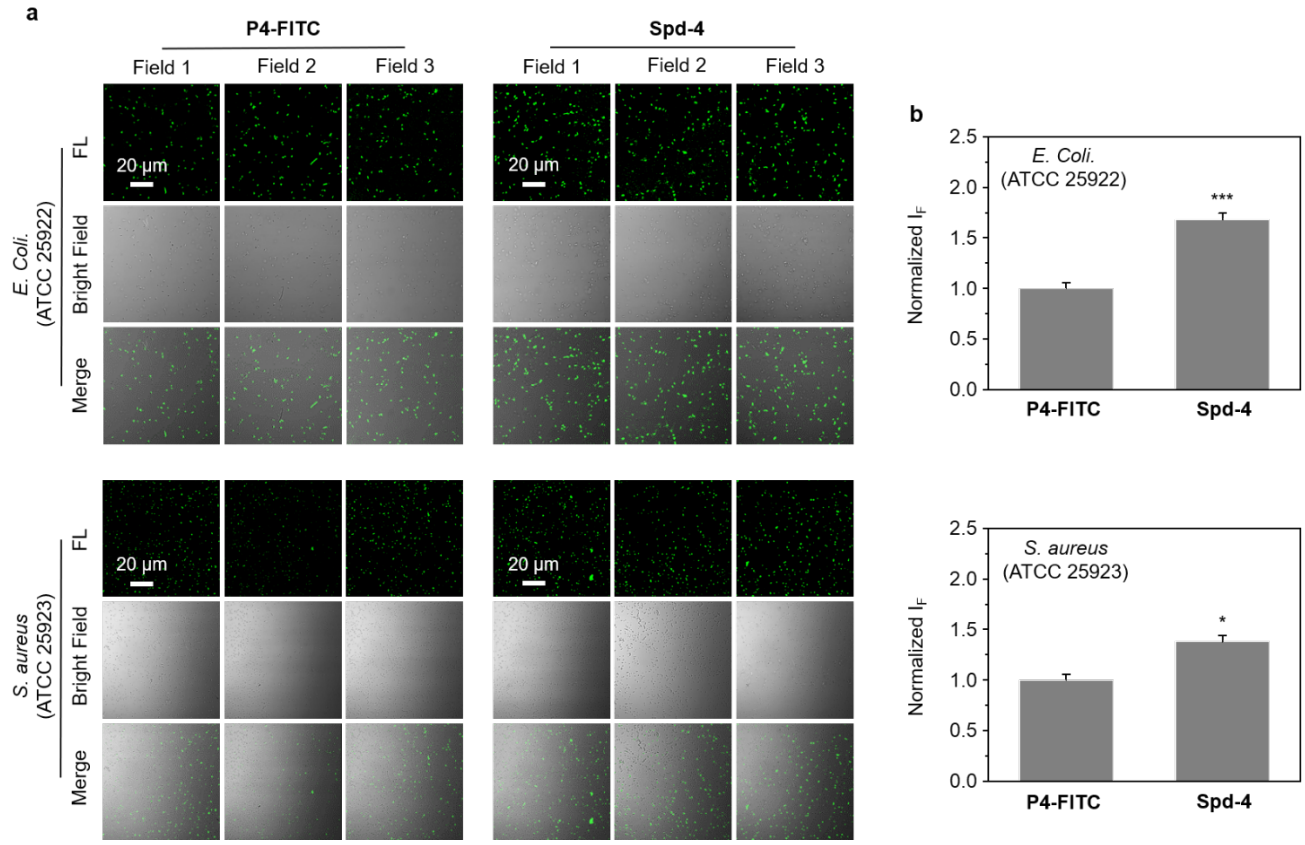


Fig. S11. Fluorescence imaging (a) and quantification (b) of *E. Coli.* (ATCC 25922) and *S. aureus* (ATCC 25923) cells treated with **P4-FITC** (1 μM) or **Spd-4** (**P4-FITC**/**DCM$_7$-*β*-CD** = 1 μM /1 μM). Excitation and emission channels for FITC are 460-490 nm and 500-550 nm, respectively. Scale bar = 20 μm (applicable to all images in Fig. S11a). ****P* < 0.001; **P*<0.05. The imaging was carried out on a Nikon AIR confocal laser-scanning microscope equipped with a 60x oil-immersion objective lens. Image-J software was used to automatically define the fluorescence intensities.

