Supporting Information

PAMAM dendrimers were evaluated for FITC loading on dendrimers following conjugation. Samples of G3.5 and G4 FITC conjugate were weighed and then dissolved in 100μL PBS pH 7.4. The samples were then read for fluorescence (λ ex./λ em. of 495/525 nm) on a fluorescent plate reader calibrated with FITC standard curves on the same plate (MD Spectramax Gemini). The FITC-loading was then quantified by calculating the concentration of FITC attached to the total mass of dendrimers. FITC was assumed to be massless compared to the dendrimer weight in the calculations. Results are shown in Figure S1.

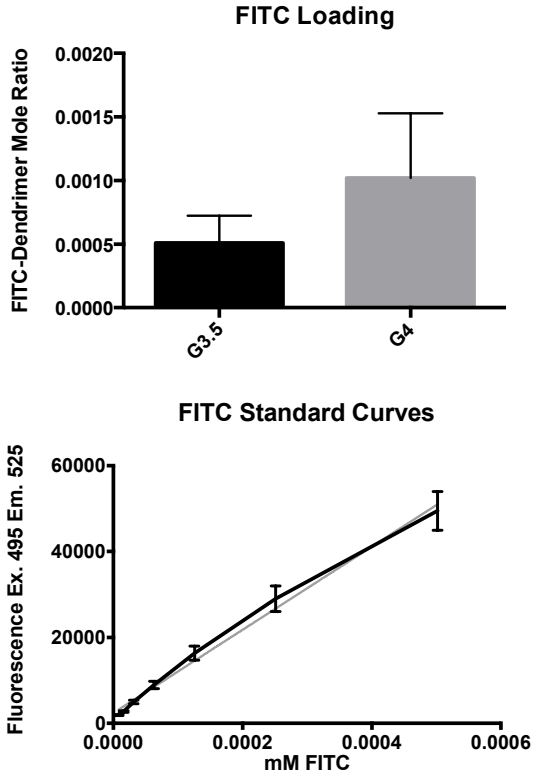


Figure S1. PAMAM Dendrimer FITC conjugate ratio. The molar ratio of FITC to dendrimer was quantified spectrophotometrically using a FITC standard curve (lower graph, grey line represents model fit, R^2=0.98) to interpolate the FITC molar ratio of dendrimer-FITC conjugate (upper graph) .

The absence of free FITC during the Ussing chamber studies was evaluated by use of size exclusion chromatography on PD-10 columns after each experiment. Basolateral solutions were removed after the experiment and stored in 4C until running on PD-10 columns. Each column was used one time to test for small molecular weight free FITC. Columns were washed prior to use with 12mL of PBS pH 7.4. 500µL of the basolateral solution was loaded onto the column followed by 1.5mL PBS. The eluted void fraction was collected (2mL) followed by 4mL fractions thereafter. Samples were collected into spectrophotometer cuvettes and analyzed for absorbance at 495nm (Pharmacia Biotech, Ultraspec 200 UV/Vis), as fluorescence signal was too weak to detect following dilution on the column. Blank elution solvent (PBS pH 7.4) was used to zero the absorption signal prior to each sample analysis and after each analysis to affirm that there was no drift in absorption signal (Fig. S2).

