Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A facile one-pot sonochemical synthesis of surface-coated mannosyl protein microspheres for detection and killing of bacteria

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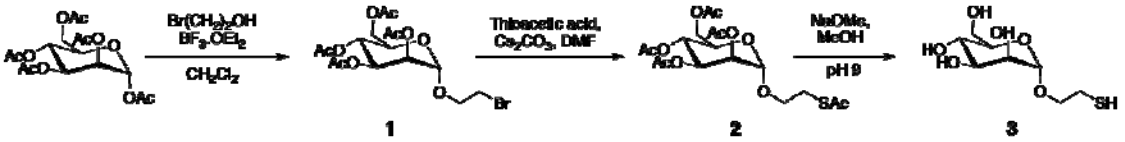
Materials and Methods: All chemicals and reagents were of analytical grade. Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich and used without further purification. Compounds were analyzed by mass spectrometry, using a Micromass QToF ESI mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a 200 MHz Bruker spectrometer.



Synthesis of 2-Bromoethyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (1): Penta-O-acetyl-D-mannose (3.29 g, 8.43 mmol) was first azeotroped with dry toluene and then dissolved in dry CH_2Cl_2 (20 mL) under Ar. To this solution 2-Bromoethanol (4.3 ml, 42 mmol) was added and the mixture was cooled on an ice bath. $BF_3.OEt_2$ (10.6 ml, 84.4 mmol) was then added dropwise for 30 min and the reaction was stirred at room temperature for 72 hours. The solution was then diluted with cold water and extracted twice with CH_2Cl_2. The organic phase was washed with 0.5 N $NaHCO_3$ and water and the organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by chromatography (n-Hex/EtOAc, 2:1) to afford a white solid in 68% yield. ^{1}H NMR (200 MHz; $CDCl_3$) δ (ppm) 1.99 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.16 (s, 3H), 3.82–4.04 (m, 2H), 4.09–4.16 (m, 1H), 4.13 (dd, J5,6=2 Hz, J6,6'=12 Hz, 1H) 4.28 (dd, J5,6'=6 Hz, J6,6'=12 Hz, 1H), 4.87 (br.s, 1H), 5.22–5.40 (m, 3H). ^{13}C NMR (200 MHz, $CDCl_3$) δ (ppm) 20.8, 21.01, 21.07, 21.19, 29.8, 62.5, 66.1, 68.6, 69.1, 69.5, 70.32, 97.9, 170.03, 170.2, 170.32, 170.5. MS: m/z calcd for: $C_{16}H_{23}BrNaO_{10}$ $[M+ Na]^{+}$: 477.05; found: 477.0.

Compound 2: Cesium carbonate (1.29 g, 3.95 mmol) was added to a solution of thioacetic acid (0.3 g, 3.95 mmol) and **1** (1.5 g. 3.3 mmol) in DMF (15 ml) at 0° C. The mixture was stirred overnight at room temperature and then extracted with ethylacetate. The organic phase was then washed with 5% of HCl, water and brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography (n-Hex/EtOAc, 3:2) to afford **2** in 66% yield. ^{1}H NMR (200 MHz; $CDCl_3$) δ (ppm) 1.99 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.16 (s, 3H), 2.36 (s, 3H), 3.12 (t, J=6.4 Hz, 2H), 4.03-4.14 (m, 2H), 4.822-4.83 (m, 1H), 5.165-5.25 (m, 1H), 5.4 (s.br, 1H). ^{13}C NMR (200 MHz, $CDCl_3$) δ (ppm): 20.8, 21.01, 21.07, 21.19, 28.7, 30.7, 62.6, 66.2, 67.05,

68.86, 69.07, 69.55, 97.67, 169.8, 169.9, 170.04, 170.66, 195. MS: m/z calcd for: $C_{18}H_{26}NaO_{11}S$ $[M+ Na]^+$: 473.12; found: 473.0.

Compound 3: To a solution of **2** (0.98 g, 2.177 mmol) in dry MeOH, NaOMe was added under N_2 atmosphere to adjust the pH to 9. The reaction was stirred overnight and then the solvent was dried under reduced pressure to afford **3** as a pure white solid (89% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) : 1.93 (s, 1H), 3.007 (t, 2H), 3.656 – 4.04 (m, 12H), 4.923 (s.br, 1H). ^{13}C NMR (200 MHz, $CDCl_3$) δ (ppm): 37.1, 60.64, 65.06, 66.43, 69.76, 70.31, 72.71, 99.55. MS: m/z calcd for: $C_8H_{16}NaO_6S$ $[M+ Na]^+$: 263.07; found: 263.0.

Preparation and characterization of BSA microspheres and mannosyl conjugated microspheres (mannosyl-CM)

BSA microspheres were generated sonochemically as described previously.[1] To prepare mannosyl coated BSA microspheres, a solution of BSA (5 mg in 30 ml double-distilled water) containing different amounts of the mannosyl derivative **3** (1:10, 1:100, 1:1000; BSA:**3** mole ratio) was overlayered with dodecane or canola oil (20 ml) in a cylindrical vessel. The tip of a high-intensity ultrasonic probe was then placed at the aqueous-organic interface, and the mixture was irradiated at an acoustic power of 150 W/cm^2 (20 kHz) for three minutes while being cooled in an ice-water bath. After synthesis, the microsphere phase was separated from the unreacted BSA and **3** by leaving the reaction mixture at 4°C for 24-36 hours and then washed twice with 25 ml of distilled water via centrifugation at 800 rpm.