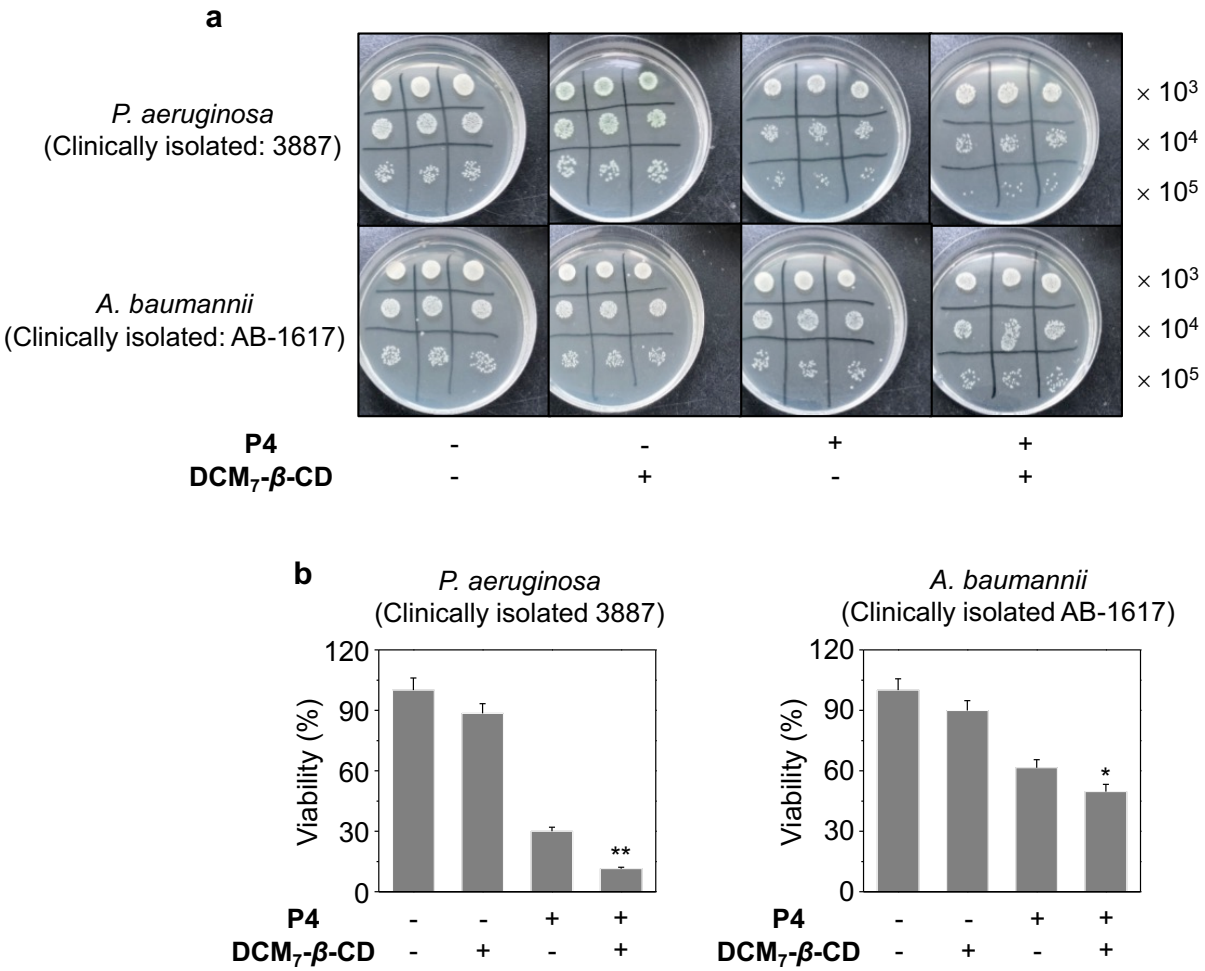


Fig. S12. (a) Bacterial cultures of clinically isolated *P. aeruginosa* (3887) and *A. baumannii* (AB-1617) with and without treatment with **P4**, **DCM$_7$-*β*-CD**, and **Spd-4**. (b) Relative bacterial viability of clinically isolated *P. aeruginosa* (3887) and *A. baumannii* (AB-1617) with and without treatment with **P4**, **DCM$_7$-*β*-CD**, and **Spd-4**. The concentrations of **P4**, **DCM$_7$-*β*-CD**, and **Spd-4** (**P4**/**DCM$_7$-*β*-CD**) were 20 μM, 20 μM and 20 μM/20 μM, respectively. $^{**}P < 0.01$; $^{*}P < 0.05$. Bacteria were $\times 10^3$, $\times 10^4$ and $\times 10^5$ diluted; viability in Fig. 12b is the ratio of the counted bacterium number ($\times 10^5$ diluted) in each group to that of the control group (bacteria alone).

