Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Carbohydrate-Coated Nanocapsules from Amphiphilic Rod-Coil Molecule: Binding to Bacterial Type 1 Pili

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Experimental section

Materials Mercury (II) cyanide (99%), *p*-toluene-sulfonyl chloride (98%) from TCI were used as received. D-(+)-Mannose, poly(ethylene glycol) (M_W = 600), *n*-butyl lithium (1.6 M solution in *n*-hexane), tetrakis(triphenylphosphine)palladium(0) (99%), DOWEX MAC-3 ION exchange resin (all from Aldrich) were used as received. Unless otherwise indicated, all starting materials and the conventional reagents were obtained from commercial suppliers (Aldrich, Lancaster, TCI, etc.). All atmosphere sensitive reactions were done under nitrogen. Flash column chromatography was carried out with Silica Gel 60 (230-400 mesh) from EM Science. 1-Bromo 2,3,4,6-tetra-*O*-acetyl-mannopyranoside, tosylated poly(ethylene glycol) and 4-biphenyl boronic acid were synthesized according to literatures.[1]-[3]

Techniques H- and ^{13}C-NMR spectra were recorded from $CDCl_3$ or DMSO solutions on a Bruker AM 250 spectrometer. The purity of the products was checked by thin layer chromatography (TLC; Merck, silica gel 60). A Perkin Elmer DSC-7 differential scanning calorimeter equipped with 1020 thermal analysis controller was used to determine the thermal translations, which were reported as the maxima and minima of their endothermic or exothermic peaks. In all cases, the heating and cooling rates were 10 ^{o}C min^{-1}. Microanalysis was performed with a Perkin Elmer 240 elemental analyzer at Organic Chemistry Research Center, Sogang University, Korea. X-ray scattering measurements were performed in transmission mode with synchrotron radiation at the 3C2 X-ray beam line at Pohang Accelerator Laboratory, Korea. In order to investigate structural changes on heating, the sample was held in an aluminum sample holder, which was sealed with the window of 7 μm thick Kapton films on both sides. The sample was heated with two cartridge heaters and the temperature of the samples was monitored by thermocouple placed close to the sample. Subtracting the scatterings from the Kapton made background scattering correction. Molecular weight distribution (M_W/M_n) were determined by gel permeation chromatography (GPC) with a Waters instrument equipped with Styragel HR 3, 4 and 4E columns, M7725i manual injector, column heating chamber and 2010 Millennium data station. Dynamic light scattering measurements were performed using a UNIPHASE He-Ne laser operating at 632.8 nm. The maximum operating power of the laser was 30 mW. The detector optics employed optical fibers coupled

to an ALV/SO-SIPD/DUAL detection unit, which employed an EMI PM-28B power supply and ALV/PM-PD preamplifier/discriminator. The Signal analyzer was an ALV-5000/E/WIN multiple tau digital correlator with 288 exponentially spaced channels. The scanning electron microscope (SEM) images were obtained from a FE-SEM (Hitachi S-4300) at an acceleration voltage of 15 kV. MALDI-TOF-MS was performed on a Perseptive Biosystems Voyager-DE STR using a 2,5-dihydroxy benzoic acid matrix. The transmission electron microscope (TEM) was performed at 120 kV using JEOL-JEM 2010. Optical absorption spectra were obtained from a Shimadzu 1601 UV spectrophotometer. The steady-state fluorescence spectra were obtained from a Hitachi F-4500 fluorescence spectrophotometer. Preparative high performance liquid chromatography (HPLC) was performed at room temperature using a 20 mm × 600 mm poly styrene column on a Japan Analytical Industry Model LC-908 recycling preparative HPLC system, equipped with UV detector 310 and RI detector RI-5.

Synthesis. The synthetic procedures used in the preparation of amphiphilic rod-coil molecule **1** is described in Scheme**1**.

Synthesis of compound 1a.

Tosylated poly(ethylene glycol) (2.5 g, 3.3 mmol), 4'-bromo-[1,1'-biphenyl]-4-ol (0.83 g, 3.3 mmol) and excess K_2CO_3 were dissolved in 100 ml of anhydrous acetonitrile. The mixture was heated at reflux for 24 h. The resulting solution was poured into water and extracted with methylene chloride. The methylene chloride solution was washed with water, dried over anhydrous magnesium sulfate, and filtered. The solvent was removed in a rotary evaporator, and the crude product was purified by column chromatography (silica gel) using methylene chloride : methanol (20:1 v/v) as eluent to yield 2.6 g of a waxy solid. yield 95.3% ; ^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ = 7.54-7.39 (m, 6Ar-*H*), 6.99 (d, 2Ar-*H* , *o* to ArBr, *J* = 6.8 Hz), 4.18 (t, 2H, CH_2O-Ar , *o* to ArO, *J* = 5.1 Hz), 3.88 (t, 2H, CH_2O, *J* = 5.1 Hz) 3.59-3.74 (m, 48H, -CH_2O).

