Supporting Information

Efficient Targeting of Adipose Tissue Macrophages in Obesity with Polysaccharide Nanocarriers

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Supporting Tables and Figures

Table S1. Experimental Conditions for Synthesis of Dextran-TR Conjugates

Sample	Amino Dextran (mg)	Free Amines (μmol)	Triethylamine (TEA) (μmol)	5-TRITC (μmol)	TRITC/Amine Ratio
D-10-TR	10	1.16	71.5	1.13	0.97
D-70-TR	10	3.14	71.5	1.13	0.36
D-500-TR	5	0.9	71.5	1.13	1.25
D-70-PEG-TR	10	0.41	71.5	1.13	2.76

Table S2. Experimental Conditions for Synthesis of Dextran-NOTA Conjugates

Sample	Amino Dextran (mg)	Free Amines (μmol)	Triethylamine (TEA) (μmol)	p-SCN-Bn -NOTA (μmol)	NOTA/Amine Ratio
D-10-NOTA	40	4.64	71.5	5.57	1.2
D-70-NOTA	40	12.57	125.7	15.1	1.2
D-500-NOTA	10	1.8	71.5	3.6	2.0
D-70-PEG-NOTA	50	2.05	71.5	5.54	2.7

Table S3. Dextran-NOTA conjugate properties

Sample	M_n (kDa)	Glucose/Dextran	NOTA/Dextran*	NOTA/total glucose units
D-10-NOTA	**10**	**62**	**1.36**	**0.02**
D-70-NOTA	**70**	**432**	**21.8**	**0.05**
D-500-NOTA	**500**	**3086**	**113**	**0.04**
D-70-PEG-NOTA	**370**	**432**	**16.9**	**0.04**

*determined by ICP-MS

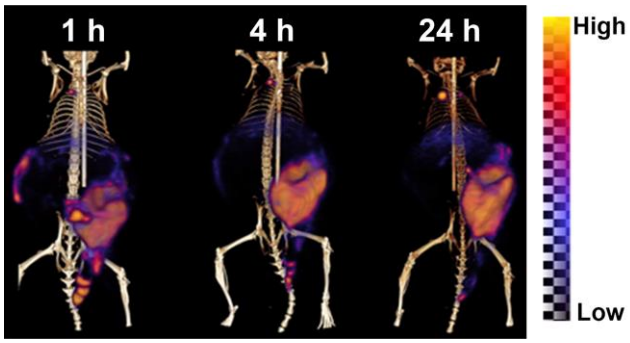


Figure S1. PET/CT images of obese C57BL/6J mice after intraperitoneal injection of D-70-rad conjugate, 1 hr, 4 hr, and 24 hr after injection.

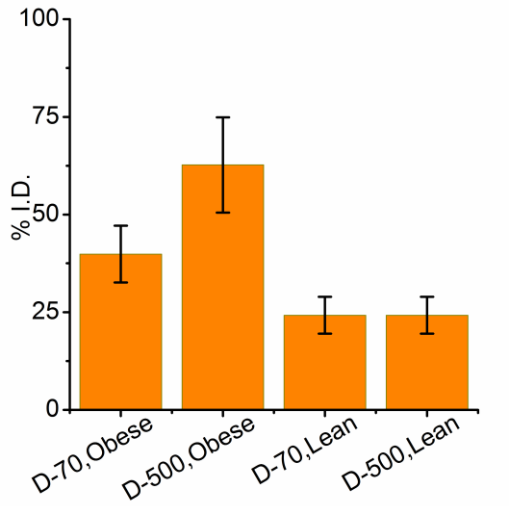


Figure S2. Biodistribution of ^{64}Cu-labeled D-70 and D-500 conjugates injected i.p. in lean and obese mice by total injected dose (% I.D.). Mesenteric AT, perirenal AT, and gonadal AT from both sides were isolated 24 hr after injection; the summed uptake amounts are plotted. n=4-5.

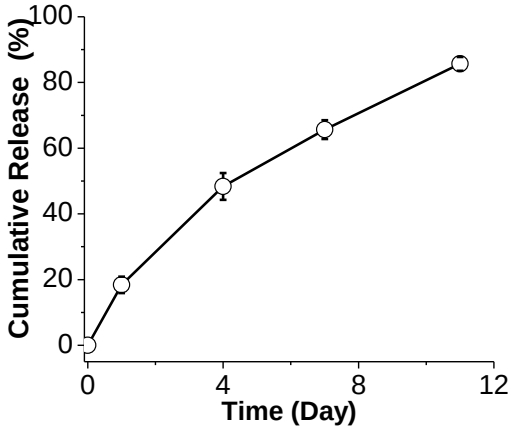


Figure S3. Time-course release of dexamethasone (and succinylated dexamethasone) from D-70-drug in PBS (pH 7.4) at 37°C. n=3.

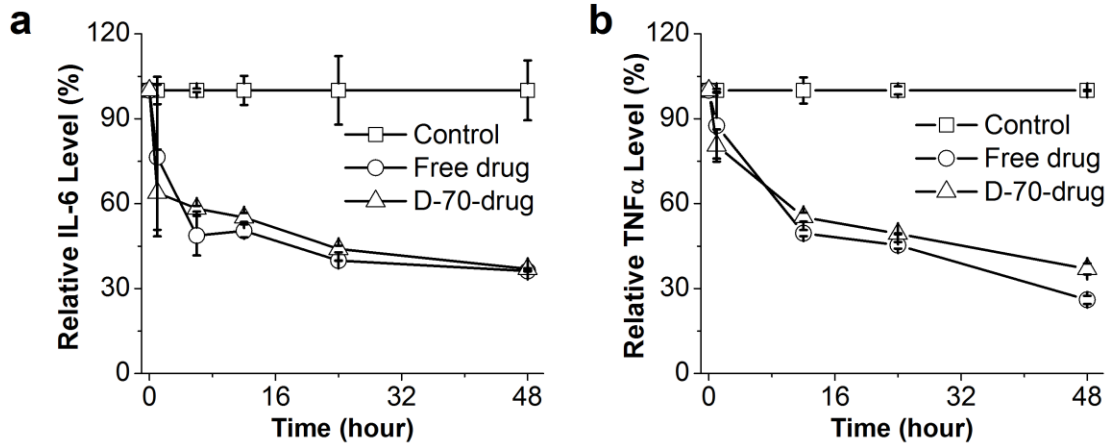


Figure S4. Relative IL-6 (a) and TNFα (b) expression in M1 macrophages in presence of vehicle control, free drug, or D-70-drug. n=2.

