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Supporting Information

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Modular Integration of Upconversion Nanocrystal-Dendrimer Composites for Folate Receptor-Specific Near Infrared Imaging and Light Triggered Drug Release

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1. Materials and Methods

Materials. All reagents and solvents were purchased from commercial suppliers including folic acid dihydrate (Sigma-Aldrich; purity 98%), folate binding protein (FBP, bovine milk), doxorubicin hydrochloride (AvaChem Scientific; purity 98%), and sulfo-Cy5 NHS ester (Thermo Scientific). These were used as received. Analytical grade solvents were purchased from Sinopharm Chemical Reagent Co, Ltd (China) that include anhydrous ethanol, chloroform, cyclohexane, methanol and ammonia. Rare earth metal oxides and reagents used for UCN synthesis such as Re_2O_3 (RE = Y, Yb, Er, Tm > 99.99%), trifluoroacetic acid (CF_3COOH, >99%), sodium carbonate anhydrous (Na_2CO_3, >99.8%) were purchased from Sinopharm Chemical Reagent Co, Ltd. Other reagents including oleic acid (OA, >90%), octadecene (ODE, >90%), sodium oleate (NaOL, >95%), (3-aminopropyl)triethoxysilane (APTES), polyoxyethylene nonylphenyl ether (NP5, Igepal^{TM} CO520, average molecular weight 441) were purchased from Sigma-Aldrich. Sodium trifluoroacetate (CF_3COONa) was prepared by dissolving Na_2CO_3 in a CF_3COOH/water (1:1; v/v) solution. Each of the $(CF_3COO)_3RE$ precursors was prepared by dissolving the corresponding RE_2O_3 in CF_3COOH/water (1:1) solutions and heating at the refluxing temperature until the solutions became clear.

Generation 5 (G5) PAMAM dendrimer G5(NH_2) was purchased as a solution in methanol (17.5 % w/w; Dendritech). The dendrimer was purified prior to use by dialysis using membrane tubing (molecular weight cut-off (MWCO) = 10 kDa) against deionized water (4 × 4L) over 3 days. Freeze-dried dendrimer appeared as a colorless glassy solid. The experimental number (n_{exptl}) of primary amines per dendrimer molecule G5$(NH_2)_n$ (n_{exptl} = 114; n_{theor} = 128[1]) was determined on a mean basis by potentiometric titration as described elsewhere.[2]

Analytical Methods. Transmission electron microscopy (TEM) images of UCNs were obtained using a JEM-2100 transmission electron microscope operated at an acceleration voltage of 200KV. Fluorescence spectra were obtained on a RF-5301PC luminescence spectrometer (Shimadzu, Japan) with an external 980 nm laser diode (1 W, continuous wave with 1 m optical fiber, Xi'an Saipling, China) as the excitation source. UV–vis absorption and fluorescence emission spectra were acquired with a Perkin Elmer Lamda 25 spectrophotometer and Fluoromax-2 fluorimeter (Horiba Scientific), respectively. NMR spectroscopy was performed with a Varian nuclear magnetic resonance spectrometer at 500

MHz for ^{1}H nuclei and at 100 MHz for ^{13}C nuclei. Chemical shift (δ) values in each spectrum are reported in ppm, and referenced to internal 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS) or tetramethylsilane (TMS), each δ = 0.00 ppm. All NMR data were collected at 297.3 K (±0.2) under standard observation conditions. Mass spectrometric characterization of low molecular weight compounds was performed by electrospray ionization mass spectrometry (ESI MS) with a Micromass AutoSpec Ultima spectrometer. Molecular weights of the G5 PAMAM dendrimers were measured by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) with a Waters TOfsPec-2E spectrometer. Samples for MALDI measurement were prepared in a matrix solution of 2,5-dihydroxybenzoic acid (10 mg/mL 50% aq acetonitrile) and spectral data was processed using Mass Lynx 3.5 software.

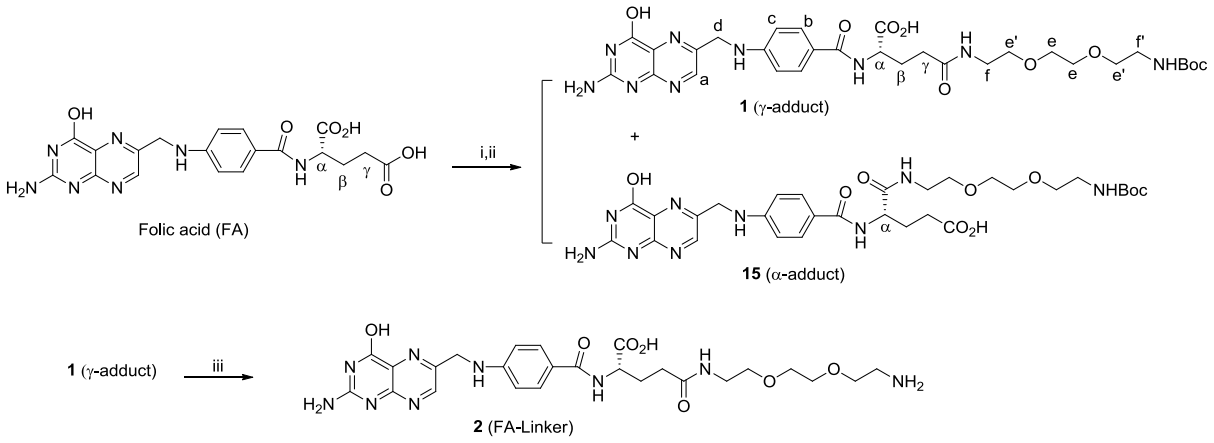
Homogeneity of small molecules and dendrimer conjugates was determined by HPLC (UPLC®) analysis performed with a Waters Acquity Peptide Mapping System equipped with a Waters photodiode array detector. Each sample was analyzed on a C4 BEH column (150 × 2.1 mm, 300 Å) connected to Waters Vanguard column. As an elution method, a linear gradient method starting with initial mobile phase composition 99:1 (v/v) water (A)/acetonitrile (B) with TFA (0.1% v/v) was employed at a flow rate of 0.2 $mLmin^{-1}$. The initial phase 1% B (0–1.4 min) was changed with linear increase to 80% B (1.4–13.4 min), linear decrease to 50% B (13.4–13.8 min) and linear decrease to 1% B (13.8–14.4 min) prior to back to 1% B (14.4–18 min).

Molecular weights and distribution of dendrimers were measured by gel permeation chromatography (GPC) experiments performed on an Alliance Waters 2695 separation module equipped with three detection modes: 1) a UV absorbance detector (Waters Corporation); 2) a multi angle laser light scattering detector (MALLS; Wyatt HELEOS); 3) an Optilab rEX differential refractometer (Wyatt Technology Corporation). GPC experiments were run under temperature control (25±0.1°C) using a citrate mobile phase (0.1 M citric acid, 0.025 wt % of sodium azide, pH 2.74), a flow rate (1 mL/min) at 3–5 mg/mL of the sample concentration. Each GPC trace was analyzed by Astra 5.3.14 software (Wyatt Technology Corporation) to determine the weight-average molecular weight (M_w) and the number-average molecular weight (M_n) which was calculated indirectly on the basis of the molecular weight distribution. A polydispersity index (PDI = M_w/M_n) value for $G5(NH_2)_{114}$ was determined as 1.09.

2. Synthesis of FA-Linker 2 (Scheme S1)

1 (FA-linker; *N*-Boc protected)**: Step i**) Anhydrous DMSO (50 mL) was added to folic acid (FA; 1.0 g, 2.27 mmol) in a reaction flask, and the mixture was sonicated shortly for 1 min and stirred until the FA was fully solubilized. To this stirred solution was added *N*-hydroxybenzotriazole (HOBt; 382 mg, 2.49 mmol) and *N*,*N*-diisopropylethylamine (DIPEA; 0.869 mL). The activation reaction was started by the addition of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP; 1.298 g, 2.49 mmol) and continued at room temp for 1.5 h.

Scheme S1



Reagents and conditions: i) PyBOP, HOBt, (*i*-Pr)$_2$EtN, DMSO, 1.5 h; then 2,2'-(ethylenedioxy)diethylamine (3 mol equiv), 18 h; ii) (Boc)$_2$O (7 mol equiv), 6 h; iii) CF_3CO_2H, CH_2Cl_2, 0.5 h.

Step ii and iii) To the activated solution of FA was added 2,2′-(ethylenedioxy)bis(ethylamine) (0.993 mL, 6.80 mmol; 3 mol equiv to FA). The mixture was stirred at room temp for 18 h, and then (Boc)$_2$O (3.463 g, 15.9 mmol) was added. The final mixture was stirred at room temp for 6 h. It was concentrated *in vacuo*, and a resulting thick solution was diluted with water (200 mL), causing the precipitation of yellow particles. The solid material was collected, dried and dissolved in methanol (10 mL). This solution was fractionated by flash silica column chromatography by eluting with a mixture of dichloromethane, methanol and triethylamine (Et_3N) at a gradient ratio of 100:30:3. After fractionation, the fractions containing the desired product were identified by mass spectrometric analysis. These fractions were combined, concentrated and refractionated by using the same flash column chromatography method. The desired isomer **1** (γ-adduct) was obtained as a yellow solid (293 mg; 19.3%) along with its regioisomer **15** (α-adduct; 100 mg; 6.6%). R_f (**1**) = 0.66 (CH_2Cl_2/MeOH/Et_3N = 100:30:3; TLC plate: silica gel 150A, Whatman, partisil K5F). MS (ESI) *m/z* (relative intensity, %) = 572.3 (100) $[M-Boc+H]^+$, 672.4 (77) $[M+H]^+$**,** 1343.8 (3) $[2M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): 8.63–8.62 (d, J = 5 Hz, 1H, H_a

(FA)), 7.67–7.62 (dd, J = 10 Hz, J = 20 Hz, 2H, H_b (FA)), 6.65–6.62 (dd, J = 5 Hz, J = 10 Hz, 2H , H_c (FA)), 4.47 (s, 2H, H_d (FA)), 4.36–4.17 (m, 1H, H_α (L-Glu)), 3.42–3.36 (m, 4H, OCH_2CH_2O), 3.19–3.14 (m, 2H, $CH_2NHC{=}O$), 3.06–3.03 (m, 2H, CH_2NHBoc), 2.73–2.69 (m, 4H, $2CH_2O$), 2.26–2.14 (m, 2H, H_γ (L-Glu)), 2.06–1.83 (m, 2H, H_β (L-Glu)), 1.36 (s, 9H, NHBoc) ppm.