Synthesis of compound 1b.

Compound **1a** (2.6 g, 3.1 mmol) and 4-biphenyl boronic acid (0.6 g, 3.1mmol) were dissolved in degassed THF (100 ml). Degassed 2 M aqueous Na_2CO_3 (70 ml) was added to the solution and then tetrakis(triphenylphosphine)palladium(0) (22 mg, 0.018 mmol) was added. The mixture was heated at reflux for 48h with vigorous stirring under nitrogen. Cooled to room temperature, the layers were separated, and the aqueous layer was then washed twice with methylene chloride. The combined organic layer was dried over anhydrous magnesium sulfate and filtered. The solvent was removed in a rotary evaporator, and the crude product was purified by column chromatography (silica gel) using methlylene chloride : methanol (8:1 v/v) as eluent to yield 1.5 g (54.8 %) of a white solid. mp 216 °C ;^{1}H-NMR

(250 MHz, $CDCl_3$, ppm) : δ = 7.72-7.02 (m, 15Ar-*H*), 6.99 (d, 2H, Ar-*H* , *J* = 6.8 Hz), 4.18 (t, 2H, CH_2O-Ar, *J* = 5.0 Hz), 3.88 (t, 2H, -CH_2OH, *J* = 5.0 Hz) 3.51-3.68 (m, 48H, -CH_2O).

Synthesis of compound 1c.

To a solution of 1-bromo 2,3,4,6-tetra-*O*-acetyl-mannopyranoside (1.8 g 4.3 mmol) in acetonitrile solution (100 ml) was added derierite (5 g), compound **1b** (0.8g, 0.82 mmol) and mercury(II) cyanide (0.22 g, 0.82 mmol). The mixture was stirred for 4 h at room temperature and then filtered. The resulting residue was dissolved chloroform (200 ml) and washed with aqueous NaCl solution. The organic layer was dried over anhydrous magnesium sulfate and filtered and concentrated. The crude product was purified by a flash column chromatography [silica gel, methylene chloride : methanol (20:1 v/v)] to yield 0.62 g (60%) of a white solid. mp 224 °C; ^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ = 7.74-7.32 (m, 15Ar-*H*), 6.99 (d, 2Ar-*H* , *o* to ArBr, *J* = 6.8 Hz), δ = 5.22-5.12 (m, 3H, H-2, H-3, H-4), 4.78 (d, 1H, H-1, *J* = 1.5 Hz,), 4.18 (d, 1H, H-6a, *J* = 5.5 Hz), 4.14(t, 2H, CH_2O-Ar, *J* = 5 Hz), 4.03-3.95 (m, 2H, H-6b, H-5), 3.84 (t, 2H, CH_2OH, *J* = 5 Hz), 3.53-3.68 (m, 48H, -CH_2O), 2.05 2.00 1.94 1.91 (s, 12H, O=CH_3).

Synthesis of compound 1.

Compound **1c** (0.62 g 0.5 mmol) in the methanol (5 ml) was treated with sodium methoxide (10 mg) and stirred at room temperature for 0.5 h. Then the solution was mixed with DOWEX MAC-3 ION exchange resin and stirred at room temperature 0.5 h, filtered and concentrated to give a pure white solid product. mp 264°C. yield 0.61 g (98%).; ^{1}H-NMR (250 MHz, DMSO, ppm) : δ = 7.79-7.64 (m, 12Ar-*H*), δ = 7.48 (t, 2Ar-*H, J* = 15 Hz), δ = 7.40 (d, 1Ar-*H, J* =15 Hz), 7.06 (d, 2Ar-*H, o* to ArBr, *J* = 10 Hz), 4.72 (d, 1H, H-1, *J* = 1.5 Hz), 4.68, 4.65, 4.53, 4.42 (d, 4H, H-2, H-3, H-4, H-5), 3.65(d, 2H, H-6), 3.60-3.22 (m, overlapped with H_2O) ^{13}C-NMR (250 MHz, DMSO, ppm) : δ = 158.6, 139.9, 139.5, 138.9, 138.1, 136.8, 129.4, 128.0, 127.3, 127.0, 126.9, 115.3, 100.3, 74.3, 71.2, 70.1, 69.8, 69.3, 67.3, 66.0 Anal. Calcd for : $C_{56}H_{80}O_{19}$: C, 63.62; H, 7.63. Found C, 63.70; H, 7.54. MALDI-TOF-MS *m/z* ($M+Na^+$) 1079.30, Calcd. 1079.59.