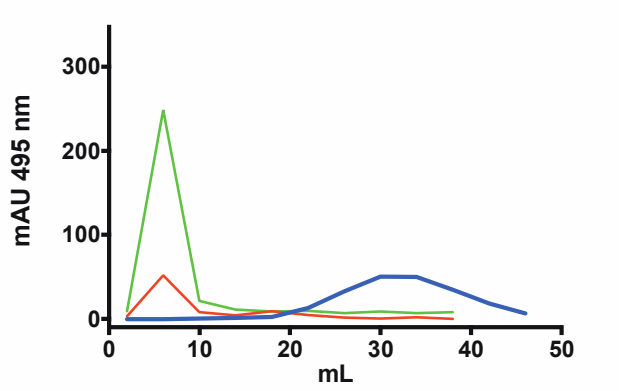
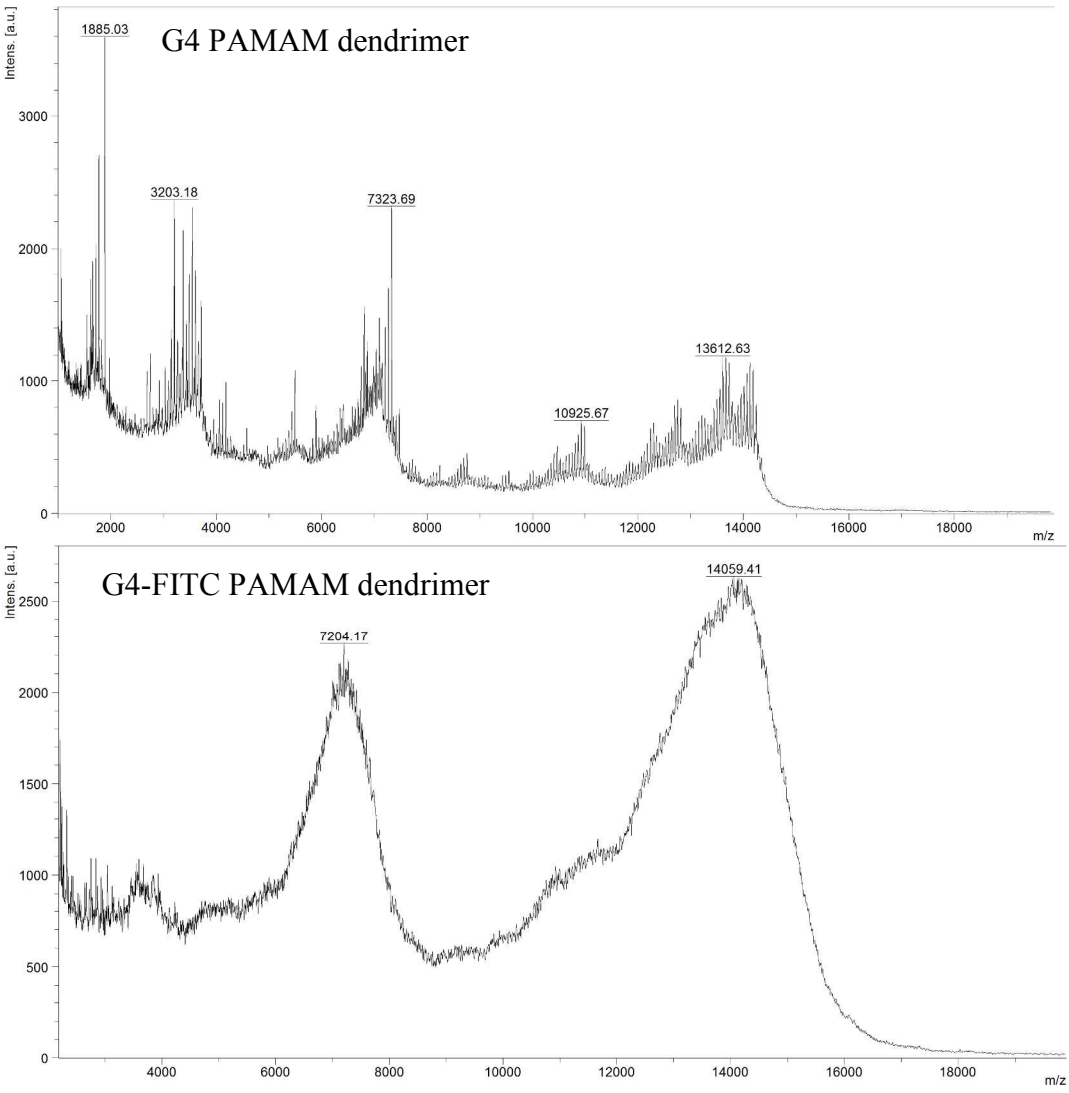


