Supporting Information

Glutathione-Responsive Multifunctional Nanoparticles Based on Mannose-Modified Pillar[5]arene for Targeted Antibiotic Delivery against Intracellular Methicillin-Resistant S. aureus

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Experimental Section

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 $\delta$ 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.

1. Syntheses of the WP5 and G

Scheme S1: Synthetic route for WP5.

Synthesis of compound 3: A mixture of $^1$H$_2$ (99 mg, 0.1 mmol), 2$^2$ (0.835 g, 2 mmol), CuSO$_4$ (32 mg, 0.2 mmol) and Sodium ascorbate (39.6 mg, 0.2 mmol) in DMF (10 mL) was stirred under N$_2$ at room temperature for 24 h. The mixture was concentrated in vacuum and the residue was soluble in water and DCM. The organic layer was separated and dried over Na$_2$SO$_4$. The filtrate was concentrated to yield the crude product, which was purified by silica column chromatography (DCM/EA, 5:1) to yield compound 3 (0.13 g, 93%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.90 (s, 10H), 6.89 (s, 10H), 5.18 (s, 30H), 4.91-4.82 (m, 30H), 4.61 (s, 20H), 4.21 - 4.20 (m, 10H), 4.11 (s, 10H), 4.03- 4.01 (m, 10H), 3.92 (s, 10H), 3.71 (s, 20H), 2.10 (s, 30H), 2.04 (s, 30H), 1.96 (s, 30H), 1.92 (s, 30H); $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 170.6, 169.9, 169.9, 169.7, 149.6, 144.6, 129.1, 128.7, 123.9, 115.7, 97.5, 69.1, 68.8, 66.4, 66.3, 65.6, 65.6, 62.1, 49.6, 20.8, 20.7, 20.6, 20.6.
Synthesis of compound WP5: A mixture of 3 (0.13 g, 0.09 mmol) in MeONa (5 mL) was stirred under N₂ at room temperature. Then a solution of MeONa in MeOH was added to mixture until the pH value reached to 11. The mixture was stirred at room temperature for 12 h and concentrated in vacuum. After removal of the solvent under reduced pressure, 10 mL of H₂O was added.
And the solution was neutralized by Amberlite IR 120 (H+ resin form), the resulted solution was filtered, and residues were washed by H₂O several times. The obtained filtrate was concentrated to afford the target host molecule WP5 (0.1 g, ~100%). ¹H NMR (400 MHz, DMSO-d₆): δ 8.24 (s, 10H), 6.98 (s, 10H), 5.06 (s, 10H), 4.74 (s, 30H), 4.63 (s, 10H), 4.56 (s, 20H), 4.49-4.48 (s, 40H), 3.93 (s, 10H), 3.78 (s, 10H), 3.64 (s, 20H), 3.57 (s, 10H), 3.40-3.37 (m, 80H), 3.22 (s, 10H); ¹³C NMR (101 MHz, DMSO-d₆): δ 149.2, 143.7, 129.2, 128.6, 124.8, 114.9, 110.2, 74.5, 71.3, 70.5, 67.2, 65.3, 61.6, 49.7, 36.2, 31.2.

Figure S3. ¹H NMR spectrum (400 MHz, DMSO-d₆, 298 K) of compound WP5.

Figure S4. ¹³C NMR spectrum (400 MHz, DMSO-d₆) of compound WP5.
Synthesis of compound 8: Compounds 4 (2.6 g, 8.3 mmol) and pyridine (2.0 g, 24.9 mmol) in DCM (100 mL) was stirred at 0 °C. The 5 was added to the solution and was stirred under N₂ for overnight. After completed, the mixture was concentrated in vacuum and washed with Et₂O to yield compound 6 without further purification. ¹H NMR (400 MHz, CDCl₃): δ 8.78 (s, 1H), 8.13 (dd, J = 8.0, 12 Hz, 1H), 7.92-7.81 (m, 1H), 7.72 (t, J = 8.0 Hz, 0.5H), 7.55 (t, J = 8.0 Hz, 0.5H), 7.45-7.38 (m, 2H), 7.30-7.15 (m, 6H), 6.81 (d, J = 8.0 Hz, 2H). The compound 7 (1.7g, 9.3 mmol) and DIPEA (9.3 mmol) in DMF (10 mL) was stirred at rt. Then added 6 to the mixture for reacting 8 h. After complected, the mixture was concentrated to yield the crude product, which was purified by silica column chromatography (DCM/EA, 5:1) to yield compound 8 (3.9 g, 73.4%). ¹H NMR (400 MHz, DMSO-d₆): δ 8.66 (d, J = 8.0 Hz, 1H), 8.44 (m, 1H), 8.10 (m, 1H), 7.87 (t, J = 8.0 Hz, 1H), 7.84-7.77 (m, 2H), 7.72 (t, J = 8.0 Hz, 3H), 7.66-7.64 (m, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.42 (m, 1H), 6.95 (s, 1H), 3.36 (t, J = 8.0 Hz, 2H), 2.97 (t, J = 8.0 Hz, 2H); ¹³C NMR (101 MHz, DMSO-d₆): δ 159.4, 158.4, 154.4, 153.4, 152.9, 152.4, 150.1, 138.3, 138.1, 135.9, 132.3, 129.7, 126.6, 125.0, 122.7, 121.7, 119.7, 119.5, 117.6, 116.2, 107.2, 60.8, 37.8. HRMS (ESI-TOF) m/z calcd for C₂₈H₂₁N₄O₃S₂ (M+H)+ 525.1050, found 525.1052.

