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Supporting Information

Bioorthogonal Chemical Handle for Tracking Multifunctional Nanoparticles

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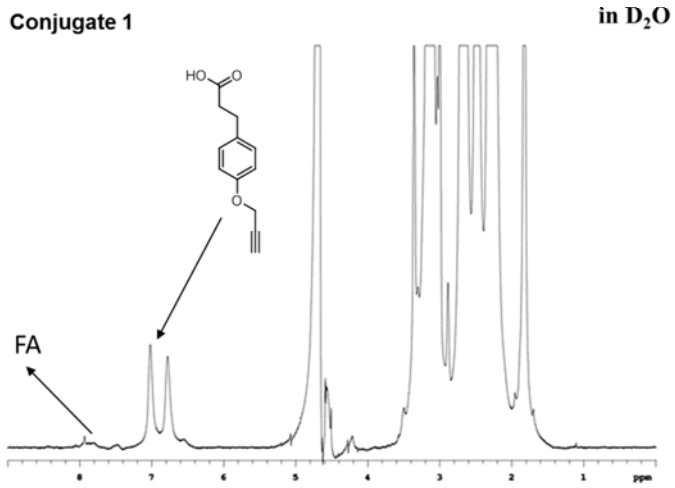
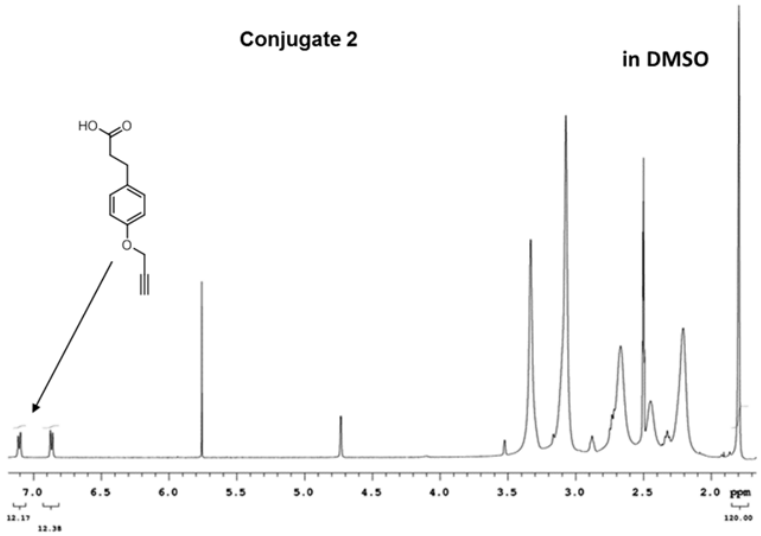
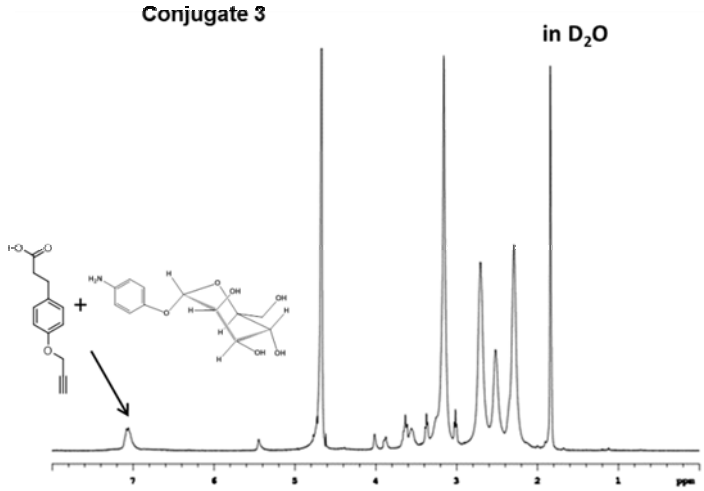
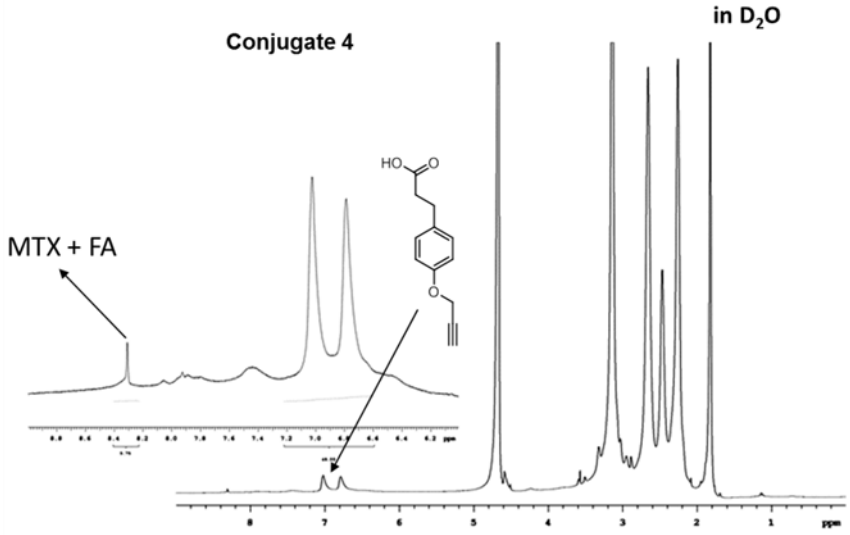
List of Contents