2 (FA-Linker): To **1** (270 mg, 0.402 mmol) in a vial was added dichloromethane (8 mL) and followed by the addition of trifluoroacetic acid (8 mL). The mixture was stirred at room temp for 30 min and concentrated *in vacuo* to about 2 mL. This solution was titrated into cold ether (30 mL), causing precipitation of the product. The precipitate was collected by centrifugation (4,500 rpm), rinsed with ether and dried under nitrogen flow, affording **2** (TFA salt) as a pale brown solid (293 mg; quantitative). HPLC analysis: t_r = 4.92 min (purity = 90%). MS (ESI) *m/z* (relative intensity, %) = 572.2 (100) $[M+H]^+$**,** 1144.5 (1) $[2M+H]^+$. HRMS (ESI) calcd for $C_{25}H_{32}N_8O_8$ $[M+H]^+$ 572.2338, found 572.2573. ^{1}H NMR (500 MHz, DMSO-D_6): 8.67–8.66 (d, J = 5 Hz, 1H, H_a (FA)), 7.67–7.64 (dd, J = 5 Hz, J = 10 Hz, 2H, H_b (FA)), 6.65–6.63 (dd, J = 5 Hz, J = 10 Hz, 2H , H_c (FA)), 4.51 (s, 2H, H_d (FA)), 4.36–4.26 (m, 1H, H_α (L-Glu)), 3.56–3.51 (m, 4H, OCH_2CH_2O), 3.42–3.36 (m, 4H, $2CH_2O$), 3.22–3.16 (m, 2H, $CH_2NHC{=}O$), 2.98–2.96 (m, 2H, CH_2NH_2),2.28–2.18 (m, 2H, H_γ (L-Glu)), 2.05–1.82 (m, 2H, H_β (L-Glu)) ppm.

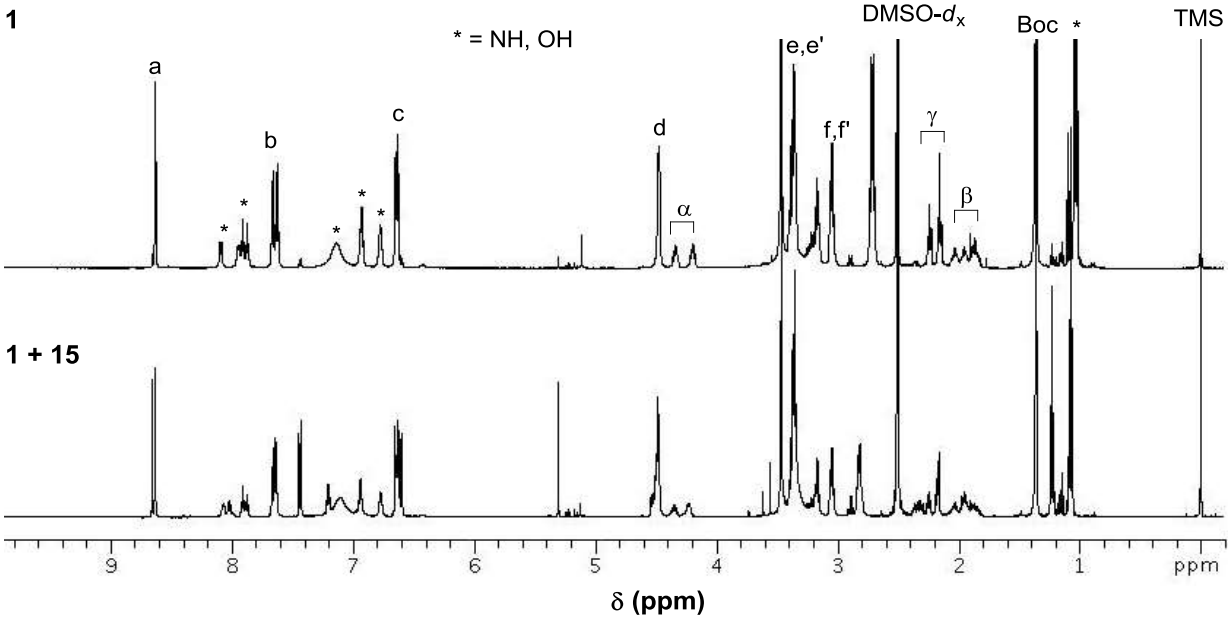
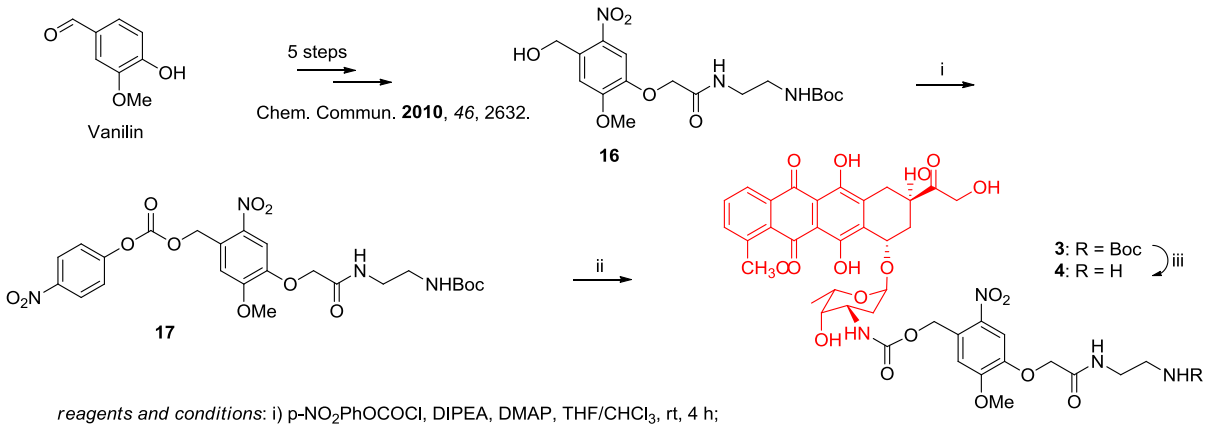


Figure S1. ^{1}H NMR spectra (500 MHz; DMSO-d_6) of **1** as a single γ-isomer (top) and its mixture with its regio α-isomer **15** (bottom).

3. Synthesis of 4 ONB-Dox (Scheme S2)

ONB-Dox **4** was synthesized according to the experimental method described in our previous communication.[3] ONB-Dox and its intermediate compounds including **3** were fully characterized by mass spectrometry, ^{1}H NMR spectroscopy and UV–vis spectrometry as summarized below.

Scheme S2



17: An *ortho*-nitrobenzyl linker **16** was synthesized from 4-formyl-2-methyoxyphenol (vanillin) and converted to its *p*-nitrophenyl carbonate ester **17** by treatment with *p*-nitrophenyl chloroformate.[3] R_f (5% MeOH/$CHCl_3$) = 0.56. ^{1}H NMR (500 MHz, CD_3OD): 8.31–8.21 (dd, J = 2.5 Hz, J = 7 Hz, 2H, H_m ($_{NO2\text{-}p}C_6H_4$)), 7.81 (s, 1H, H_a (ONB)), 7.43–7.41 (dd, J = 2.5 Hz, J = 7 Hz, 2H, H_o ($_{NO2\text{-}p}C_6H_4$)), 7.23 (br, NH), 7.17 (s, 1H, H_b (ONB)), 5.72 (s, 2H, H_c), 4.60 (s, 2H, H_d), 4.04 (s, 3H, OCH_3), 3.52–3.48 (m, 2H, H_e), 3.38–3.30 (m, 2H, H_f), 1.42 (s, 9H, NHBoc) ppm.

