Supporting Information

Pillar[5]arene-based, Dual pH and Enzyme Responsive Supramolecular Vesicles for Targeted Antibiotic Delivery against Intracellular MRSA

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Experimental Section

1. Experimental Procedures

1.1 Instrumentation and chemicals

Chemicals were used as received without special purification unless stated otherwise. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a 400 MHz NMR spectrometer (100 MHz for ^{13}C NMR). NMR results were reported in δ units, parts per million (ppm), and were referenced to $CDCl_3$ (7.26 or 77.0 ppm) as the internal standard. The coupling constants J are given in Hz. Transmission electron microscope (TEM) investigations were carried out on a JEM-2100 instrument. Dynamic light scattering (DLS) measurements were carried out on a Brookhaven BI-9000AT system (Nano ZS90; Malvern Instruments, Malvern, UK), using a 200-mW polarized laser source (λ = 514 nm). Zeta-potential measurements were performed at 25 ^{o}C on a Zetasizer Nano Z apparatus (Malvern Instruments Ltd., UK) using the Smoluchowski model for the calculation of the Zeta-potential from the measured electrophoretic mobility.

1.2 HPLC analysis of vancomycin

An HPLC system consisting of a Waters 510 pump (Waters, Milford, MA) and 717 plus autosampler (Waters) was equipped with C18 column (Extend-C18 column, 250×4.6 mm, 5 μm, 100 Å; Agilent Technologies). The autosampler was cooled to 10 ^{o}C. The column was maintained at room temperature. EZChrom Elite chromatography data system (Scientific Software, Pleasanton, CA) was used to quantify the peak heights. Acetonitrile-0.05 M potassium dihydrogen phosphate buffer (9.5: 90.5) was as the mobile phase. The flow rate was 1.0 mL/min and the UV detection wavelength was 280 nm.[1] The sample volume for one sample was 20 μL and the running time for one sample was 25 min. Chromatographic procedures were performed at room temperature.[2]

1.3 Van loading and release of Man@AP5 and mPEG@AP5

Vancomycin was loaded by mixing Man@AP5 or mPEG@AP5 (vesicles 0.5 mg/mL) with Vancomycin at different ratios of weight. The mixture was ultrasonic for 30 min and standing overnight. The Vancomycin-loaded vesicles was purified by dialysis (Da 8000) against Milli-Q water for one day to remove unloaded Vancomycin and was kept germ-free throughout. To determine the loading efficiency of Vancomycin or PI, 1 mL of the purified vesicles were freeze-dried, weighed and acidified with 2 mL of HCl (1 N), then stood still overnight. The content was measured by HPLC. The Van encapsulation and loading efficiency were calculated by the following equations:

drug loading content (%) = (mVan-loaded/mVan) 100 (1)

drug loading efficiency (%) = (mVan-loaded/mvesicle)100 (2)

mVan-loaded, mVan, and mvesicle are mass of Van encapsulated in vesicles, Van added, and Van-loaded vesicles, respectively. Propidium iodide (PI) was loaded in the same way

1.4 Interaction of vancomycin and Man@AP5 or mPEG@AP5

To determine the nature of Man@AP5 or mPEG@AP5-Van interactions, Man@AP5-Van or mPEG@AP5-Van NPs were incubated in 1.54 M NaCl or 5 M urea solution at RT for 2 h with constant agitation, and the released vancomycin was determined with HPLC.

1.5 Localization of Man@AP5-PI and mPEG@AP5-PI in RAW264.7 cells

For the intracellular localization studies, PI or PI-loaded Man@AP5 or mPEG@AP5 (1 μg/mL of PI, final concentration) were incubated with Raw264.7 cells ($1x10^5$) were in 0.5 mL complete DMEM culture medium for 1 hour. After incubation at 37 ^oC for 2 h, the medium was decanted and the cells were quickly washed twice with PBS and then incubated with 0.2 mL DAPI for 5 minutes. Subsequently, the cells were washed with PBS three times and fixed with 4% PFA for 20 minutes. Finally, the cells were rinsed with cold PBS and viewed under a confocal laser scanning microscope (Leica TCS SP8; Leica Microsystems).

1.6 Accumulation of Man@AP5 and mPEG@AP5 in RAW264.7 cells

PI or PI-loaded Man@AP5 or mPEG@AP5 (1 μg/mL of PI, final concentration) were incubated with Raw264.7 cells ($1x10^6$) in 2.0 mL complete DMEM culture medium for 2, 4, 6 and 8 h. The fluorescence intensity of PI at RAW264.7 cell was then monitored by Flow Cytometer (FCM), which was further quantitatively analyzed by integrated fluorescence density.

In the competitive inhibition assay, cells were incubated with D−mannosamine hydrochloride at a final concentration of 50 mM for 1 h before the addition of PI or Man@AP5-PI or mPEG@AP5- PI. After further incubation at 37 ^oC for 1 h, cells were trypsinized, washed twice with PBS, resuspended in 200 μL of PBS and subjected to flow cytometric analysis.

1.7 In vitro release of Van

The in vitro release profile of Van from Man@AP5-Van or mPEG@AP5-Van was determined in three different media: 1) PBS at pH 5.0, 6.0 and 7.4; 2) Cathepsin B from human placent in citrated PBS (CPBS) at pH 7.4; 3) Cathepsin B from human placenta at pH 5.0, 6.0 and 7.4. The media were incubated at 37 ^oC with continuous shaking at 80 rpm in a shaker bath (WE-1 Shaking water bath; Tianjin Honor Inc.). At different time intervals, 200 μL aliquots of the samples were collected and analyzed by HPLC.

1.8 Determination of the inhibitory effect of Vancomycin-loaded Man@AP5 or mPEG@AP5 to MRSA WHO-2 strain

Vancomycin, Man@AP5-Van or mPEG@AP5-Van at predetermined concentrations in TSB solution (200 μL) were added to 96-wells plates, then 2 μL of a MRSA WHO-2 bacterial suspension with an OD_{600} of 0.5 were added. After 24 h, the OD_{600} of the bacterial suspension was recorded using a BioTek microplate reader.

1.9 Intracellular survival of MRSA inside macrophages treated with Man@AP5 in vitro

RAW264.7 cells were plated at a density of 4×10^5 cells/well and infected with MRSA WHO-2 at a ratio of 10-20 bacteria per macrophage. Macrophage cultures were maintained in growth media supplemented with 50 μg mL^{-1} of gentamycin to inhibit the growth of extracellular bacteria.[3] The infected macrophages were incubated with Man@AP5, Man@AP5-Van, mPEG@AP5 or mPEG@AP5-Van (5, 10, 15, 20 and 30 μg/mL equiv. Van) for 24 h or incubated with Man@AP5, Man@AP5-Van, mPEG@AP5 or mPEG@AP5-Van (10 μg/mL equiv. Van) for different time points. At each time point, the medium was

decanted and the cells were quickly washed twice with PBS. Then, the cells were lysed with Hanks Buffered Saline Solution (HBSS) supplemented with 0.1% BSA (w/v) and 0.1% Triton-X (v/v), and serial dilutions of the lysate were made in a PBS solution containing 0.05% Tween-20 (v/v). The number of surviving intracellular bacteria was determined by plating on tryptic soy agar plates with 5% defibrinated sheep blood (v/v). In this experiment, macrophage cultures were maintained in growth media supplemented with 50 μg/mL gentamycin to inhibit the growth of extracellular bacteria.

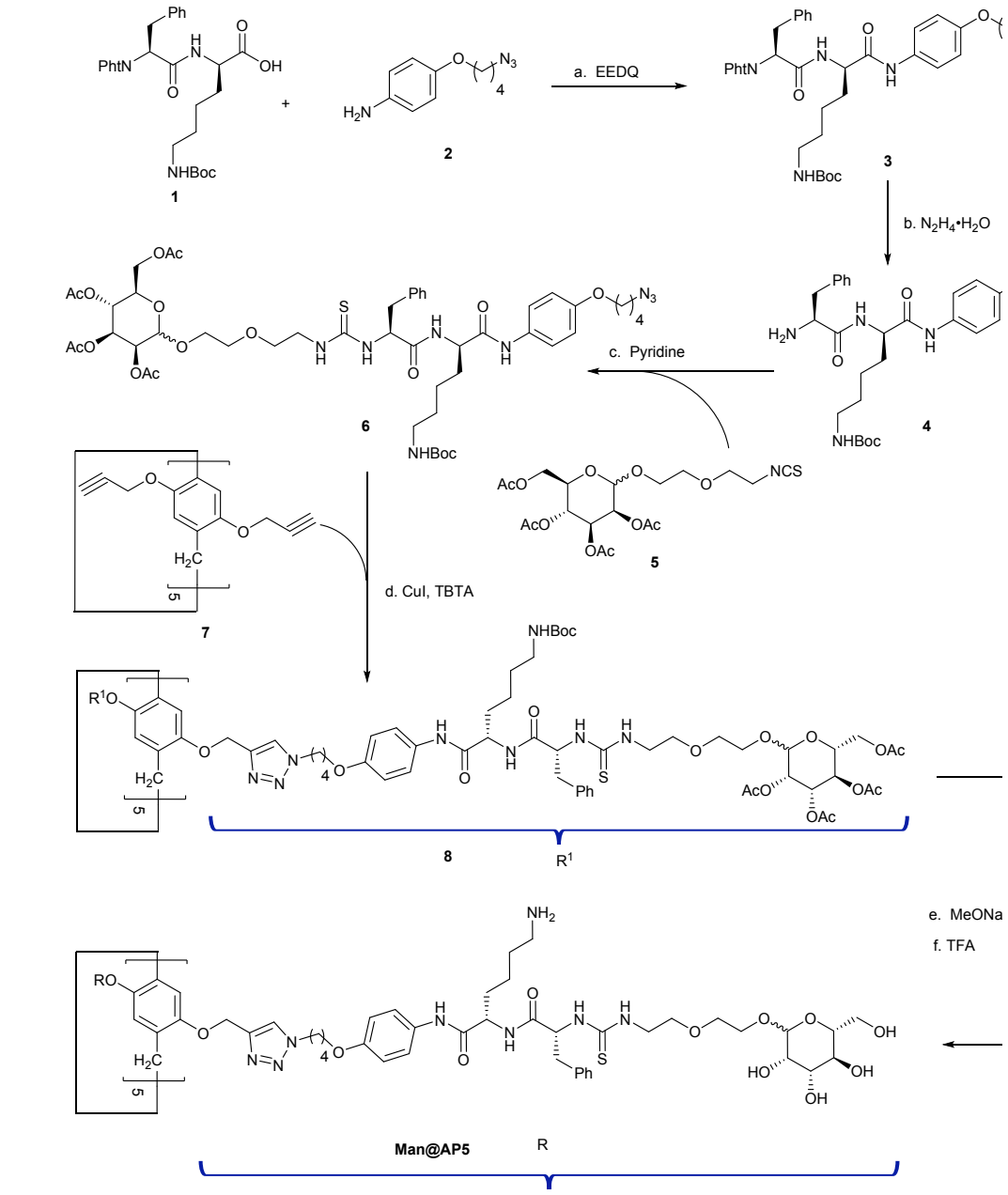
1.10 Quantification of released antibiotic inside the macrophages

RAW264.7 cells were infected in 6-well tissue culture dishes as described above for the determination of intracellular MRSA survival. Infected cells were incubated with Van or Man@AP5-Van or mPEG@AP5-Van at Vancomycin concentration of 5, 10, 15, 20 μg/mL (equiv. Van) for 24 h or incubated with Van or Man@AP5-Van or mPEG@AP5-Van at Vancomycin concentration of 10 μg/mL (equiv. Van) for 2, 4, 8, 12 and 24 h. At each time point, the medium was decanted and the cells were quickly washed five times with PBS and the washing media was measured by HPLC, confirming that extracellular Van was efficiently removed. The cells were lysed with HBSS supplemented with 0.1% BSA (w/v) and 0.1% Triton-X (v/v), and the dilutions of the lysate were made in 0.5 mL of 1 N HCl solution, the mixture incubated for 1 h. The diluents were lyophilized by evaporation under a Termovap Sample Concentrator (Shanghai Joyn, Shanghai, People's Republic of China), reconstituted in 100 μL of 1 N HCl solution, filtered using a 0.45 μm syringe filter, and analyzed by HPLC.

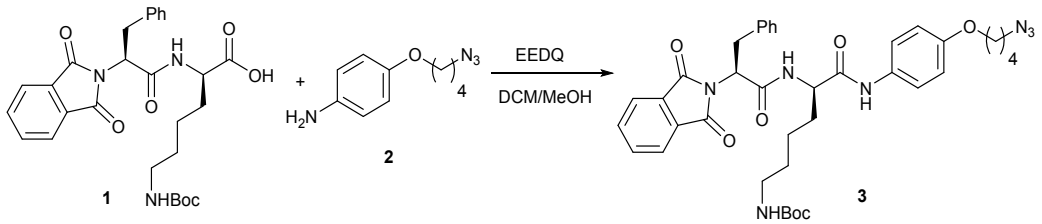
1.11 In vitro cytotoxicity assay

The cytotoxicity of Man@AP5, Man@AP5-Van, mPEG@AP5 or mPEG@AP5-Van was evaluated by the tetrazolium-based colorimetric (MTT) assay. Cells were seeded in 96-well plates at a density of 5×10^3 per well and incubated for 72 hours with either vehicle (DMSO) or desired concentrations of Man@AP5, Man@AP5-Van, mPEG@AP5 or mPEG@AP5-Van at 37 ^{o}C in a 5% CO_2 incubator. Then, the medium was replaced with 100 μL of fresh medium without serum and 10 μL of MTT (5 mg/mL; Sangon Biotech) solution and incubated for an additional 4 hours at 37 ^{o}C in a 5% CO_2 incubator. Subsequently, the medium was removed and 100 μL of DMSO was added. The absorbance at 490 nm was measured using a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA). Cell viability was calculated as the ratio of the absorbance of the treated cells to that of the control group cells.

2. Syntheses of Man@AP5 and mPEG@AP5



Scheme S1: Synthetic route for **Man@AP5**.



Synthesis of compound **3**: A mixture of **1** (5.24 g, 10 mmol), 4-(4-azidobutoxy)aniline 2 (2.06 g, 10 mmol) and EEDQ (7.42 g, 30 mmol) in DCM/MeOH (100 mL, 4:1) was stirred under N_2 at room temperature for 16 h. The mixture was concentrated in vacuum and the residue was partitioned between 150 mL 1 N HCl and 150 mL DCM. The organic layer was separated and dried over Na_2SO_4. The filtrate was concentrated to yield the crude product, which was purified by silica column chromatography (DCM/MeOH, 50:1) to yield compound **3** (6.9 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (s, 1H), 7.89 (s, 1H), 7.52 (s, 2H), 7.46 (s, 2H), 7.34 (d, J = 8.0Hz, 2H), 7.04-7.01 (m, 5H), 6.56-6.55 (m, 1H), 5.14 (s, 2H), 4.57 (s, 1H), 3.79-3.62 (m, 3H), 3.42 (t, J = 12Hz, 1H), 3.30-3.27 (m, 2H), 3.07-2.98 (m, 2H), 1.72 (s, 6H), 1.40-1.18 (m, 13H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.93, 169.17, 168.06, 156.17, 155.39, 136.65, 134.03, 131.28, 131.15, 128.96, 128.42, 126.75, 123.16, 121.17, 114.40, 78.83, 67.23, 54.95, 54.10, 51.15, 40.26, 35.01, 31.21, 29.27, 28.42, 26.48, 25.70, 23.22. HRMS (ESI-TOF) m/z caled for $C_{38}H_{46}N_7O_7$ $(M+H)^+$ 712.3459, found 712.3458.

