SUPPORTING INFORMATION

Light-Controlled Active Release of Photocaged Ciprofloxacin for Lipopolysaccharide-Targeted Drug Delivery using Dendrimer Conjugates

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I. Materials and Analytical Methods

Materials. Reagents and solvents were purchased primarily from Sigma-Aldrich including ciprofloxacin (≥98.0%), lipopolysaccharides (from *Escherichia coli* 0127-B8), epibromohydrin (98%), glycidol (96%), ethanolamine (≥98%) and fluorescein 5(6)-isothiocyanate (FITC; purity ~90%). Polymyxin B sulfate (>6500 IU/mg) was purchased from AK Scientific, Inc. SPR sensor chip (CM5) and HBS-EP buffer were purchased from GE Healthcare. Generation 5 (G5) poly(amido amine) (PAMAM) dendrimer was purchased as a solution in methanol (17.5% w/w; Dendritech, Inc.), and prior to use, it was partially purified by membrane dialysis (MWCO 10 kDa) to remove lower generation impurities.[1,2] Reaction progress involving small molecules was monitored by thin layer chromatography (TLC) using a glass plate coated with silica gel (150A, Whatman, partisil K5F) in which the product is identified by its value of retention factor (R_f).

NMR and UV–vis Spectroscopy. NMR spectra were acquired at 500 MHz for ^{1}H nuclei and at 100 MHz for ^{13}C nuclei using a Varian NMR spectrometer. Chemical shifts (δ) in ^{1}H NMR spectrum were recorded in standard units (ppm) with residual solvent peaks or an internal reference compound 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS; δ = 0.00 ppm). UV–vis spectra were acquired with a Perkin Elmer Lambda 20 spectrophotometer.

UPLC. Purity of the dendrimer conjugate was analyzed by ultra-performance liquid chromatography (UPLC) performed on an Acquity Peptide Mapping System equipped with a photodiode array detector (Waters).[3] Each sample was prepared at a concentration of 0.1–1.0 mg·mL^{-1} in water or 50% aqueous acetonitrile, and analyzed on a C4 BEH column (150 × 2.1 mm, 300 Å) at a flow rate of 0.2 mL·min^{-1}. A linear gradient method was used starting with 99:1 (v/v) water/acetonitrile with TFA (0.1 v/v%) (eluent A and B respectively). The intial gradient, 1% B (0–1.4 min), was increased to 80% B (1.4–13.4 min), decreased to 50% B (13.4–13.8 min), decreased to 1% B (13.8–14.4 min) and 1% B (14.4–18 min).

Mass spectrometry. Molecular weights (M_r) of small molecule compounds were measured by electrospray ionization mass spectrometry (ESI MS) with a Micromass AutoSpec Ultima spectrometer. Characterization of M_r for G5 PAMAM dendrimer conjugates was performed by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a TOfsPec-2E spectrometer (Waters).

Gel permeation chromatography (GPC). This was performed in an Alliance 2695 separation module (Waters) equipped with a HELEOS Multi Angle Laser Light Scattering detector and an Optilab rEX differential refractometer (Wyatt Technology Corporation). Each GPC sample was prepared at 3–5 mg·mL^{-1} in 0.1 M citric acid (pH 2.7) for GPC analysis. Its data were analyzed with Astra 5.3.14 software (Wyatt Technology Corporation) to extract the values of weight-average molecular weight (M_w), number-average molecular weight (M_n) and polydispersity index (PDI = $M_w \cdot M_n^{-1}$). Unmodified G5 PAMAM dendrimer has a molecular weight of M_w = 26,600 g·mol^{-1} with a PDI value of 1.010.

Dynamic Light Scattering (DLS) and Zeta Potential (ZP). DLS and ZP measurements were performed on a Zetasizer Nano-ZS (Malvern Instruments Ltd.) using a same dendrimer sample prepared in 1 mM HEPES (pH 7) as described previously.[4] Errors reported for the size and ZP measurements refer to standard deviation values.

Surface plasmon resonance (SPR) spectroscopy. SPR experiments were carried out in a Biacore® X instrument (Pharmacia Biosensor AB). A CM5 sensor chip (Pharmacia Biosensor) immobilized with lipopolysaccharides (LPS) was prepared as described previously.[5] This immobilization was achieved by the injection of an amine-modified LPS solution (5 mg·mL^{-1}, pH 9; 35 µL) to flow cell 1 (Fc1) preactivated with EDC/NHS (1:1 mixture of 0.4 M EDC and 0.1 M NHS; 70 µL). A reference cell was prepared by the treatment of flow cell 2 in the same manner as above but without the injection of the LPS solution.

SPR sensorgrams were acquired by the injection (50 µL) of analyte solutions, each prepared in HBS-EP buffer, at a flow rate of 30 µL/min. The chip surface was regenerated by treatment with 10 µL of a 10 mM glycine-HCl (pH 2.5) buffer at the end of each dissociation phase (t ≥ 600 s). Prior to kinetic analysis, each sensorgram in Fc1 (RU$_1$) was corrected for the contribution of non-specific adsorption by the subtraction of the reference sensorgram in Fc2 (RU$_2$): ΔRU (corrected) = RU$_1$ − RU$_2$. Global fitting analysis was performed for each corrected sensorgram to extract dissociation (off) rate constant k_{off} and association (on) rate constant k_{on} using a Langmuir binding model.[6] Dissociation constant (K_D) was calculated by using two kinetic parameters—association (on) rate constant k_{on} and dissociation (off) rate constant k_{off}: $K_D = (k_{off} \div k_{on})$. Each K_D value refers to a mean of four or more independent measurements (n ≥ 4).

II. Synthesis of 3 G5(ONB-Cipro)$_m$ and 4 G5(PMB)$_n$(ONB-Cipro)$_m$

II.1. Synthesis of 2 ONB-Cipro. Compound S2: To a solution of **S1** ONB-OH (50 mg, 0.125 mmol)[3] dissolved in anhydrous chloroform (1 mL) was added DIPEA (47 μL, 0.262 mmol) and then *p*-nitrophenyl chloroformate (26 mg, 0.131 mmol) dissolved in anhydrous THF (1 mL). The mixture was stirred at room temp under nitrogen atmosphere in dark overnight. A second portion of *p*-nitrophenyl chloroformate (26 mg, 0.131 mmol) and dimethylaminopyridine (DMAP) (16 mg, 0.131 mmol) were added and stirred at room temp in dark for 4 h. The reaction progress was monitored by TLC (R_f = 0.44 in ethyl acetate/hexane). When the reaction was complete, the mixture was evaporated and the residue was diluted with ethyl acetate (15 mL) and washed with 1M H_3PO_4 (to remove DIPEA and DMAP; 3 × 10 mL), water (10 mL) and cold $NaHCO_3$ (10 mL), and brine (10 mL). The organic layer was collected, dried over Na_2SO_4 and evaporated to dryness, yielding the product **S2** as pale yellow solid (96 mg). MS (ESI) *m/z* (relative intensity, %) = 465.1 (100), 587.1 [M+Na]$^+$. ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.25–8.24 (dd, J$_1$= 2 Hz, J$_2$ = 7 Hz, 2H, ArH(*p*NP)), 7.81 (s, 1H, ArH (ONB), ortho to NO$_2$), 7.314–7.296(dd, J$_1$=2 Hz, J$_2$ = 7 Hz, 2H, ArH(*pNP*)), 7.17 (s, 1H, ArH (ONB), meta to NO$_2$), 5.72 (s, 2H, ArCH$_2$OC(=O)), 4.90 (br, 1H, NH), 4.60 (s, 2H, ArOCH$_2$C(=O)), 4.04 (s, 3H, OCH$_3$), 3.51–3.48 (m, 2H, CH$_2$N), 3.34–3.32 (m, 2H, CH$_2$N), 1.42 (s, 9H, *N*-Boc) ppm.