3 ONB-Dox (*N*-Boc protected): For conjugation with doxorubicin, **17** (277 mg, 0.491 mmol) was added to a solution of doxorubicin hydrochloride (300 mg, 0.517 mmol) in DMF (30 mL) containing triethylamine (216 μL, 1.55 mmol). After stirring in the dark at room temp for 16 h, the mixture was concentrated *in vacuo*, affording a red residue. It was purified by flash column chromatography (silica gel) by eluting with MeOH/$CHCl_3$ (3 to 10%). The desired product **3** was obtained as a brick red solid (196 mg; 39%). R_f (10% MeOH/$CHCl_3$) = 0.45. HPLC analysis: t_r = 9.88 min (purity = 98%). MS (ESI) *m/z* (relative intensity, %) = 991.4 (23) $[M+Na]^+$, 455.2 (100). ^{1}H NMR (500 MHz, $CDCl_3$): 8.06–8.04 (d, J = 10 Hz, 1H, H_1 (dox)), 7.82–7.78 (t, J = 10 Hz, 1H, H_2 (dox)), 7.70 (s, 1H, H_a (ONB)), 7.41–7.39 (d, J = 10 Hz, 1H, H_3 (dox)), 7.15 (br, NH), 7.00 (s, 1H, H_b (ONB)), 5.52–5.25 (m, 3H, $H_{1'}$(anomeric, dox) and H_c (ONB)), 4.76 (s, 2H, H_d (ONB)), 4.53 and 4.50 (s, 2H, H_{14} (dox)), 4.19–4.04 (m, 2H, $H_{3'}$, $H_{5'}$(dox)), 4.09 (s, 3H, OCH_3 (C_4, dox)), 3.97 (s, 3H, OCH_3 (ONB)),

3.92–3.86 (m, 1H, $H_{4'}$(dox)), 3.48–3.44 (m, 2H, H_e), 3.34–3.28 (m, 2H, H_f), 3.28 (m, 0.5H, H_{10} (dox)), 3.06–3.02 (m, 0.5H, H_{10} (dox)), 2.40–2.30 (m, 0.5H, H_8 (dox)), 2.22–2.12 (m, 0.5H, H_8 (dox)), 1.98–1.72 (m, 2H, $H_{2'}$ (dox)), 1.41 (s, 9H, NHBoc)), 1.31–1.29 (m, 3H, CH_3 ($C_{6'}$, dox)) ppm. UV–vis (10% aq MeOH): λ_{max} = 235 (ε = 2108 $M^{-1}cm^{-1}$), 291 (ε = 694 $M^{-1}cm^{-1}$), 349 (ε = 456 $M^{-1}cm^{-1}$), 499 (ε = 531 $M^{-1}cm^{-1}$) nm.

4 ONB-Dox: To deprotect the *N*-Boc group, **3** (181 mg, 0.155 mmol) was dissolved in dichloromethane (4 mL) and followed by the addition of trifluoroacetic acid (4 mL). After stirring for 30 min at room temp, the mixture was concentrated *in vacuo* (~1 mL), and titrated into cold ether (20 mL), causing precipitation of the product. The precipitate was collected by centrifugation (4,500 rpm), rinsed with ether, and dried under nitrogen atmosphere. **4** ONB-Dox (TFA salt) was isolated quantitatively as a red brick solid (198.6 mg). MS (ESI) *m/z* (relative intensity, %) = 869.4 (100) $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): 8.25 (t, *J* = 10 Hz, NH), 7.93–7.92 (d, *J* = 5 Hz, 1H, H_1 (dox)), 7.73 (br s, 1H, H_a (ONB)), 7.68–7.66 (t, *J* = 10 Hz, 1H, H_2 (dox)), 7.34–7.20 (m, 2H, H_3 (dox), H_b (ONB)), 5.42–5.28 (m, 2H, H_c (ONB)), 5.06–5.12 (m, 1H, $H_{1'}$(anomeric, dox)), 4.64 (d, 2H, H_d (ONB)), 4.58–4.54 (s, 2H, H_{14} (dox)), 4.00 (s, 3H, OCH_3 (C_4, dox)), 3.95 (s, 3H, OCH_3 (ONB)), 3.94–3.93 (m, 2H, $H_{3'}$, $H_{5'}$ (dox)), 3.4 (br s, 3H, H_e (ONB), $H_{4'}$ (dox)), 3.07–2.93 (dd, 2H, H_{10} (dox)), 2.90 (m, 2H, H_f (ONB)), 2.19–1.98 (m, 2H, H_8 (dox)), 1.90–1.53 (m, 2H, $H_{2'}$ (dox)), 1.08–1.05 (m, 3H, CH_3 ($C_{6'}$, dox)) ppm.

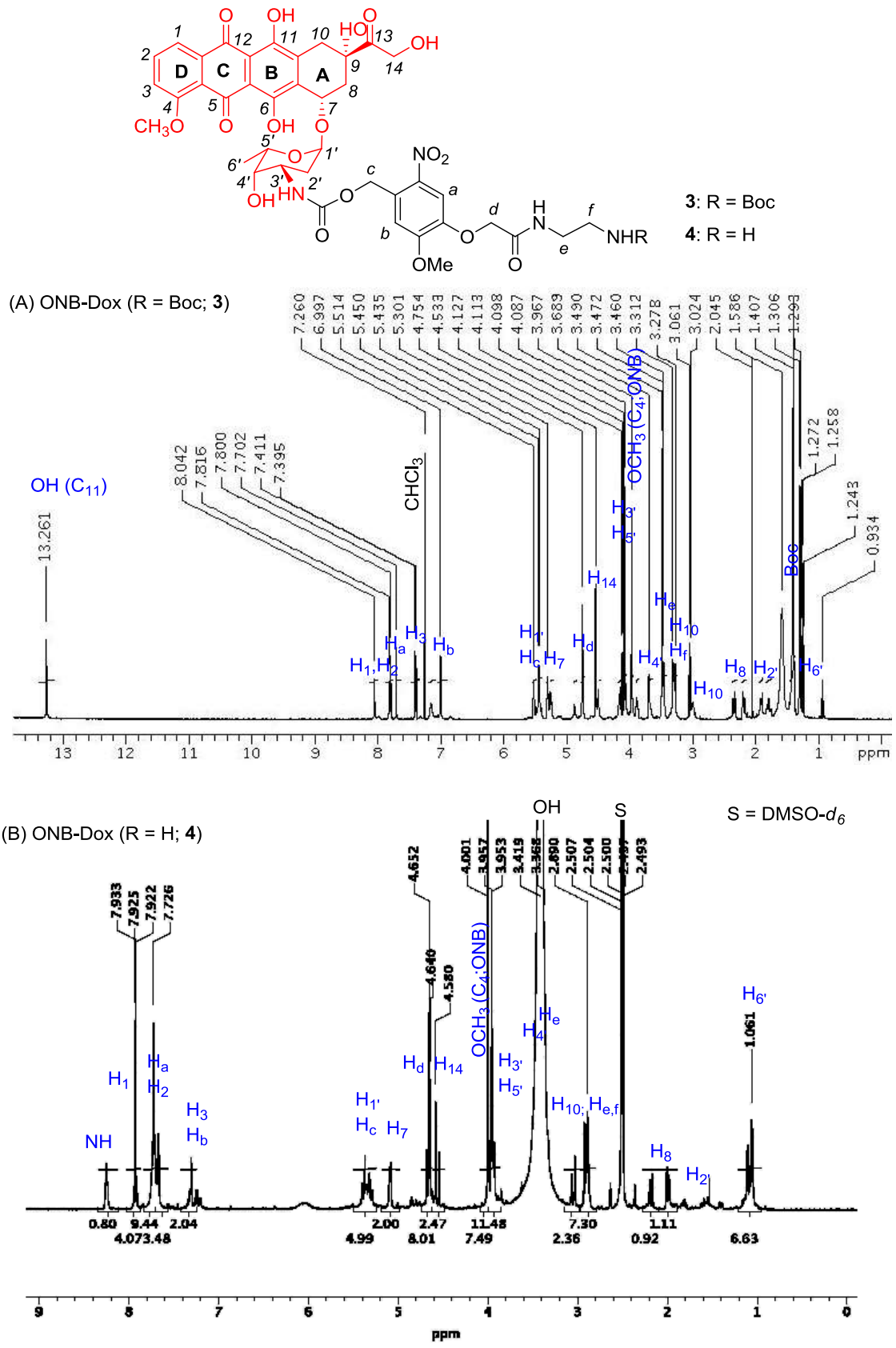


Figure S2. ^{1}H NMR spectra (500 MHz) of ONB-Dox. (A) **3** (R = Boc; $CDCl_3$); (B) **4** (R = H; DMSO-d_6). Details of proton assignment are also given in the text.

4. Synthesis of G5 PAMAM dendrimer conjugates 7 G5(FA)$_6$ and 8 G5(FA)$_6$(ONB-Dox)$_{6.9}$ (Scheme 1C)

6 G5(GA): Primary amines in the periphery of G5 PAMAM dendrimer **5** were exhaustively derivatized with glutaryl amide (GA) by treatment with glutaric anhydride, yielding G5(GA).[3]

7, **8** G5(FA)$_n$(ONB-Dox)$_m$: To a glass vial containing **6** G5(GA) (250 mg), *N*-hydroxysuccinimide (NHS; 155 mg, 1.34 mmol) and *N*,*N*-dimethyl-4-aminopyridine (DMAP; 205 mg, 1.68 mmol) was added DMF (18 mL) and followed by the addition of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC; 321 mg, 1.68 mmol). This mixture was stirred at room temp overnight. During the reaction, the dendrimer which was initially in suspension was slowly solubilized and the mixture became a homogenous solution. To this solution was added a solution of **2** FA-linker (68 mg, 0.0746 mmol) dissolved in DMF (1 mL) containing *N*,*N*-diisopropylethylamine (DIPEA; 0.13 mL). The mixture was stirred at room temp for 7 h, and divided into two vials, each in equal amount. One vial was not further treated, and **7** G5(FA)$_n$ contained in the vial was purified as described below. For synthesis of **8** G5(FA)$_n$(ONB-Dox)$_m$, **4** ONB-Dox (46 mg, 0.0466 mmol) was dissolved in DMF (1 mL) containing DIPEA (0.041 mL), and it was added to the solution in the other vial. This mixture was stirred at room temp overnight. For purification, each of the reaction solution was concentrated *in vacuo*, and the residue was dissolved in water (5 mL). This solution was dialyzed using a membrane dialysis tubing of 10 kDa molecular weight cutoff (MWCO) against deionized water (2 L × 1), PBS pH 7.4 (2 L × 2) and deionized water (2 L × 2) over 2 days in the dark. Freeze-drying of each dialyzed solution afforded **7** as a pale yellow solid (121 mg) or **8** as a pale red brick solid (130 mg), respectively.