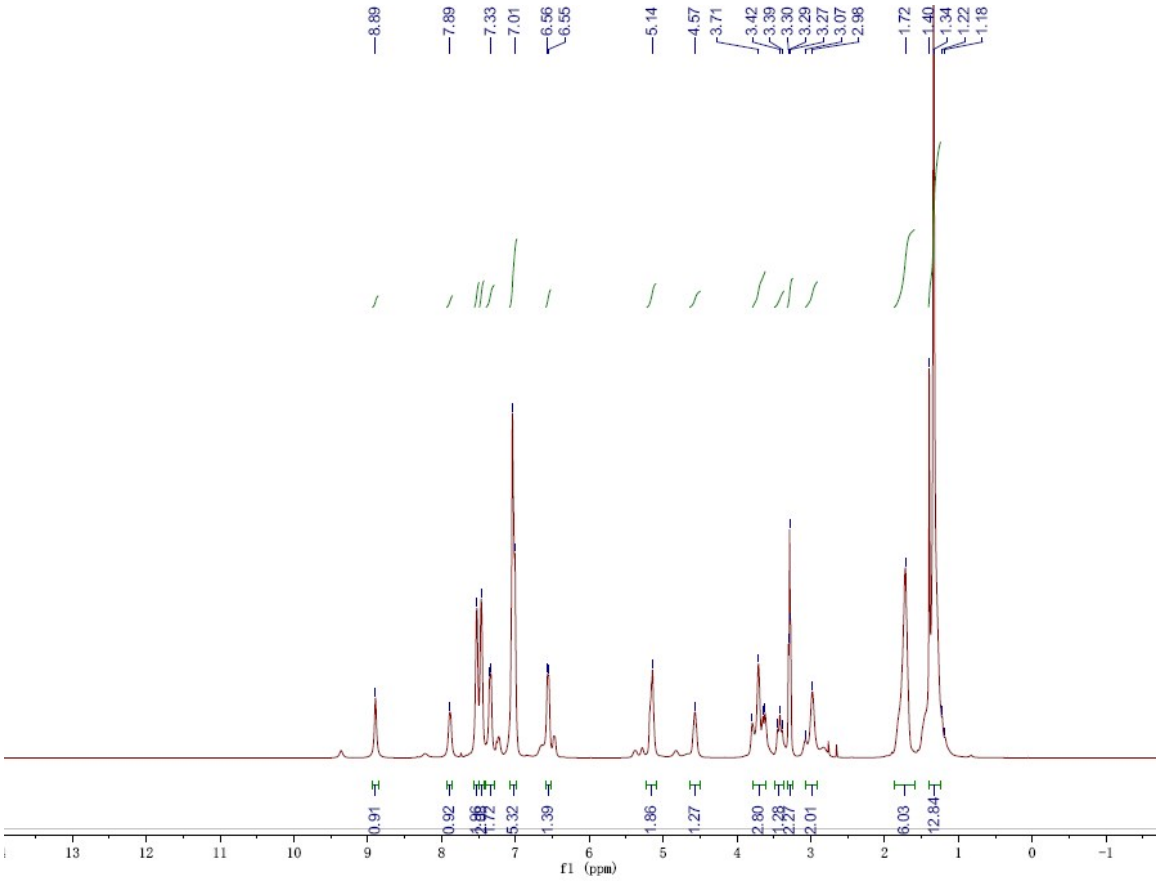


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **3**.

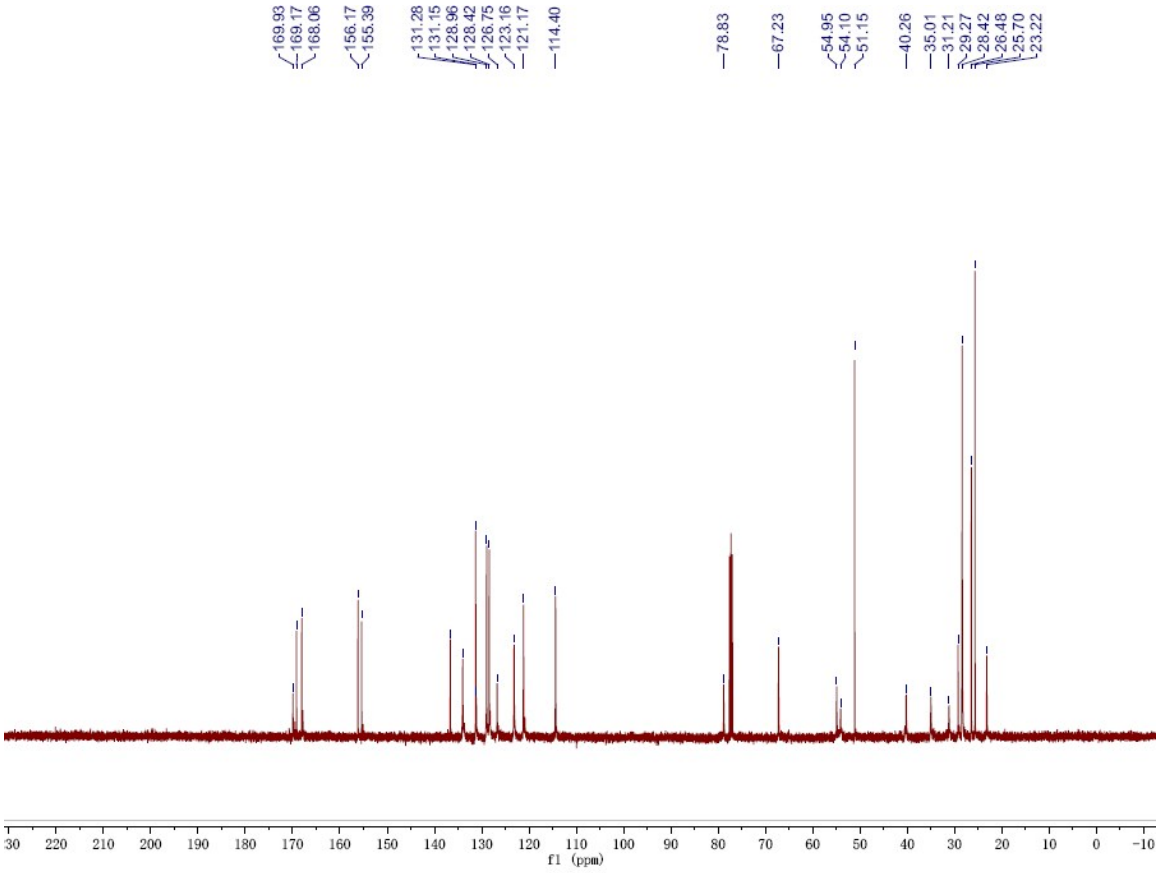
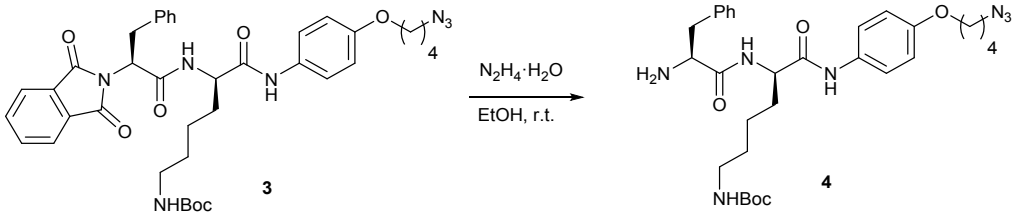


Figure S2. ^{13}C NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **3**.



Synthesis of compound **4**: A mixture of **3** (6.4 g, 9.0 mmol), hydrazinehydrate (0.90 g, 18.0 mmol) in EtOH (100 mL) was stirred under N_2 at room temperature for overnight. The mixture was concentrated in vacuum and the crude product was purified by silica column chromatography (DCM/MeOH, 40:1) to yield compound 4 (5.0 g, 96%). ^1H NMR (400 MHz, $CDCl_3$): δ 9.34-9.30 (m, 1H), 8.02 (dd, J = 8.0Hz, 1H), 7.43 (d, J = 8.0Hz, 2H), 7.23-7.09 (m, 5H), 6.76-6.67 (m, 2H), 4.96 (s, 1H), 4.64 (s, 1H), 3.88-3.81 (m, 2H), 3.60-3.58 (m, 1H), 3.29 (t, J = 8.0Hz, 2H), 3.16-3.03 (m, 3H), 2.72-2.54 (m, 2H), 1.89-1.67 (m, 6H), 1.46-1.37 (m, 13H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.09, 175.02, 169.89, 155.51, 137.61, 131.30, 129.22, 129.15, 128.63, 126.76, 121.66, 114.59, 67.37, 56.47, 53.42, 51.11, 46.07, 41.06, 40.91, 40.17, 32.00, 29.59, 28.41, 26.45, 25.67, 22.75. HRMS (ESI-TOF) m/z calcd for $C_{30}H_{44}N_7O_5$ $(M+H)^+$ 582.3404, found 582.3407.

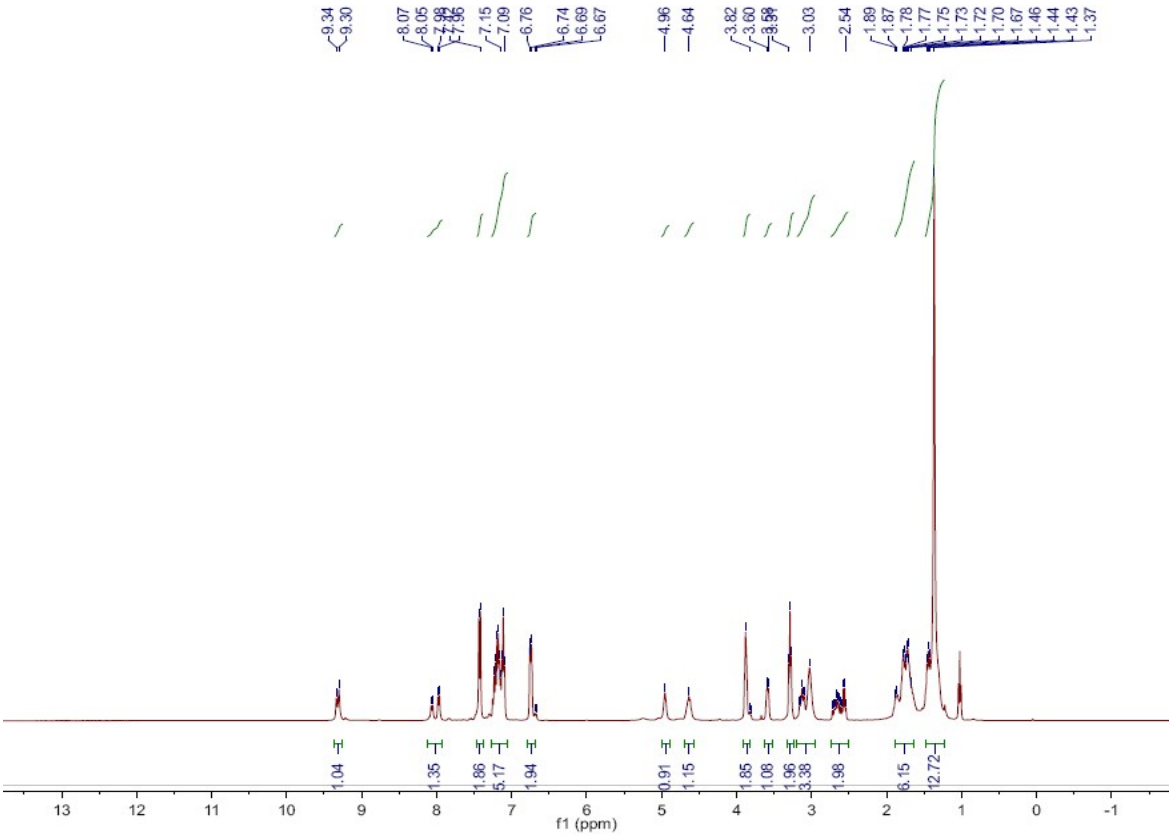


Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **4**.

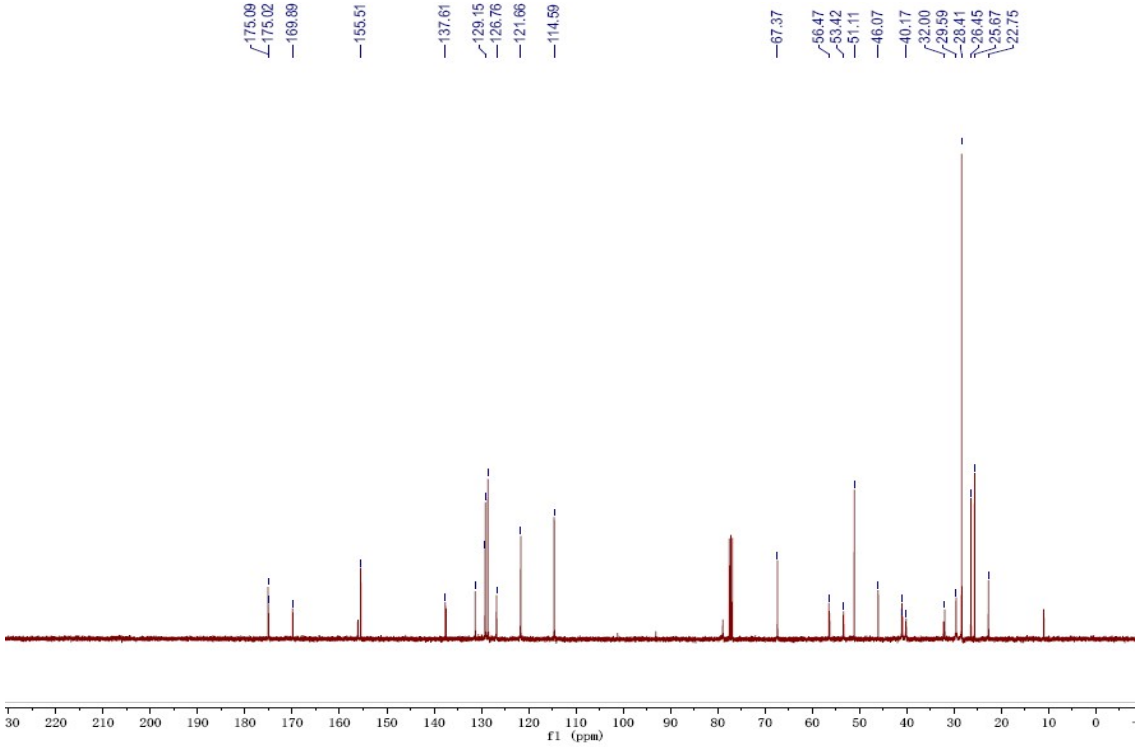
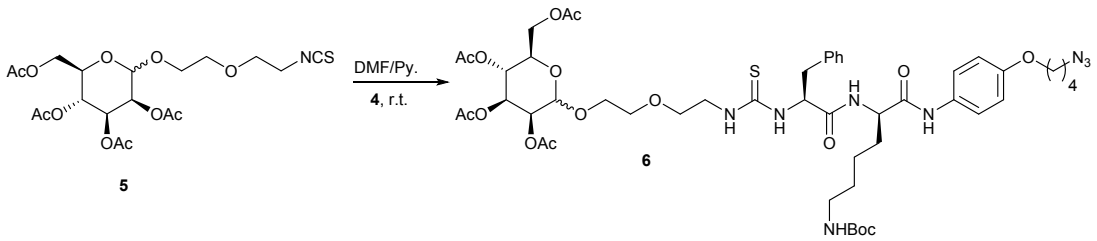


Figure S4. ^{13}C NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **4**.