Figure S1. ^{1}H NMR spectra of dendrimer conjugates
Figure S2. UPLC chromatograms of dendrimer conjugates.
Figure S3. In vitro flow cytometry comparison of traditional and bioorthogonal reporters
Figure S4. In vitro flow cytometry comparison of bioorthogonal reporter strategy with and without a copper catalyst.
Figure S5. In vitro flow cytometry comparison of washes post-CuAAC ligation with fluorescent reporters.
Figure S6. Schematic illustration of click efficiency tests using 3-azido-7-hydroxy coumarin fluorescent assay.
Supplemental Table 1 Click efficiency tests using 3-azido-7-hydroxy coumarin fluorescent assay






Conjugate 3
in D_2O


Conjugate 4
in D_2O
MTX + FA

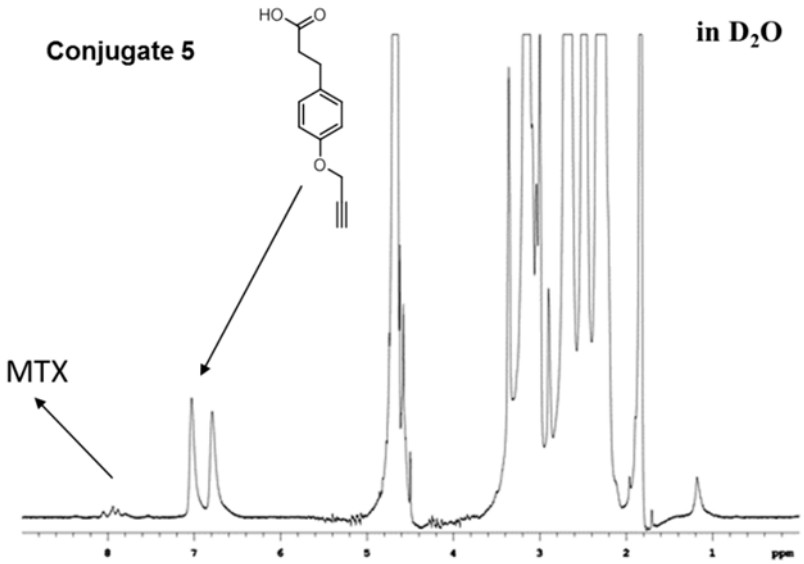
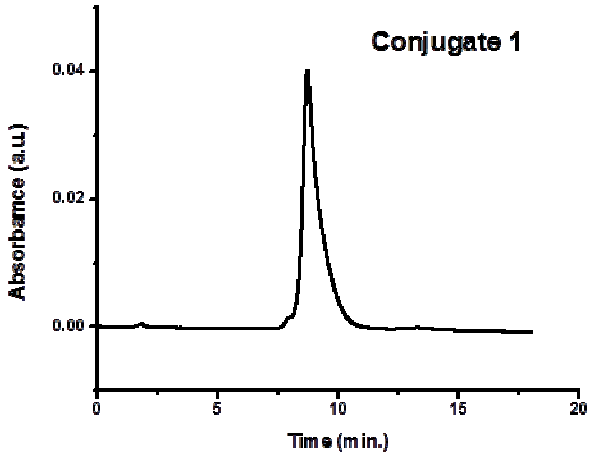
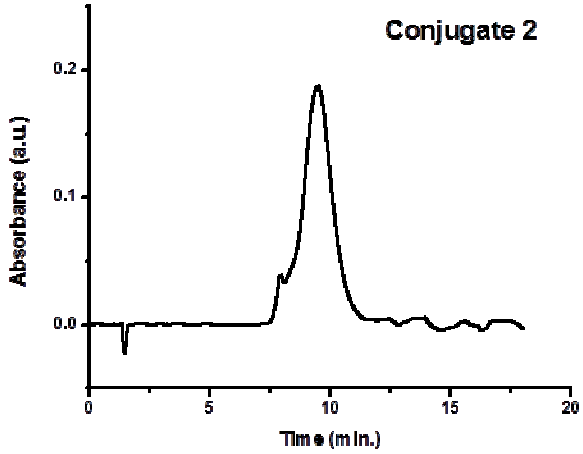
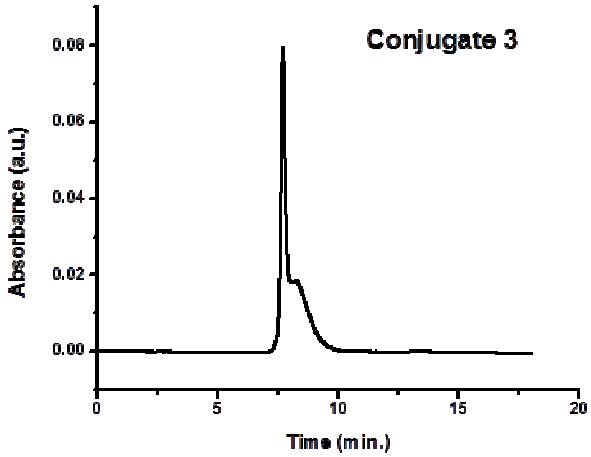

Conjugate 5
in D_2O
MTX

Figure S1. ^{1}H NMR spectra of dendrimer conjugates 1) G5-FA-Alkyne; 2) G5-Alkyne; 3) G5-Mannose-Alkyne; 4) G5-FA-MTX-Alkyne; 5) G5-MTX-Alkyne.