Both bacterial strains were isolated from Huashan Hospital, Fudan University. An antibiotic-sensitivity assay revealed that *A. baumannii* (AB-1617) is resistant to levofloxacin (MIC = 256 μg mL^{-1}), tetracycline (MIC > 512 μg mL^{-1}), aureomycin (MIC > 512 μg mL^{-1}), cefotaxime (MIC > 512 μg mL^{-1}), meropenem (MIC = 128 μg mL^{-1}), cissomicin sulfate (MIC = 512 μg mL^{-1}), neomycin (MIC = 512 μg mL^{-1}), gentamicin (MIC = 512 μg mL^{-1}) and ceftriaxone (MIC > 512 μg mL^{-1}).

S2. Experimental Section and Copies of Original Spectra

General remarks. All chemicals and reagents were purchased commercially and were analytical grade. Peptide probes were customized from Shanghai MuJin BioTech. Dynamic light scattering (DLS) was carried out on a Horiba LB-550 Dynamic Light Scattering Nano-Analyzer. Transmission electron microscopy (TEM) images were obtained on a FEI TACNAI G2 F20 transmission electron microscope operating at an accelerating bias voltage of 200 kV.

Reactions of **DCM_7-*β*-CD** were monitored by TLC on silica gel 60F-254 plates with detection by UV (254 nm or 365 nm) or by spraying with 10% H_2SO_4 in EtOH and heating for about 30 s at 400-600 °C. ^1H and ^{13}C-NMR spectra were recorded on a JEOL ECS-400 spectrometer. The TMS and the residual solvent signals were taken as the references for the ^1H and ^{13}C NMR spectra, respectively. HRMS (ESI) spectra of **DCM_7-*β*-CD** were recorded on a Q-TOF mass spectrometer by the "Fédération de Recherche" ICOA/CBM (FR2708) platform. Optical rotation was measured using a JASCO P-2000 polarimeter at 589 nm where no absorption occurred.

Preparation of Spds. DCM_7-*β*-CD (DMSO solution) was added to a PBS solution containing the chosen peptide (**P1**-**P4**) (PBS (phosphate buffered saline) solution (0.1 M, pH 7.4)). This solution was then mixed for 10 min to 30 min and then was used for the subsequent experiments.