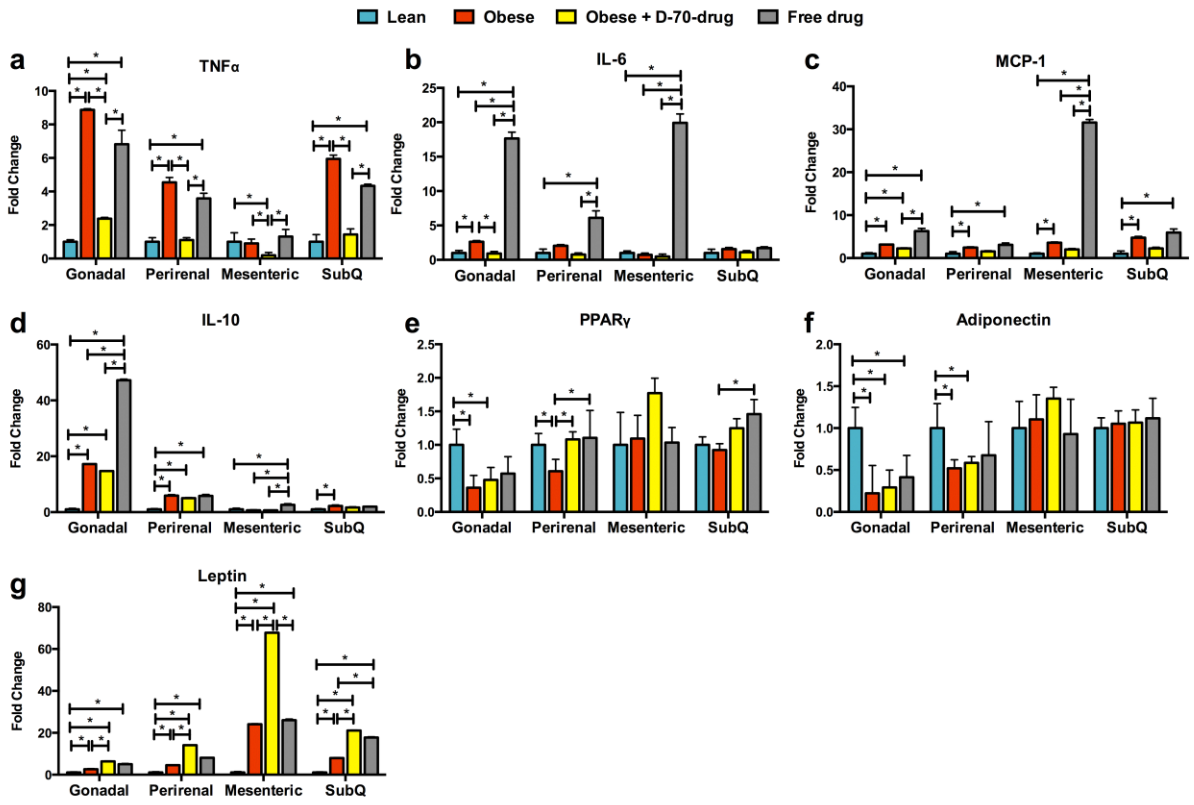


Figure S5. *In vivo* anti-inflammatory effects of free drug and D-70-drug in obese mouse VAT. (a-g) Relative mRNA-level expression of genes in 4 adipose depots, comparing lean mice (relative expression = 1) with obese mice and obese mice treated with either D-70-drug or free drug. All data are shown as in Figure 4 of the main text, but with the *obese + free drug* group added for comparison. Values of significance are indicated as *lean vs*. *obese*, *obese vs*. *obese + D-70-drug, lean* vs. *obese + free drug, obese + D-70-drug* vs. *obese + free drug, obese vs. obese + free drug* for $p < 0.05$.

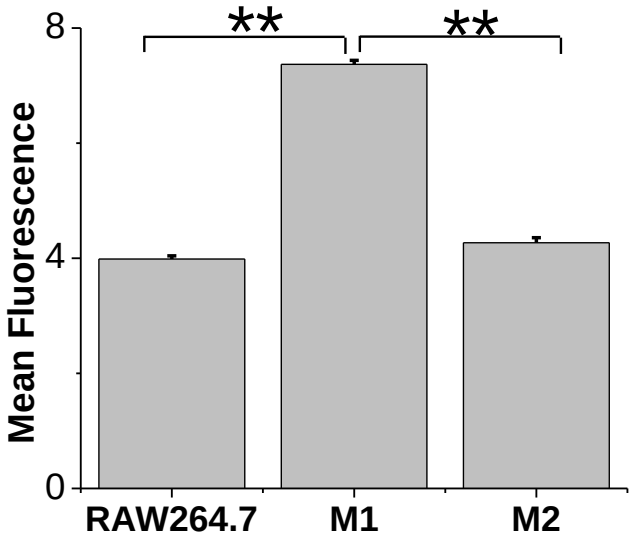


Figure S6. *In vitro* cell uptake of D-70-fluor by M1, M2 and nonactivated RAW 264.7 cells was measured as mean fluorescence of treated cells *via* flow cytometry. (**) indicates $p < 0.01$.

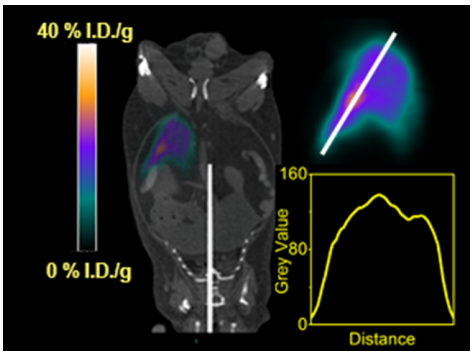


Figure S7. Obese C57BL/6J mice injected i.p. with D-70-rad conjugate. After 24 hours, coronal section of PET/CT image showed even distribution in adipose tissue (see grey value plot in bottom right inset).