Scheme S1
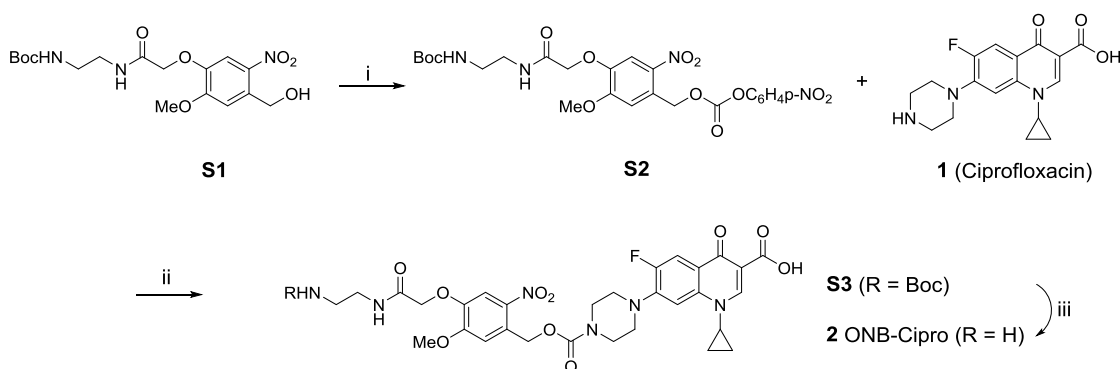
reagents and conditions: i) p-Nitrophenyl chloroformate (1.1 eq), Et$_3$N, DMAP, CH$_2$Cl$_2$, 0°C to rt, 12 h; ii) ciprofloxacin (1.1 eq), Et$_3$N, CH$_2$Cl$_2$, rt, 18 h; iii) CF$_3$CO$_2$H, CH$_2$Cl$_2$, rt, 30 min.

Compound S3 *N*-Boc ONB-Cipro: To **S2** prepared above (70 mg, 0.125 mmol) was added anhydrous dichloromethane (5 mL), and added a solution of dichloromethane (2 mL) containing ciprofloxacin (46 mg, 0.138 mmol) and trimethylamine (174 μL; 1.25 mmol, 10 equiv). The

mixture was stirred for 18 h in the dark at room temp and the reaction progress was monitored by TLC. After full consumption of starting materials, the mixture was diluted with dichloromethane (20 mL), and washed with water (3 × 20 mL). The organic layer was collected, dried over Na_2SO_4 and evaporated to dryness, yielding the crude product as pale yellow solid. It was purified by flash column chromatography by eluting with 10% methanol/ethyl acetate. The product **S3** was obtained as a white solid (51 mg). R_f = 0.4 in 15% methanol/ethyl acetate; R_f = 0.55 in 10% methanol/dichloromethane. MS (ESI) *m/z* (relative intensity, %) = 757.6 (100) [M+H]$^+$, 657.6 (79) [M−Boc+H]$^+$. HRMS (ESI) calcd for $C_{35}H_{41}FN_6O_{12}$ [M+H]$^+$ 757.2839, found 757.2844; [M+Na]$^+$ 779.2659, found 779.2655. ^1H NMR (500 MHz, $CDCl_3$): δ 8.73 (s, 1H, C=CHN (cipro)), 8.00–7.98 (d, J = 10 Hz, 1H, ArH (cipro)), 7.71 (s, 1H, ArH (ONB), ortho to NO_2), 7.37–7.36 (d, J = 5 Hz, 1H, ArH (cipro)), 7.20 (br, 1H, NH), 7.03 (s, 1H, ArH (ONB), meta to NO_2), 5.52 (s, 2H, $ArCH_2OC$(=O)), 4.92 (br, 1H, NH), 4.56 (s, 2H, $ArOCH_2C$(=O)), 4.00 (s, 3H, OCH_3), 3.76–3.74 (m, 4H, $2CH_2N$ (cipro)), 3.55 (s, 1H, CHN (cipro)), 3.50–3.46 (m, 2H, CH_2N (ONB)), 3.32–3.31 (m, 6H, $2CH_2N$ (cipro) and CH_2N (ONB)), 1.41 (s, 9H, *N*-Boc), 1.22–1.20 (m, 4H, $2CH_2C$ (cipro)) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 187.99, 167.80, 166.80, 154.52, 153.80, 147.55, 140.23, 112.44, 111.96, 109.97, 108.14, 105.19, 68.88, 64.49, 56.41, 49.63, 40.24, 35.32, 28.28 ppm.

2 ONB-Cipro: To a solution of the *N*-Boc protected ONB-Cipro **S3** (40 mg) dissolved in dichloromethane (2 mL) was added 2 ml of TFA. The mixture was stirred at room temp for 30 min, and evaporated to dryness, yielding a pale orange residue. It was dissolved in water (2 mL), and lyophilized to afford the product **2 ONB-Cipro** (TFA salt) as a pale beige solid (51 mg). HPLC analysis: t_r = 9.5 min (purity ≥95%). MS (ESI) *m/z* (relative intensity, %) = 657.3 (100) [M+H]$^+$. ^1H NMR (500 MHz, CD_3OD): δ 8.83 (s, 1H, C=CHN (cipro)), 7.99–7.97 (d, J = 10 Hz, 1H, ArH (cipro)), 7.82 (s, 1H, ArH (ONB), ortho to NO_2), 7.63–7.62 (d, J = 5 Hz, 1H, ArH (cipro)), 7.23 (s, 1H, ArH (ONB), meta to NO_2), 5.52 (s, 2H, $ArCH_2OC$(=O)), 4.67 (s, 2H, $ArOCH_2C$(=O)), 4.01 (s, 3H, OCH_3), 3.77–3.73 (m, 5H, CHN (cipro) and $2CH_2N$ (cipro)), 3.59–3.57 (t, 2H, J = 10 Hz, CH_2N (ONB)), 3.39 (m, 4H, $2CH_2N$ (cipro)), 3.12–3.10 (t, J = 10 Hz , 2H, CH_2N (ONB)), 1.42–1.38 (m, 2H, CH_2C (cipro)), 1.23–1.20 (m, 2H, CH_2C (cipro)) ppm. UV–vis (10% aq. methanol; 0.13 mM): λ_{max} = 280 (ε = 1.38 × 10^4 $M^{-1}cm^{-1}$).