7: HPLC analysis: t_R = 6.58 min (polymer purity ≥94%); UV–vis (PBS pH 7.4): λ_{max} = 286 (ε = 110,400 $M^{-1}cm^{-1}$; FA), 354 (ε = 62,800 $M^{-1}cm^{-1}$; FA) nm. MALDI-TOF (*m/z*): 38,600 $gmol^{-1}$. GPC: M_p = 42,000 $gmol^{-1}$, M_n = 23,300 $gmol^{-1}$, PDI = 3.36. ^{1}H NMR (500 MHz, D_2O): weak peaks: δ 8.8 (br s*), 8.3 (br s), 8.1 (br s), 7.8–7.9 (br m), 6.8–7.0 (br m); strong peaks: 3.5–3.3 (br s), 3.3 (s), 3.2–2.7 (br m), 2.7 (m), 2.6–2.5 (br s), 2.4 (m), 2.26–2.24 (t), 2.23–2.21 (t), 1.85–1.79 (m) ppm. *Acronyms: br (broad), s (singlet), m (multiplet). The number of FA (**2**) attached to **7** G5(FA)$_n$ (ligand valency = n = 6.0 ± 0.22) was determined on a mean basis by comparative analysis of UV–vis absorbance (A_{300}) values measured at three serially diluted concentrations (2.87–11.4 μM in PBS pH 7.4) to a standard calibration plot for FA: [FA] = (5.66 × 10^{-5}) × A_{300} (R^2 = 0.997). In addition, it was estimated by a NMR

integration method in which H_c (2H; FA) signal (δ 6.8–7.0 ppm) was compared to a reference group of -HNC(=O)CH_2**CH_2**CH_2C(=O) at δ 1.85–1.79 ppm (108 CH_2 residues per dendrimer), yielding n = 6.0 (±1.0).

8: HPLC analysis: t_R = 7.53 min (polymer purity ≥96%); UV–vis (PBS pH 7.4): λ_{max} = 285 (ε = 268,000 $M^{-1}cm^{-1}$; FA), 355 (ε = 163,300 $M^{-1}cm^{-1}$; FA), 490 (ε = 121,000 $M^{-1}cm^{-1}$; Dox) nm. MALDI-TOF (*m/z*): 40,400 $gmol^{-1}$. ^{1}H NMR (500 MHz, D_2O): weak peaks: δ 8.3 (br), 8.1–8.0 (br), 7.8–7.0 (br), 6.9–6.6 (br), 5.4–5.2 (br); strong peaks: 3.8 (br), 3.7–3.6(br), 3.5–3.3 (br), 3.2–2.8 (br), 2.7 (br), 2.6–2.4 (br s), 2.4 (m), 2.3–2.2 (br m), 2.2–2.1 (br m), 1.9 (br), 1.9–1.8 (br m) ppm. The number (m = 6.9 ± 0.69) of **4** ONB-Dox attached to **8** G5$(FA)_6(ONB\text{-}Dox)_m$ was determined on a mean basis by analysis of UV–vis absorbance (A_{480}) values measured at three serially diluted concentrations (2.53–10.1 μM in PBS pH 7.4) to a standard calibration plot for Dox: $[Dox]_{calibration} = (5.74 \times 10^{-5}) \times A_{480}$ (R^2 = 0.993). Analysis of NMR integration was also performed by comparison of the signals of H_c (ONB) and $H_{1'}$ (Dox) at δ 5.4–2.2 ppm to a reference group of -HNC(=O)CH_2**CH_2**CH_2C(=O) at δ 1.85–1.79 ppm (108 CH_2 residues per dendrimer), yielding n = 6.6 (±1.6). Here, the values of FA or Dox valency determined by these two methods (UV–vis; NMR) are similar but the NMR values are associated with greater standard deviation (±SD) because of line broadening and signal overlaps. Thus the values of valency (n, m) cited for G5$(FA)_{n=6}(ONB\text{-}Dox)_{m=6.9}$ are based on those by the UV–vis method.

Table S1. Summary of molecular weights, distribution and ligand valency of conjugates **7**, **8**

Dendrimer Conjugate	M_r [a]	M_p [b] (PDI[c])	UV–vis (λ_{max}, nm)	Ligand/Drug Valency
G5$(NH_2)_{114}$	27,600	24,100 (1.09)		-
7 G5$(FA)_n$	38,600	42,000 (3.36)	286, 354	n = 6.0±0.22[d] (6.0±1.0[e])
8 G5$(FA)_n(ONB\text{-}Dox)_m$	40,400	nd[f]	355, 490	n = 6.0;[d] m = 6.9±0.69[d] (6.6±1.6[e])

[a] molecular weight of peak maxima (MALDI-TOF MS), g mol^{-1}; [b] molecular weight of peak maxima (GPC), g mol^{-1}; [c] polydispersity index (PDI) = M_w/M_n (GPC); [d] mean value determined by UV–vis method; [e] mean value determined by UV–vis method; [f] not determined due to insufficient solubility in a GPC running buffer (0.1 M citric acid, pH 2.74)

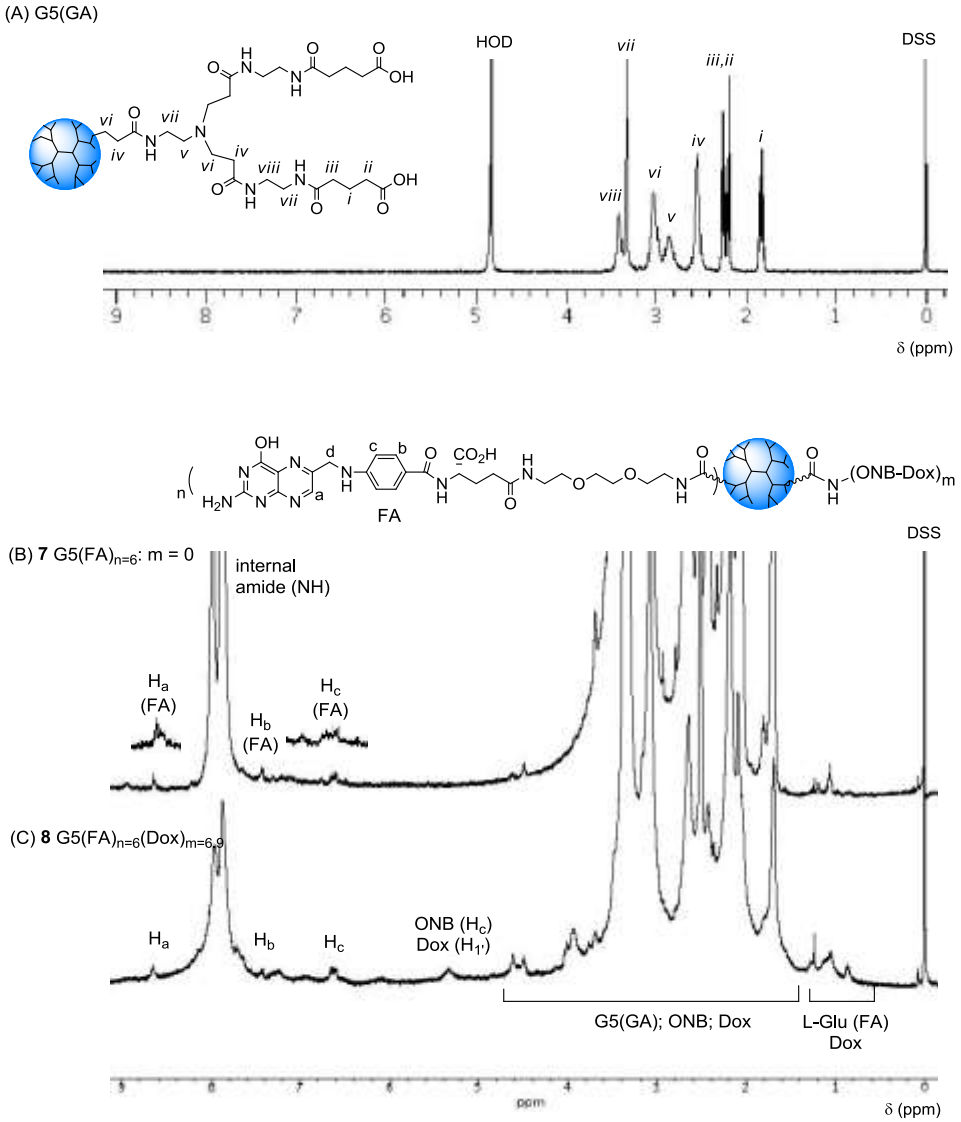


Figure S3. ^{1}H NMR spectra of G5 PAMAM dendrimer G5(GA) (A) and its conjugates G5(FA)$_n$(ONB-Dox)$_m$ (B, C). **7** (n = 6, m = 0); **8** (n = 6; m = 6.9). Each NMR spectrum was acquired in D_2O (5 mg/mL). DSS = 4,4-dimethyl-4-silapentane-1-sulfonate