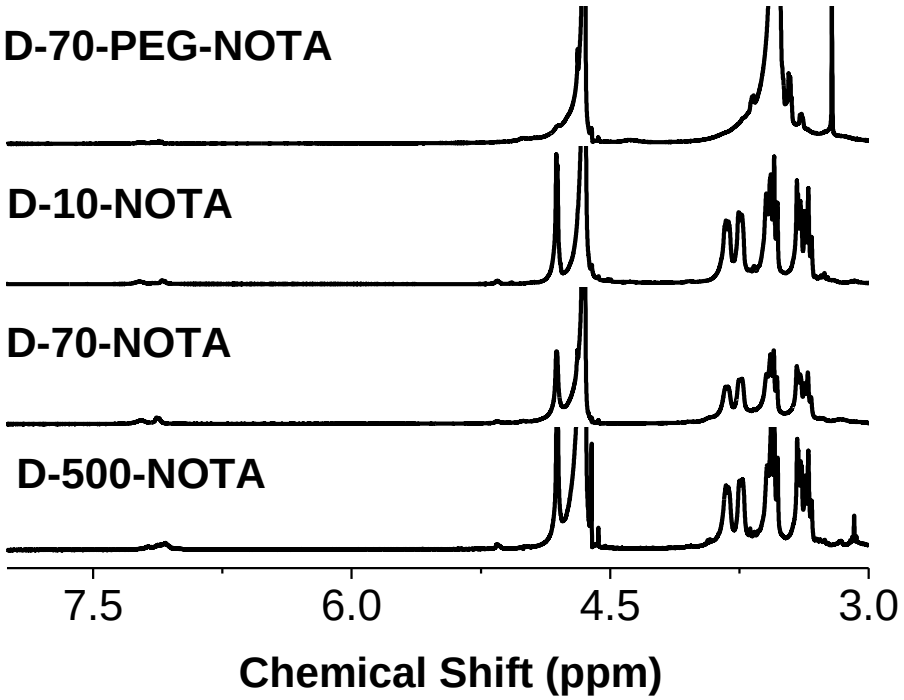


Figure S8. ^{1}H NMR of NOTA-dextran conjugates in D_2O.

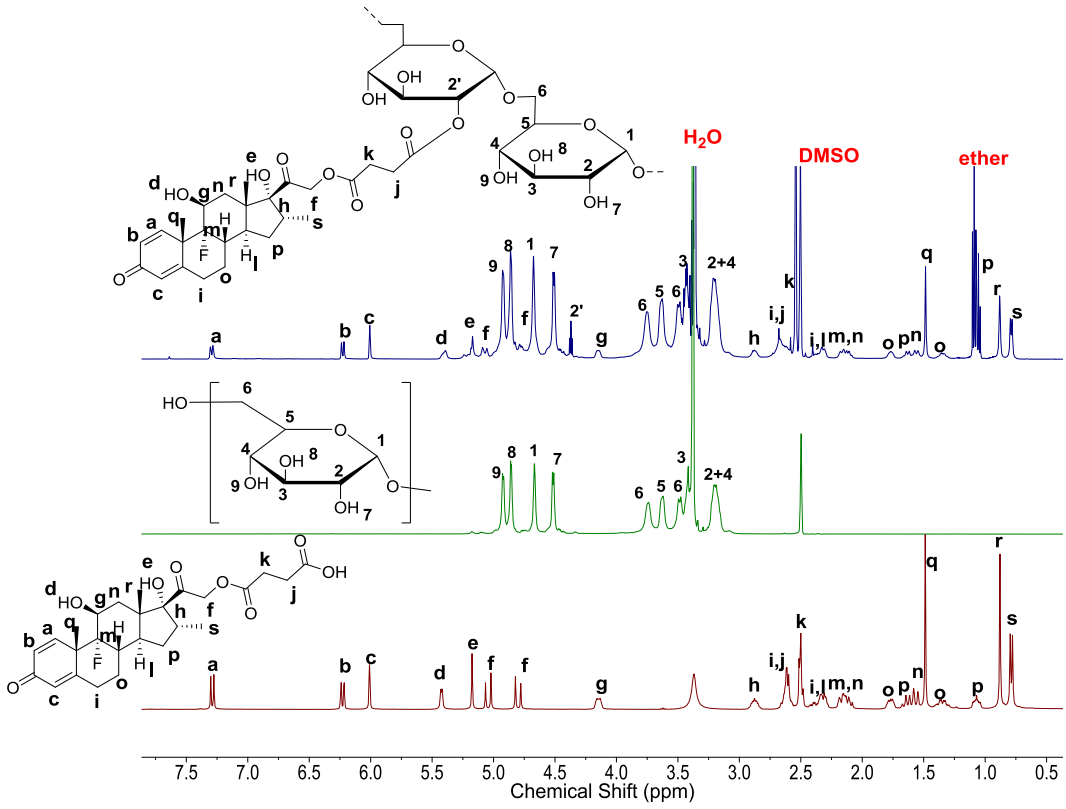


Figure S9. ^{1}H NMR spectra of D-70-drug conjugate, dextran and DSA (from top to bottom) in DMSO-d_6.

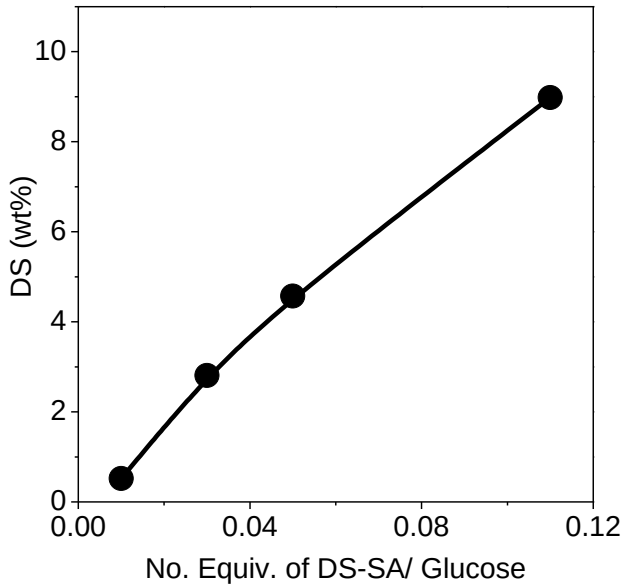


Figure S10. Weight percentage of dexamethasone in D-70-drug with different feeding ratios of dexamethasone succinic acid (DSA) and dextran (D-70) determined by UV-Vis spectroscopy.