II.2. Preactivation of ONB-Cipro. (step i) To a solution of glycidol (2.0 mg, 21.7 μmol) in dichloromethane was added bis-NHS ester (6.0 mg, 21.7 μmol) and *N,N*-diisopropylethylamine (DIPEA; 8 μL, 43.5 μmol). The mixture was stirred at room temp overnight and then concentrated. It was purified by flash column chromatography by eluting with 10% methanol/dichloromethane to give glycidol NHS activated ester as a clear oil (5.6 mg). R_f = 0.52 in 10% methanol/dichloromethane. MS (ESI) *m/z* (relative intensity, %) = 160 (100), 238.1 (39) [M+Na]⁺.

<div align="center">Scheme S2. Preactivation of ONB-Cipro</div>
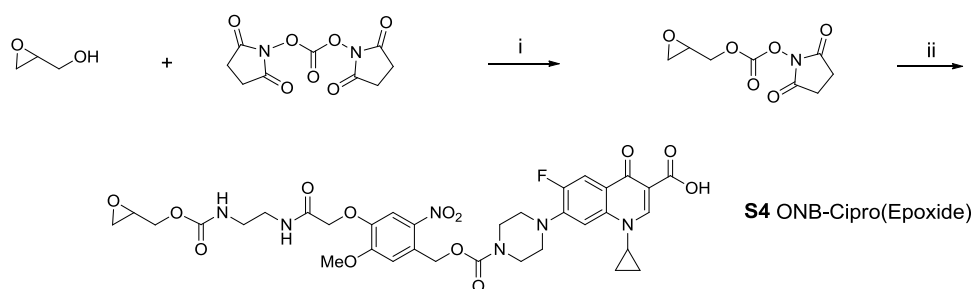
reagents and condition: i) DIPEA, CH₂Cl₂, rt; ii) **2** ONB-Cipro, DIPEA, MeOH, rt

(step ii) **S4** ONB-Cipro(Epoxide). To a separate solution of **2** ONB-cipro (TFA salt; 14 mg, 18.1 μmol) dissolved in methanol (1.0 mL) was added DIPEA (8 μL, 43.5 μmol), and then the solution of the glycidol NHS activated ester prepared above. The colorless mixture was stirred at room temp for 6 h, and showed a single product identified by TLC analysis: R_f (5% methanol/dichloromethane) = 0.85. This solution was dried *in vacuo*, yielding the product **S4** as a solid residue. It was resuspended in methanol (2 mL) and used immediately for conjugation with dendrimer as described below.

II.3. Dendrimer conjugation. **S5** G5(ONB-Cipro)(Epoxide): To a solution of G5(NH₂) (50 mg, 1.81 μmol) dissolved in methanol (10 mL) was added DIPEA (19 μL, 0.11 mmol) and then acetic anhydride (10 μL, 0.11 mmol). The mixture was stirred at room temp for 30 min prior to the addition of the solution of **S4** ONB-Cipro(Expoxide) solution prepared above. The final mixture was shaken in a water bath at 45°C for 16 h and cooled to room temp. To this solution was added epibromohydrin (87.7 μL, 91 μmol), and the mixture was stirred at room temp for 22 h.

Scheme S3. Dendrimer conjugation
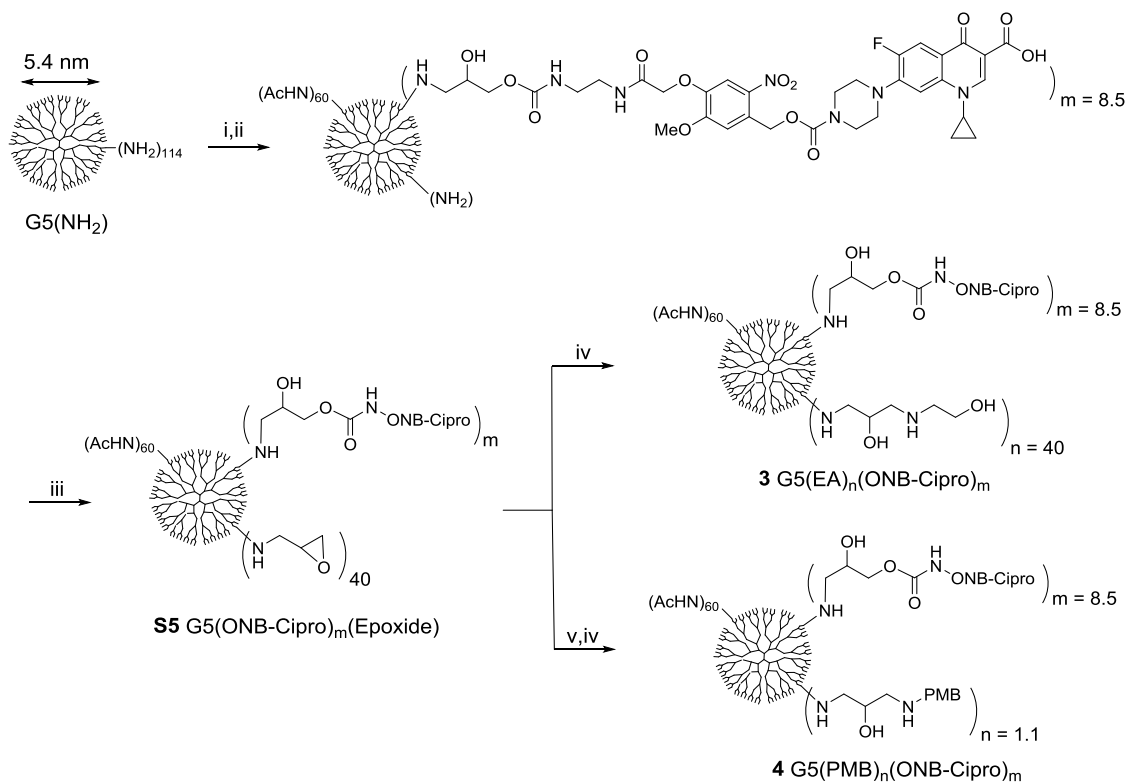
reagents and condition: i) Ac$_2$O (60 equiv), DIPEA, rt, 30 min; ii) **S4** ONB-Cipro(Epoxide), 45°C; iii) epibromohydrin (40 equiv), rt, 24 h; iv) ethanolamine (200 equiv), 45°C, 24 h; v) polymyxin B (PMB) (10 equiv), 45°C, 24 h

3 G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$ and 4 G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$: After this reaction, the mixture was divided into two vials, each containing ~4.8 mL and 7.2 mL. To the smaller solution (4.8 mL) was added ethanolamine (22 µL, 0.362 mmol), and the mixture was shaken at 45°C overnight, yielding a crude product **3** G5(EA)$_{40}$(ONB-Cipro)$_m$. To the larger solution (7.2 mL) was added polymyxin B (15 mg, 10.8 µmol) predissolved in 0.1 M NaOH (0.5 mL). This solution was shaken at 45°C for 12 h prior to the addition of ethanolamine (22 µL, 0.362 mmol). The final mixture was shaken at 45°C overnight, yielding a crude product **4** G5(PMB)$_n$(ONB-Cipro)$_m$.

Dendrimer purification and characterization. Each solution of the crude conjugate product was concentrated *in vacuo*, and the residue was dissolved in 10 mL of phosphate buffered saline (PBS, pH 7.4). This solution was loaded in membrane dialysis tubing (MWCO 10 kDa), and

dialyzed against PBS (2 × 2 L) and deionized water (3 × 2 L) over two days. Lyophilization of the dialyzed solution afforded **3** (20 mg) and **4** (27 mg), each as pale beige solid.