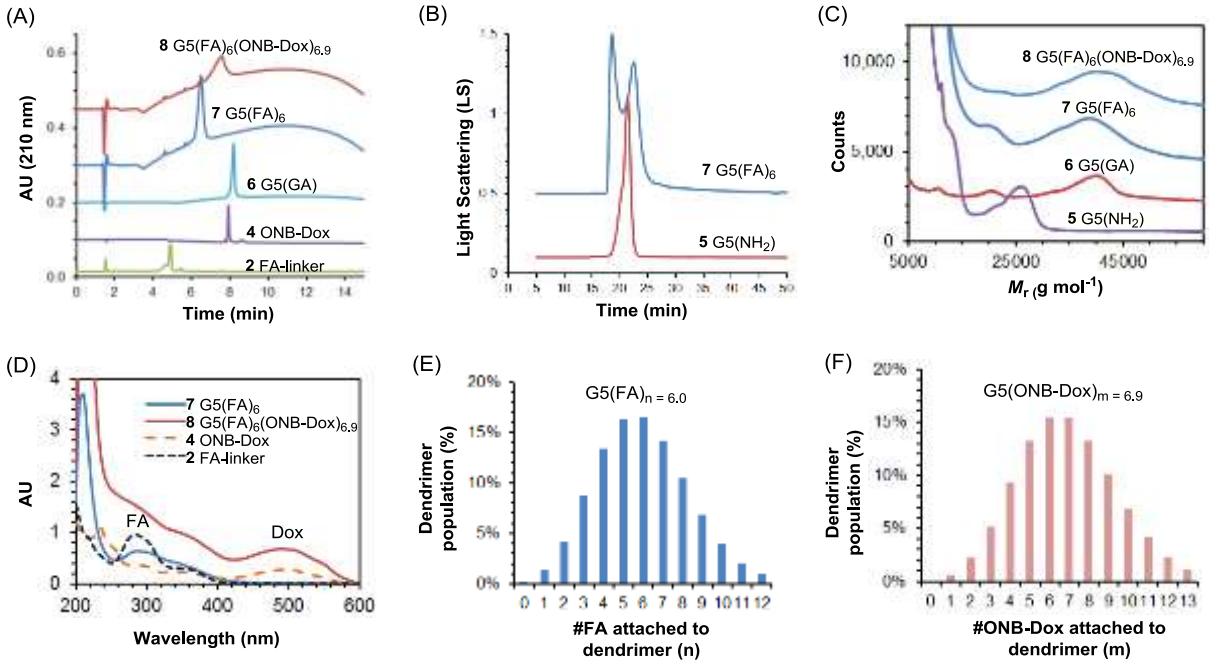


Figure S4. (A) Comparison of UPLC traces of dendrimers **6**–**8** with FA-linker **2** and photocaged ONB-Dox **4**. (B) Gel permeation chromatography (GPC) traces of **5** G5(NH_2) (PDI = 1.09) and **7** G5$(FA)_6$ (PDI = 3.36). For the GPC trace of **8** G5$(FA)_6$(ONB-Dox)$_{6.9}$, see Figure 3E in the main text. (C) MALDI-TOF mass spectra of dendrimers **5**–**8**. (D) Overlay of UV–vis spectra of **2** (17.1 μM in 50% aq MeCN), **3** ONB-Dox (41.3 μM in MeOH), **7** G5$(FA)_6$ (13 μM in PBS) and **8** G5$(FA)_6$(ONB-Dox)$_{6.9}$ (12 μM in PBS). (E, F) Poissonian simulation[4] for the distribution of G5$(FA)_{n\,=\,6.0}$ and G5$(ONB\text{-}Dox)_{m\,=\,6.9}$, each having the mean valency of n = 6.0 (FA) or m = 6.9 (ONB-Dox), respectively.

5. Synthesis of UCN_x and $UCN_x@SiO_2(NH_2)$ (x = 1, 2; Figure 2)

The β-$NaYF_4$: Yb/Er (UCN_1) was prepared following a hot injection approach as previously reported.[5] CF_3COONa (350 mg, 2.5 mmol), $(CF_3COO)_3Y$ (333.8 mg, 0.78 mmol), $(CF_3COO)_3Yb$ (102 mg, 0.2 mmol), and $(CF_3COO)_3Er$ (10.2 mg, 0.02 mmol) were added into a 25 mL flask with oleic acid (OA; 2.5 mL) and octadecene (ODE; 2.5 mL) at room temp to prepare the first solution. In a separate container, a second solution was prepared by mixing ODE (10 mL), OA (9.7, 9.5 and 9.3 mL respectively for preparation of UCN_1 with diameter of 50 nm, 30 nm and 20 nm) and NaOL (288, 384 and 576 mg respectively for UCN_1 with diameter of 50 nm, 30 nm and 20 nm). Each mixture was stirred at 100°C for 30 min under vacuum to remove water. Then the second solution was heated to 290°C under dry nitrogen, followed by the dropwise injection of the first solution into the second one. After the injection, the mixed solution was heated to 330°C and stirred for an additional 20 min. Then the mixture

was cooled down to room temp, and the UCN_1 product was precipitated with ethanol. UCN_1 was collected, and then washed with chloroform and methanol 4 times.

$NaYF_4$: Yb/Tm (UCN_2) was prepared in the same manner except that those reagents in the first solution were replaced with CF_3COONa (350 mg, 2.5 mmol), $(CF_3COO)_3Y$ (319.7 mg, 0.75 mmol), $(CF_3COO)_3Yb$ (128.1 mg, 0.25 mmol), and $(CF_3COO)_3Tm$ (1.5 mg, 0.003 mmol).

Surface modification of each UCN_x with a thin amine-containing silica layer $SiO_2(NH_2)$ was performed following a non-ionic water-in-oil microemulsion method.[6] Each of the UCN_x was dispersed in cyclohexane (110 mL) by sonication and polyoxyethylene (5) nonylphenylether (NP5; M_n = 441; 6 mL) was added while stirring the solution for 1 h. To this suspension was added ammonia (1.2 mL) and APTES (400 μL) in a sequence, and the final solution was stirred at room temp for two days. $UCN_x@SiO_2(NH_2)$ was collected by centrifugation, washed with ethanol for 4 times, and freeze dried.

6. Surface modification of UCN@$SiO_2(NH_2)$ with dendrimer and photocaged Dox (Scheme 2)

9 UCN_1@(G5FA) and **10** UCN_1@(Cy5)(G5FA): **7** $G5(FA)_6$ (3.3 mg, 0.0855 μmol) was added to a mixture of NHS (1.3 mg, 11.6 μmol) and DMAP (2.8 mg, 22.9 μmol) loaded in a plastic vial (2 mL size). To this mixture was added 1.0 mL of anhydrous DMF and then EDC (2.2 mg, 11.5 μmol). The mixture was shaken at room temp for 18 h. To a new plastic vial was loaded 10 mg of $UCN_1@SiO_2(NH_2)$ (d = 50 nm) and followed by addition of DMF (0.6 mL) and triethylamine (3.2 μL). This $UCN_1@SiO_2(NH_2)$ was gently shaken and sonicated for 30 s prior to the addition of the activated **7** $G5(FA)_6$ solution prepared above (0.6 mL). The mixture was shaken at room temp for 13 h, and divided into two vials, each in equal amount. For preparation of **9** UCN_1@(G5FA), the solution in one vial was treated with ethylenediamine (92 μL, 1.38 μmol) dissolved in DMF (0.2 mL) to quench the solution and the mixture was shaken at room temp for an additional 2 h. For preparation of **10** UCN_1@(Cy5)(G5FA), a solution of sulfo-Cy5 NHS ester (1.0 mg, 1.31 μmol) was prepared in DMSO (0.5 mL) and added to the solution in the other vial. The mixture was shaken at room temp for 24 h prior to quenching of the reaction with a solution of ethylenediamine (92 μL, 1.38 μmol) in DMF (0.2 mL) as described above.

For purification of UCN-dendrimer conjugate, each reaction mixture was diluted with methanol (0.5 mL) and spun down at 5,000 rpm (Fisher Scientific, microcentrifuge) for 15

min. The supernatant was removed by a micropipette and methanol (1 mL) was replenished to the residue. The mixture was sonicated for 30 s and spun at 5,000 rpm for 15 min. The supernatant was carefully removed and the rinsing procedure was repeated five more times. Each dendrimer-coated UCN_1 was dried under gentle nitrogen flow for 3 h, yielding **9** UCN_1@(G5FA) as a pale brown powder (4.0 mg) and **10** UCN_1@(Cy5)(G5FA) as a light blue powder (3.7 mg), respectively. Each modified UCN_1 was characterized by UV–vis absorption spectrometry: [**9** or **10**] = 1 mg dispersion per mL water (Scheme 3C). Presence of surface labeled sulfo-Cy5 in **10** UCN_1@(Cy5)(G5FA) was confirmed by fluorescence emission spectrometry: [**10**] = 0.25 mg dispersion per mL MeOH; λ_{ex} = 600 nm (Cy5), λ_{em} = 667 nm (Scheme 3C, top inset).

Treatment of UCN@$SiO_2(NH_2)$ with epibromohydrin. To 20 mg of UCN_x@$SiO_2(NH_2)$ (x = 1 or 2; d = 50 nm) dispersed in methanol (1.0 mL) was added epibromohydrin (0.031 mL, 0.362 mmol). This mixture was shaken at room temp for 14 h and spun down at 5,500 rpm for 15 min. The supernatant was carefully removed, and the collected UCN was resuspended in methanol (1.5 mL). The UCN was dispersed by sonication for 30 s, and spun down. The supernatant was removed and the remaining UCN was rinsed again using methanol (1 mL) containing 0.1 M NaOH (0.1 mL) by centrifugation and dried under nitroge.