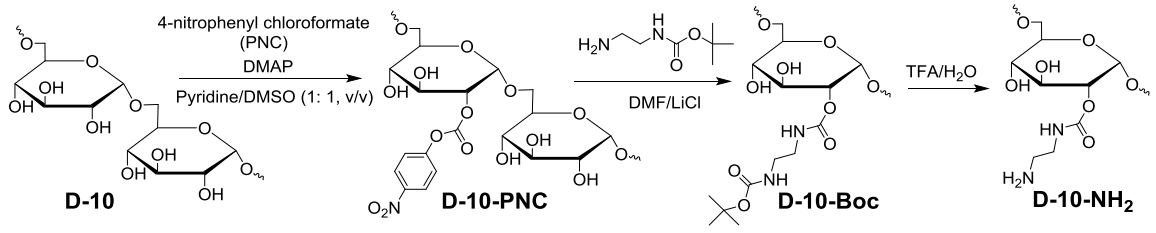
Supporting Notes

Note S1: Materials

Commercial Sources. Dextran (MW = 10 kDa and MW = 70 kDa), 4-(dimethylamino)pyridine (DMAP), 4-Nitrophenyl chloroformate (PNC), lithium chloride (LiCl), trifluoroacetic acid (TFA), triethylamine (TEA), succinic anhydride, ethylenediaminetetraacetic acid (EDTA) and fluorescamine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous dimethyl sulfoxide (DMSO) was purchased from Macron Fine Chemicals (Center Valley, PA, USA). Anhydrous pyridine was purchased from EMD Chemicals. Anhydrous dimethylformamide (DMF) was purchased from Alfa Aesar (Ward Hill, MA, USA). CH_3O-PEG-NH_2 (MW = 2 kDa) and H_2N-PEG-NH-Boc (MW = 3 kDa) were purchased from Rapp Polymere (Tuebingen, Germany). Amino dextran (MW = 70 kDa and MW = 500 kDa) was purchased from Life Technologies (Grand Island, NY, USA). Dexamethasone was purchased from Cayman Chemical Company (Ann Arbor, Michigan, USA). Tetramethylrhodamine-5-isothiocyanate (5-TRITC) was obtained from Setareh Biotech (Eugene, OR, USA). p-SCN-Bn-NOTA was purchased from Macrocyclics (Dallas, TX, USA). Spectra/Por RC dialysis tubing with a molecular weight cut-off (MWCO) of 1 kDa and 2 kDa was purchased from Spectrum Laboratories (Rancho Dominguez, CA, USA). Dextran and LiCl were dried at 80°C under vacuum for 4 hours before use. All other solvents were used as received. Mono-boc protected diethylamine (BocEDA) was prepared according to the literature.[1]

Dextran Conjugates 1: D-10 Conjugates

D-10 conjugates were synthesized from D-10-NH_2, which was synthesized as depicted in Supporting Scheme 1.[2-4]



Supporting Scheme 1. Synthetic route for D-10-NH_2

D-10-PNC. D-10 (2 g) was added to DMSO/Pyridine (80 mL, v/v, 1/1) with stirring at 0°C, followed by slow addition of DMAP (72 mg, 589.3 µmol) and PNC. The reaction mixture was

stirred at 0°C for 4 hr, and the product was precipitated in excess ethanol/ether (800 mL, v/v, 1/1). D-10-PNC was then collected by filtration, washed with excess ethanol and diethyl ether, and dried under vacuum. Yield: 1.8 g (80%). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.5 and 8.3 (dd, aromatic protons), 5.3 and 5.5 (s, dextran glucosidic protons at positions which have nitrophenyl substituents), 4.50, 4.84, and 4.91 (s, dextran hydroxyl protons), 4.66 (s, dextran anomeric proton), 3.10 – 3.89 (m, dextran glucosidic protons).

D-10-Boc. D-10-PNC (0.8 g) was dissolved in DMF (10 mL) containing LiCl (1 w/v %). BocEDA (64 mg, molar ratio of amine to PNC group = 6.5) was dissolved in DMF (1 mL) and added to the dextran solution. The reaction was stirred for 12 hr at room temperature. D-10-Boc was recovered by precipitation in ethanol, washed several times with ethanol and ether, and finally dried under vacuum. Yield: 0.6 g (80%). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.45 and 8.28 (dd, aromatic protons), 4.50, 4.84, and 4.91 (s, dextran hydroxyl protons), 4.66 (s, dextran anomeric proton), 3.10 – 3.89 (m, dextran glucosidic protons), 3.33 – 3.52 (m, -CH_2-CH_2-), 1.37 (s, -C(CH_3)$_3$).

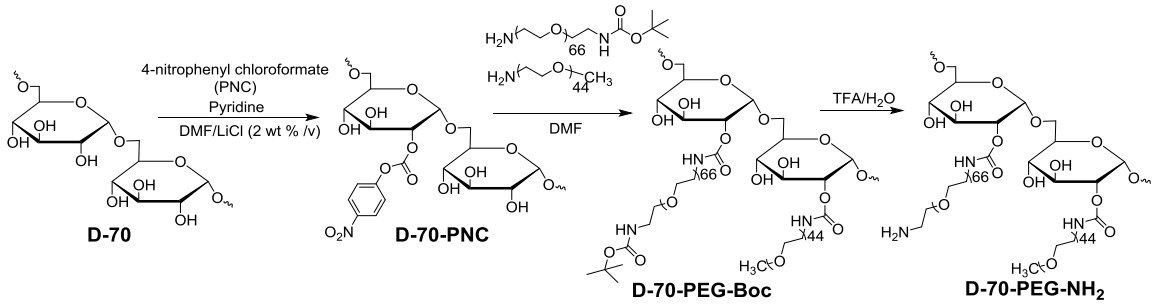
D-10-NH$_2$. D-10-Boc (0.8 g) was deprotected with 0.75 mL of TFA in 8.0 mL deionized water. The reaction was allowed to proceed overnight under N_2. After neutralizing with 4.0 M NaOH to pH 7.0, the mixture was dialyzed against water (MWCO 2 kDa). D-10-NH$_2$ was obtained as a solid white product after lyophilization. Yield: 0.16 g (20%). ^{1}H NMR (D_2O, 500 MHz): δ 4.81 (s, dextran anomeric proton), 3.31 – 3.89 (m, dextran glucosidic protons), 2.97 and 3.46 (m, -CH_2-CH_2-).