Figure S2. Stability of the FITC-dendrimer conjugates was monitored by size exclusion chromatography (PD-10) following fluxes across jejunal mucosae. FITC labeled dendrimers

showed no peaks corresponding with free FITC at 120 minutes as detected by absorbance at 495nm (Free FITC – blue, G4 dendrimers – red, G3.5 dendrimers – green).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) was used to characterize the FITC-labeled dendrimers. The mass spectral data shown was collected using delayed ion extraction mode on a Bruker's ultrafleXtreme™ MALDI-TOF/TOF mass spectrometer. Samples were spotted using dried-droplet method. A fresh solution of saturated 2′,4′,6′-Trihydroxyacetophenone monohydrate (THAP) in a solvent system of 50:50 water:acetonitrile 0.1% TFA was prepared by thoroughly mixing the matrix powder with 0.5 mL of solvent in a 1.7 mL eppendorf tube, and then centrifuged to pellet of un-dissolved matrix. The supernatant of this matrix solution was used for sample preparation for MALDI analysis. Samples (0.5 uL of 1 pmol/uL) were loaded onto a stainless steel target plate and mixed on the target with 0.5 μL of supernatant of saturated matrix solution. Then the sample spot was air-dried and allowed for co-crystallization of the mixture and peptide sample, then the spot was ablated with a 1 kHz smartbeam-II™ laser technology from the plate while the sample was simultaneously desorbed, ionized, and then accelerated into a flight tube. The MALDI spectrum was acquired in linear mode, which was operated a mass range from 1000 to 120000 Da. Results show no significant shift in M/Z ratio (Fig S3).


G4 PAMAM dendrimer
Intens. [a.u.]
1885.03
3203.18
7323.69
10925.67
13612.63
m/z
G4-FITC PAMAM dendrimer
Intens. [a.u.]
7204.17
14059.41
m/z