Synthesis of compound **6**: The compounds **5** (4.3 g, 9.0 mmol) and **4** (4.9 g, 8.4 mmol) in DMF/Pyridine (60 mL, 1:1) was stirred under N_2 at room temperature for 12 h. The mixture was concentrated in vacuum and the residue was partitioned between 100 mL water and 150 mL DCM. The organic layer was separated and dried over Na_2SO_4. The filtrate was concentrated to yield the crude product, which was purified by silica column chromatography (DCM/MeOH, 30:1) to yield compound **6** (7.6g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.98 (s, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 1H), 7.24-7.21 (m, 3H), 7.15-7.12 (m, 2H), 6.80-6.69 (m, 4H), 5.36-5.17 (m, 4H), 5.10-5.08 (m, 1H), 4.95-4.86 (m, 1H), 4.64 (s, 1H), 4.48-4.36 (m, 1H), 4.25-4.20 (m, 1H), 4.13-4.08 (m, 1H), 4.02-4.01 (m, 1H), 3.95-3.89 (m, 2H), 3.71-3.52 (m, 8H), 3.33 (q, J = 8.0 Hz, 2H), 3.15-2.99 (m, 2H), 2.14 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.85-1.71 (m, 4H), 1.41 (s, 9H), 1.36-1.23 (m, 4H), 1.04-1.02 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.84, 170.85, 170.23, 169.71, 169.25, 162.57, 156.13, 155.54, 136.29, 131.03, 129.26, 128.63, 127.02, 126.86, 121.92, 114.47, 97.24, 79.09, 70.39, 70.17, 68.92, 68.36, 67.36, 66.80, 66.23, 62.57, 53.92, 51.15, 44.58, 39.99, 37.97, 36.48, 31.43, 31.26, 29.62, 29.47, 28.43, 26.47, 25.69, 22.49, 21.00, 20.73, 20.67. HRMS (ESI-TOF) m/z caled for $C_{49}H_{70}N_8NaO_{16}S$ $(M+Na)^-$ 1081.4534, found 1081.4535.

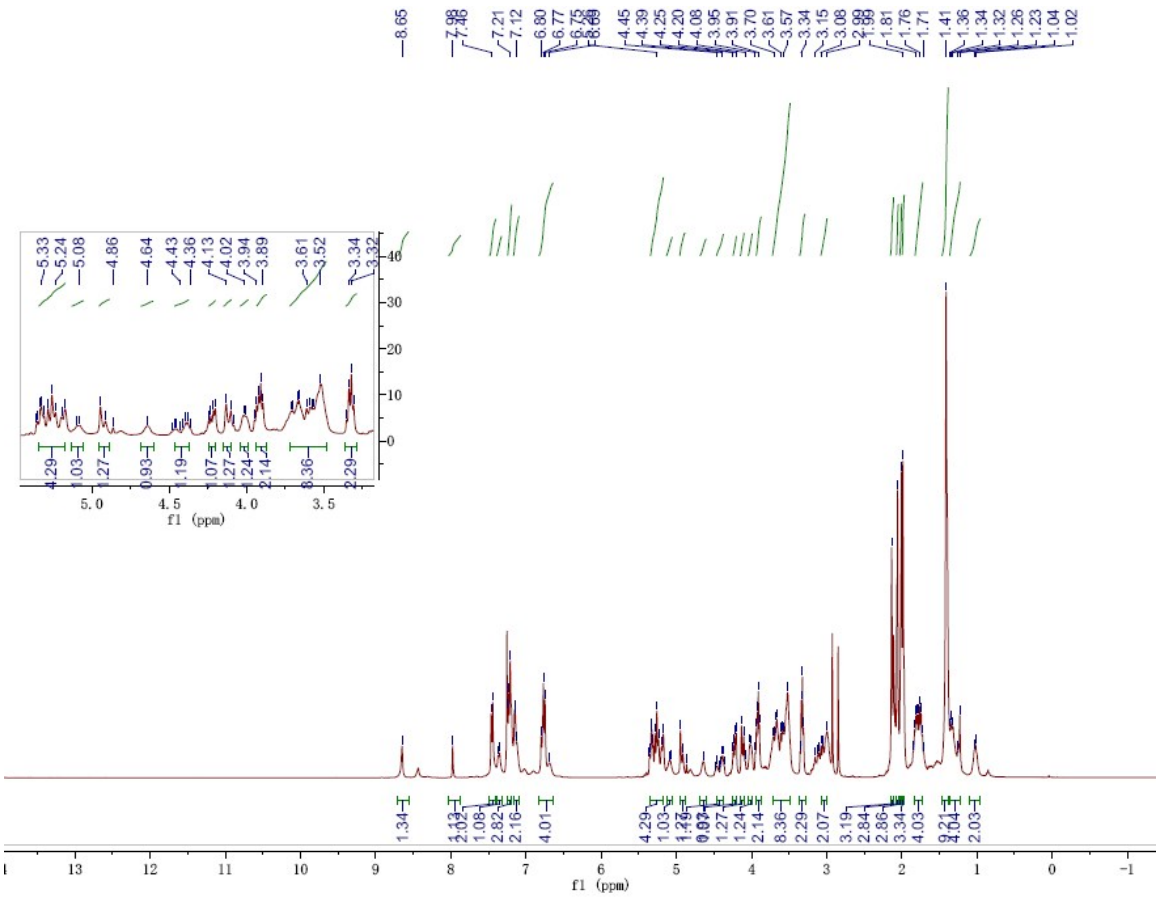


Figure S5. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **6**.

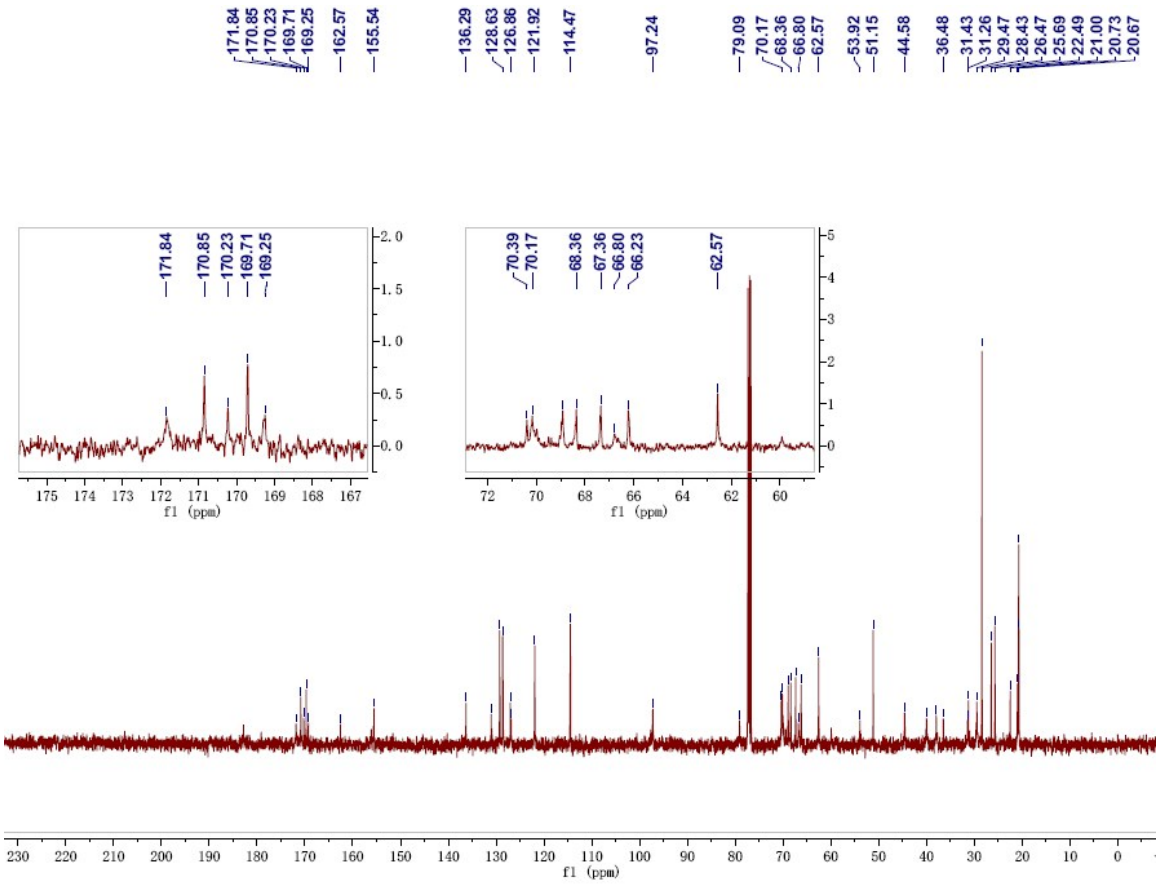
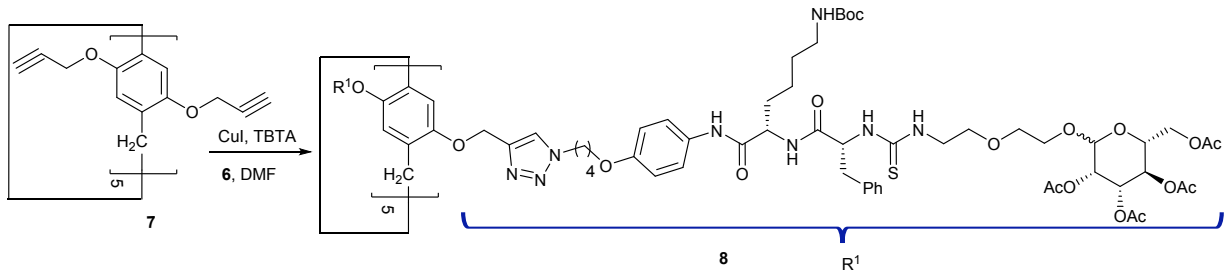


Figure S6. ^{13}C NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **6**.



Synthesis of compound **8**: A mixtures of **7** (198.2 mg, 0.2 mmol), **6** (2.3 g, 2.2 mmol), CuI (76.2 mg, 0.4 mmol) and TBTA (0.23 g, 0.4 mmol) in DMF (20mL) were stirred under N2 at room temperature for 24 h. The mixture was concentrated in vacuum and the residue was partitioned between 100 mL of 1 N HCl and 150 mL DCM. The organic layer was separated and dried over Na_2SO_4. The filtrate was concentrated to yield the crude product, which was purified by silica column chromatography (DCM/MeOH, 10: 1) to yield compound **8** (1.4 g, 61%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.89 (s, 10H), 7.28 (s, 10H), 7.20 (s, 30H), 7.09 (s, 30H), 6.90 (s, 10H), 6.57 (s, 20H), 5.44 (s, 10H), 5.27-5.21 (m, 40H), 5.04-5.00 (m, 10H), 4.85 (s, 20H), 4.31-4.21 (m, 20H), 4.08-4.03 (m, 20H), 3.98 (s, 10H), 3.71-3.52 (m, 90H), 3.09-2.98 (m, 40H), 2.66 (s, 20H), 2.08 (s, 30H), 2.04 (s, 30H), 1.98 (s, 30H), 1.94 (s, 30H), 1.63 (s, 20H), 1.35 (s, 130H), 1.21-1.18 (m, 20H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.74, 170.69, 170.28, 170.00, 169.98, 169.94, 169.68, 169.66, 156.20, 156.14, 155.43, 144.00, 136.51, 130.94, 129.30, 129.27, 128.52, 128.45, 126.82, 123.44, 122.24, 114.28, 97.46, 78.88, 69.77, 69.61, 69.05, 69.02, 68.37, 67.00, 66.96, 66.10, 62.46, 53.33, 49.94, 44.31, 40.18, 31.85, 31.45, 29.61, 29.28, 27.09, 26.21, 22.62, 20.87, 20.84, 20.73, 20.67, 14.06. MALDI-TOF-MS. Calcd. for $C_{555}H_{750}N_{80}NaO_{170}S_{10}$ $[M+Na]^+$ 11597.961, found 11597.969.

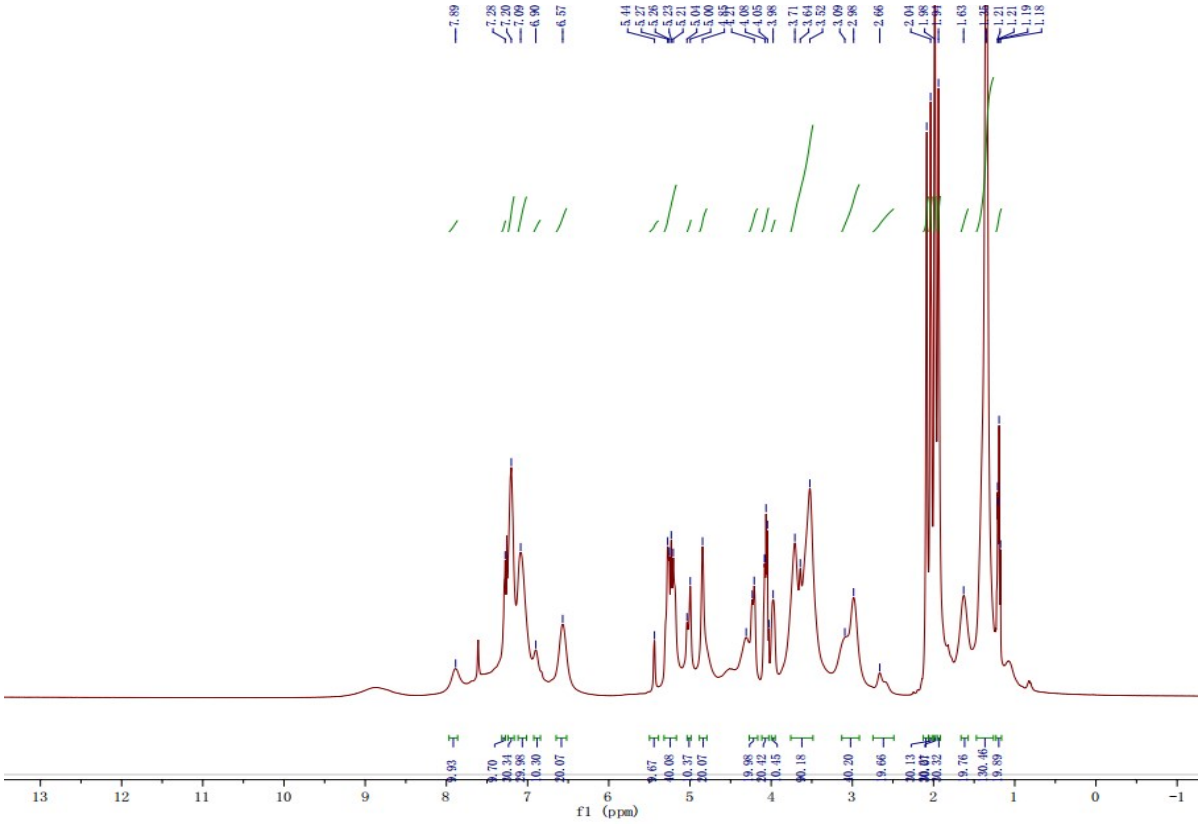


Figure S7. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **8**.

