

SUPPORTING INFORMATION

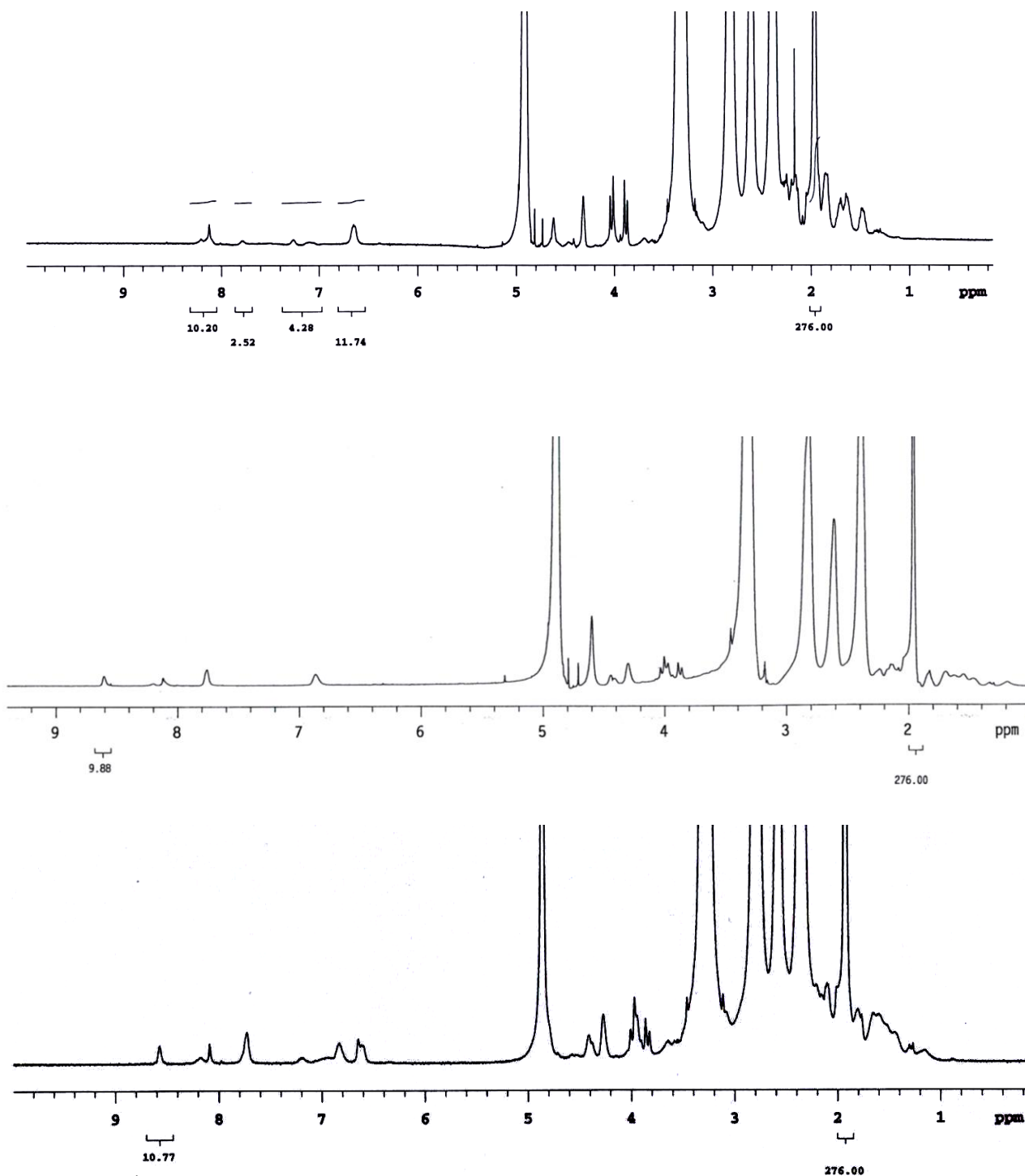


Figure S1. ^1H NMR of G5-5T₃ (A), G5-MTX₁₀ (B) and G5-5T₃-MTX₁₀ (C). The acetamide proton at 1.84 ppm was integrated as an internal reference to 276 that corresponded to 92 acetamide groups. The aromatic proton integration of 5T in panel A indicated there were 3

molecules of the 5T per dendrimer molecule. MALDI-Tof spectra for G5-5T₃, G5-MTX₁₀, and G5-5T₃-MTX₁₀ indicate that their molecular weights (g/mol) are 36,075, 38,808, and 40,302, respectively. The pteridine ring proton integration at 8.61ppm was 9.56 indicating that there was about 10 MTX in the G5-MTX₁₀ (panel **B**). Similarly, the G5-5T₃-MTX₁₀ had 10.77 molecules MTX and 3 molecules of 5T (panel **C**).