UPLC analysis of conjugate **3** showed a broad peak with t_r of 8.0–10.0 min (polymer purity >95%). GPC: M_w = 27,400 gmol^{-1}, PDI = 1.081. MALDI-TOF mass spectrometry (m/z; gmol^{-1}): 31,300. UV–vis (PBS, pH 7.4): λ_{max} = 275 nm (ε = 7,463 M^{-1}cm^{-1}), 324 nm (ε =3,386 M^{-1}cm^{-1}), 337 nm (ε = 3,315 M^{-1}cm^{-1}). ^{1}H NMR (500 MHz, DMSO-d_6): 8.68 (weak s), 7.97–7.92 (strong br m), 7.69 (weak s), 7.61 (weak m), 7.23 (weak s), 5.42 (weak s), 4.63 (weak s), 4.16 (weak m), 3.95 (weak s), 3.43–3.16 (strong m), 3.07 (strong s), 2.86 (s), 2.65 (strong s), 2.58 (s), 2.43 (s), 2.20 (strong s), 1.92 (weak s), 1.86 (weak s), 1.80 (strong s), 1.69–1.67 (weak m), 1.38–1.18 (weak m) ppm. (abbreviation: br = broad; s = singlet; m = multiplet, t = triplet)

UPLC analysis of conjugate **4** showed a broad peak with t_r of 7.7–9.2 min (polymer purity >95%). GPC: M_w = 33,500 gmol^{-1}, PDI = 1.131. MALDI-TOF mass spectrometry (m/z; gmol^{-1}): 31,500. UV–vis (PBS, pH 7.4): λ_{max} = 275 nm (ε = 7,689 M^{-1}cm^{-1}). 323 nm (ε = 3,966 M^{-1}cm^{-1}), 335 nm (ε = 3,857 M^{-1}cm^{-1}). ^{1}H NMR (500 MHz, DMSO-d_6): 8.67 (weak s), 7.97–7.86 (strong br m), 7.69 (weak s), 7.60 (weak m), 7.23 (weak s), 5.43 (weak s), 4.63 (weak s), 4.15 (weak m), 3.95 (weak s), 3.53–3.23 (m), 3.17 (br), 3.07 (strong s), 2.85 (s), 2.65 (strong s), 2.58 (s), 2.43 (s), 2.30 (br) 2.19 (strong s), 1.92 (weak s), 1.86 (weak s), 1.80 (strong s), 1.67 (weak s), 1.33–1.13 (weak m) ppm.

Drug valency (m = 8.5 ± 0.6) for ONB-Cipro in **3** G5(EA)$_{40}$(ONB-Cipro)$_m$ and **4** G5(PMB)$_n$(ONB-Cipro)$_m$ was determined on a mean basis by their UV–vis absorption values to an ONB-Cipro calibration curve ([**2** ONB-Cipro] = slope × $A_{271\ nm}$; slope = 1.34 × 10^{-4}). PMB valency (n = 1.1) of **4** G5(PMB)$_n$(ONB-Cipro)$_m$ was estimated using a NMR integration method as reported previously[5] by comparison of the (D)-Phe (PMB) signal (δ 7.3–7.1 ppm) to a reference peak of NHAc (δ 1.8 ppm; 60 Ac residues per dendrimer), yielding n = 1.1 (±0.8).

III. Light-controlled Release Kinetics

Kinetics of drug release controlled by UV light was performed with a Spectroline® UV lamp device (XX-15A; maximal emission intensity at 365 nm).[3,7,8] Typically, a solution of **2** ONB-Cipro (0.13 mM in methanol) or **3**–**4** G5(PMB)(ONB-Dox) (0.032 mM in water) was exposed to

a light source at the distance of ~5 cm. A series of aliquots were taken as a function of exposure time, and analyzed by UPLC and UV–vis spectrometry to determine the rate of ciprofloxacin release from photocaged ciprofloxacin.

IV. Confocal Microscopy

E. Coli (XL-1) was grown in LB broth overnight in a 37°C shaker. Cells were washed with fresh LB, and 5×10^7 CFU were incubated with 5 μM of the FITC-labeled control dendrimer, "FITC-(Ac)G5" or drug conjugate, "FITC-G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$" in LB broth at room temp for 40 min on an orbital shaker. Cells were spun at 9000 rpm in a microfuge for 10 min and washed twice with PBS. Cells were fixed in 4% paraformaldehyde in PBS for 10 min, washed with PBS, and stained with 1 μM of Syto59 in PBS for 10 min at room temp. Cells were washed twice with PBS, and then resuspended in Prolong Gold (Life Technologies) and mounted onto a coverglass slide for imaging. Confocal fluorescence microscopy was performed on a Leica Inverted SP5X confocal microscope (Leica Biosystems). FITC fluorescence was imaged at $\lambda_{ex} = 488$ nm; $\lambda_{em} = 500\text{-}600$ nm, and Syto59 fluorescence at $\lambda_{ex} = 610$ nm; $\lambda_{em} = 653\text{-}695$ nm.