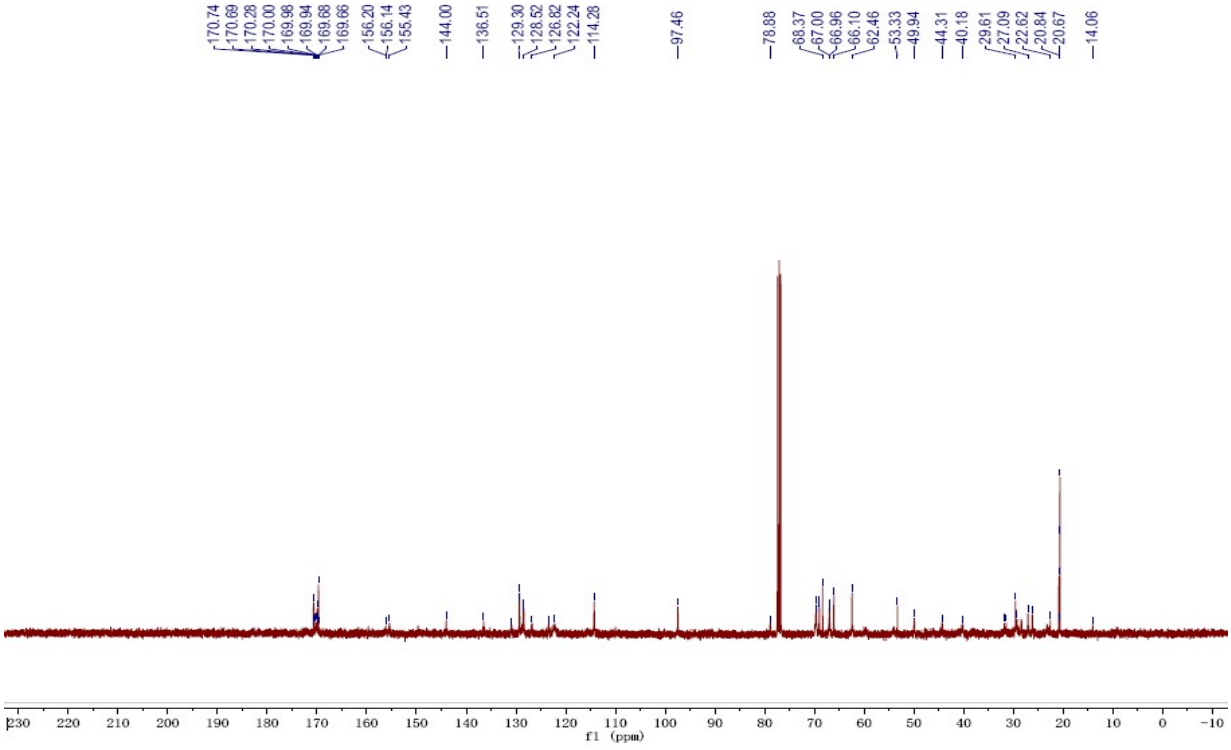
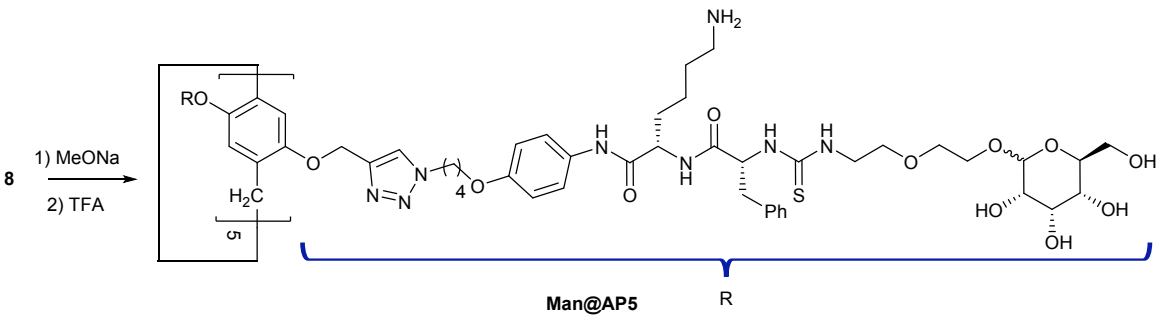


Figure S8. ^{13}C NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **8**.



Man@AP5

Synthesis of compound **Man@AP5**: A mixture of **8** (1.2 g, 0.1 mmol) in MeOH (30 mL) was stirred under N_2 at room temperature and 30% MeONa in MeOH adjusts the pH to 9. The mixture was concentrated in vacuum. The crude product was dissolved in TFA/DCM (20 mL, 1:1) and stirred under N_2 at room temperature for 5 h. The mixture was concentrated in vacuum to afford crude product **Man@AP5**. Then purified with a dialysis bag (5000Da) which afford product **Man@AP5**. (0.86 g, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.49-10.42 (m, 10H), 10.12 (s, 10H), 9.66 (s, 10H), 9.40 (s, 10H), 8.31 (s, 20H), 7.83-7.75 (m, 20H), 7.47-7.40 (m, 20H), 7.26-7.20 (m, 10H), 7.08 (s, 10H), 6.93 (s, 10H), 6.84 (s, 20H), 5.04 (s, 10H), 4.71-4.34 (m, 80H), 3.85-2.71 (m, 190H), 1.87 (m, 10H), 1.77 (m, 10H), 1.58-1.50 (m, 60H), 1.36-1.25 (m, 20H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.22, 159.17, 158.84, 155.48, 149.23, 143.66, 131.64, 130.76, 130.55, 129.24, 128.55, 124.40, 121.51, 121.30, 114.97, 100.40, 77.66, 74.30, 71.37, 70.68, 67.31, 66.75, 66.09, 61.64, 60.18, 53.16, 49.53, 38.85, 31.08, 31.01, 28.23, 27.04, 26.96, 26.19, 21.98, 21.72, 15.57, 14.49. MALDI-TOF-MS. Calcd. For $C_{425}H_{590}N_{80}O_{110}S_{10}$, $[M]^+$ 8894.024, found 8894.187.

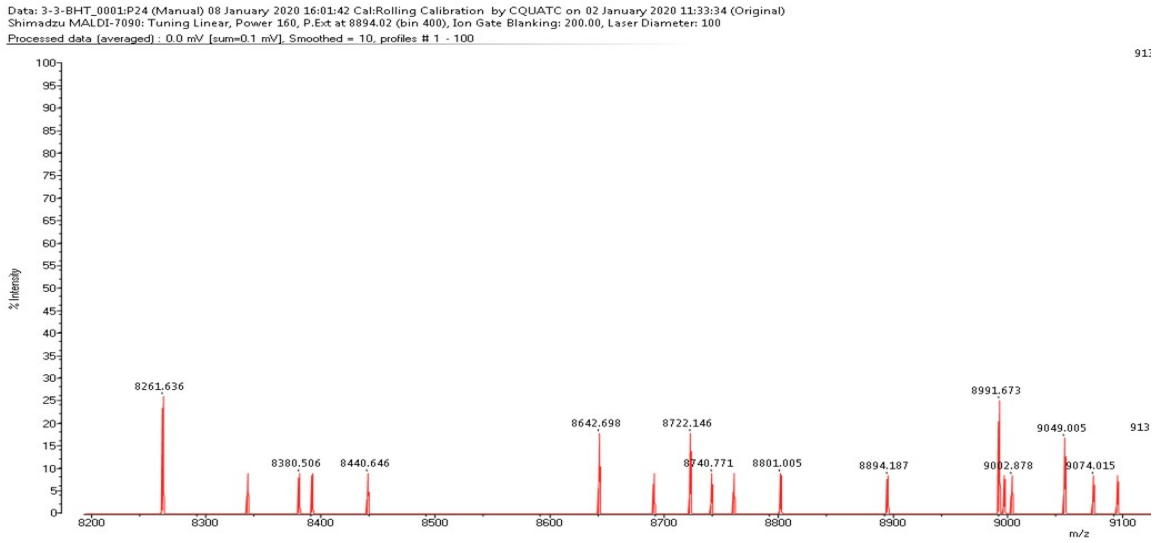


Figure S9. MALDI-TOF-MS of **Man@AP5**

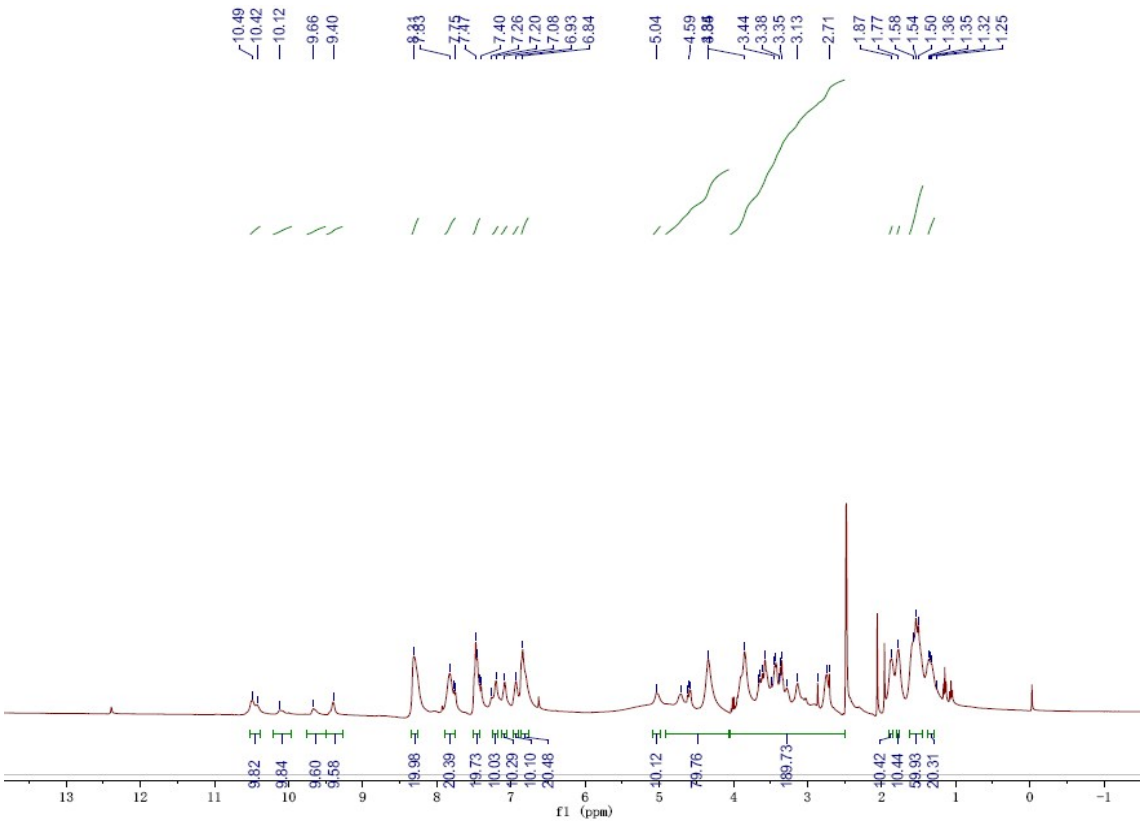


Figure S10. [1]H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **Man@AP5.**

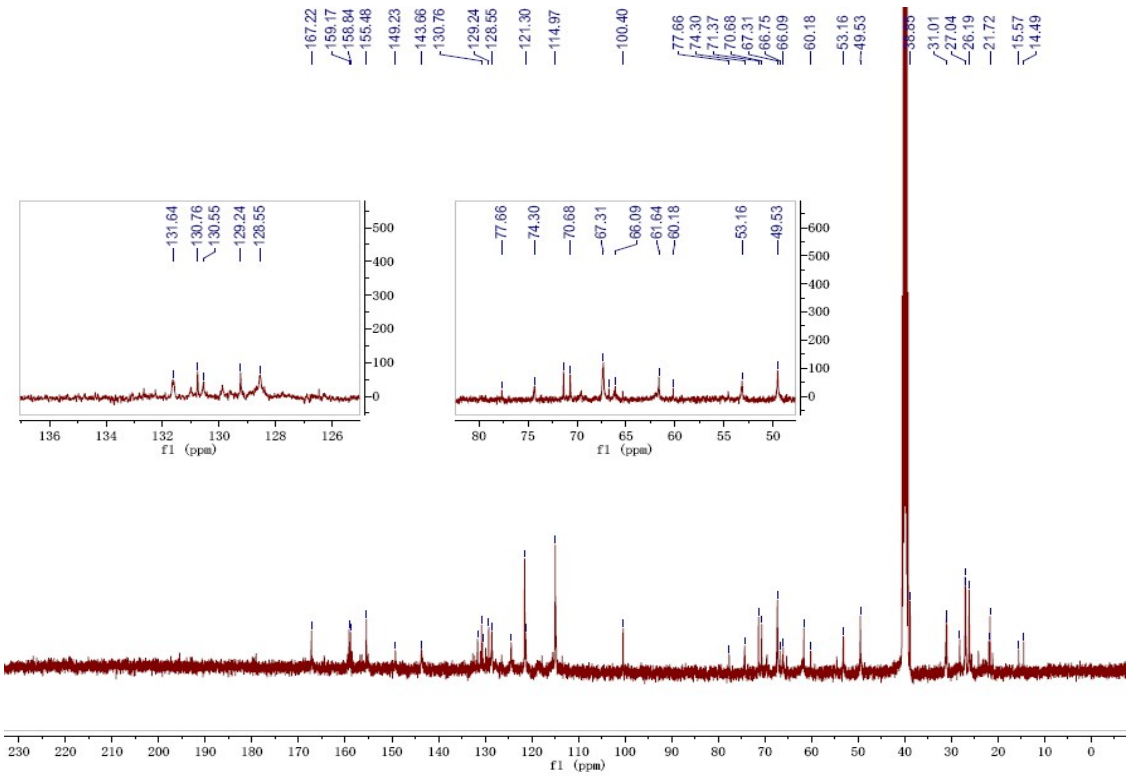
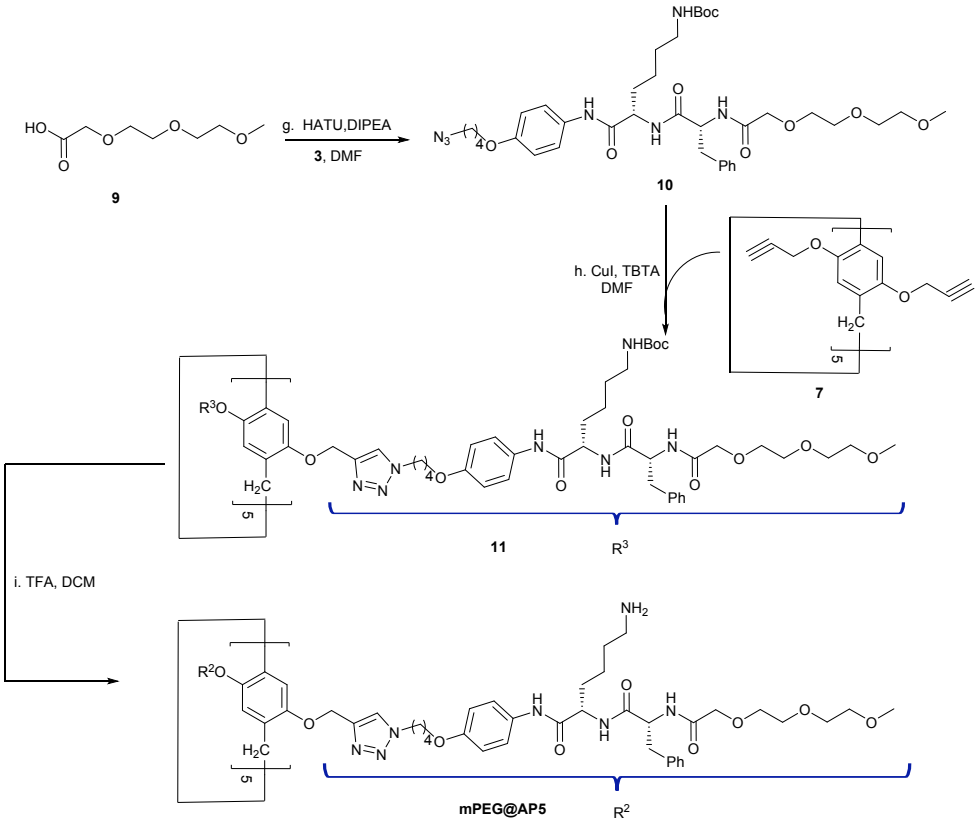
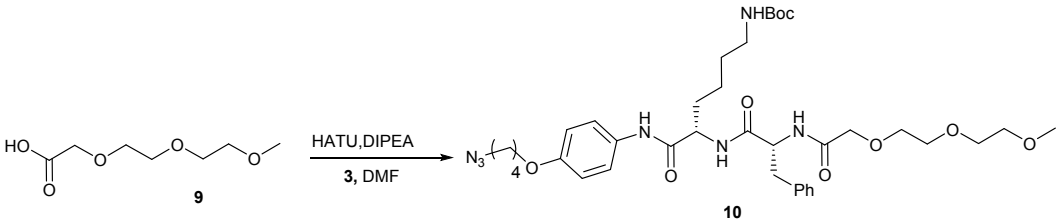


Figure S11.[13]C NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **Man@AP5**.