12 UCN_2@(ONB-Dox). **4** ONB-Dox (TFA salt; 2.0 mg, 2.3 μmol) was weighed in an Eppendorf vial, and dissolved in methanol (1.0 mL). In order to neutralize its TFA salt, 0.1 M NaOH (0.069 mL) was added to the solution, which caused a color change from red to dark blue purple. A small aliquot (0.02 mL) of this solution was taken out and diluted with PBS pH 7.4 (1.0 mL). This diluted aliquot was set aside as a reference solution for quantitative analysis of **4** ONB-Dox consumed in the conjugation reaction with UCN_2. To the epibromonhydrin-treated UCN_2@$SiO_2(NH_2)$ pellet prepared above was added this neutralized solution (1.05 mL). The UCN_2 pellet was dispersed well in the solution and sonicated for 30 s. The mixture was shaken at room temp for 24 h, and followed by incubation in a water bath (45^{o}C) for an additional 48 h. After reaction, the mixture was spun at 5,500 rpm for 15 min. An aliquot (0.02 mL) was taken out from the supernatant (while leaving the excess supernatant in the reaction vial) and diluted with PBS pH 7.4 (1.0 mL). This diluted aliquot was analyzed later by UV–vis spectrometry along with the first aliquot solution prepared above. To quench the reaction, an excess amount of ethanolamine (0.044 mL, 0.0728 mmol) was added to the entire content (the supernatant and UCN_2 pellet) in the vial such that any epoxide residues remaining on the surface of UCN_2 reacted with the added amine. The

mixture was incubated at 45°C for 24 h and spun down at 5,500 rpm for 20 min. Its supernatant was carefully removed, and the UCN_2 pellet was rinsed five times with methanol (1.5 mL each) by centrifugation (5,500 rpm, 20 min). The pellet was dried under gentle nitrogen flow, resulting in **12** UCN_2@(ONB-Dox) as pale red powder (15 mg). To determine the amount of **4** ONB-Dox covalently attached to the UCN_2, UV–vis absorption was measured for each of the ONB-Dox aliquots taken before and after its conjugation reaction with UCN_2: A_{495} = 0.2073 (before); A_{495} = 0.1049 (after). On the basis of decrease in the absorption (ΔA= 0.1024; 49%), the total amount of **4** attached to UCN_2 was estimated as ~0.98 mg (%w/w (**4**/**12**) ≈ 6.5%). UV–vis (1.0 mg dispersion in 1.0 mL MeOH): λ_{max} = 234, 253, 498 (Dox), 533 (Dox) nm (Scheme 3D).

14 UCN_2@(ONB-Dox)(G5FA). A solution of DMF (0.5 mL) containing **7** G5(FA)$_6$ (2 mg; 51.8 nmol), NHS (1.0 mg, 8.89 μmol) and DMAP (1.1 mg, 8.89 μmol) was prepared in an Eppendorf vial. This mixture was sonicated for 30 s, and followed by addition of EDC (1.7 mg, 8.89 μmol) as solid. The final mixture was shaken at room temp for 12 h. An aliquot (0.03 mL) was taken out from this EDC-treated dendrimer solution and diluted with PBS pH 7.4 (1.0 mL) for comparative analysis by UV–vis spectrometry later. The remaining EDC-treated G5(FA)$_6$ solution (0.47 mL) was added to another plastic vial containing **12** UCN_2@(ONB-Dox) (15 mg). The mixture was shaken at room temp for 24 h and at 45°C for an additional 24 h. The mixture was centrifuged at 5,500 rpm for 20 min. An aliquot (0.03 mL) was taken out from the supernatant, and diluted with PBS pH 7.4 (1.0 mL) for UV–vis analysis. The supernatant solution was carefully removed by a micropipette, and the pellet was rinsed with methanol (2 × 1.0 mL) by centrifugation. This rinsing process was repeated three more times (1 × 50% aq methanol; 2 × methanol). The resulting pellet was dried under nitrogen atmosphere, affording **14** UCN_2@(ONB-Dox)(G5FA) as pale purple powder (14.8 mg). UV–vis analysis of the two aliquot samples, each taken before and after the reaction, showed ~9% decrease in absorption: ΔA_{355} = 0.2114 (before) − 0.1922 (after) = 0.01915. This analysis suggests ~9% of **7** G5(FA)$_6$ (total added in the reaction) conjugated to the UCN_2: %w/w (**7**/**14**) = 1.2%; 0.31 nmol of **7** per mg of **14**. UV–vis (1.0 mg dispersion in 1.0 mL MeOH): λ_{max} = 233, 253, 498 (Dox), 534 (Dox) nm (Scheme 3D).

11 UCN_1@(ONB-Dox): UCN_1@$SiO_2(NH_2)$ (18 mg) treated with epibromohydrin reacted with **4** ONB-Dox (1.8 mg) as described above for **12**. UCN_1@(ONB-Dox) was isolated as a pale red powder (15 mg). UV–vis comparative analysis of the two reaction aliquots showed ~29% decrease in absorption (A_{495} = 0.2017; A_{495} = 0.1487; Δ = 0.1487), suggesting that the

total amount of **4** ONB-Dox consumed for the conjugation to UCN was ~0.52 mg (%w/w (**4**/**11**) ≈ 3.5%). UV–vis (1.0 mg dispersion in 1.0 mL water): λ_{max} = 289, 331, 496 (Dox) nm.

13 UCN_1@(ONB-Dox)(G5FA): **11** UCN_1@(ONB-Dox) reacted with an EDC-activated solution of **7** $G5(FA)_6$ in the same way as described above for **14**, yielding UCN_1@(ONB-Dox)(G5FA) as a pale red powder. UV–vis (1.0 mg dispersion in 1.0 mL water): λ_{max} = 275, 496 (Dox) nm.

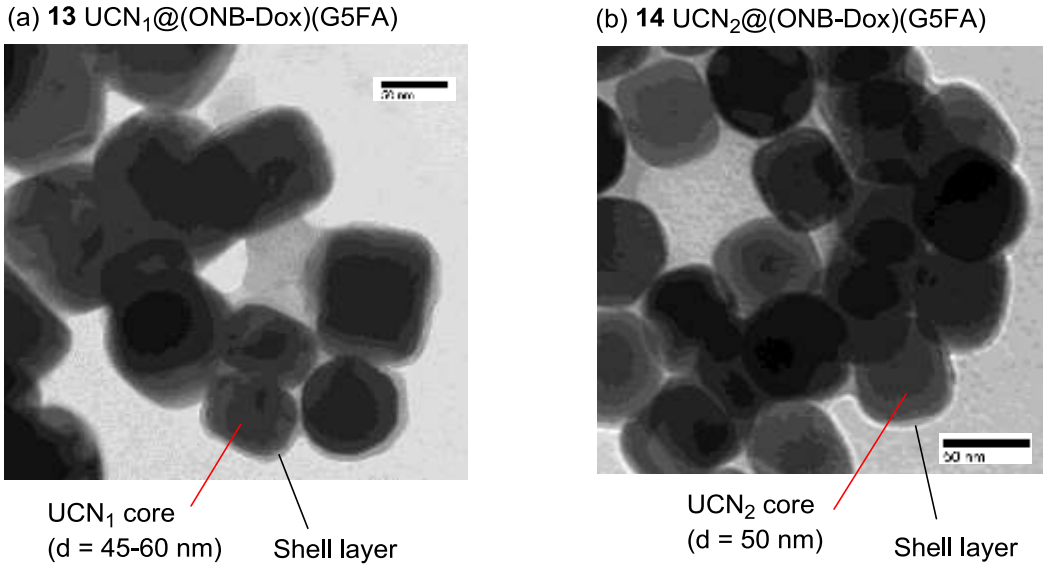


Figure S5. Transmission electron microscope (TEM) images of **13** UCN_1@(ONB-Dox)(G5FA) (A) and **14** UCN_2@(ONB-Dox)(G5FA) (B). For TEM imaging, a suspension of **13** or **14**, each dispersed in ethanol at 1.0 mg/mL was dropped onto the carbon film supported on the copper grid (3 mm in diameter; 300 mesh) and was dried at room temperature. This copper grid was mounted into the specimen chamber, and the TEM images were acquired with a JEOL 1400plus electron microscope (operated at 80 kV).

7. Surface Plasmon Resonance (SPR) Spectroscopy

The SPR experiments were carried out with a Biacore® X instrument (Pharmacia Biosensor AB). A CM5 sensor chip (Pharmacia Biosensor) coated with a carboxymethylated dextran layer (~100 nm) was immobilized with folate binding protein (FBP) in flow cell 1 (Fc1). This protein immobilization was achieved by the injection of the protein solution (1.67 mg/mL; 35 μL) to the Fc1 pretreated with EDC/NHS (1:1 mixture of 0.4 M EDC and 0.1 M NHS; 70 μL). The protein immobilization led to an increase in the response units (RU = 13087 ≈ 13.1 ng FBP/mm^2) relative to flow cell 2 (Fc2), a reference surface which was treated in the same way without FBP (Figure S6).