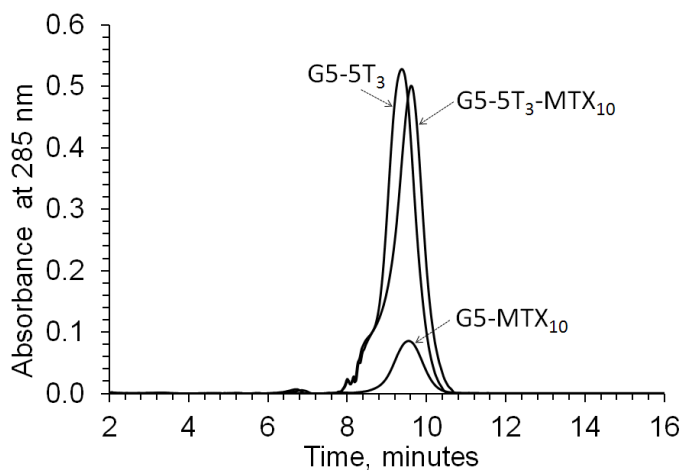


Figure S2. UPLC profile of G5-5T₃, G5-MTX₁₀ and G5-5T₃-MTX₁₀. 3 μ l each of 1 mg/ml solutions of the conjugates were injected in the UPLC system and analyzed as given in the Methods. The elution peak times for G5-5T₃, G5-MTX₁₀ and G5-5T₃-MTX₁₀ were 9.31, 9.48 and 9.55 minutes, respectively.

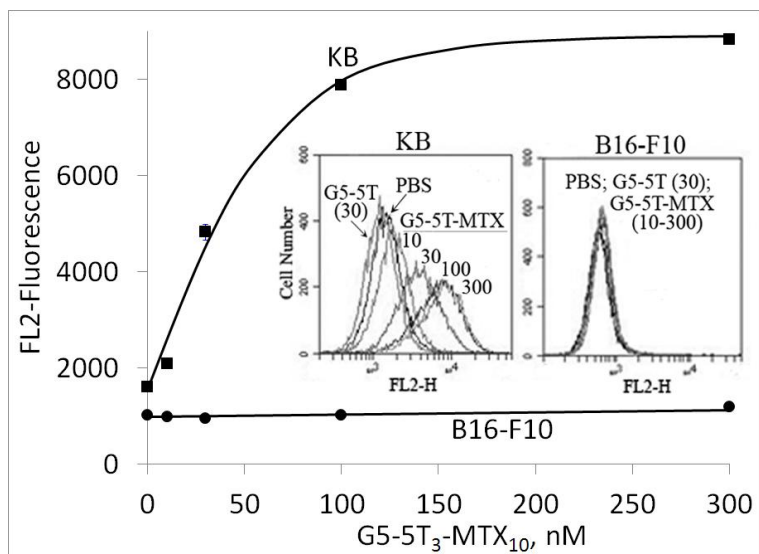


Figure S3. Uptake of G5-5T₃-MTX₁₀ in KB (FR-positive) and B16-F10 (FR-negative) cells. Cells were treated with G5-5T₃-MTX₁₀ for 20 hours and the mean fluorescence of gated live cells was quantified as given in Methods. The data represents the Mean ± SE of 3 cell samples, with similar data obtained in an independent experiment. ***Inset:*** Representative histograms showing the FL2 fluorescence of 10,000 cells. At 30 nM conjugate concentrations, the mean FL2 fluorescence of KB cells treated with vehicle, G5-5T₃, and G5-5T₃-MTX₁₀, were 1604 ± 11, 1456 ± 48, and 4822 ± 164, respectively. In the B16-F10 cells, there were no significant increases in the fluorescence over PBS control under any treatment conditions.