V. Turbidity Assay

E. Coli (XL-1) cells were grown in LB broth in a 5 mL culture overnight in a 37°C shaker. CFUs were counted by plating. *E. Coli* at a concentration of 5×10^6 CFU/mL was treated with increasing concentrations of each dendrimer conjugate or unconjugated Cipro in LB media in a 96 well plate. Final concentrations for the conjugates are expressed as dendrimer concentration, and as Cipro concentration for unconjugated drug. The initial turbidity (Abs$_{650nm}$) was measured immediately upon addition of conjugate using a 96-well plate spectrophotometer. Bacteria were incubated for 24 h at 37°C, and the turbidity was measured again. The initial turbidity was subtracted from the 24 h reading to account for inherent turbidity arising from the conjugates, and the relative viability was determined in realtion to untreated bacteria at 24 h.
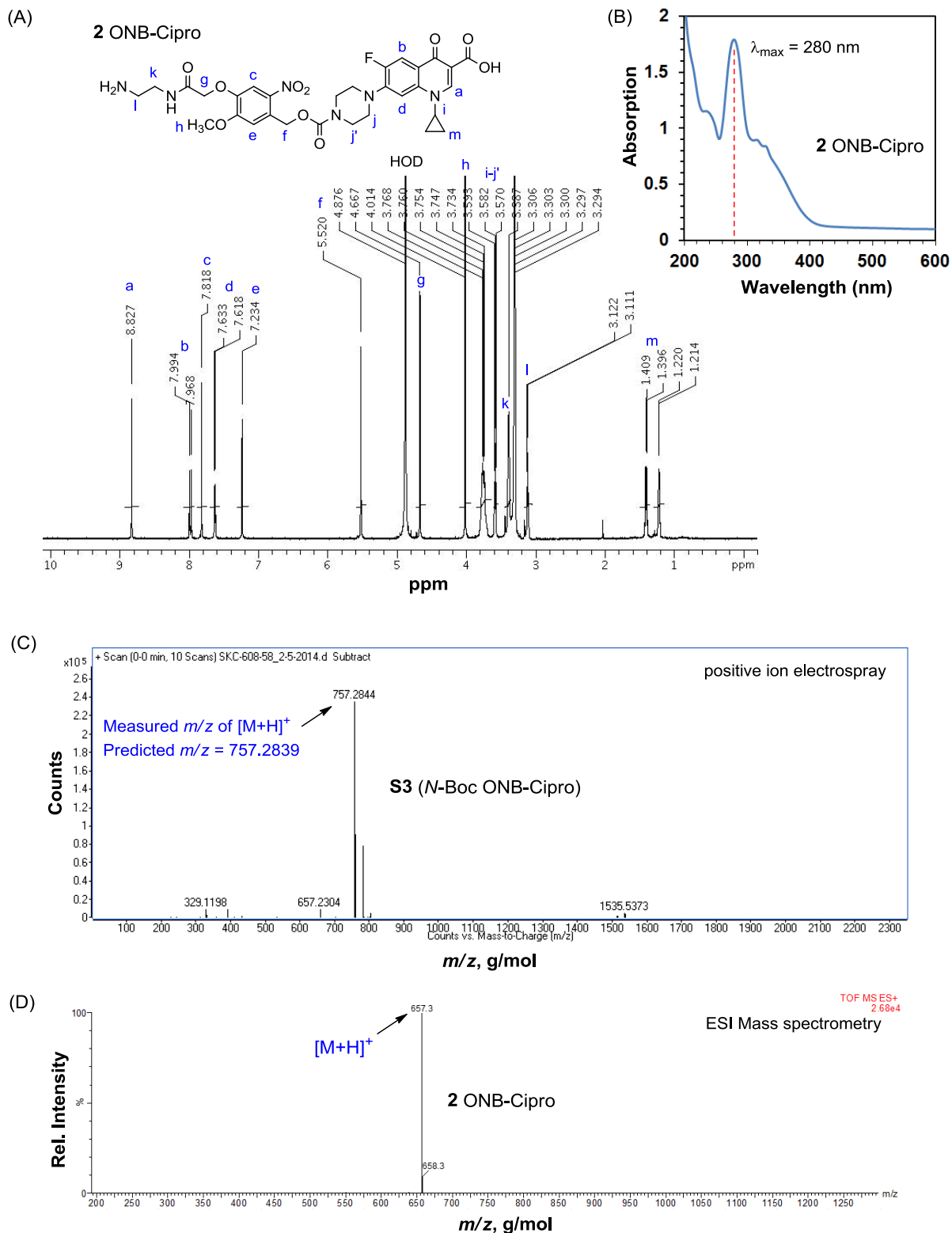
Figure S1. ^{1}H NMR (500 MHz, CD$_3$OD) spectrum (A) and UV–vis (B) of **2** (ONB-Cipro) measured in 10% aqueous methanol (0.1 mg/mL); high resolution mass (HRMS) spectrum of **S3** (C) and ESI mass spectrum of **2** ONB-Cipro (D).
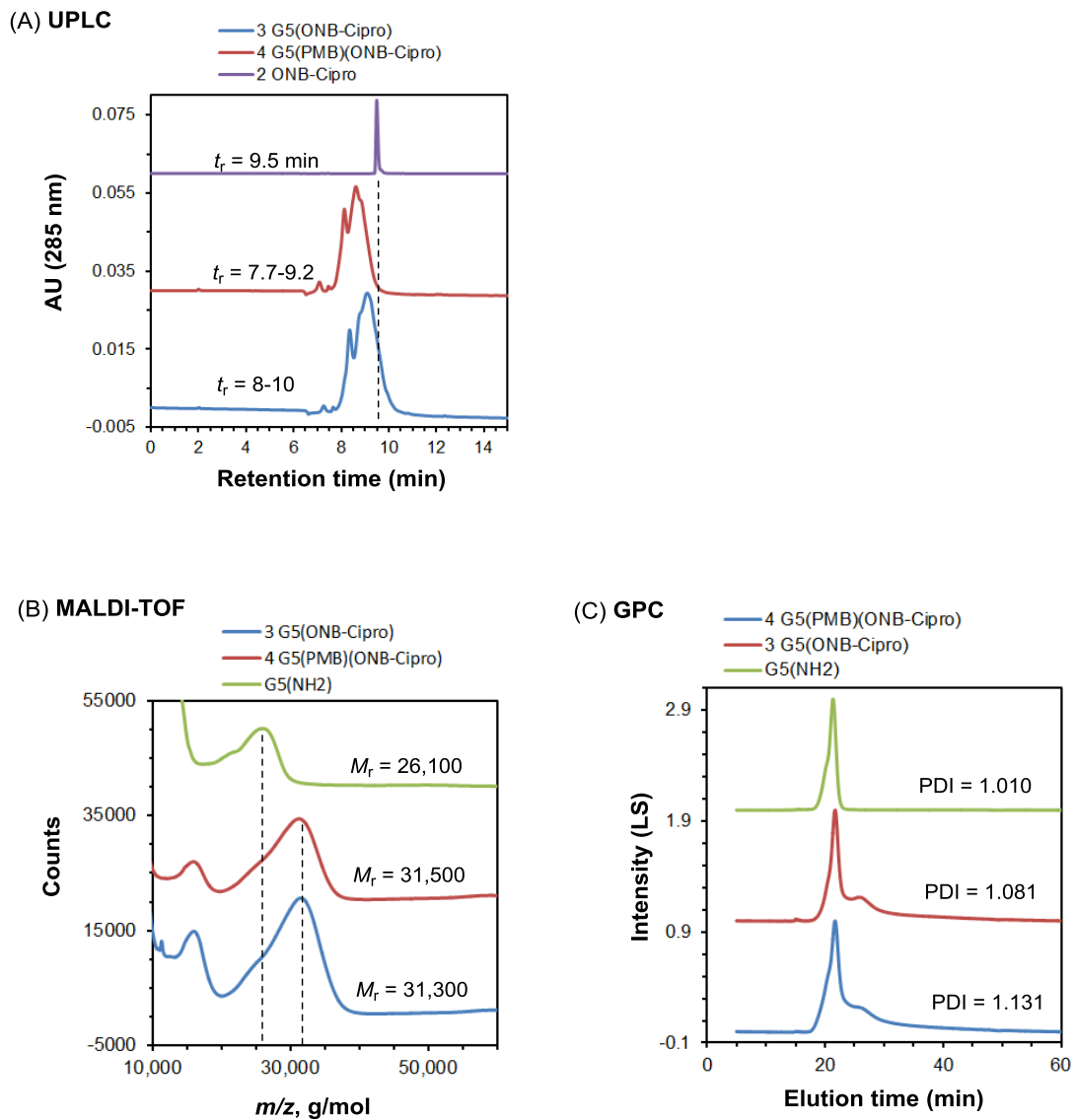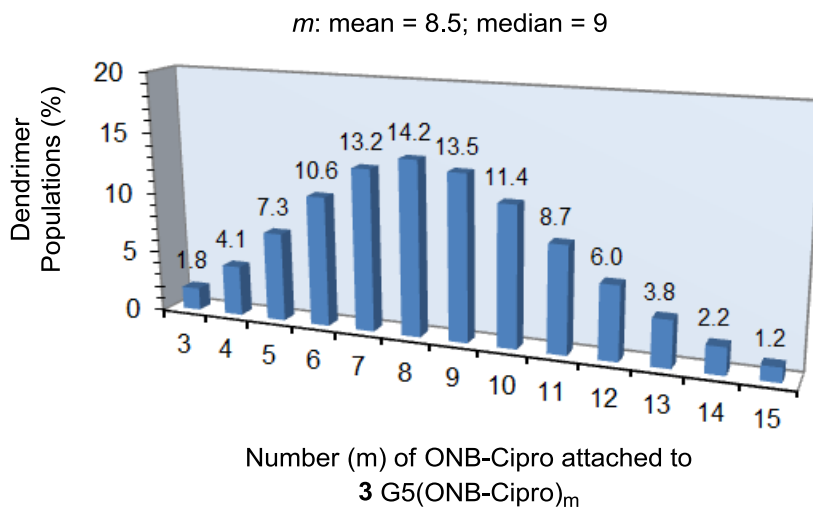
Figure S2. UPLC traces (A), MALDI-TOF mass spectra (B), and GPC (C) traces of **3** G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$ and **4** G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$.
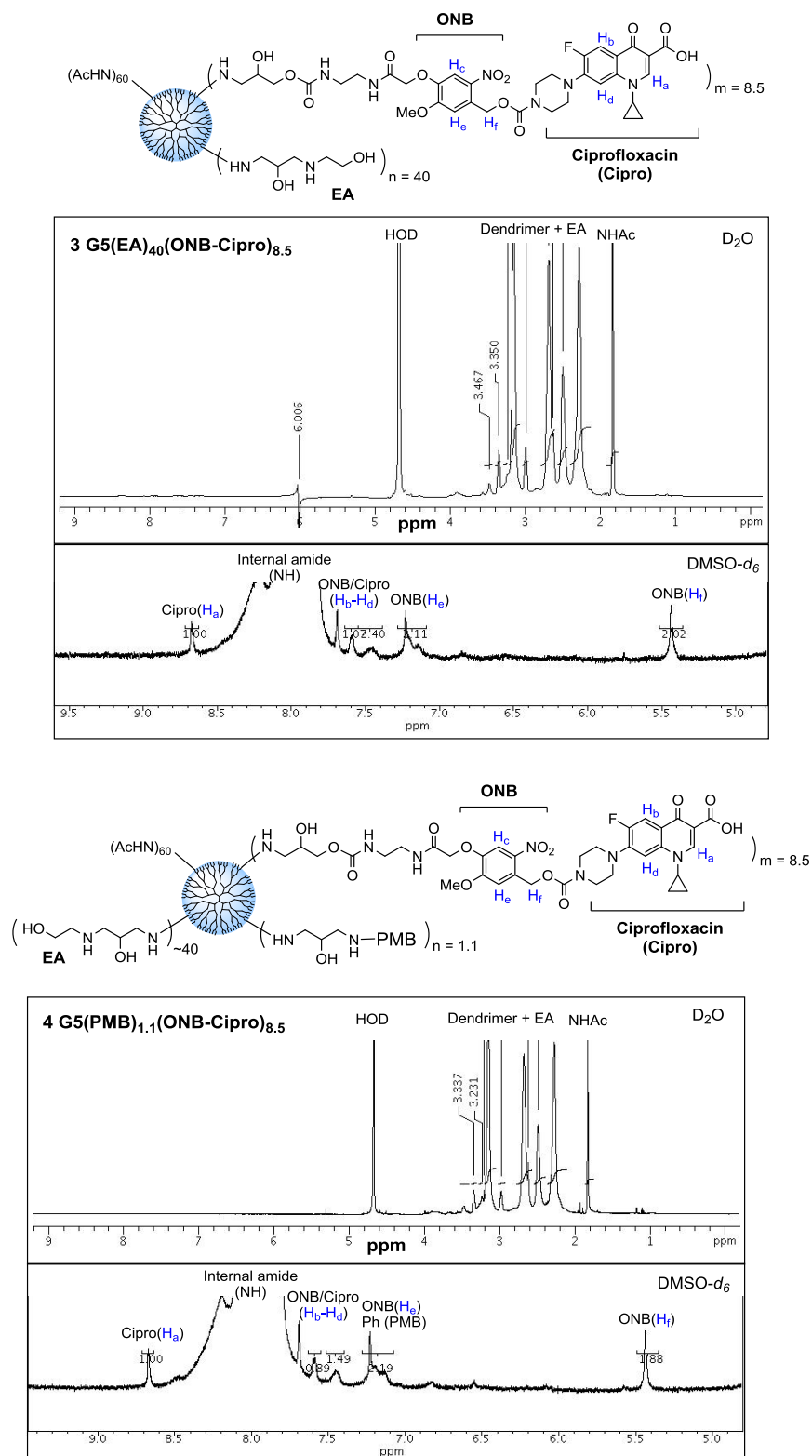
Figure S3. ^{1}H NMR (500 MHz) spectra of **3** G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$ (top) and **4** G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$ (bottom), each taken in D$_2$O and DMSO-d_6 (downfield).
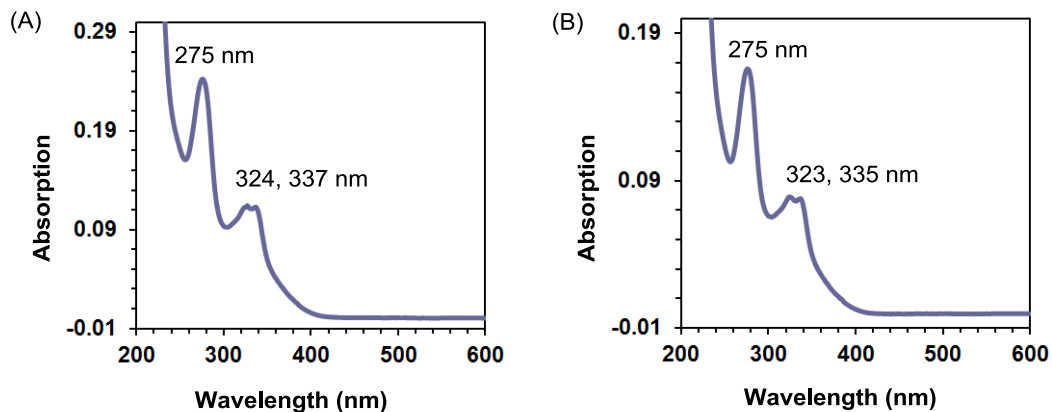
Figure S4. UV–vis absorption spectra of **3** G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$ (A) and **4** G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$ (B), each measured in water (31.7 μM).