Each sensorgram was acquired by an injection of an analyte solution (50 μL) prepared in HBS-EP buffer at a flow rate of 30 μL/min. Data collection was performed until the end of the dissociation phase (~600 s after injection), and the chip surface was regenerated with 10 μL of 10 mM glycine–HCl (pH 2.5). For SPR data analysis, response unit (RU_1) recorded in Fc1 (FBP surface) was corrected to reduce the contribution made by the non-specific adsorption and changes in refractive index (the bulk effect). This correction was made by subtracting the value of RU_2 in Fc 2 (non-FBP reference): ΔRU (corrected) = $RU_1 - RU_2$. Kinetic analysis was performed to extract the rate of association (k_a), and the rate of dissociation (k_d) by a global fitting of each corrected sensorgram to a Langmuir model.[7] Equilibrium dissociation constant ($K_D = k_d/k_a$) was determined as a mean value (n ≥ 4 independent measurements) ± a value of standard deviation (SD).

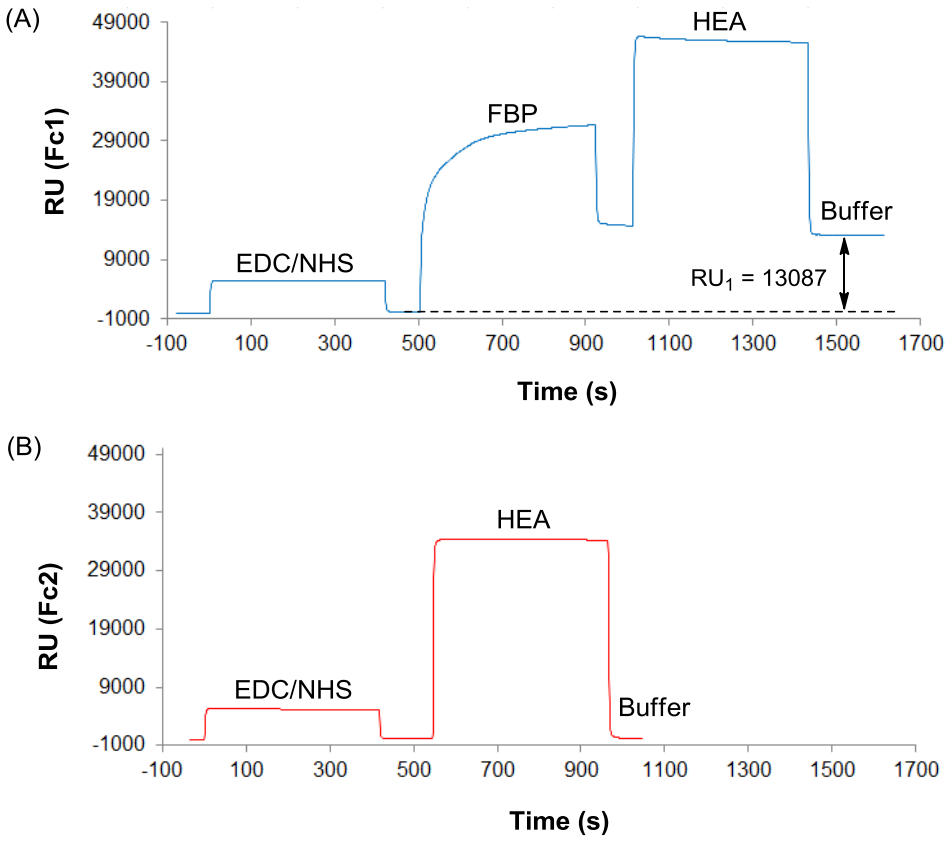


Figure S6. Preparation of a CM5 sensor chip immobilized with folate binding protein (FBP) for surface plasmon resonance (SPR) experiments. In this chip, the surface of flow cell 1 (Fc1) was immobilized with FBP (RU_1 = 13,090 ≈ 13 ng protein/mm^2) (A), and the surface of flow cell 2 (Fc2; reference surface) was treated in the same manner but without an injection of FBP ($RU_2 \approx 0$) (B). Abbreviations: EDC = *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, NHS = *N*-hydroxysuccinimide, HEA = 2-hydroxyethylamine.

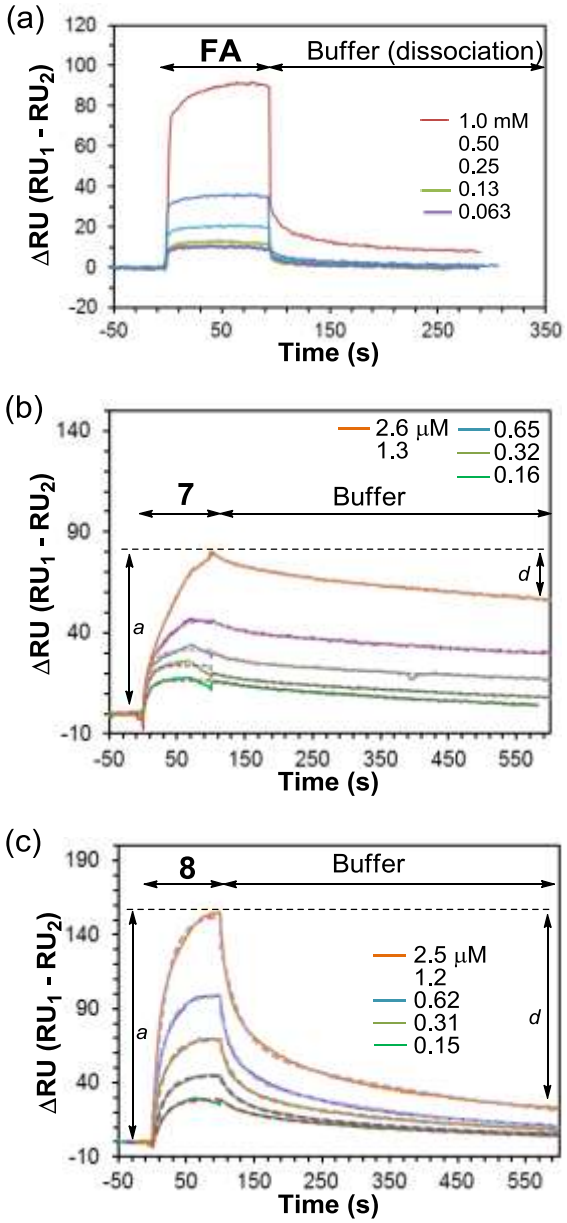


Figure S7. Surface plasmon resonance (SPR) sensorgrams of folic acid FA (a), **7** G5(FA)$_6$ (b) and **8** G5(FA)$_6$(ONB-Dox)$_{6.9}$ (c) with a CM5 sensor chip immobilized with folate binding protein (FBP). ΔRU (Response Unit) = RU_1 (Fc1) – RU_2 (Fc2): solid line (experimental); dotted line (simulated global fit). Abbreviation: a = adsorption; d = desorption

8. Light-controlled Drug Release

Kinetic studies of drug release by UV light exposure were performed by using a Spectroline® UV lamp device (XX-15A; emission wavelength with a maximal intensity at 365 nm).[3,8] Typically, a solution of **3** ONB-Dox or **8** G5(FA)$_6$(ONB-Dox)$_{6.9}$ was prepared in 10% aq MeOH (1.03×10^{-4} M) or water (2.48×10^{-5} M in water), respectively, and exposed to light at a distance of 5 cm. A series of aliquots were taken as a function of exposure time, and analyzed by HPLC (Figure 3), UV–vis spectrometry (Figure S8) or GPC (Figure 3) to determine the rate of Dox release from photocaged Dox.

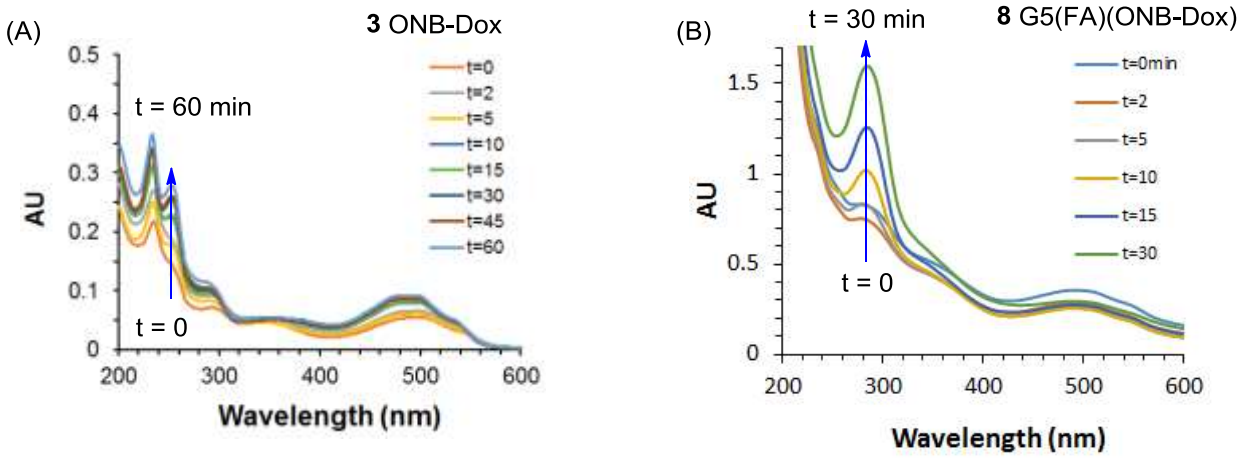


Figure S8. UV–vis spectral traces of **3** ONB-Dox (1.03×10^{-4} M in 10% aq methanol) and **8** $G5(FA)_6(ONB\text{-}Dox)_{6.9}$ (2.48×10^{-5} M in water) recorded after UV (365 nm) exposure as a function of exposure time.