To estimate the amount of **3** incorporated onto the shell of the microspheres, samples from the aqueous layer, generated following the microspheres preparation, were injected to HPLC and the amount of **3** was calculated from a standard curve made by injecting different amount of **3** to HPLC.

Microspheres were also loaded with tetracycline (TTCL) by dissolving different amounts of TTCL (10 and 60 mg) directly in dodecane or canola oil (20 ml) and sonocating the solution in the presence of an aqueous solution of BSA or BSA-**3** mixture as described.[2] To determine the amount of encapsulated TTCL, known volumes of mannosyl-CM were destroyed by centrifuging the samples (10,000 g) for 10 min. The amount of the TTCL (mg/ml of microspheres) in each microsphere preparation was then determined spectroscopically.

Table S1. Loading efficacy of TTCL in BSA microspheres and mannosyl-CM

Amount of TTCL used in microspheres preparation (mg/ml)	Amount of TTCL encapsulated in BSA microspheres (mg/ml)	Amount of TTCL encapsulated in mannosyl-CM (mg/ml)
1.2	0.48	0.38
0.20	0.12	0.11

The shape and morphology of the microspheres were characterized by optical-fluorescence microscopy (Apo-Tome AxioImager.z1 microscope, Zeiss, Germany), scanning electron microscopy (SEM, FEI Quanta™ 200 FEG, Hillsboro, Oregon) as well as confocal microscopy (Leica-SPE microscope, Mannheim, Germany). For SEM analyses a sample (10 µl) of the microsphere was spotted onto a glass wafer, followed by drying and gold sputtering. The samples were then analyzed by SEM. The size and the size distribution of the microspheres were measured with an optical microscope **(× 100) by counting 500 microspheres**, and processed by Scion image software.[3]

Binding capability of Con A to the mannosyl-CM

A suspension of mannosyl-CM (80 µl, containing 10 fold increasing amounts of **3**) were incubated under constant agitation (750 rpm) with FITC-Con A (20 µl, 0.1 mg/ml) in Tris buffer (0.1 M, pH 7.4) for 6 hours at 4°C. After the incubation, samples were diluted with cold Tris buffer (100 µl) and centrifuged at 1000 rpm for 5 min. A sample (100 µl) of the solution was then removed and filtered through a 0.22 µm filter to remove the remaining microspheres. The fluorescence of the filtrate was then determined by a plate reader (Infinite M200, Tecan, Switzerland), using the excitation and emission wavelengths of 485 and 520 nm, respectively. The remaining microspheres were studied by fluorescence microscopy or FACS for Con A binding.

Cell growth and conditions:

Two different strains of *E. coli,* a mannose-binding strain, K-12, and a strain that does not bind mannose, 1317, were used. Cells were grown in LB media overnight at 37 °C until they reached an approximate OD_{600} of 1.0. To test the clustering effect of the microspheres on the bacteria, *E. coli* strains were incubated with the microspheres (1:1000; BSA:**3** mol ratio) in PBS for 30 min and studied by an optical microscope (Fig. S1).

In order to determine the targeting effect of the mannosyl-CM or BSA microspheres on the antibacterial activity of encapsulated TTCL, a microdilution antibacterial assay was employed.[4] In brief, overnight-grown inoculum of each isolate was diluted to an approximate concentration of $1\text{-}5\times10^5$ cfu/mL in Mueller-Hinton Broth II (MHB). The bacterial solution (0.9 ml) was then dispensed into the 24 well plates containing increasing amount of the TTCL-encapsulated microspheres (0-60 µl) and total volume was adjusted to 1 ml by MHB. The plates were then incubated at 37ºC for 20 hours under gentle agitation. The turbidity of the samples was then determined by measuring the absorption of the wells at 595 nm, using the Tecan plate reader.

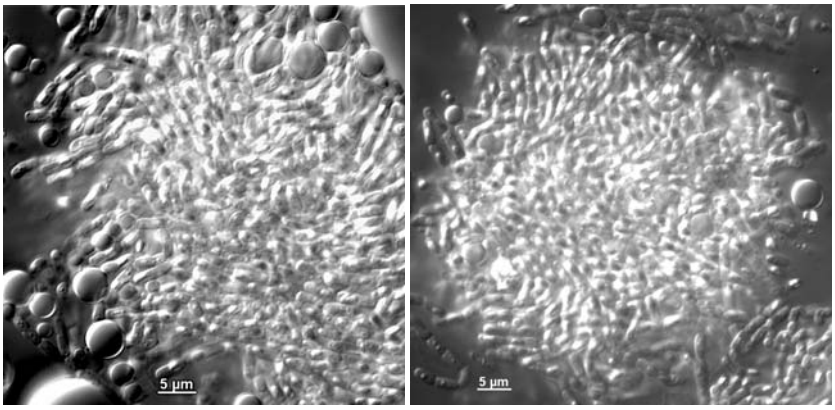


Fig. S1. Agglutination of FimH expressing *E.coli* induced by mannosyl-CM.

Reference:

1 (a) K. S. Suslick and M. W. Grinstaff, *J. Am. Chem. Soc.*, 1990, **112**, 7807-7809; (b) M. W. Grinstaff and K. S. Suslick, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 7708-7710.

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3 V. B. Scion image software program, Frederick, MD and (USA).

4 in *National Committee for Clinical Laboratory Standards.* , Villanova, Pa., 1997.