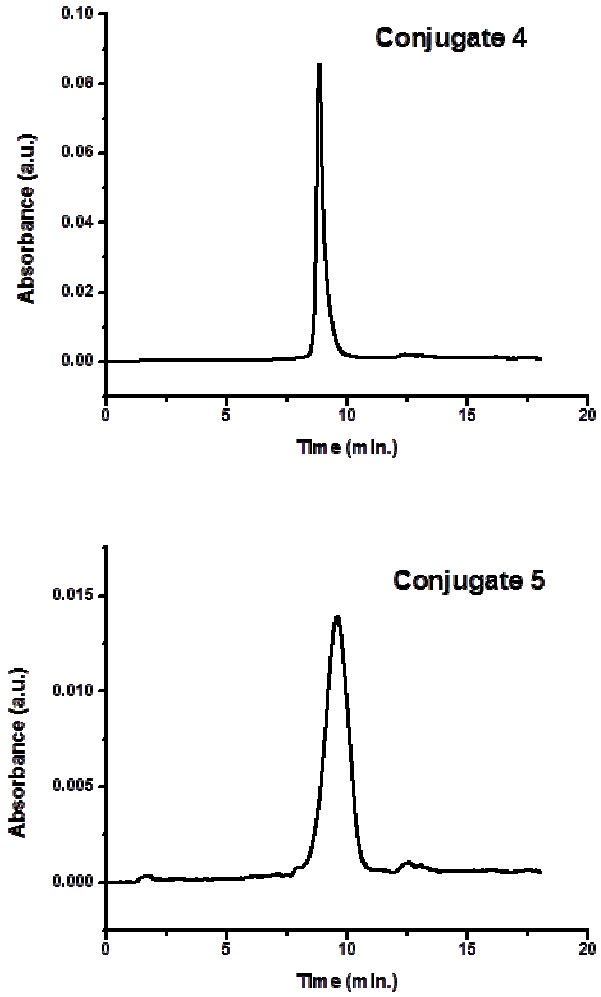


Figure S2. UPLC chromatograms of dendrimer conjugates. Chromatograms were adjusted using solvent control to reduce background. 1) G5-FA-Alkyne; 2) G5-Alkyne; 3) G5-Mannose-Alkyne; 4) G5-FA-MTX-Alkyne; 5) G5-MTX-Alkyne.

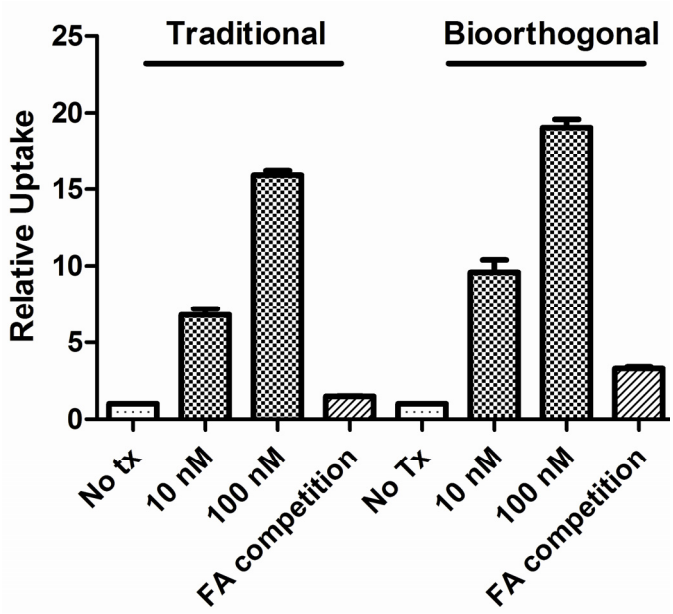


Figure S3. In vitro flow cytometry comparison of traditional and bioorthogonal reporters. KB cells were treated with increasing doses of traditional FITC labeled G5-FA and the G5-FA-Alkyne **1** for 1 hour. Uptake of the conjugates was assessed using flow cytometry. Cell fluorescence was indexed to the background fluorescence of the no treatment control group and represented as relative uptake. To demonstrate folate receptor specificity, KB cells were preincubated with 100 nM of FA for 30 min prior to 1 hour treatment with 100 nM of FA-targeted conjugates. Results are representative of two independent experiments.