2.2 The synthetic procedures for mPEG@AP5 was shown in Scheme S2.



Scheme S2: Synthetic route for **mPEG@AP5**.



Synthesis of compound **10**: The compound **3** (2.33 g, 4.0 mmol), compound **9** (0.91 g, 4.1 mmol), HATU (2.9 g, 5.0 mmol) in DMF (20 mL) was stirred under N_2 at 0 °C and then added the DIPEA (0.65 g, 5.0 mmol). The mixture was stirred under N_2 at room temperature for overnight. The mixture was concentrated in vacuum and the residue was partitioned between 50 mL 1 N HCl and 100 mL DCM. The organic layer was separated and the solvent was evaporated in vacuo. the residue was purified by preparative TLC on GF254 (DCM/MeOH, 40:1) to afford the desired product **10** (2.9 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83-8.81 (m, 1H), 7.42 (d, J = 8.0Hz, 2H), 7.15 (d, J = 8.0Hz, 5H), 6.70-6.65 (m, 2H), 4.57-4.52 (m, 1H), 4.37-4.34 (m, 1H), 4.20-4.00 (m, 1H), 3.87-3.81 (m, 2H), 3.54-3.44 (m, 10H), 3.25 (s, 3H), 3.07-2.81 (m, 3H), 2.78-2.68 (m, 2H), 1.71-1.68 (m, 4H), 1.35-1.14 (m, 13H), 0.97 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.15, 170.74, 169.50, 162.55, 156.01, 155.32, 136.32, 131.43, 129.23, 128.52, 121.52, 114.42, 78.80, 71.78, 70.88, 70.43, 70.29, 70.11, 68.84, 68.43, 67.29, 63.69, 60.29, 58.81, 55.11, 53.65, 51.08, 39.96, 38.53, 37.55, 36.41, 31.13, 29.39, 28.38, 26.41, 25.63, 22.54. HRMS (ESI-TOF) m/z calcd for $C_{37}H_{55}N_7NaO_9$ $(M+Na)^+$ 764.3953, found 764.3953.

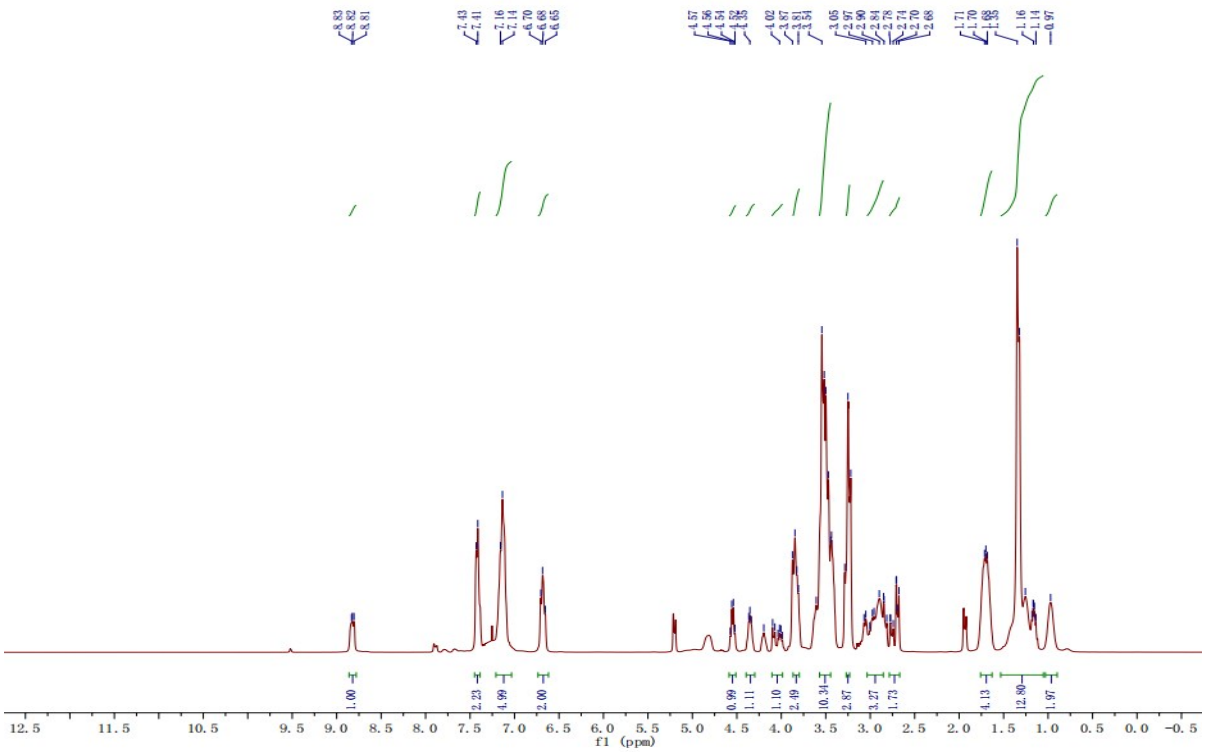


Figure S12. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **10**.

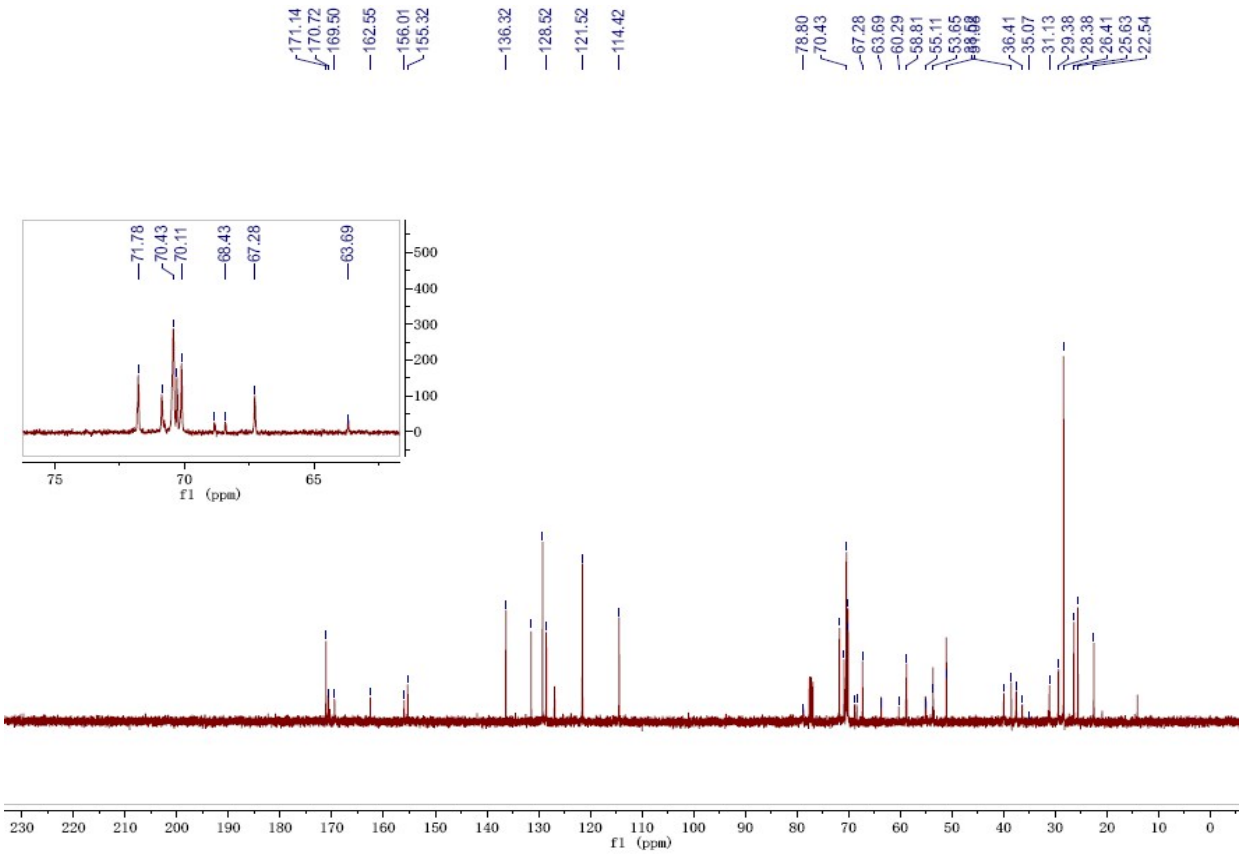
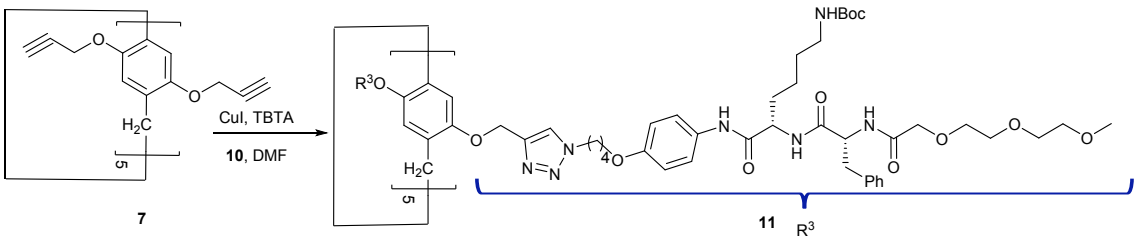


Figure S13. ^{13}C NMR spectrum (400 MHz, $CDCl_3$ 298 K) of compound **10.**



Synthesis of compound **11**: Pillar[5]arene **7** (99.1 mg, 0.1 mmol), compound **10** (0.86 g, 1.1 mmol), CuI (38.1 mg, 0.2 mmol) and TBTA (106.1 mg, 0.2 mmol) in DMF (10 mL), at room temperature for 24 h. The mixture was concentrated in vacuum and the residue was partitioned between 50 mL water and 100 mL DCM. The organic layer was separated and dry with the Na_2SO_4. The solvent was evaporated in vacuo. the residue was purified by preparative TLC on GF254 (DCM/MeOH, 15: 1) to afford the desired product **11** (794 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.98 (s, 10H), 7.95 (s, 10H), 7.69-7.51 (m, 20H), 7.35-7.29 (m, 20H), 7.25-7.21 (m, 40H), 6.59 (s, 20H), 4.92 (s, 10H), 4.67 (s, 10H), 4.44 (s, 10H), 4.30-4.19 (m, 20H), 3.88 (s, 20H), 3.70 (s, 20H), 3.58 (s, 50H), 3.51-3.42 (m, 50H), 3.29 (s, 30H), 3.14 (s, 10H), 3.06-2.96 (m, 20H), 1.93 (s, 20H), 1.61 (s, 20H), 1.39-1.16 (m, 130H), 1.04 (s, 20H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.29, 170.76, 169.85, 158.00, 157.70, 156.10, 155.29, 155.05, 144.28, 136.42, 131.35, 131.28, 129.31, 129.02, 128.58, 126.98, 121.80, 114.36, 78.88, 71.75, 70.78, 70.37, 70.27, 70.05, 56.90, 55.10, 55.05, 53.77, 53.35, 50.04, 49.99, 40.15, 37.71, 31.43, 29.64, 29.48, 28.45, 27.08, 26.17, 22.68. MALDI-TOF-MS. Calcd. for $C_{435}H_{600}N_{70}NaO_{100}$, [M+Na]+ 8427.391, found 8427.397.

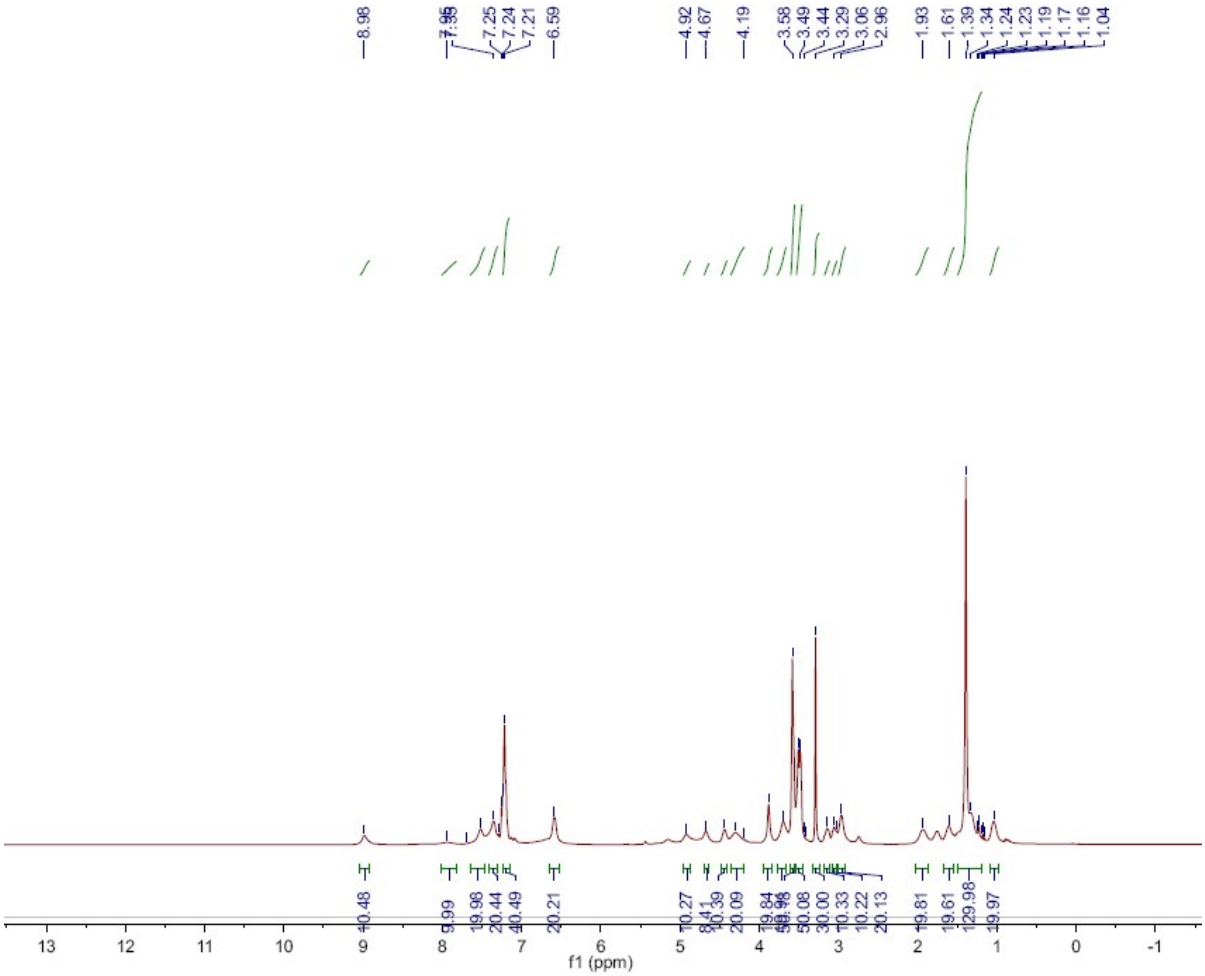


Figure S14. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **11**.