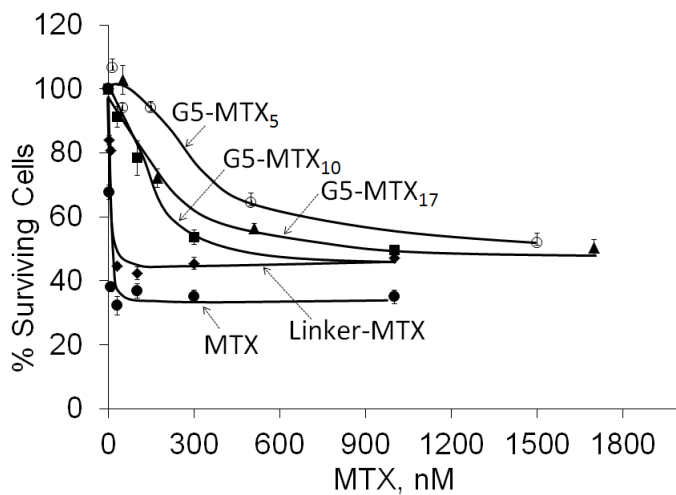


Figure S4. Cytotoxicity of G5-MTX_n conjugates in FR-positive KB cells, with the concentrations of the conjugates expressed as nanomolar of MTX. Cells were incubated for 48 hours with the indicated conjugates, and the cytotoxicity was determined by XTT assay as given in Methods. The data represents the Mean \pm SE of 4 cell samples with similar data obtained in an independent experiment. Other details are as in Figure 5.

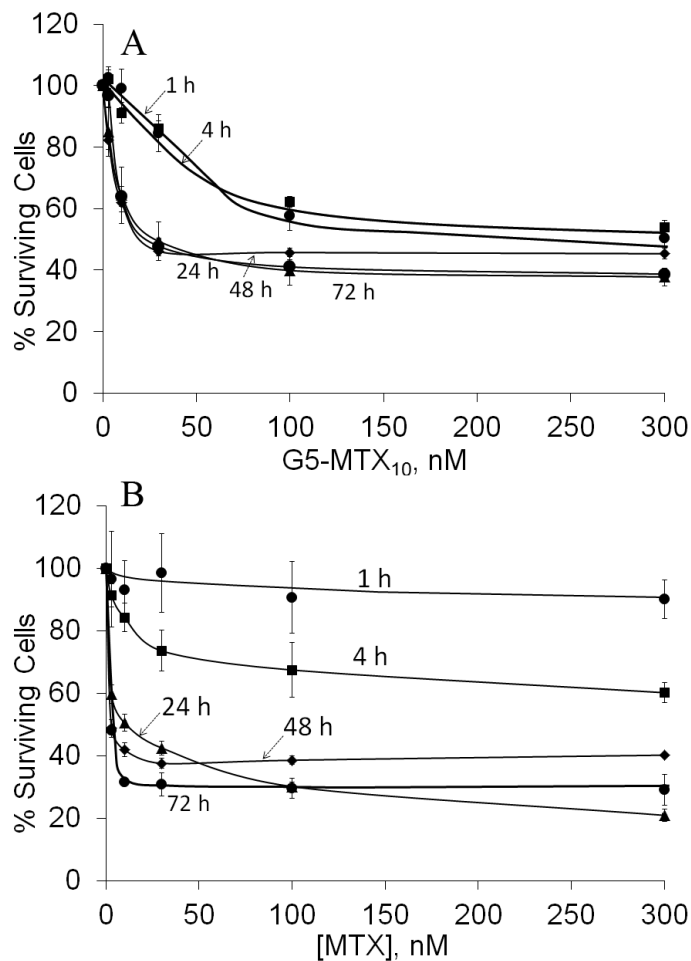


Figure S5. Time-dependent cytotoxicity of G5-MTX₁₀ (**A**) and MTX (**B**) in KB cells. Cells were incubated for the indicated time periods, the cells were rinsed, and fresh medium was added in the absence of any drugs and incubated for a total of 48 hours (including the time of incubation with the drug). For the 72-hour data points, the samples were incubated continuously for a total of 72 hours. The data represents the Mean \pm SE of 6 replicate cell samples obtained from 2 independent experiments. The cytotoxicity was determined by XTT assay, as given in Methods.