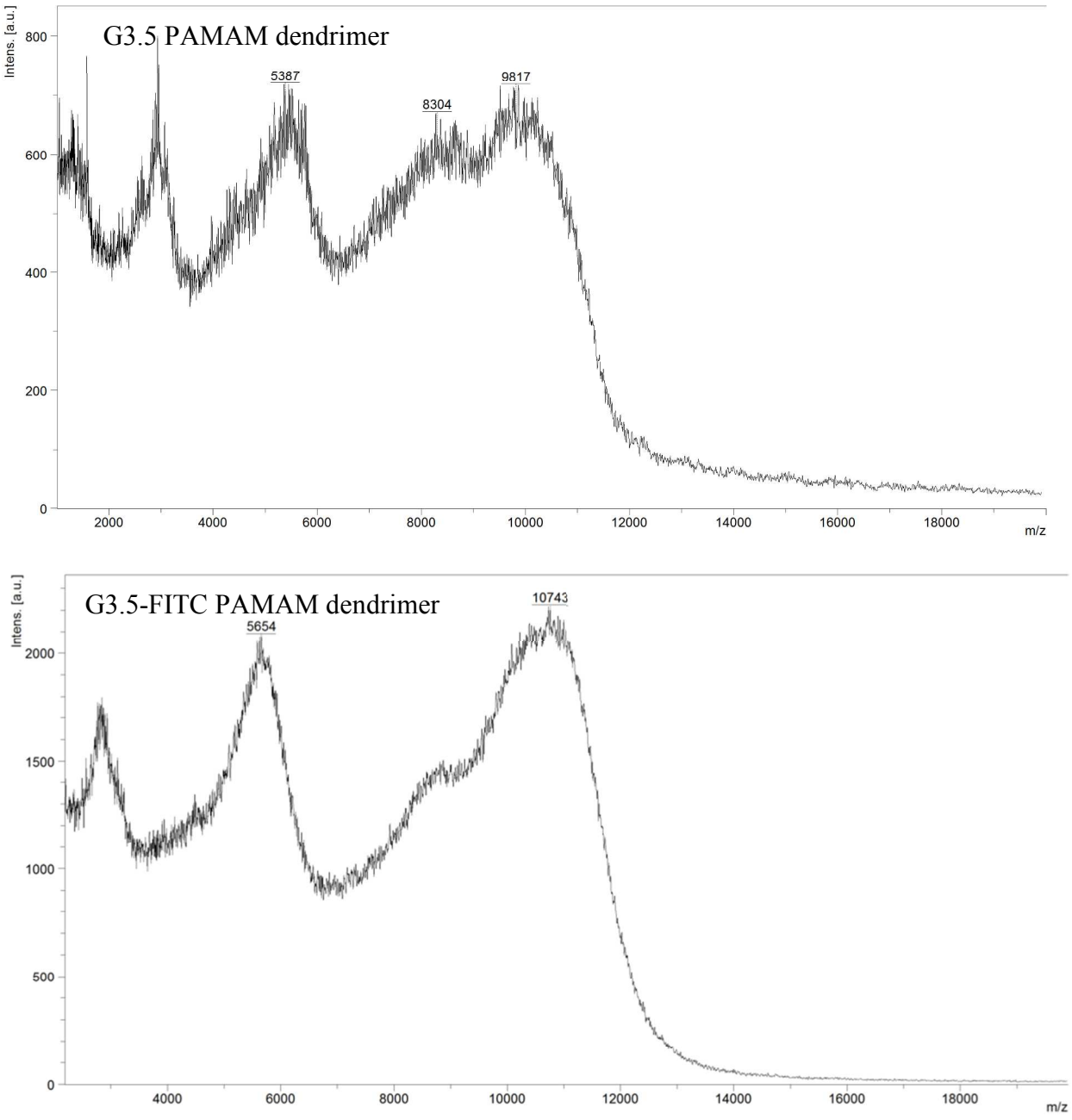


Figure S3. MALDI-TOF of FITC-labeled dendrimers. PAMAM dendrimers in a THAP matrix were evaluated by MALDI-TOF. A significant increase in molecular weight was not observed due to the broad peaks.

G3.5 PAMAM dendrimers that were modified with tert-butyl N-(2-aminoethyl) carbamate were dissolved in CD_3COD and analyzed by nuclear magnetic resonance (H1-NMR). Spectra

were obtained on a Varian Mercury 400MHz instrument. NMR spectra were also obtained for the amine modified G3.5-ethylenediamine dendrimers after TFA deprotection as well (Fig. S4-S7). FITC loading was quantified by integration of H1-NMR spectra of the G3.5 and G4 FITC labeled dendrimers in CD_3COD (Fig. S8-S16). Internal standards were the residual methanol (δ 3.31) and water (δ 4.78 peaks).