Dextran Conjugates 2: D-70 Conjugates

D-70 conjugates were synthesized from D-70-NH$_2$, which was purchased from a commercial source and used after narrowing of molecular weight distribution. A 40 mg/mL solution was prepared by dissolving 400 mg amino-dextran in 900 µL water and 100 µL 10X PBS. The solution was centrifuged at 7,000 *g* for 10 min and then filtered (0.22 µm pore size). Gel filtration chromatography was performed (flow rate: 0.5 mL/min, mobile phase: 1X PBS), with fractionation collection, and partial sample loop filling was used for sample loading. A 250 µL sample was loaded in a 500 µL sample loop and 5-8 fractions were combined each time. A total of 160 mL product was obtained, which was concentrated and desalted using an Amicon filter with molecular weight cutoff (MWCO) of 30 kDa. A solid white product was obtained after lyophilization.

Dextran Conjugates 3: D-PEG Conjugates

D-PEG conjugates were synthesized from D-70, as depicted in Supporting Scheme 2.



Supporting Scheme 2. Synthetic route for D-70-PEG-NH_2

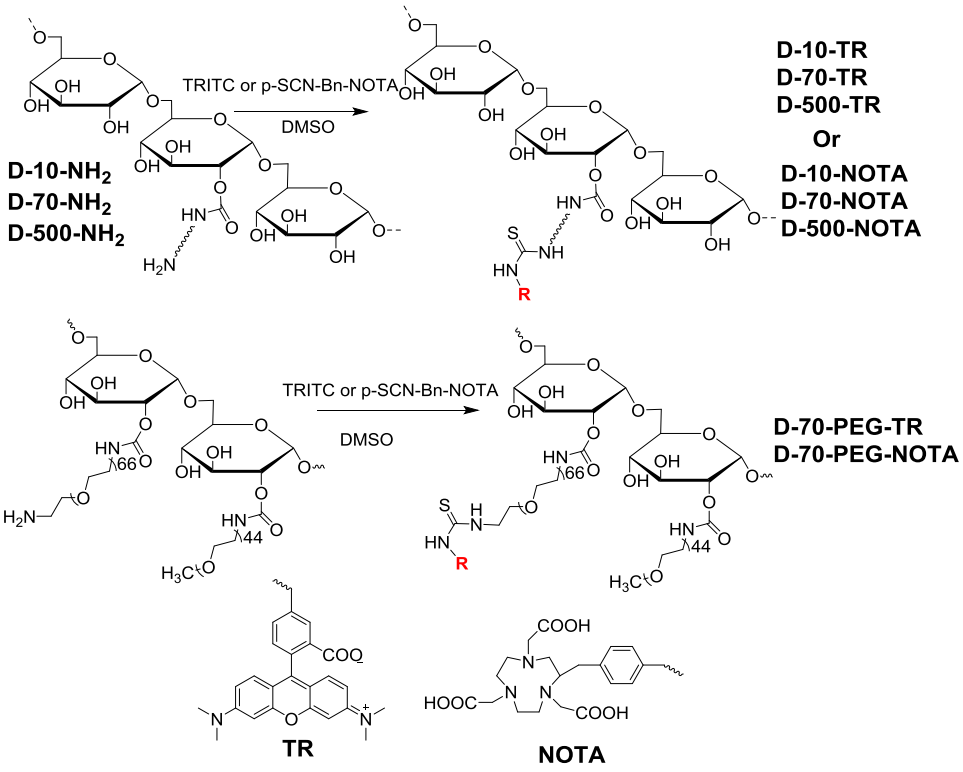
D-70-PNC. D-70 (0.2 g) was suspended in 20 mL DMF (dry) and LiCl (0.4 g, 2 w/v % of DMF) was added. The suspension was heated to 90°C until the solution became clear. Then the reaction mixture was cooled to 0°C and pyridine (249 µL, 3.08 mmol) was added, followed by PNC (622 mg, 3.08 mmol). The reaction mixture was stirred at 0°C for 4 hr, and D-70-PNC was obtained by precipitation from cold ethanol, washed with diethyl ether, and dried under vacuum.[5] Yield: 0.4 g (50%). M_n = 150 kDa; M_w/M_n = 1.24. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.51 and 8.28 (b, aromatic protons), 5.33 and 5.52 (s, dextran glucosidic protons at positions which have nitrophenyl substituents), 4.9, 4.8, and 4.5 (s, dextran hydroxyl protons), 4.7 (s, dextran anomeric proton), 3.10 – 3.89 (m, dextran glucosidic protons).

D-PEG-Boc. D-70-PNC (50 mg) was dissolved in anhydrous DMF (4 mL) under N_2. Then a solution of CH_3O-PEG-NH_2 (MW = 2 kDa, 367.8 mg) and H_2N-PEG-NH-Boc (MW = 3 kDa, 55.17 mg) in 1 mL DMF was added in one portion. The mixture was stirred at room temperature for 24 hr. The product was purified using an Amicon filter (MWCO 10 kDa) and freeze-dried to give the product D-70-PEG-Boc. Yield: 130 mg (30%). M_n = 370 kDa; M_w/M_n = 1.23. ^{1}H NMR (D_2O, 500 MHz): δ 4.81 (s, dextran anomeric proton), 3.31 – 3.89 (m, dextran glucosidic protons), 3.53 – 3.57 (b, -O-CH_2-CH_2-), 3.22 (s, -O-CH_3), 1.28 (s, -C(CH_3)$_3$).