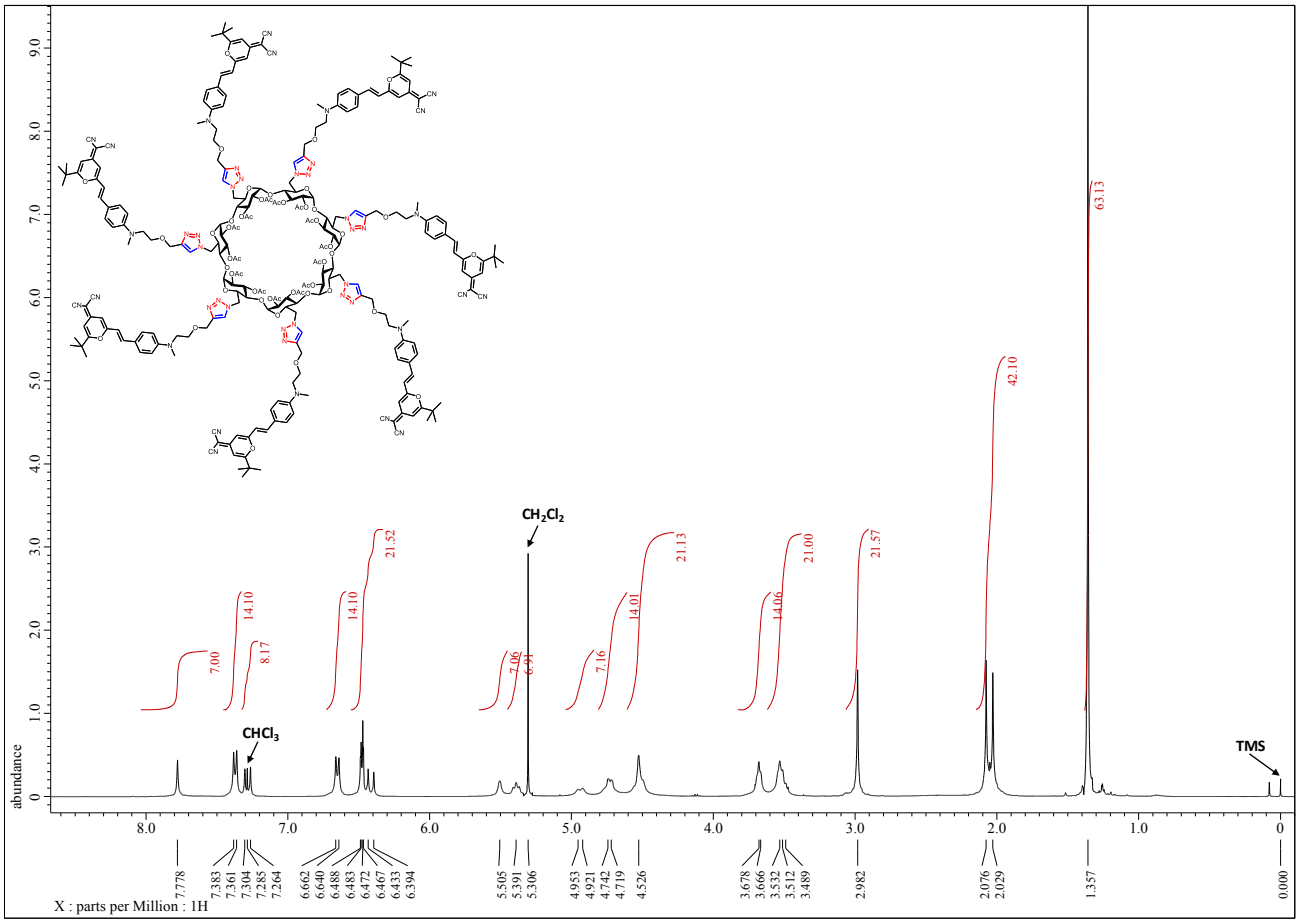
Cell culture and establishment of an apoptotic cell model. Hep-G2 cells were cultured in HG-DMEM containing 10% fetal bovine serum. Then, cells (2×10^5/well) were seeded on a black 96-well microplate with an optically clear bottom (Greiner bio-one, Germany) overnight. The cells were treated with 500 μM of palmitic acid (PA) that was dissolved in 10% BSA for 24 hours to induce apoptosis. The cells were incubated with peptide probes or **Spd**s for 30 min. Then, the nuclear staining reagent Hoechst (5 μg mL^{-1}) was added to cells at 37 °C in a humidified atmosphere of 5% CO_2 for 5 min. The cells were then washed three times with PBS (phosphate buffered saline). Fluorescence images were recorded using an Operetta high content imaging system (PerkinElmer, US) and quantified and plotted using the Columbus analysis system (PerkinElmer, US). Excitation and emission channels for FITC are 460-490 nm and 500-550 nm, respectively. Excitation and emission channels for the nucleus stain are 360-400 and 410-480 nm, respectively.

Fluorescence imaging of Hep-G2 cells treated with different concentrations of PA. Hep-G2 cells were cultured in HG-DMEM containing 10% fetal bovine serum. After being seeded, the cells were treated with palmitic acid (PA) for 12 h at 0.125, 0.5 and 1 mM. Then, **Spd-1** was added and incubated for 30 min. The cells were then washed with PBS three times. Fluorescence images were recorded using an Operetta system and quantified and plotted using the Columbus analysis system.