Encapsulation of Calcein : Compound **1** (5 mg) was dissolved in a small amount of methylenechloride. The solution was rotary-evaporated in vial to yield thin film. A thin film was dried in vacuo overnight. The dried film was then suspended in 1 ml pure water containing 50 mM calcein. The suspension was sonicated for 5 min. at room temperature using a bath sonicator. Untrapped free calcein was removed by gel filtration from the suspension of molecule **1**, accomplished by passing the suspension through Sephadex G-50 while eluting with pure water. The fraction (early fractions) was collected and used directly in the release study. The solution was pipetted into a quartz cuvette and the fluorescence spectrum was recorded at excitation wavelength of 520 nm.

Release of Entrapped Calcein : An excitation wavelength of 490 nm and an emission wavelength of 520 nm were used. A solution of molecule **1** was aliquoted from a fraction collected after Sephadex gel filtration. Immediately, the initial fluorescence intensity (F_0) of one of aliquoted solutions was measured using the fluorescence spectrometer (value measured = F_0). And then the fluorescence intensity (F_t) was measured as a function of time. After 40 hr when the fluorescence intensity still had not changed, appropriate amount of Triton-X 100 (1 mg Triton-X 100/1 mg water) was added to the solution and then the fluorescence intensity was measured (F_∞). The release percentage of the solution was calculated using the following formula,

$$\text{Release percentage (\%)} = (F_t - F_0) / (F_\infty - F_0) \times 100$$

where F_0 is initial fluorescence intensity, F_t is fluorescence intensity at time t and F_∞ is fluorescence intensity after adding a drop of Triton-X 100.

Binding of compound 1 to *E.coli* type 1 pili.: The *E.coli* K-12 stains ORN 178 and ORN 208 (kindly provided by Prof. P.E. Orndorff) were grown in LB medium at 37 ^oC to an optical density of 0.7 at 600nm (approximately 10^9 cells per ml). Bacteria from 200 μL culture was precipitated by centrifugation at 3000g for 5 min, redissolved in 200 μl binding buffer (water) and followed by adding 10 μl nanoparticle solution. The resulting bacteria and nanoparticle mixtures were incubated at temperatures (37 ^oC) with mild shaking for 30 min. After washing three times with binding buffer, bacteria were redissolved in 10-20 μl binding buffers.

Transmission Electron Microscopy (TEM) measurement: Approximately each sample of 5 μl was placed onto a carbon-coated grid, and the suspension was allowed to settle for 1 to 2 min before excess liquid was removed with a paper wick. A drop of 1% phosphotungastic acid was then used to negatively stain the sample for 40 seconds. The stained cells were examined by JEOL-JEM 2010 TEM.

Field-Emission Scanning Electron Microscopy (FE-SEM) measurement: A drop of aqueous solution of **1** (2×10^{-4} g ml^{-1}) was put on glass, and dried under reduced pressure. The sample was then coated with gold in the ion coater for 30 sec.

Dynamic laser light scattering : The dynamic laser light scattering experiments were performed with the aqueous solution of **1** (2×10^{-4} g ml^{-1}) at scattering angle of 90 ^oC.