Figure S5. ¹H NMR spectrum (400 MHz, DMSO-d₆, 298 K) of compound 8.
Synthesis of compound G: A mixture of 8 (0.39 g, 0.74 mmol), 9 (0.29 g, 0.74 mmol) in MeOH (10 mL) were stirred under N\textsubscript{2} at room temperature for 8 h. The mixture was concentrated in vacuum and the residue was washed with MeOH and Et\textsubscript{2}O respectively to yield compound G (0.46 g, 59%). \textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}): \(\delta\) 8.70 (d, \(J\) = 8.0 Hz, 1H), 8.10-8.08 (m, 1H), 8.04 (d, \(J\) = 8.0 Hz, 1H), 7.90 (t, \(J\) = 8.0 Hz, 1H), 7.77-7.71 (m, 4H), 7.59 (t, \(J\) = 8.0 Hz, 1H), 7.46 (d, \(J\) = 16.0 Hz, 1H), 7.19 (d, \(J\) = 8.0 Hz, 1H), 7.00-6.99 (m, 1H), 3.37-3.28 (m, 4H), 3.23 (t, \(J\) = 8.0 Hz, 2H), 3.01 (s, 9H), 2.84 (t, \(J\) = 8.0 Hz, 2H), 2.77 (t, \(J\) = 8.0 Hz, 2H), 2.02 (t, \(J\) = 8.0 Hz, 2H), 1.61 (s, 2H), 1.44 (s, 2H), 1.21 (s, 12H); \textsuperscript{13}C NMR (101 MHz, DMSO-d\textsubscript{6}): \(\delta\) 172.7, 158.5, 154.4, 153.4, 153.0, 152.4, 138.2, 135.9, 132.2, 129.7, 126.6, 125.1, 122.7, 119.8, 119.5, 117.5, 116.2, 107.2, 65.7, 65.4, 60.7, 52.5, 38.2, 37.8, 37.6, 35.8, 29.3, 29.2, 29.1, 28.9, 26.2, 25.7, 22.5. HRMS (ESI-TOF) m/z calcd for C\textsubscript{39}H\textsubscript{51}BrN\textsubscript{5}O\textsubscript{4}S\textsubscript{2} (M+H)\textsuperscript{+} 796.2560, found 796.2561.
Figure S7. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound G.

Figure S8. $^{13}$C NMR spectrum (400 MHz, DMSO-$d_6$) of compound G.
2. Results

2.1 Host-guest complexation of WP5 and G

Figure S9. $^1$H NMR (400 MHz, D$_2$O, 298 K) spectra: (a) WP5 (2.0 mM), (b) WP5 (2.0 mM) and G (2.0 mM).

2.2 Host-guest complexation of WP5 and Gm

Figure S10. $^1$H NMR spectra (400 MHz, D$_2$O, and 298 K) of Gm at a constant concentration of 4.0 mM with different concentrations of WP5 (mM): (a) 0.0, (b) 1.0, (c) 2.0, (d) 3.0, (e) 4.0, (f) 5.0, (g) 6.0, (h) 7.0, (i) 8.0, (j) 10.0, (k) 12.0 and (l) WP5 (4.0 mM).

2.3 Critical aggregation concentration (CAC) determination of WP5 $\supseteq$ G
Figure S11. Plot of the surface tension of water vs. the concentration of WP5⊃G. There are two linear segments in the plot and a sudden decrease of the slope, implying that the CAC of WP5⊃G is approximately 18 μM.

2.4 Emission spectra of G and DCM

Figure S12. Emission spectra of G and DCM in a DMSO/PBS solution (50/50, v/v, pH7.4, 10 mM) at 37 °C, λex= 490 nm.

2.5 The DLS and TEM images of WP5⊃G nanoparticles in PBS (pH 7.4)
Figure S13 (a) DLS of WP5⊃G in PBS (pH 7.4) at scattering angle of 90°; (b) TEM images of WP5⊃G in PBS (pH 7.4); (c) DLS of LZD-WP5⊃G in PBS (pH 7.4) at scattering angle of 90°; (d) TEM images of LZD-WP5⊃G in PBS (pH 7.4);

2.6 CLSM images of RAW 264.7 cells cultured with WP5⊃G nanoparticles
2.7 Flow cytometry analysis of RAW 264.7 cells cultured with WP5⊃G nanoparticles

2.8 Competitive experiments
Figure S16. Cellular fluorescence intensity of Raw264.7 cells incubated with DCM or WP5⊃G (1 μg·mL⁻¹, equiv. G) in the absence (a) or presence of mannosamine (50 mM).

2.9 The standard curve of LZD

Figure S17. The standard curve of LZD

2.10 Stability of WP5⊃G and LZD-WP5⊃G in H₂O
2.11 Release of LZD from LZD-WP5 ⊃G

**Figure S19.** The in vitro release profile of LZD from LZD-WP5 ⊃G was determined in PBS with different concentrations of GSH.

2.12 In vitro cytotoxicity assay

**Figure S20** In vitro cytocompatibility of WP5 ⊃G and LZD-WP5 ⊃G nanoparticles (1-30 μM, equiv. G) against cells after incubation for 48 h. (a) 293T and (b) RAW264.7 respectively. Data represent the mean ±SD of three independent experiments in triplicate.
3 Reference