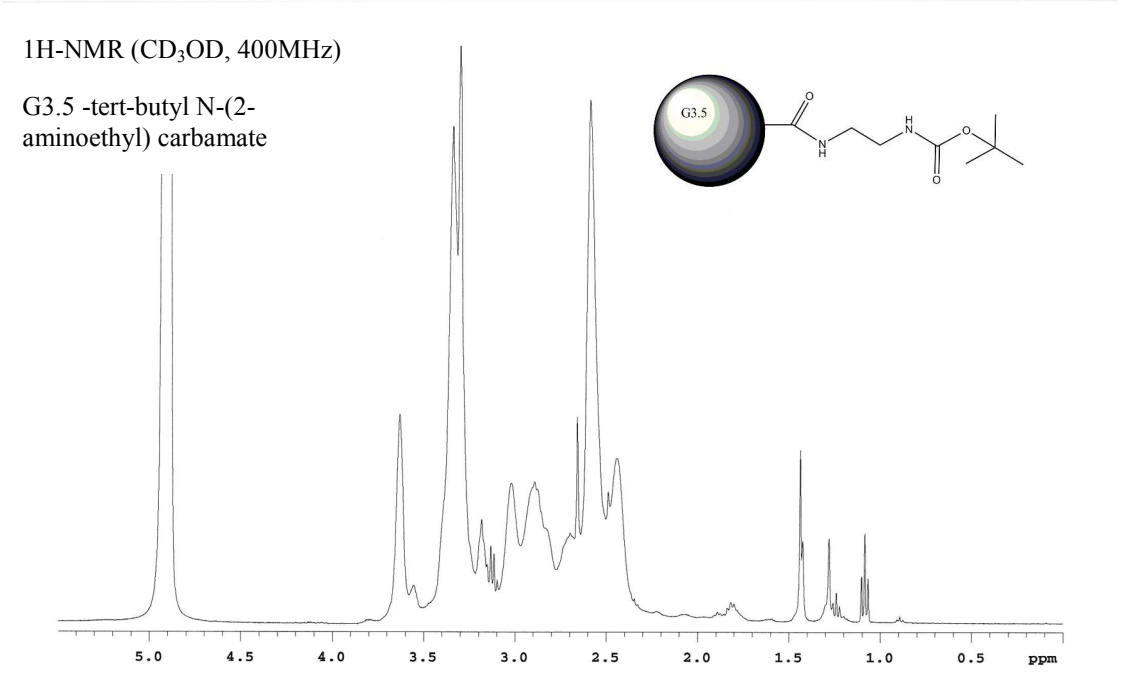


Figure S4. H1-NMR spectra of G3.5-tert-butyl N-(2-aminoethyl) carbamate. The appearance of peaks at 1.5ppm correlated with the addition of tert-butyl N-(2-aminoethyl) carbamate. The solvent was CD_3OD.

1H-NMR (CD_3OD, 400MHz)

tert-butyl N-(2-aminoethyl) carbamate

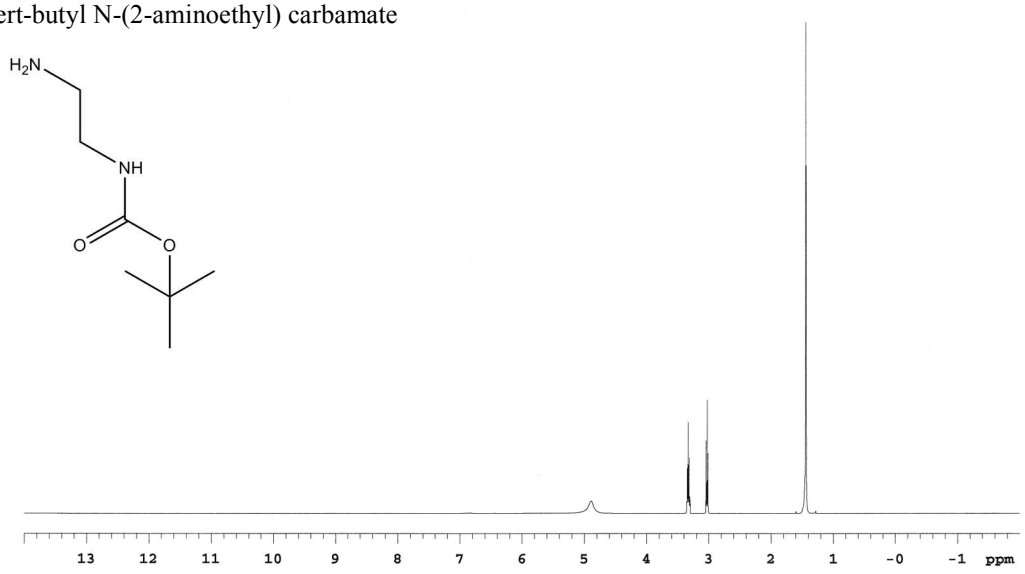


Figure S5. H1-NMR spectra of tert-butyl N-(2-aminoethyl) carbamate. CD_3OD was the solvent.