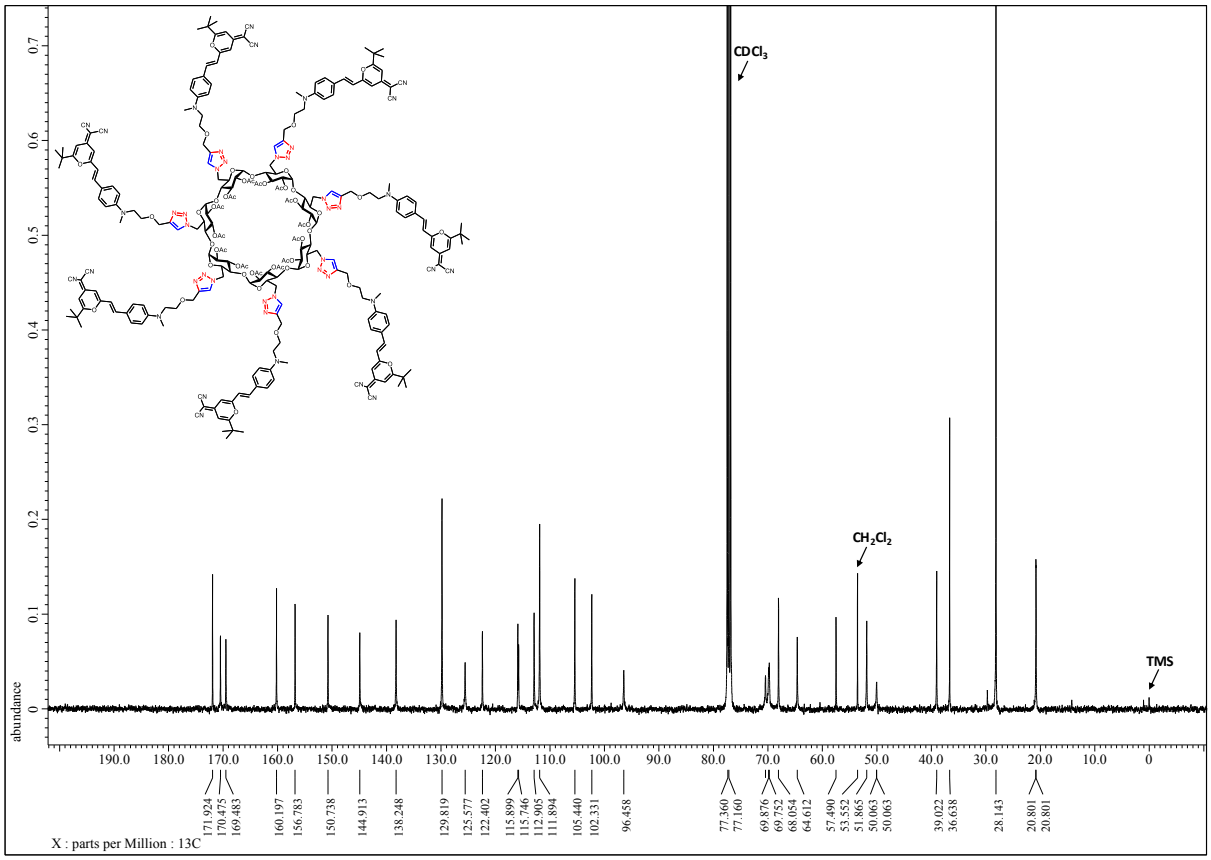
Western blot analysis. Protein samples were extracted for western blot after 24 h of PA treatment with 1×loading (1 g SDS, 50 mg BPB, 5 mL glycerin and 0.77 g DTT dissolved in H_2O to 50 mL volume). A known quantity of 20-100 μg of protein per lane was loaded onto a Tricine SDS page gel and then transferred to a NC membrane (Amersham Biosciences). The membranes were processed for immunoblotting. The following primary antibodies were used. Cleaved caspase-3 were purchased from Cell Signaling Technology and used in 1:1000 dilutions; anti-actin primary antibody was purchased from Sigma and used at 1:10000. The immunoblot bands were visualized by enhanced chemiluminescence (Amersham Biosciences).

Fluorescence-activated cell sorting (FACS) analyses. For FACS analyses, Hep-G2 cells were cultured on a 12-well plate and apoptosis was induced per the above protocol. Cells were trypsinized and the resulting single cell suspensions were stained with **P-1** or **P-2** or with **Spd-1** or **Spd-2** for 30 min at room temperature. Cells were analyzed with a Guava flow cytometer (Millipore, St. Charles, MO, USA) with the data being collected using the FlowJo software. HeLa cells stably expressing mCherry-α-tubulin were cultured on 12 wells overnight, then the cells were incubated with **P3** with or **Spd-3**. FACS analysis and imaging were carried out per the above procedures.