Figure S5. Poissonian distribution[9] of dendrimer species included in **3** G5(EA)$_{40}$(ONB-Cipro)$_m$ with mean drug valency $m = 8.5$ of ONB-Cipro. The distribution based on the valency of ONB-Cipro ($m = 3–15$; total 98%) is plotted as % population.
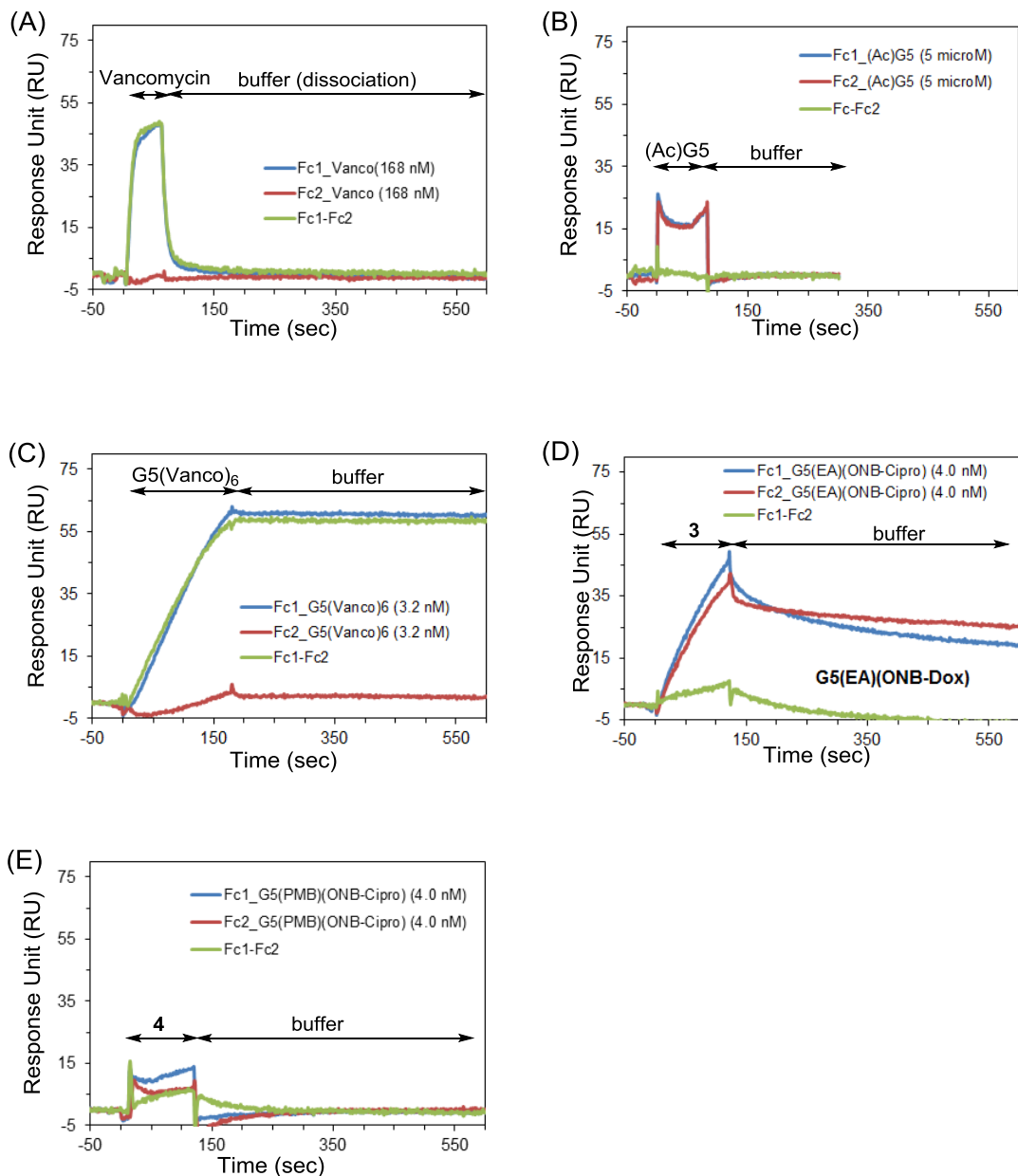
Figure S6. Surface plasmon resonance (SPR) sensorgrams recorded on a model surface for Gram-positive bacteria. (A) Vancomycin; (B) (Ac)G5 (a negative control); (C) G5(Vacno)$_6$[10] (a positive comparator); (D) **3** G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$ (a negative comparator); (E) **4** G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$ (a negative comparator). Here, flow cell 1 (Fc1) on a CM5 sensor chip was immobilized with N^α-Ac-Lys-(D)-Ala-(D)-Ala peptide as the bacterial model surface while flow cell 2 (Fc2) was treated in the same manner without the peptide to serve as a reference surface.[10] The concentration of the injected analyte is indicated on each plot.