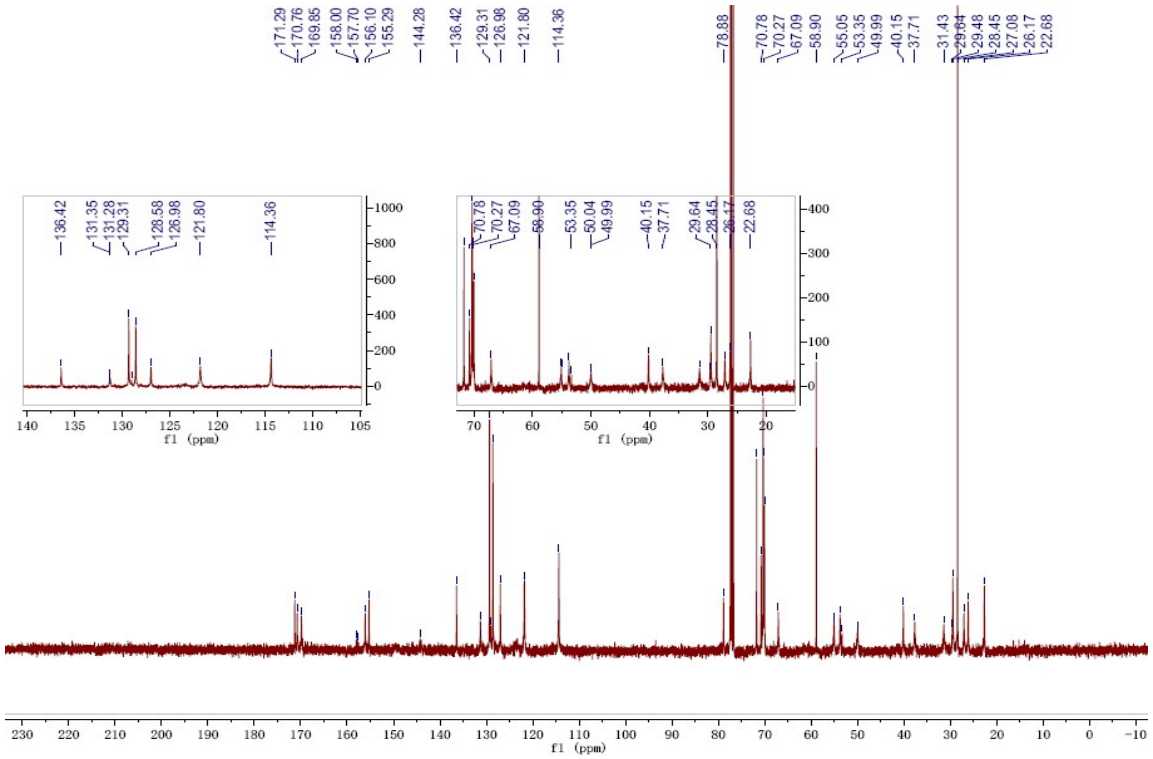
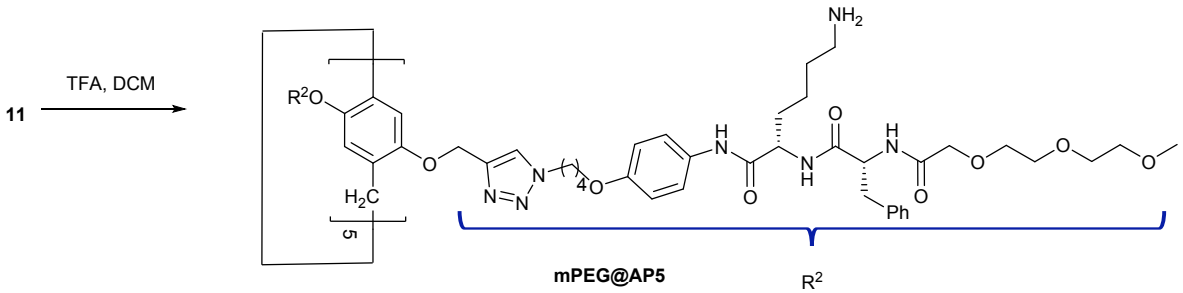


Figure S15. ^{13}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **11**.



Synthesis of compound **mPEG@AP5**: The compound **11** (700 mg, 0.08 mmol) was dissolved in TFA/DCM (10 mL, 1:1) and stirred under N_2 at room temperature for 5 h. The mixture was concentrated in vacuum to afford the desired product **mPEG@AP5** (628 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 10H), 8.46 (d, J = 8.0Hz, 10H), 7.80-7.73 (m, 40H), 7.53 (d, J = 8.0Hz, 20H), 7.23-7.17 (m, 50H), 6.81 (d, J = 8.0Hz, 20H), 4.64 (q, J = 8.0Hz, 10H), 4.33-4.27 (m, 40H), 3.84 (q, J = 16Hz, 40H), 3.47-3.35 (m, 100H), 3.17 (s, 30H), 3.08-2.97 (m, 10H), 2.93-2.84 (m, 10H), 2.71-2.70 (m, 20H), 1.86 (s, 20H), 1.68-1.47 (m, 60H), 1.16-1.07 (m, 20H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.29, 170.22, 169.82, 158.39, 158.05, 155.05, 155.00, 149.07, 137.67, 132.35, 132.21, 129.70, 129.12, 128.47, 126.78, 121.19, 114.72, 109.99, 79.33, 71.64, 70.57, 70.20, 70.14, 69.94, 67.21, 58.36, 54.17, 53.43, 49.73, 46.09, 39.06, 38.21, 31.68, 27.05, 27.01, 26.17, 22.65. MALDI-TOF-MS. Calcd. for $C_{385}H_{520}N_{70}NaO_{80}$, $[M+Na]^+$ 7426.867, found 7427.934.

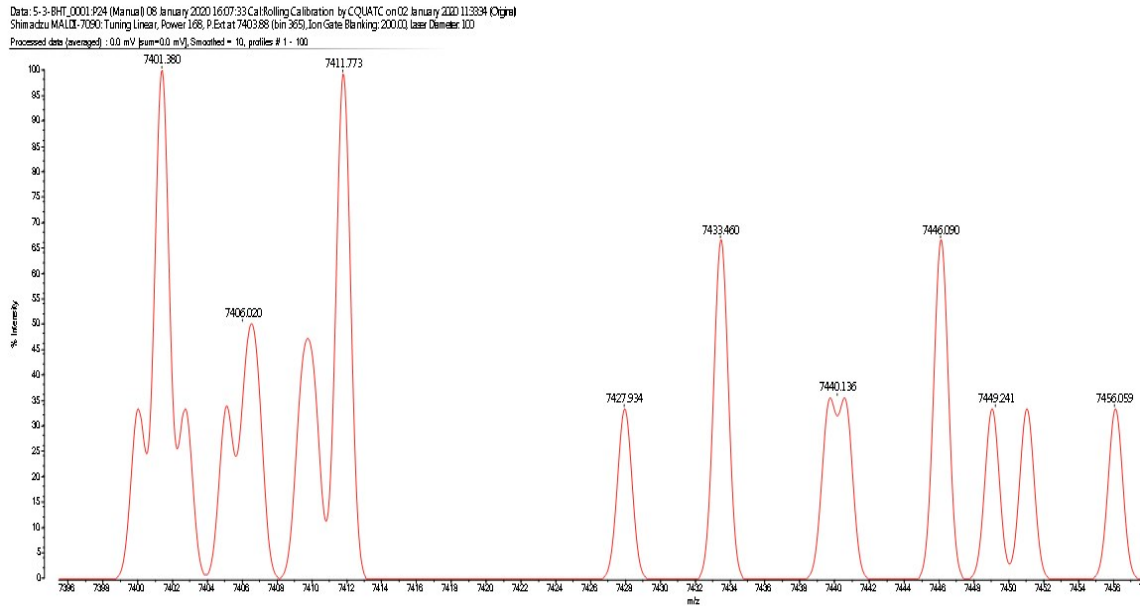


Figure S16. MALDI-TOF-MS of **mPEG@AP5**

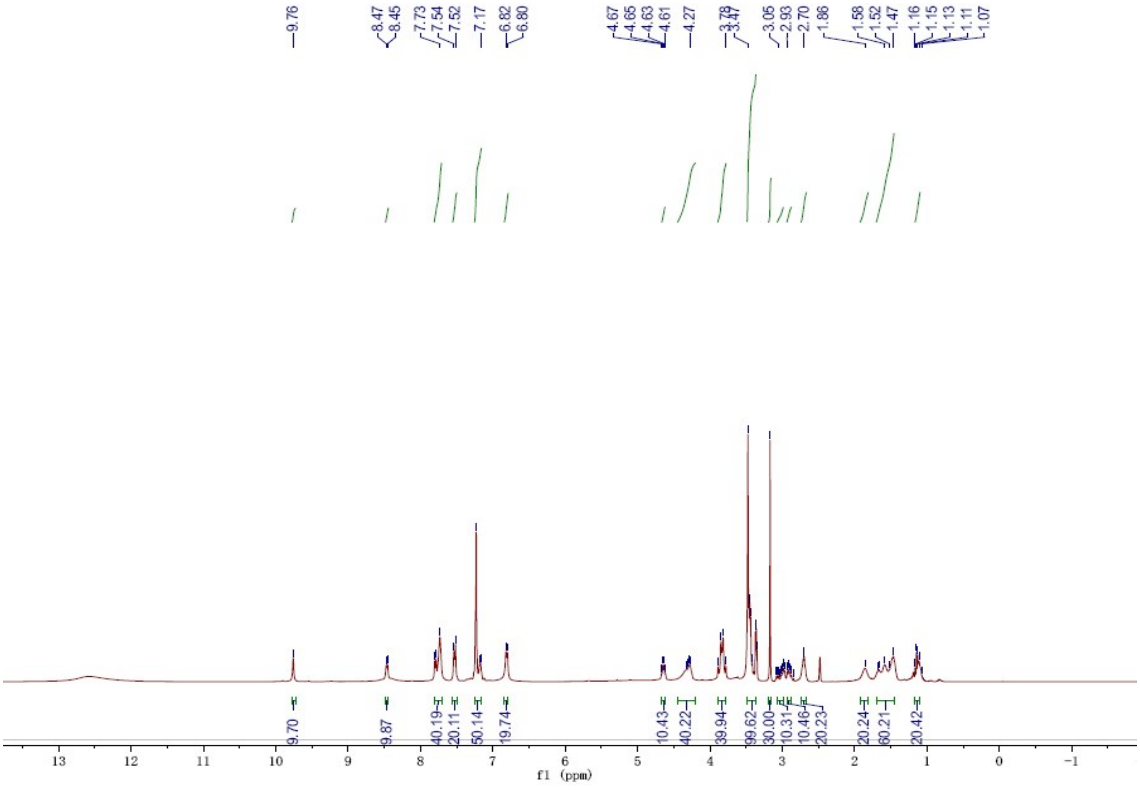


Figure S17. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **mPEG@AP5**.

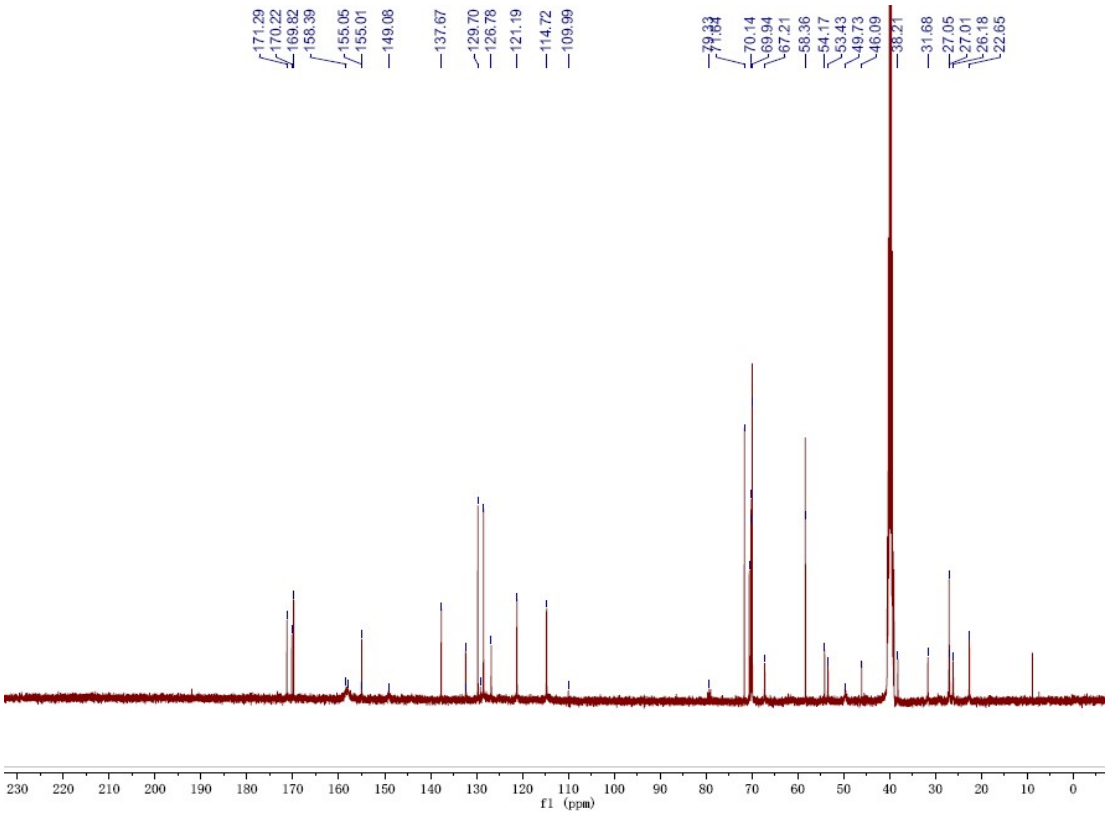


Figure S18. ^{13}C NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **mPEG@AP5.**