D-PEG-NH_2. D-70-PEG-Boc (130 mg) was deprotected with TFA (0.3 mL) in 3.1 mL of deionized water overnight under N_2. After neutralization with 4.0 M NaOH to pH = 7.0, the mixture was purified by using an Amicon filter (MWCO 10 kDa). D-70-PEG-NH_2 was obtained as a solid white product after lyophilization. Yield: 80 mg (80%). ^{1}H NMR (D_2O, 500 MHz): δ 4.81 (s, dextran anomeric proton), 3.31 – 3.89 (m, dextran glucosidic protons), 3.53 – 3.57 (b, -O-CH_2-CH_2-), 3.22 (s, -O-CH_3).

Dextran Conjugates 4: D-fluor Conjugates

D-fluor conjugates were synthesized from D-NH_2, as depicted in Supporting Scheme 3.[6, 7]



Supporting Scheme 3. Synthetic route for D-fluor (D-TR) and D-NOTA conjugates

D-70-NH_2 (10 mg, 3.14 μmol free amine) was dissolved in 1 mL anhydrous DMSO with TEA (10 μL, 71.5 μmol) for 4 hr. Then 5-TRITC (0.5 mg, 1.13 μmol, 0.36 equivalents of amines) in 0.5 mL DMSO was added. The mixture stirred for 14 hr in the dark. The polymer was purified by repeated concentration and dilution (at least 8 times) with an Amicon filter (MWCO 30 kDa) in the dark. The tetramethylrhodamine (TR) conjugate was obtained after lyophilization (8 mg). Syntheses of other dextran-TR conjugates were similar except dialysis (MWCO 1 kDa) was used for purification. Detailed experimental conditions for synthesis of other dextran-TR conjugates are provided in Table S1.

Dextran Conjugates 5: D-NOTA Conjugates

D-NOTA conjugates were synthesized from D-NH_2, as depicted in Supporting Scheme 3. D-70-NH_2 (40 mg, 12.47 μmol free amine) was dissolved in 2 mL anhydrous DMSO, and anhydrous TEA (17.53 μL, 125.7 μmol) was added. Then p-SCN-Bn-NOTA (8.4 mg, 15.1 μmol, 1.2 equivalents of amines) in 500 μL DMSO was added and the mixture was left at room

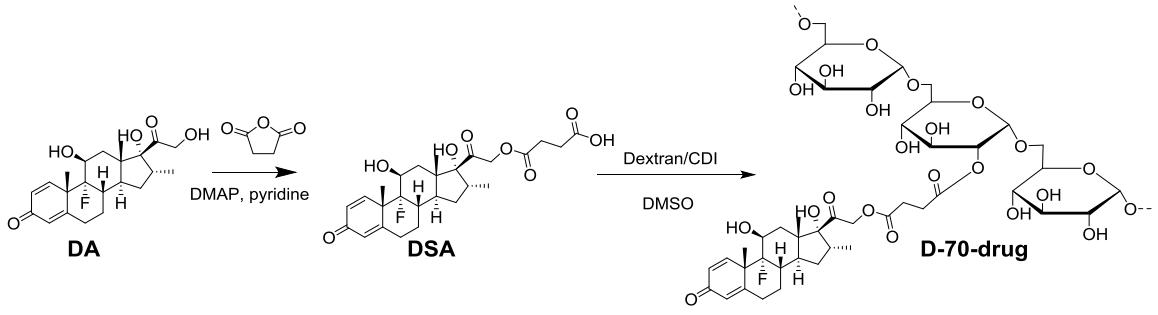
temperature for 16 hr. The product was purified using an Amicon filter (MWCO 30 kDa) and the solid D-70-NOTA was obtained after lyophilizaiton. Yield: 30 mg (70%). ^{1}H NMR (D_2O -d_6, 500 MHz): δ 7.1 and 7.2 (dd, aromatic protons), 4.8 (s, dextran anomeric proton), 3.3 – 3.9 (m, dextran glucosidic protons). For other NOTA conjugates, dialysis (MWCO 1 kDa) was used for purification and solid samples were obtained after lyophilization. Detailed experimental conditions for synthesis of other dextran-NOTA conjugates are provided in Table S2.

Dextran Conjugates 6: D-rad Conjugates

A solution of D-NOTA in NH_4OAc buffer (100 µL, 5 mg/mL, pH = 5.5, 0.1 M) was mixed with a solution of ^{64}Cu chloride (750 µCi, obtained from Washington University at St. Louis, MO, USA). The mixture was incubated for 10 min at 60°C before nonradioactive copper (equivalent molar amount to NOTA in the dextran conjugate) was added to saturate remaining NOTA. The mixture was then incubated for 20 min at 60°C. EDTA (2 equivalent of total copper amount) was added to scavenge nonspecifically bound copper or free copper and incubated at 60°C for another 10 min. Free copper (^{64}Cu and nonradioactive Cu), EDTA, and EDTA-copper chelates were removed and buffer was exchanged to 1X PBS using an Amicon filter (MWCO 3 kDa). To assess sample purity, a small aliquot (10 µCi) of solution was reacted with a large excess of EDTA (1000: 1 molar excess) and incubated at 60°C for 15 min. Solutions were then analyzed by radio-TLC in an EtOH/Ammonium Acetate (1:1, v/v) mobile phase. R_f (^{64}Cu-EDTA): 0.84; R_f (^{64}Cu-D-10): 0.16; R_f (^{64}Cu-D-70): 0.03; R_f (^{64}Cu-D-500): 0.07, R_f (^{64}Cu-D-70-PEG): 0.04. Samples with radiochemical purity (RCP) greater than 90% were used for imaging experiments.

Dextran Conjugates 7: D-drug Conjugates

D-drug conjugates were synthesized from dexamethasone succinic acid and dextran, as depicted in Supporting Scheme 4.