Confocal laser scanning microscopy (CLSM). HeLa cells stably expressing mCherry-α tubulin were seeded on poly-L-lysine-coated 12-mm glass coverslips. After 24 h, cells were incubated with **Spd-3** for 30 min. Then, cells were washed with PBS three times and fixed in 4% paraformaldehyde for 10 min. Coverslips were mounted in mounting medium (Dako). Images were obtained by CLSM (Leica SP 8, Plan Apochromat 63X, NA 1.4 oil immersion objective). Excitation channels for DNA (stained by Hoechst), FITC and mCherry are 405, 488 and 552 nm, and emission channels for DNA, FITC and mCherry are 430-480, 490-540 and 570-640 nm, respectively.



^{1}H NMR spectrum of **DCM$_7$-*β*-CD** in $CDCl_3$ at 400 MHz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TMS
abundance
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0.2
0.3
0.4
0.5
0.6
0.7
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
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77.160
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68.054
64.612
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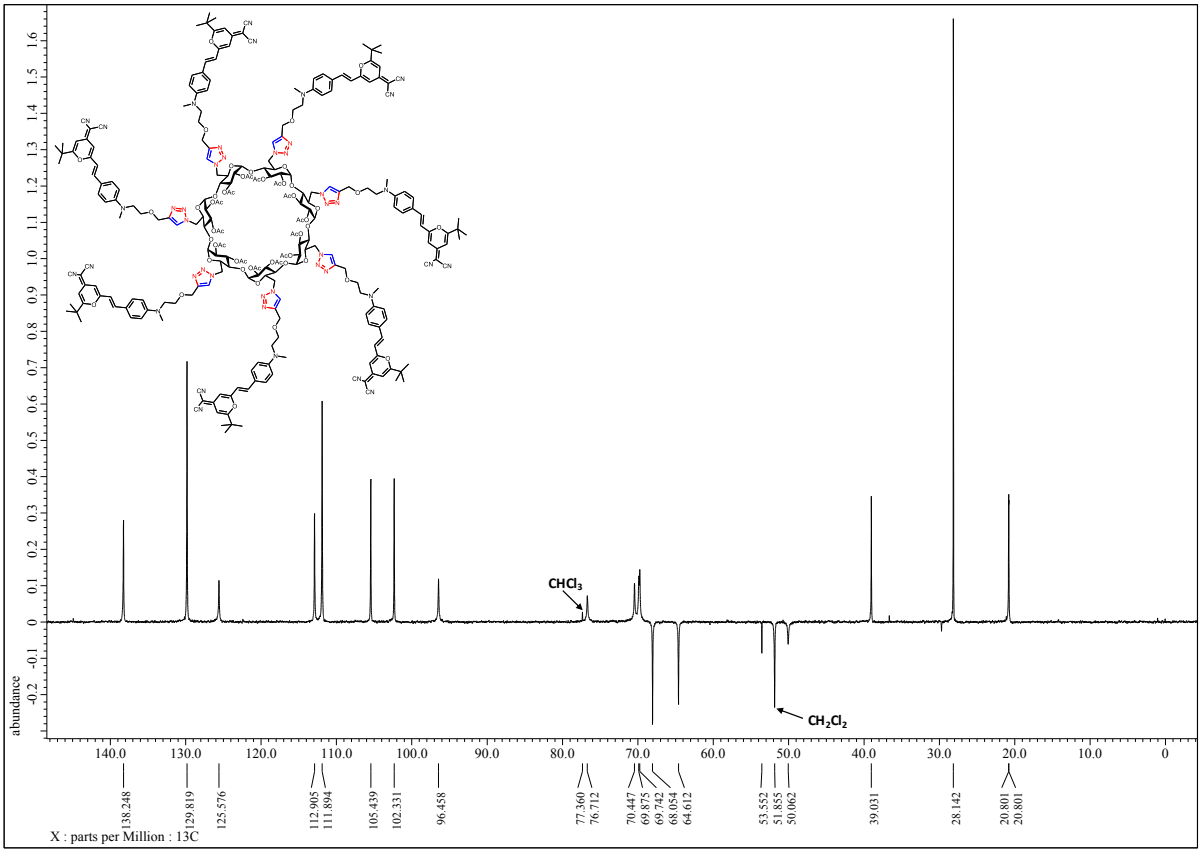
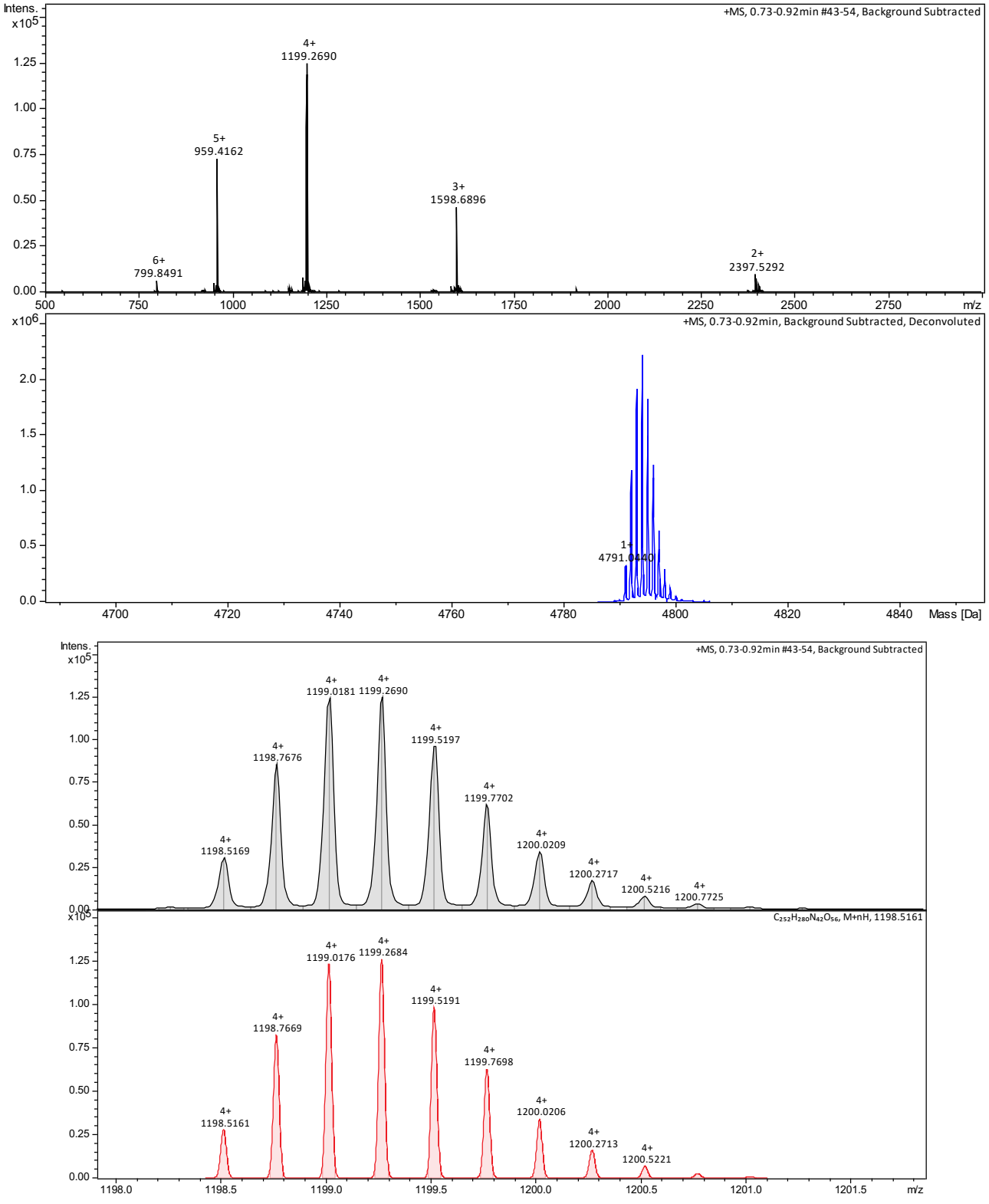
20.801
20.801
X : parts per Million : 13C



^{13}C NMR and DEPT135 spectra of **DCM$_7$-*β*-CD** in $CDCl_3$ at 100 MHz



ESI-HRMS spectra of **DCM$_7$-β-CD**