3. Results

3.1 Zeta-potentials of the aggregates formed from Man@AP5 and mPEG@AP5

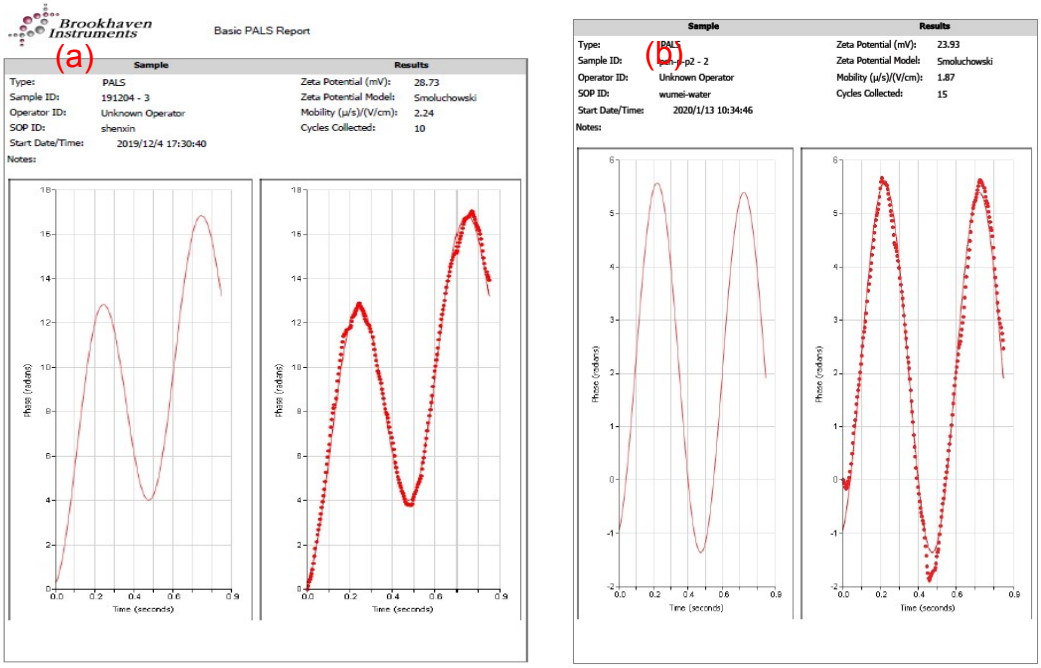


Figure S19. Zeta-potentials of the aggregates formed by (a) Man@AP5 and (b) mPEG@AP5

3.2 Critical aggregation concentration (CAC) determination for Man@AP5 and mPEG@AP5

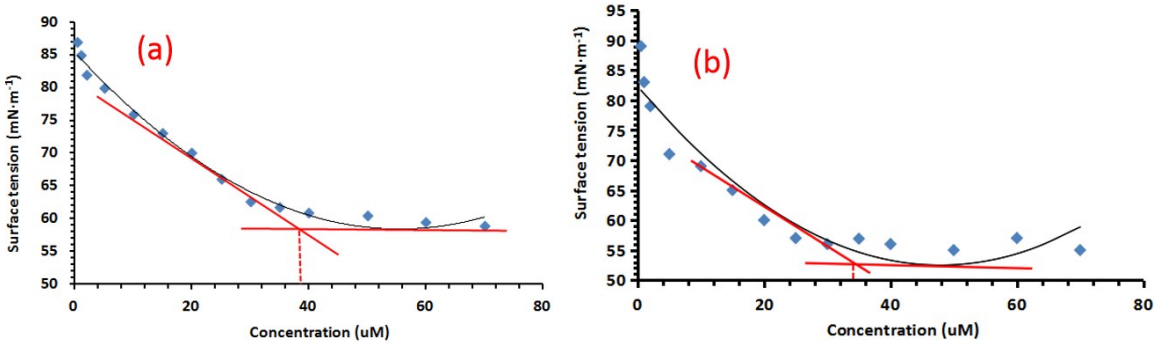


Figure S20. Plot of the surface tension of water vs. the concentration of Man@AP5 or mPEG@AP5. There are two linear segments in the plot and a sudden decrease of the slope, implying that the CAC of Man@AP5 and mPEG@AP5 is approximately 39 μM and 34 μM.

3.3 DLS and TEM for the vesicles formed from mPEG@AP5 and mPEG@AP5-Van

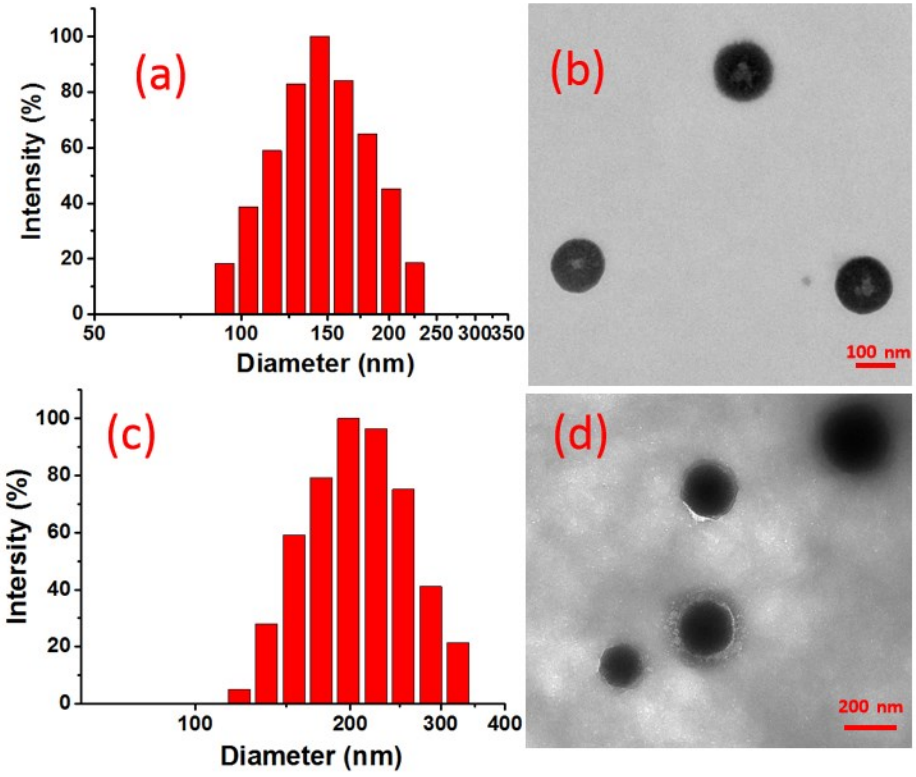


Figure S21. (a). DLS results at a scattering angle of 90° for the vesicles formed by mPEG@AP5 in water; (b) TEM images of aggregates of **mPEG@AP5** in water; (c) DLS results at a scattering angle of 90° for the vesicles formed by mPEG@AP5-Van in water; (b) TEM images of aggregates of mPEG @AP5-Van in water

3.4 The lengths of mPEG@AP5 and Man@AP5 monomers

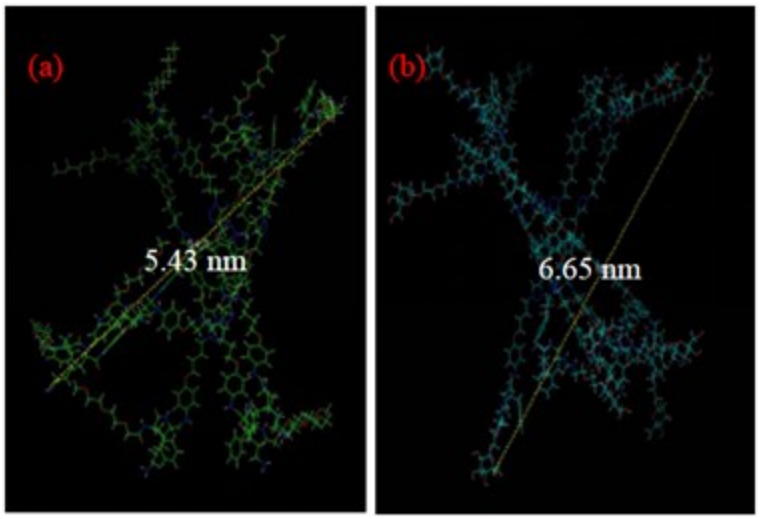


Figure S22. The lengths of mPEG@AP5 and Man@AP5. (a) mPEG@AP5; (b) mPEG@AP5

3.5 The standard curve of Van and PI

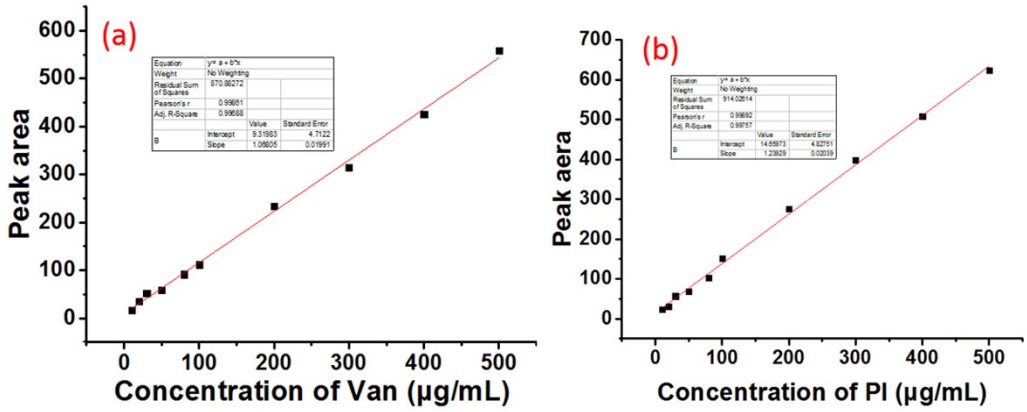


Figure S23. (a) the standard curve of Van; (b) the standard curve of PI

3.6 The drug loading content (DLC) and drug loading efficiency (DLE) of the Man@AP5 or mPEG@AP5

Table S1. The drug loading content (DLC) and drug loading efficiency (DLE) of the Man@AP5 or mPEG@AP5

vesicle	Feed weight ratio[a]	DLE (%)	DLC (%)
Man@AP5	3: 10	55.1	14.2
Man@AP5	5: 10	47.9	19.3
Man@AP5	8: 10	41.6	25.0
Man@AP5	10: 10	32.7	24.6
Man@AP5	12: 10	28.3	22.1
Man@AP5	15: 10	24.5	20.0
mPEG@AP5	8: 10	34.1	25.4

[a] ratio of weight was the Vancomycin to vesicle

3.7 The release of Van from Man@AP5-Van was incubated with 1.5 M NaCl solution and 5.0 M urea solution.

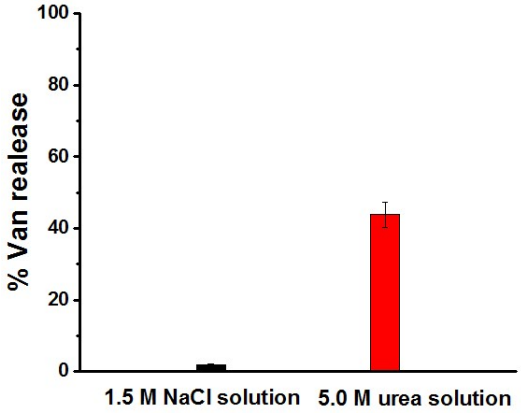


Figure S24. The vancomycin release of Man@AP5-Van nanoparticles in 1.5 M NaCl solution and 5.0 M urea solution.

3.8 In vitro release of Van from Man@AP5-Van or mPEG@AP5-Van was incubated with different pH and cathespin B.

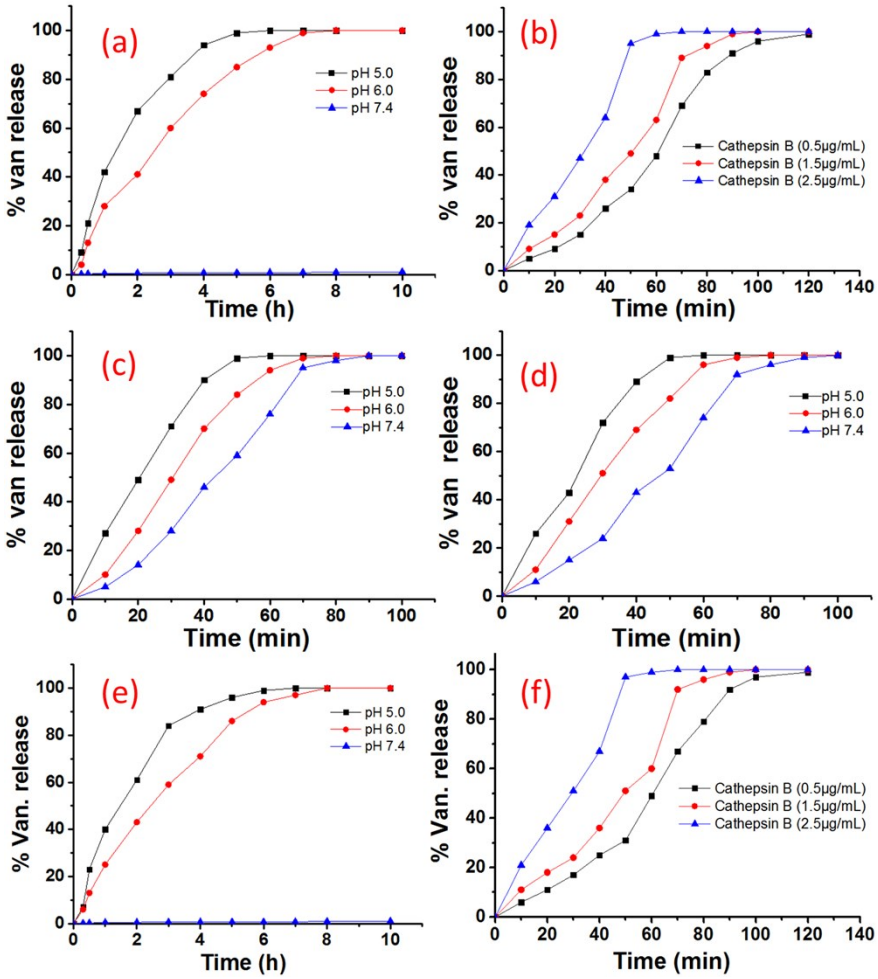


Figure S25. *In vitro* release of Van at 37 °C (a) Man@AP5-Van at different pH; (b) Man@AP5-Van with cathepsin B at pH 7.4; (c) Man@AP5-Van with 1.5 μg•mL^{-1} of cathepsin B at different pH; (d) mPEG@AP5-Van with 1.5 μg•mL^{-1} of cathepsin B at different pH; (e) mPEG@AP5-Van at different pH; (f) mPEG@AP5-Van with cathepsin B at pH 7.4.