9. *In vitro* Cell Studies

Cell Culture. The FAR-overexpressing (+) KB human epithelial carcinoma cell line (ATCC) was maintained in RPMI 1640 medium without folic acid (Life Technologies) in a 37°C incubator under 5% CO_2. This culture medium was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 IU penicillin, and streptomycin (100 µg/mL). All studies were performed with cells maintained in this FA deficient medium. The FAR(−) B16-F10 mouse melanoma cell line (ATCC) was maintained in the same medium with folic acid**.**

Confocal Microscopy. Confocal microscopy was performed on a Leica Inverted SP5X confocal microscope (Leica Biosystems).[9,10] FAR(+) KB cells were grown in a FA-free medium and seeded at a density of 5×10^4 cells/well on an 8-well chambered cover glass slide. Conjugates **9** and **10** were added to the cells in FA-free media at final concentrations of 50, 200, and 500 µg/mL and incubated for 2 h at 37°C. The treated cells were washed twice with PBS pH 7.4, fixed with 4% paraformaldehyde for 10 min, and then mounted in prolong gold with 4,6'-diamidino-2-phenylindole (DAPI: λ_{ex} = 350 nm; λ_{em} = 430–460 nm). Cy5 fluorescence was imaged at λ_{ex} = 640 nm; λ_{em} = 660–680 nm with a white light laser, and the UCN core was imaged by excitation with a NIR laser (980 nm) and the emission was measured at both 500–550 nm and 640–650 nm.

Flow Cytometry. Cellular association (binding and uptake) of **10** UCN_1@(Cy5)(G5FA) was determined by flow cytometry. A stock solution of **10** UCN_1@(Cy5)(G5FA) was

prepared in the FA free media at 1 mg/mL. FAR(+) KB or FAR(−) B16-F10 cells were trypsinized and resuspended in FA free media at a density of 2×10^5 cells/mL. The cells were treated with **10** at concentrations of 0, 5, 10 or 50 μg/mL, and incubated at 37°C for 1 h. The cells were collected and washed twice with FACS buffer (PBS, 0.1% BSA, 0.1% sodium azide) and then resuspended in 200 μL of FACS buffer. These cells were kept on ice and analyzed on a BD Accuri C6 flow cytometer (BD Accuri Cytometers, MI). The mean fluorescence intensity for the Cy5 channel was determined for the population of viable cells.

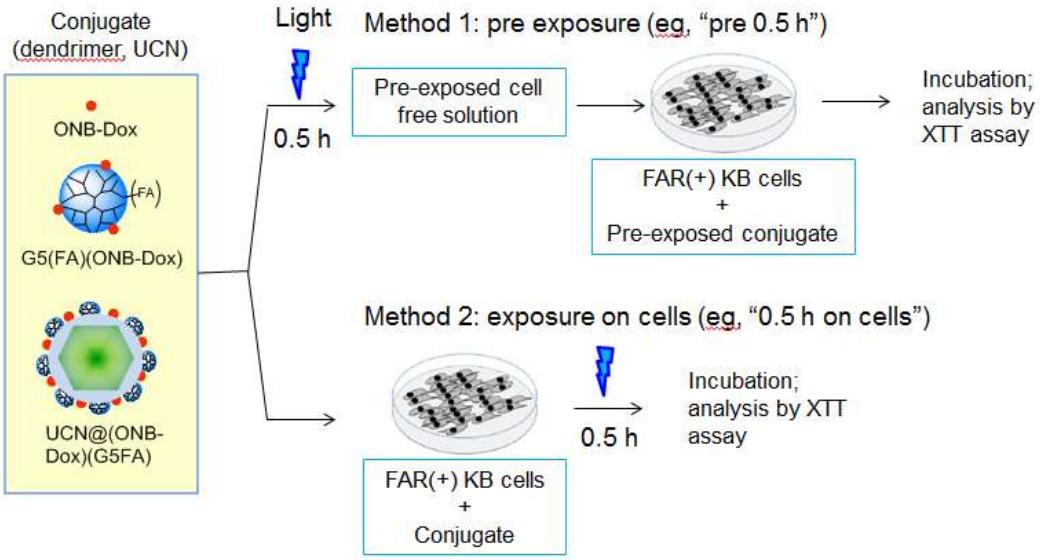


Figure S9. Illustration of light exposure conditions. (A) "Pre-exposure": each test solution was exposed to light for a certain duration prior to 1.5 h incubation with the cells; (ii) "Exposure on cells": cells treated with each test solution were incubated for a certain duration and then exposed to light for a specified amount of time.

***In Vitro* Light-controlled Cytotoxicity.** The cytotoxicity of Dox, **4** and the various UCNs (**8**, **13**, **14**) towards FAR(+) KB cells was determined by an XTT cell viability assay (Roche Life Science).[11,12] Briefly, KB cells (3×10^3) were seeded for two nights in FA-free RPMI 1640 media in 96-well microtiter plates. Cells were treated with the conjugates under three different treatment conditions as illustrated in Figure S9. For the "pre-exposure" condition, each test solution was exposed to light prior to addition to the cells. A solution of each test conjugate in in PBS/1% BSA (PBSB) (1 mL) was placed in a 24 well plate and the plate was exposed directly to a UV lamp (365 nm) at a distance of ~5 cm for 30 min. KB cells were rinsed once with PBSB, and each treated solution (50 μL) was added to the cells and incubated at 37°C for 1.5 h. Fresh medium (150 μL) was added to the cells to dilute the

conjugate by four, and the cells were incubated for an additional 24h. The conjugate containing media was then removed and replaced with fresh medium. The cells were allowed to incubate at 37^{o}C for 4 additional days prior to determination of cell viability. For the "exposure on cells" condition, cells were rinsed once with PBSB and the non-UV treated test conjugates in PBSB (50 μL) were to the cells. The cells in the test solution were exposed directly to a UV lamp at a distance of ~5 cm for 0.5 h or 1 h. The cells were allowed to incubate in an incubator for an additional 1 h or 0.5 h at 37^{o}C, respectively, before adding fresh media (150 μL). After further incubation for 24 h, the conjugate containing medium was replaced with fresh medium and incubated at 37^{o}C for 4 additional days prior to cell viability determination. Lastly, for the "near infrared (NIR) exposure on cells" condition, cells were rinsed once with PBSB, and **13** or **14** in PBSB (50 μL) was added at the indicated concentrations. The cells were incubated for 30 min at 37^{o}C, and the cells in the test solution were exposed directly to NIR laser light (980 nm, power output = 1 W; CNI Optoelectronics Tech) at room temp for 1 h. A fresh medium (150 μL) was immediately added and the cells were incubated for 24 h at 37^{o}C. Conjugate containing medium was removed and replaced with fresh medium. The cells were incubated at 37^{o}C for 4 additional days, prior to measurement of viability.

To measure viability, the medium in each test well was replaced with PBS buffer and an XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate) assay was performed according to a manufacturer's protocol (Roche Molecular Biochemicals, Indianapolis, IN).

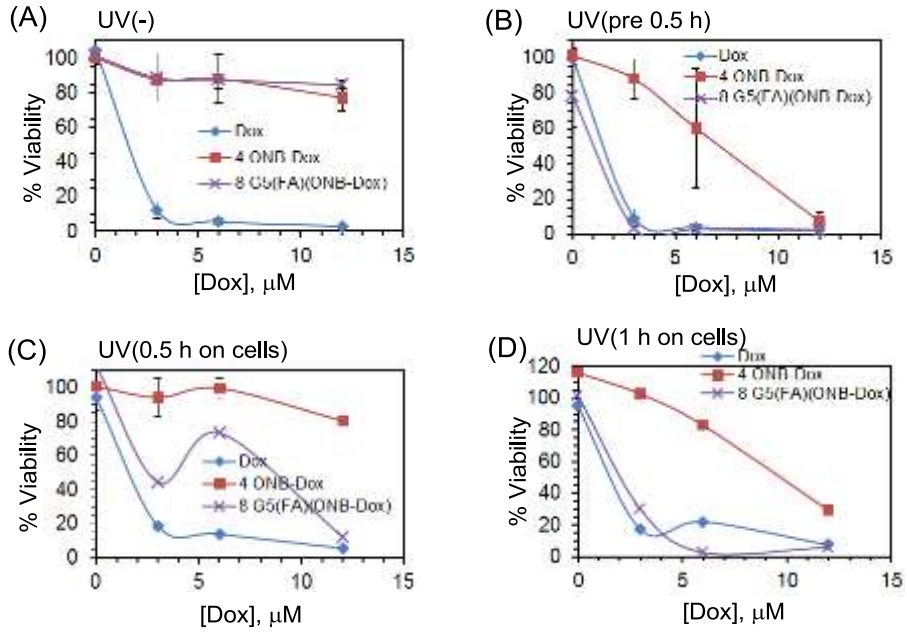


Figure S10. Light-controlled cytotoxicity of **4** ONB-Dox and **8** G5(FA)$_6$(ONB-Dox)$_{6.9}$ in FAR(+) KB cancer cells. (A–D) Viability (cell survival, %) of cells treated with free doxorubicin (Dox), **4** and **8** under various UV (365 nm) exposure conditions: no UV (A); pre 0.5 h (B); 0.5 h (C) and 1 h (D) on cells. The exposure condition labeled as "pre 0.5 h" refers to pre-exposure of the test solution to UV for 0.5 h prior to addition to and incubation with the cells. The condition labeled as "0.5 h or 1 h on cells" refers to the condition in which the cells were incubated with each test solution for 30 min prior to exposure of the cells with the test solution to UV for the indicated length of time (0.5 or 1 h). Under all conditions, cells were exposed to conjugate for a total of 1.5 h prior to dilution step and further overnight incubation.

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