Supporting Scheme 4. Synthetic route for D-70-drug conjugates

Dexamethasone Succinic Acid (DSA). Dexamethasone (1.0 g, 2.55 mmol) was dissolved in pyridine (25 mL). A solution of succinic anhydride (0.77 g, 7.65 mmol) and DMAP (31 mg, 0.255 mmol) in pyridine (10 mL) was added to the dexamethasone solution. The reaction was stirred overnight under nitrogen at room temperature. Pyridine was then removed under vacuum and 40 mL water was added into the residue. Then mixture was stirred for 10 min and then centrifuged. The resulting precipitate was washed again with H_2O. DSA was collected as a white powder[8]. Yield: 1.0 g (80%). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.24 (dd, 1H, -CH=C*H*-CH_2-), 6.19 (dd, 1H, -C*H*=CH-CH_2-), 5.97 (t, 1H, -C*H*=C<), 5.41 (s, 1H, -O*H*), 5.13 (s, 1H, -O*H*), 5.00 (dd, 2H, -CH_2-O-), 4.76 (dd, 1H, -CH_2-O-), 4.10 (dd, 1H, >C*H*-OH), 2.83 (t, 1H, -C-C*H*<), 2.60 – 2.48 (m, 2H, -CH_2-CH_2-, -CO-CH_2-CH_2-), 2.47 – 2.41 (s, 2H, -CO-CH_2-CH_2-), 2.41 – 2.21 (m, 2H, -CH_2-CH_2-,-C*H*-CH-), 2.18 – 2.01 (m, 2H, HO-CH-CH_2-, -CH-CH_2-), 1.73 (dd, 1H, -CH_2-CH_2-), 1.65 – 1.42 (m, 5H, -CH_3, -CH-CH_2-CH_2-, HO-CH-CH_2-), 1.30 (m, 1H, -CH_2-CH_2-), 1.03 (dd, 1H, -CH-CH_2-CH_2-), 0.84 (s, 3H, >C-CH_3), 0.74 (dd, 3H, >CH-CH_3).

D-70-drug. DSA (0.5 mmol, 0.246 g) and 1,1′-carbonyldiimidazole (0.162 g, 1 mmol) were dissolved in anhydrous DMSO (2.5 mL). The resulting mixture was stirred for 1 hr and then a solution of dextran (0.75 g, 5%, w/v) in anhydrous DMSO (15 mL) was added. TEA (1.5 mL) was added to catalyze the reaction. The solution was sealed under nitrogen and allowed to react for 36 hr. The dextran conjugate was precipitated by dilution with 30 mL ethanol/ether (50: 50, v/v). The precipitate was dispersed in ethanol and centrifuged to collect the polymer, which was then washed with anhydrous ether three times and dried under vacuum. The polymer was purified by redissolution in anhydrous DMSO (10 mL) and precipitation from ethanol (30 mL). The product was washed with ethanol and ether and dried under vacuum to yield a white powder[9]. Yield: 0.9 g (90%).

^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.50, 4.84, and 4.91 (s, dextran hydroxyl protons), 4.66 (s, dextran anomeric proton), 3.10 – 3.89 (m, dextran glucosidic protons), 7.24 (dd, -CH=C*H*-CH_2-, 1H), 6.19 (dd, -C*H*=CH-CH_2-, 1H), 5.97 (t, -C*H*=C<, 1H), 5.41 (s, -O*H*, 1h), 5.13 (s, -O*H*, 1H), 5.00 (dd, -CH_2-O-, 1H), 4.76 (dd, -CH_2-O-, 1H), 4.10 (dd, >C*H*-OH, 1H), 2.83 (t, -C-C*H*<, 1H), 2.60 – 2.48 (m, -CH_2-CH_2-, -CO-CH_2-CH_2-, 2H), 2.47 – 2.41 (m, -CH_2-CH_2-, -CO-CH_2-CH_2-, 2H), 2.41 – 2.21 (m, -CH_2-CH_2-,-C*H*-CH-, 2H), 2.18 – 2.01 (m, HO-CH-CH_2-, -CH-CH_2-, 2H), 1.73 (dd, -CH_2-CH_2-, 2H), 1.65 – 1.42 (m, -CH_3, -CH-CH_2-CH_2-, HO-CH-CH_2-, 3H), 1.30 (m, -CH_2-CH_2-, 1H), 1.03 (dd, -CH-CH_2-CH_2-, 1H), 0.84 (s, >C-CH_3, 3H), 0.74 (dd, >CH-CH_3, 3H). To tune the dexamethasone content, different feeding ratios of DSA and dextran were used (see Supporting

Figure S10). A conjugate with 4.57 wt% dexamethasone was used in the reported therapeutic studies.

Note S2: Conjugate Characterization

Amination Analysis. Quantitative analysis of primary amine incorporation into dextran was performed using a fluorescamine assay. Briefly, a 10 mg/mL solution of fluorescamine in anhydrous DMSO was freshly prepared, and glycine standards (0 – 1.0 mM) were prepared in deionized water. The assay was initiated by mixing 380 µL water, 70 µL fluorescamine solution, 50 µL sample or standard, and 50 µL of 500 mM sodium borate buffer (pH 8.5). After 20 min of reaction in the dark, the fluorescence intensity at 470 nm, with 380 nm excitation was measured using a fluorescence microplate reader (BioTek Synergy HT).

Verification of NOTA Content. D-70-NOTA (5 mg, 1.571 µmol of NOTA) was dissolved in 1 mL NH_4OAc buffer (pH 5.5). Then 40 mM $CuCl_2$ (100 µL, 4 µmol) was added and the reaction mixture was stirred overnight at room temperature. Free copper was removed by dialysis against distilled water (MWCO 1 kDa). The sample was then lyophilized and weighed, and ICP-MS was performed on a sample of known weight.

Note S3: Animal Studies

PET/CT Imaging. Mice were injected i.p. with D-rad conjugates with different sizes (notated as D-10-rad, D-70-rad, D-500-rad, D-PEG-rad; each mouse received ~100 µCi radioactivity). Micro-PET/CT imaging was performed with a small animal dedicated Inveon PET-CT system (Siemens Healthcare, USA). Mice were anesthetized with isoflurane (1-3 %) and placed on the imaging bed and anesthesia was maintained for the duration of the imaging procedure. Static micro-PET scans (15 min acquisition time) were acquired at selected time points (1, 4, and 24 hr post-injection) in combination with micro-CT scans for anatomical co-registration. The obtained micro-PET and micro-CT images were reconstructed using ordered subset expectation maximization (OSEM) and cone-beam algorithms with existing commercial software (Inveon Acquisition Workspace and Cobra Exxim, respectively).