3.9 Stability of vesicles and Van-loaded vesicles

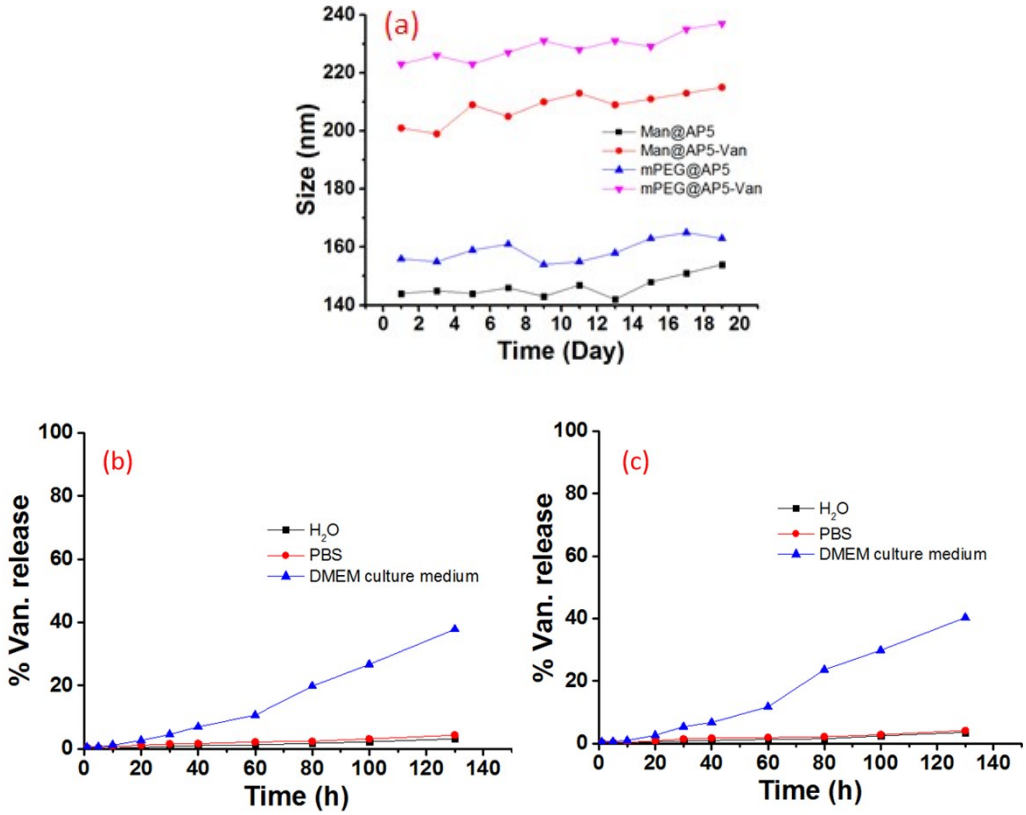


Figure S26. (a) Time-dependent size changes of Man@AP5 or mPEG@AP5 vesicles and Man@AP5-Van or mPEG@AP5-Van nanoparticles in water. Time-dependent Van release of vesicles under different conditions (b) Man@AP5-Van; (c). mPEG@AP5-Van

3.10 Extracellular antibacterial activities

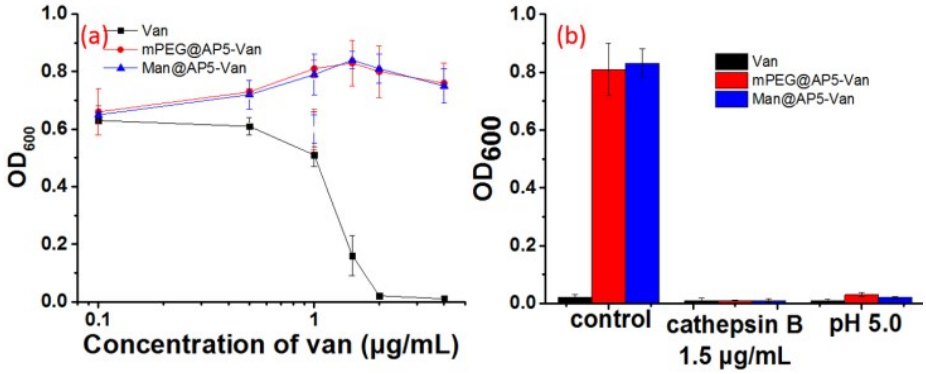


Figure S27. Extracellular antibacterial activities. (a) Van, mPEG@AP5-Van, Man@AP5-Van at 5 μg·mL^{-1} for 24 h; (b) Van, mPEG@AP5-Van, Man@AP5-Van at 5 μg·mL^{-1} in the presence of 1.5 μg·mL^{-1} cathepsin B or pH 5. (equal. van, bacterial: MRSA WHO-2). Data represent the mean ±SD of three independent experiments in triplicate.

3.11 CLSM images of Raw264.7 cells cultured with PI, mPEG@AP5-PI or Man@AP5-PI

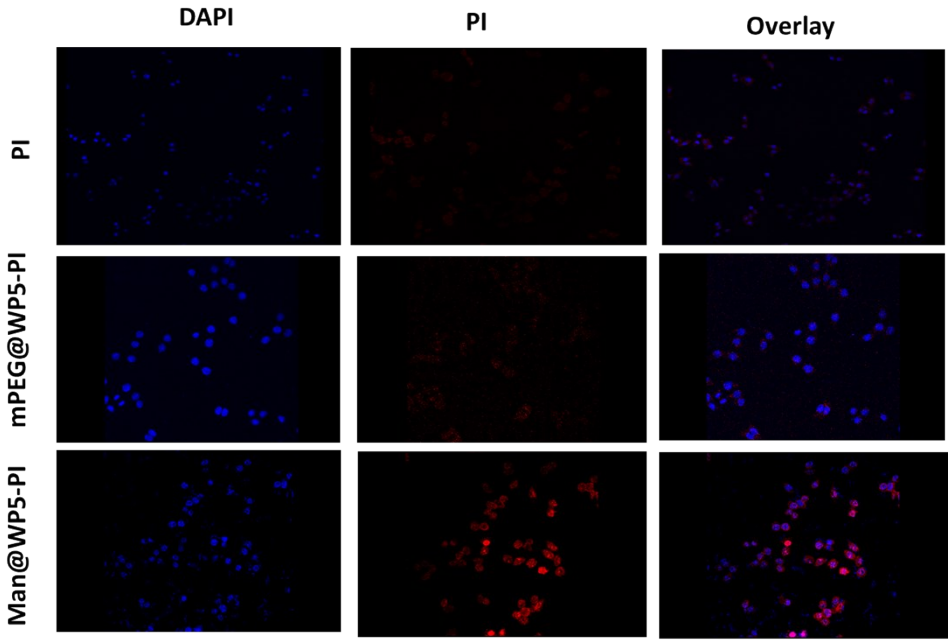


Figure S28. CLSM images of Raw264.7 cells cultured with PI, mPEG@AP5-PI or Man@AP5-PI (1 mg mL

1, equiv. PI) for 2 h. The nucleus was stained with DAPI. PI: propidium iodide. Scale bars: 20 mm.

3.12 Cellular fluorescence intensity of Raw264.7 cells incubated with PI, mPEG@AP5-PI, or Man@AP5-PI for different time.

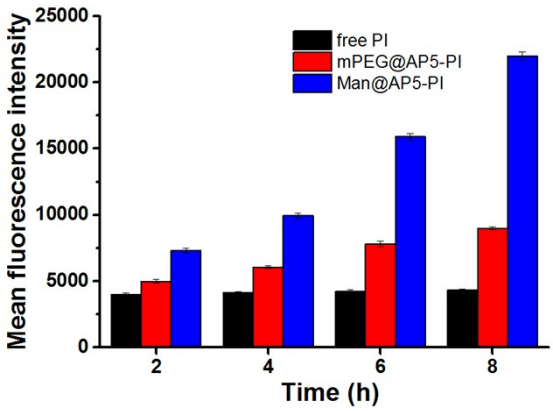


Figure S29. Cellular fluorescence intensity of Raw264.7 cells incubated with PI, mPEG@AP5-PI, or Man@AP5-PI (1 μg•mL^{-1}, equiv. PI) for different time

3.13 Competition experiments

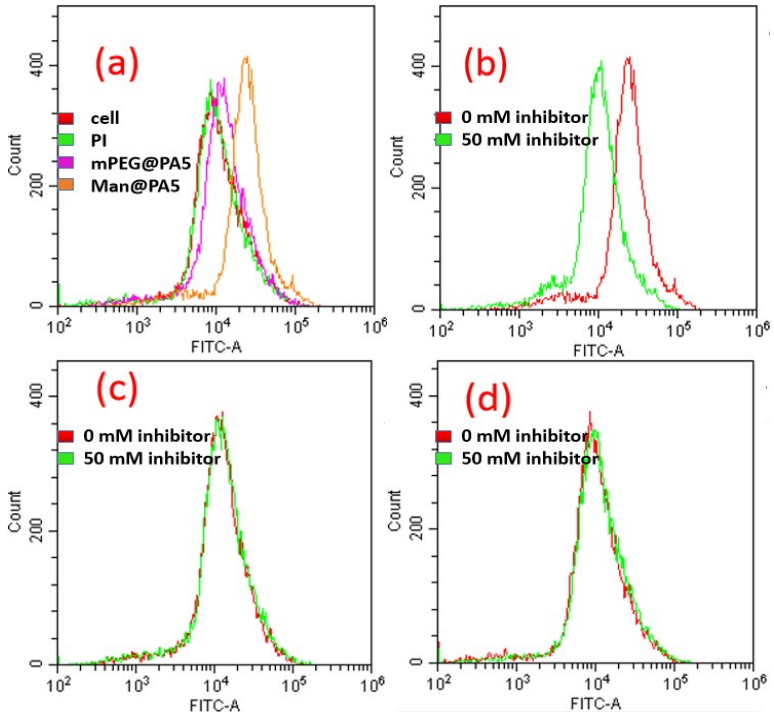


Figure S30. Cellular fluorescence intensity of Raw264.7 cells incubated with PI, mPEG@AP5-PI, or Man@AP5-PI (1 μg·mL^{-1}, equiv. PI) in the absence (a) or presence of mannosamine (50 mM) (b, c and d, with Man@AP5, mPEG@AP5-PI and PI, respectively).

3.14 Intracellular concentration of Van

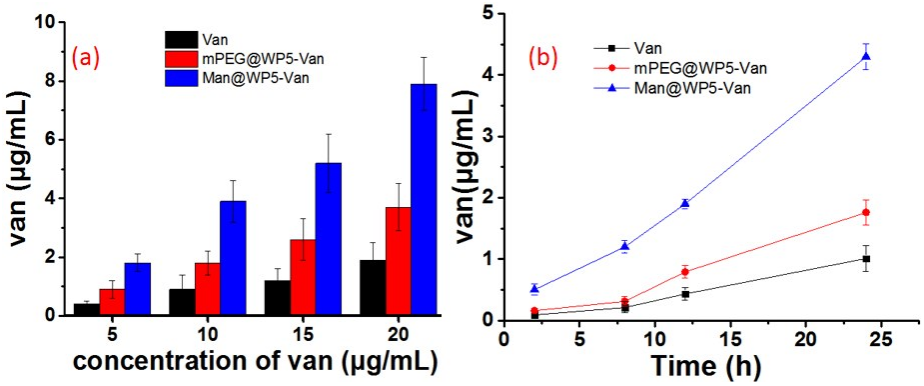


Figure S31. (a) Intracellular concentration of van. RAW264.7 cells were cultured with free Van, mPEG@AP5-Van and Man@AP5-Van (5, 10, 15, 20 μg·mL^{-1}, equiv. van) for 24 h; (b) Intracellular concentration of Van. RAW264.7 cells were cultured with free Van, mPEG@AP5-Van and Man@AP5-Van (10 μg·mL^{-1}, equiv. Van) for different times.

3.15 In vitro cytocompatibility of vesicles

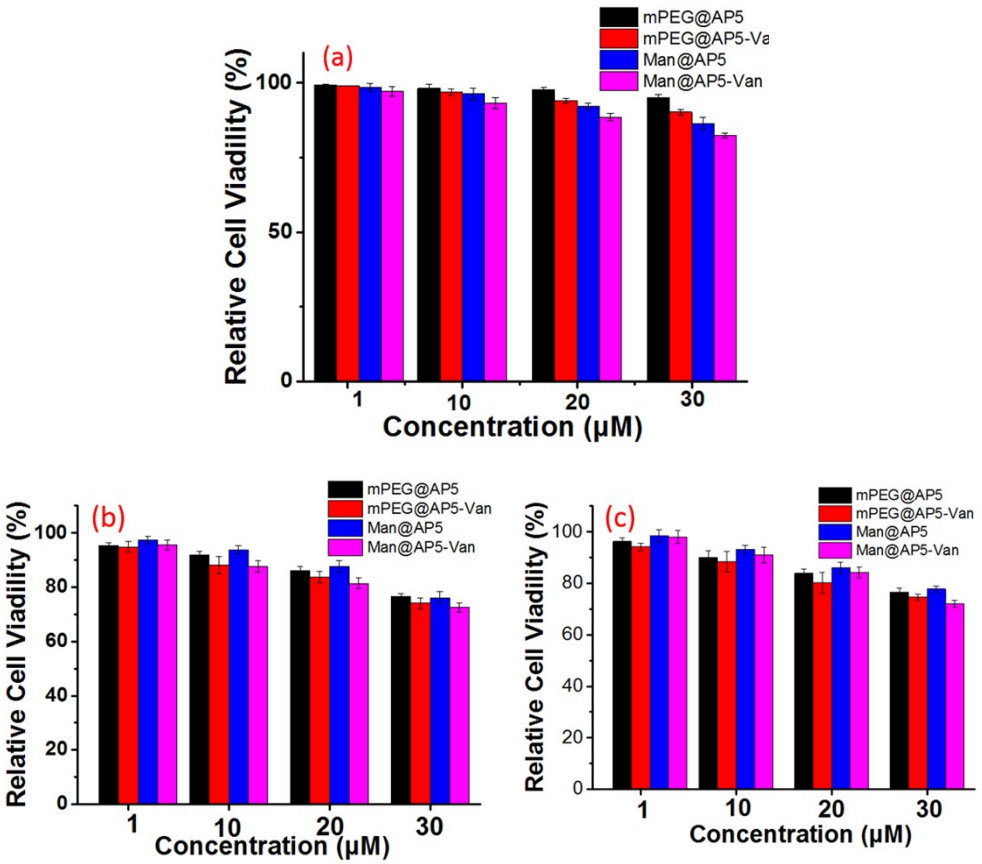


Figure S32. *In vitro* cytocompatibility of Man@AP5, Man@AP5-Van, mPEG@AP5 and mPEG@AP5-Van vesicles against cells after incubation for 48 h. (a) 293T; (b) HUVEC and (c) RAW264.7 respectively.

4 Reference

1. Y. Pei, M. F. Mohamed, M. N. Seleem and Yoon Yeo, *J. Control. Release*, 2017, **267**, 133-143.
2. M. Hagihara, C. Sutherland and D. P. Nicolau, *J. Chromatogr. SCI.*, 2013, **51**, 201–207.
3. S. Lehar, T. Pillow, M. Xu, L. Staben, K. Kajihara, R. Vandlen, L. DePalatis, H. Raab, W. Hazenbos, J. H. Morisaki, J. Kim, S. Park, M. Darwish, B-C. Lee, H. Hernandez, K. Loyet, P. Lupardus, R. Fong, D. Yan, C. Chalouni, E. Luis,Y. Khalfin, E. Plise, J. Cheong, J. Lyssikatos, M. Strandh, K. Koefoed, P. Andersen, J. Flygare, M. W. Tan, E. Brown and S. Mariathasan, *Nature*, 2015, **527**, 323-328.