S14
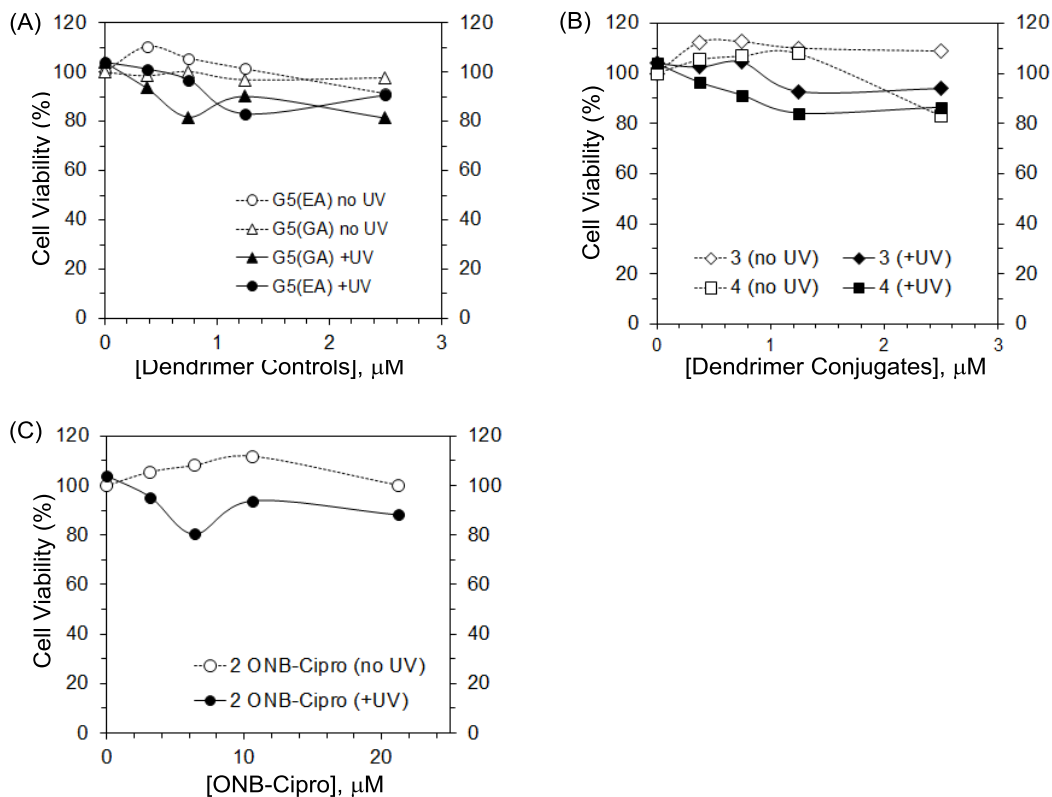
Figure S7. Effects of dendrimer conjugates and photocaged ciprofloxacin on the viability of human KB cells: (A) dendrimer controls G5(EA) and G5(GA); (B) conjugates **3** G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$ and **4** G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$; (C) **2** ONB-Cipro. KB cells, a subline of HeLA cells, were treated in a dose dependent manner with or without exposure to UVA (365 nm) for 30 min, and their viability (%) was determined by conducting a standard XTT assay as reported earlier.[11]
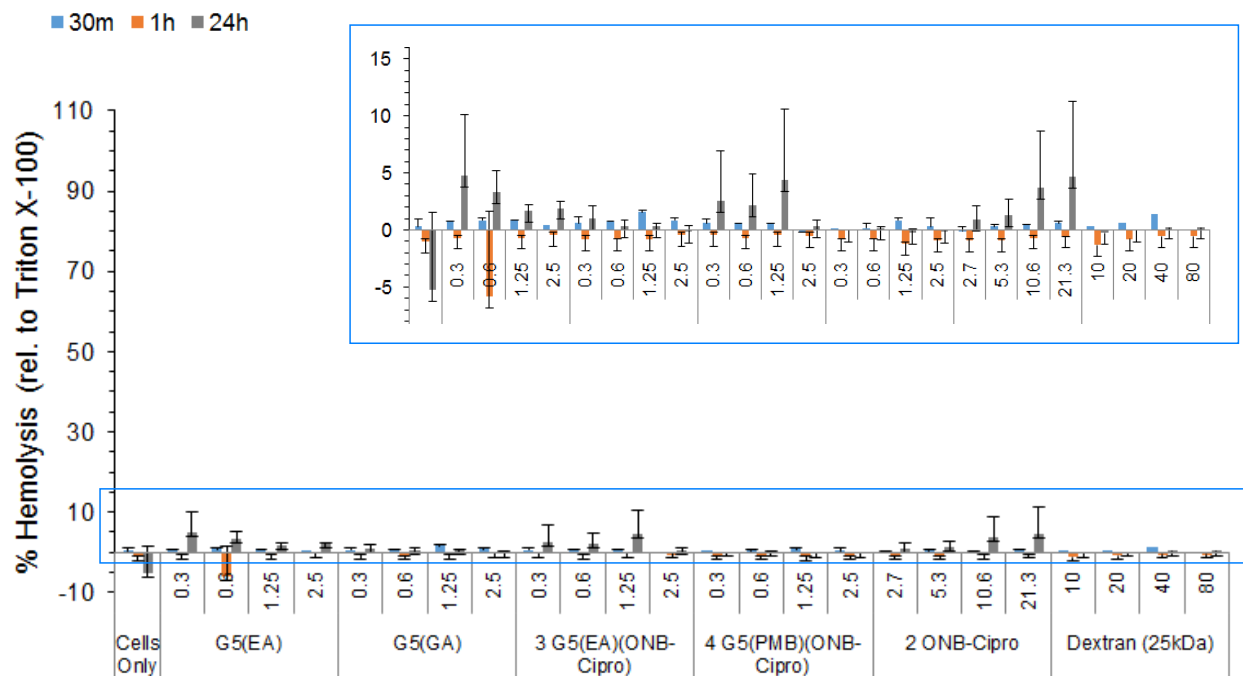
Figure S8. Effects of dendrimer conjugates on the hemolysis of red blood cells (RBCs). Conjugates and compounds tested: G5(EA), G5(GA), **3** G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$, **4** G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$, **2** ONB-Cipro and dextran (a negative control). Unit for doses = μM. Each data point refers to a mean value (±SD). Inset is an expanded plot for clarity.