1H-NMR (CD_3OD, 400MHz)

tert-butyl N-(2-aminoethyl) carbamate

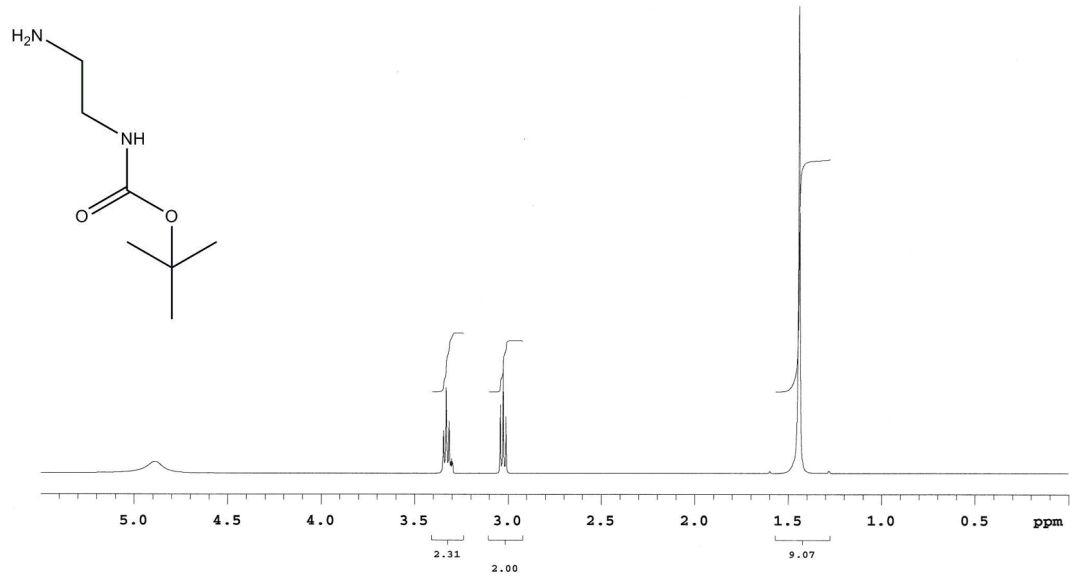


Figure S6. H1-NMR Spectra of tert-butyl N-(2-aminoethyl) carbamate (zoom with integration).