Tissue Radioactivity Measurements *via* Gamma-Counting. Post-mortem measurements of radioactivity were conducted using a Wizard2 Automatic γ-counter (Perkin-Elmer, USA). Mice

were euthanized and dissected after the final micro-PET/CT imaging session (24 hr after injection). All major organs and tissues were collected, weighed and measured for radioactivity using the appropriate energy window centered at 511 keV for ^{64}Cu. Raw counts were corrected for background, decay, and weight. Corrected counts were converted to micro-curie (μCi) per gram using a previously determined calibration curve based on ^{64}Cu aliquot standards. Activity in each collected tissue sample was calculated as percentage of injected dose per gram of tissue (%I.D./g). For this calculation, the radioactivity in tissue was corrected for decay at the time of γ-well counting. Data were presented as %I.D./g (mean ± standard error).

Note S4: Drug Release Kinetics

Drug conjugates were dissolved in 1X PBS (pH 7.4) and incubated at 37°C in a Pur-A-Lyzer (MWCO 3.5 kDa) (Aldrich, Catalog No. PURD35050-1KT), immersed in 20 mL buffer solution. After specific time intervals (0, 1, 4, 7, and 11 days), 1 mL of the buffer solution was withdrawn and 1 mL of fresh buffer solution was added to maintain a constant volume. The total concentration of dexamethasone and dexamethasone succinic acid was measured by UV-Vis absorption. Each experiment was repeated three times. The cumulative drug release percentage (E_r) was calculated using the following equation:

$$E_r(\%) = \frac{V_t \sum_1^{n-1} C_i + V_0 C_n}{m_{Dex}} \times 100\%$$

Where m_{Dex} represents the mass of dexamethasone in the conjugate, V_0 is the total volume (V_0 = 20 mL), V_t is the volume of the replaced media (V_t=1.0 mL), and C_n is the total concentration of dexamethasone and dexamethasone succinic acid in the sample.

Note S5: Cell Culture Studies

Flow Cytometric Analysis of Macrophage Endocytosis of Dextran Conjugates. RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) at 37°C with 5% CO_2. To induce M1 or M2 polarization, cells seeded in 12-well plates (3×10^5 cells/well) were grown in the presence of 100 ng/mL LPS (Sigma Aldrich, Product No. L2880) or 20 ng/mL IL-4 (PeproTech, Rocky Hill, NJ), respectively, for 24 hr. Then dextran-Texas Red (MW = 70 kDa, Life Technologies, Catalog No. D1830, 100 μL, 10 mg/mL) was added to the wells and the cells

were incubated for an additional 4 hr. Cells were then washed, gently harvested using a cell scraper, and transferred to microcentrifuge tubes on ice. The cells were washed with 500 µL cold wash buffer (PBS, 2% FBS, 0.1% sodium azide) and collected by centrifugation. This was repeated three times and then the cells were resuspended in 500 µL ice cold flow cytometry buffer (PBS, 10% FBS, 0.1% sodium azide). At least 10,000 cells/sample were analyzed using a BD LSRFortessa^{TM} cell analyzer (BD Biosciences).

***In vitro* Cellular Anti-Inflammatory Phenotype Analysis.** RAW 264.7 cells were seeded on 24-well plates at 2×10^4 cells/well and cultured for 24 hr. The culture medium was exchanged with complete medium supplemented with 100 ng/mL LPS to induce M1 polarization. After 24 hr, the medium was exchanged with freshly prepared complete medium supplemented with 100 ng/mL LPS and 1 µM of either free dexamethasone solution, D-drug conjugate solution (1 µM free drug concentration) or vehicle as a control. Conditioned medium was collected at different time points (1, 6, 12, 24, 48 hr) for quantification of extracellular TNFα or IL-6 production by enzyme-linked immunosorbent assays (ELISA, R&D, USA). The impact of drugs was measured as the percent of TNFα or IL-6 detected divided by the percent detected in control cells which did not receive drug treatment.

Note S6: Histologic Evaluation

Portions of VAT were removed at the time of euthanasia and placed in 10% neutral buffered formalin for 24 hr at a volume of at least ten parts formalin to one part tissue. Tissues were then transferred to 80% ethanol for storage at 4°C until trimming. Tissues were processed in graded alcohol through xylene, then imbedded in paraffin blocks. Tissues were sectioned at 3 µm thickness and placed on slides, deparaffinized and stained with Hematoxylin/Eosin using an automated staining procedure. Slides were evaluated subjectively in blinded fashion by a board-certified veterinary pathologist (Wallig) for the presence or absence of lesions, including adipocyte degeneration / necrosis and interstitial inflammation, characterized by increased interstitial cellularity due to the presence of macrophages, lymphocytes and/or neutrophils.

Note S7: Instrumentation

NMR spectra were recorded using a Varian U500 or VXR500 500 MHz NMR spectrometer. UV-Vis absorption spectra were recorded using a Cary 5000 UV-Vis-NIR spectrophotometer from Agilent Technologies (Santa Clara, CA, USA). Size-exclusion chromatography (SEC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS multi-angle laser light scattering (MALLS) detector, and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (50 Å, 100 Å, 500 Å, and 10^3 Å Phenogel columns, 5 µm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60°C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene with no need for calibration using polymer standards and can be used for the determination of the absolute molecular weights (MWs). The MW was determined from the d*n*/d*c* value calculated offline by means of an internal calibration system processed by ASTRA V software (Version 5.1.7.3, Wyatt Technology). Gel filtration chromatography (GFC) was performed using a chromatography system from GE Healthcare (ÄKTApurifier NPC 10, Pittsburgh, PA, USA) equipped with a HiPrep 16/60 Sephacryl S-500 HR column using a mobile phase with a flow rate of 0.5 mL/min. The typical sample injection volume was 100 µL. Detection was by eluent absorption at 254 nm. For purification, a HiPrep 16/60 Sephacryl S-500 HR column was used for amino dextran (MW 500 kDa) and a Superose 6 10/300 GL column was used for amino dextran (MW 70 kDa). Elemental analysis was performed with a PerkinElmer Optima 2000DV ICP–optical emission spectrometer in the Microanalysis Laboratory at the University of Illinois.

Supporting References

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