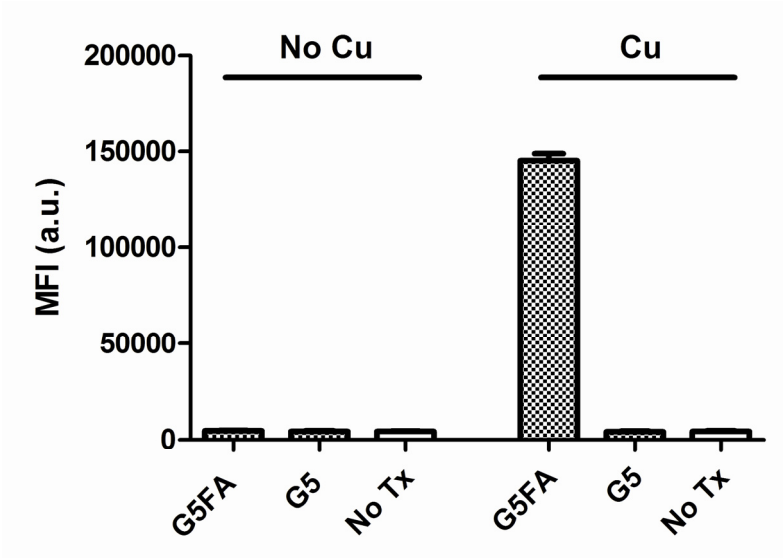


Figure S4. In vitro flow cytometry comparison of bioorthogonal reporter strategy with and without a copper catalyst. KB cells were treated with 100 nM of G5-FA-Alkyne **1** and G5-Alkyne **2** for 1 hour. Cells were harvested and the internalized dendrimer conjugates were reacted with AF647 using the CuAAC staining solution with or without copper. Results are representative of two independent experiments.

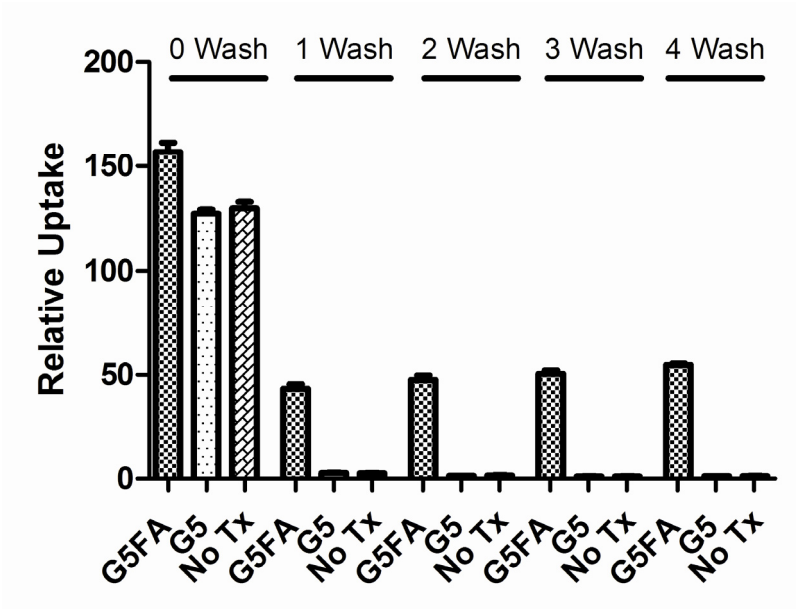


Figure S5. In vitro flow cytometry comparison post-CuAAC fluorescent reporter ligation washes on fluorescent signal. KB cells were treated with 100 nM of G5-FA-Alkyne **1** and G5-Alkyne **2** for 1 hour. Cells were harvested and the internalized dendrimer conjugates were reacted with AF647 using the CuAAC and analyzed by flow cytometry after each wash step. Cell fluorescence was indexed to the background fluorescence of a no treatment/no reporter control group and results are represented as relative uptake. Results are representative of two independent experiments.

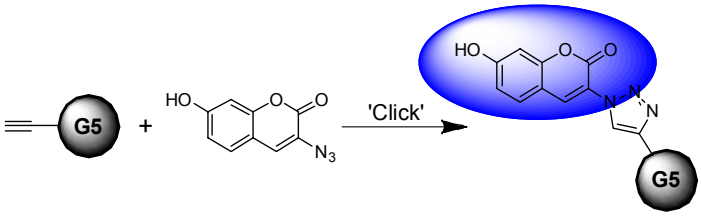


Figure S6. Schematic illustration of click efficiency tests using 3-azido-7-hydroxy coumarin fluorescent assay.

Supplement Table 1.

Sample	λ_{em} (nm)	Intensity[a,b]
G5- FA-Alkyne **1**	458	0.85
G5- Mannose-Alkyne **3**	460	0.92
G5- Alkyne **2**	458	1.00

Fluorescent data of G5 dendrimers and 3-azido-7-hydroxy coumarin click products. [a]The fluorescent intensities were normalized to G5-Alkyne **2** click product. [b]The fluorescence intensity was measured by summing the total fluorescence emission from 400 to 600 nm.