References

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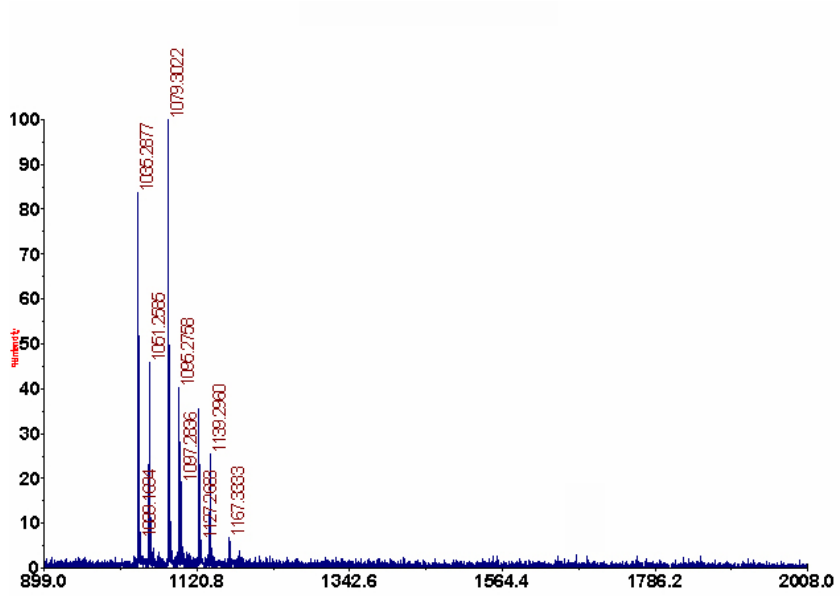
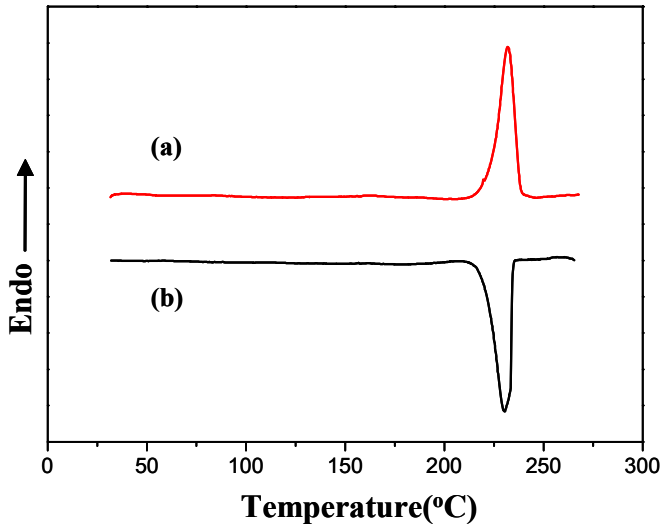


Figure S1. MALDI-TOF-MS of compound **1.**

Figure S2. DSC trace recorded during the heating scan (a) and the cooling scan (b) of **1.**



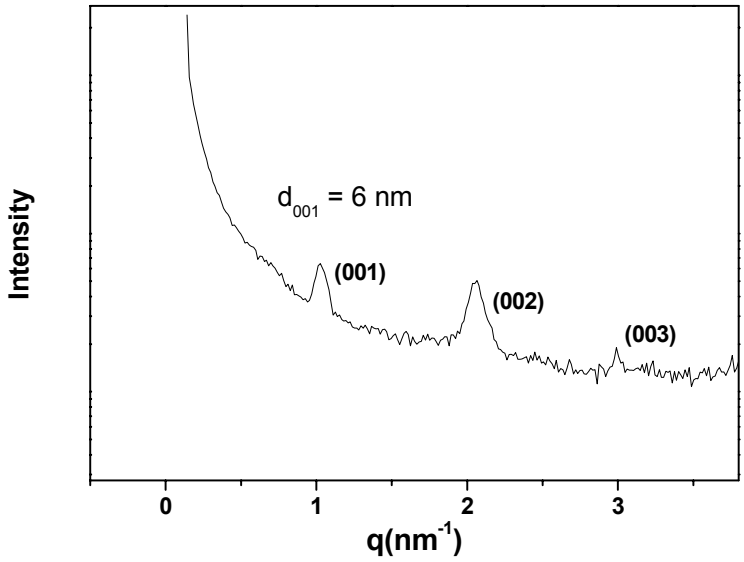


Figure S3. Small-angle X-ray diffraction of molecule **1.**

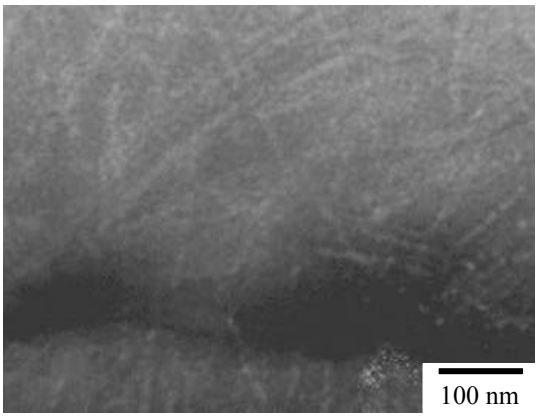


Figure S4. TEM image of the *E. coli* ORN 208 strain deficient of the FimH gene without nanocapsule of molecule **1** binding.