Assay protocol: Sheep RBCs (2%, Rockland Immunochemicals, PA) in PBS (pH 7.4) were treated with each conjugate at various doses for 30 min, 1 h and 24 h, and their hemolysis (%) was determined by the established assay protocol reported in literature.[12] Typically, after incubation, the treated cells were centrifuged, and the supernatant was measured for its absorption at 550 nm for the quantification of hemoglobin which is released following the membrane rupture. Hemolysis values (%) are reported relative to Triton X-100 (1% v/v) as a positive control which is set as 100%.

Table S1. A summary of molecular weights, polydispersity index (PDI) and drug/ligand valency of **3** G5(EA)$_n$(ONB-Cipro)$_m$ and **4** G5(PMB)$_n$(ONB-Cipro)$_m$

Dendrimer	$M_r{}^a$	$M_w{}^b$ (PDI^c)	Ligand/Drug Valency
G5(NH$_2$)$_{114}$	26,100	26,600 (1.010)	-
3 G5(EA)$_n$(ONB-Cipro)$_m$	31,300	27,400 (1.081)	n = 40; m = 8.5^d
4 G5(PMB)$_n$(ONB-Cipro)$_m$	31,500	33,500 (1.131)	n = 1.1; m = 8.5^d

^{a} Relative molar mass measured by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF; gmol^{-1}) mass spectrometry
^{b} Weight-averaged molecular weight determined by gel permeation chromatography (GPC; gmol^{-1})
^{c} Polydispersity index (PDI) = $M_w \div M_n$
^{d} Determined by UV–vis spectrometry (ONB-Cipro)

Table S2. Hydrodynamic size (diameter, Z$_{ave}$) and zeta potential (ZP) of G5 PAMAM dendrimer and its conjugates **3**–**4**.^{a}

Dendrimer	Z$_{ave}$, nm^b	PDI^c	ZP (±SD), mV
G5(NH$_2$)$_{114}$	106.3	0.29	5.2 (±3.8)
G5(EA)$_{40}$	147.5	0.40	31.9 (±5.5)
3 G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$	38.6	0.46	24.2 (±4.25)
4 G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$	26.8	0.55	17.7 (±5.3)

^{a} Measured in 1 mM HEPES pH 7.0 ([dendrimer] = 2.5 μM)
^{b} Determined by dynamic light scattering (DLS); refer to the apparent size of hydrated nanoparticles in non-spherical aggregates
^{c} Polydispersity index (PDI) = $(\sigma/Z_{ave})^2$ (σ = standard deviation)

Table S3. Kinetic rate constants (k_{on} = association rate constant, k_{off} = dissociation rate constant), and equilibrium dissociation constants K_D (= k_{off}/k_{on}) determined by surface plasmon resonance (SPR) spectroscopy for the binding of dendrimers **3–4** to the lipopolysaccharide (LPS)-immobilized sensor chip as a bacterial cell wall model

	k_{on} ($M^{-1}s^{-1}$) $\times 10^{-4}$	k_{off} (s^{-1}) $\times 10^{4}$	K_D (M),[a] nM	β^{b}
Polymyxin B (PMB)	5.9 ($\pm$ 4.4)	40 ($\pm$ 25)	150 ($\pm$ 68)[ref 5]	1
3 G5(EA)$_{40}$(ONB-Cipro)$_{8.5}$	3.0 ($\pm$ 0.98)	2.1 ($\pm$ 1.0)	7.0 ($\pm$ 2.9)	21
4 G5(PMB)$_{1.1}$(ONB-Cipro)$_{8.5}$	3.9 ($\pm$ 1.7)	2.3 ($\pm$ 1.2)	5.7 ($\pm$ 0.66)	26

[a] Mean value $\pm$ standard error of the mean (N = 5)
[b] β = multivalent binding enhancement = $K_D^{Polymyxin\ B} \div K_D^{dendrimer}$

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