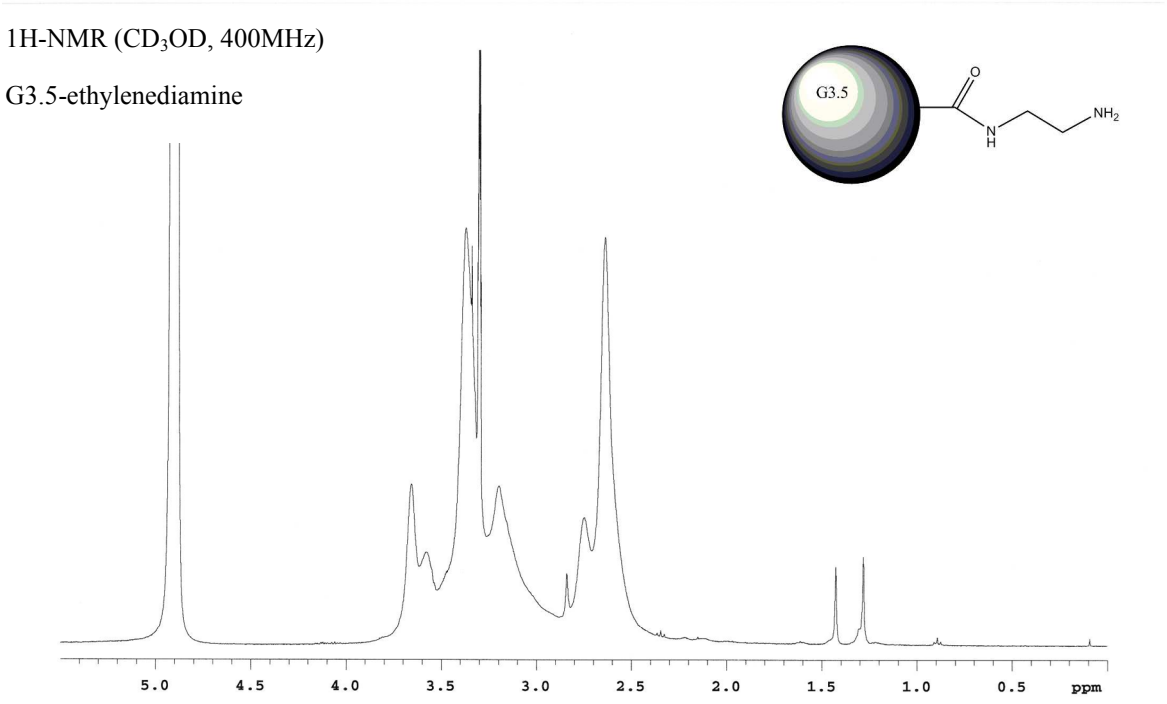


Figure S7. H1-NMR spectra of G3.5-ethylenediamine. After G3.5 modification with tert-butyl N-(2-aminoethyl) carbamate, the reaction was deprotected using TFA. The resultant product was dissolved in CD_3OD and analyzed. Spectra was obtained with peak at 1.5ppm noted to signify the presence of ethylenediamine.

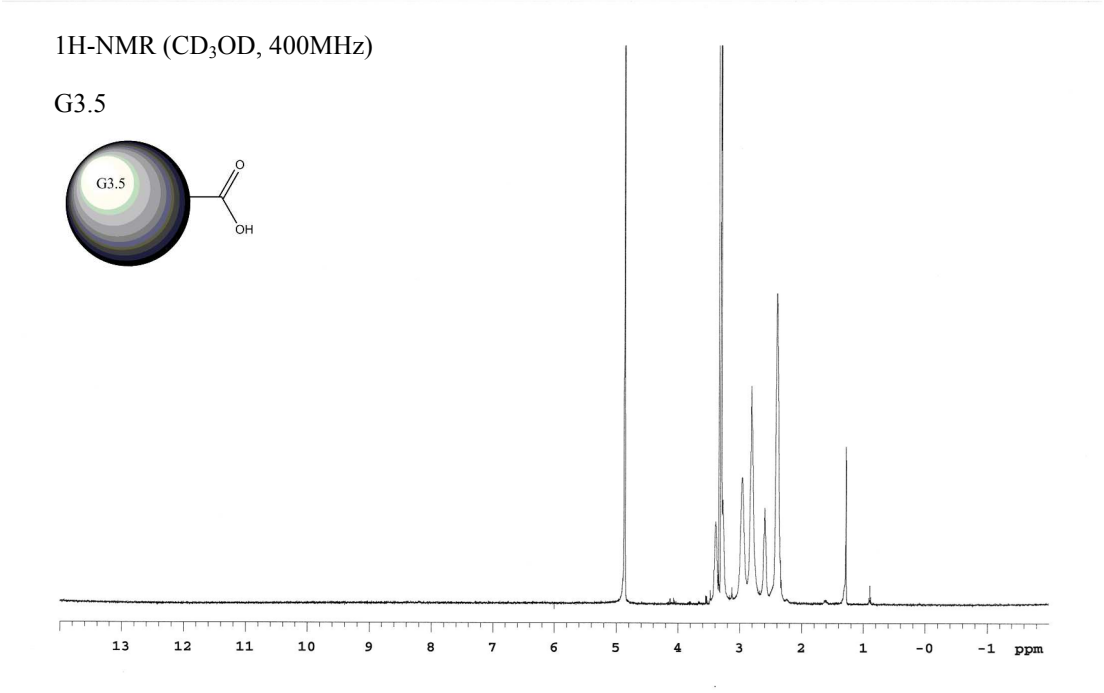


Figure S8. H1-NMR Spectra of G3.5 PAMAM dendrimer. Solvent was CD_3OD.

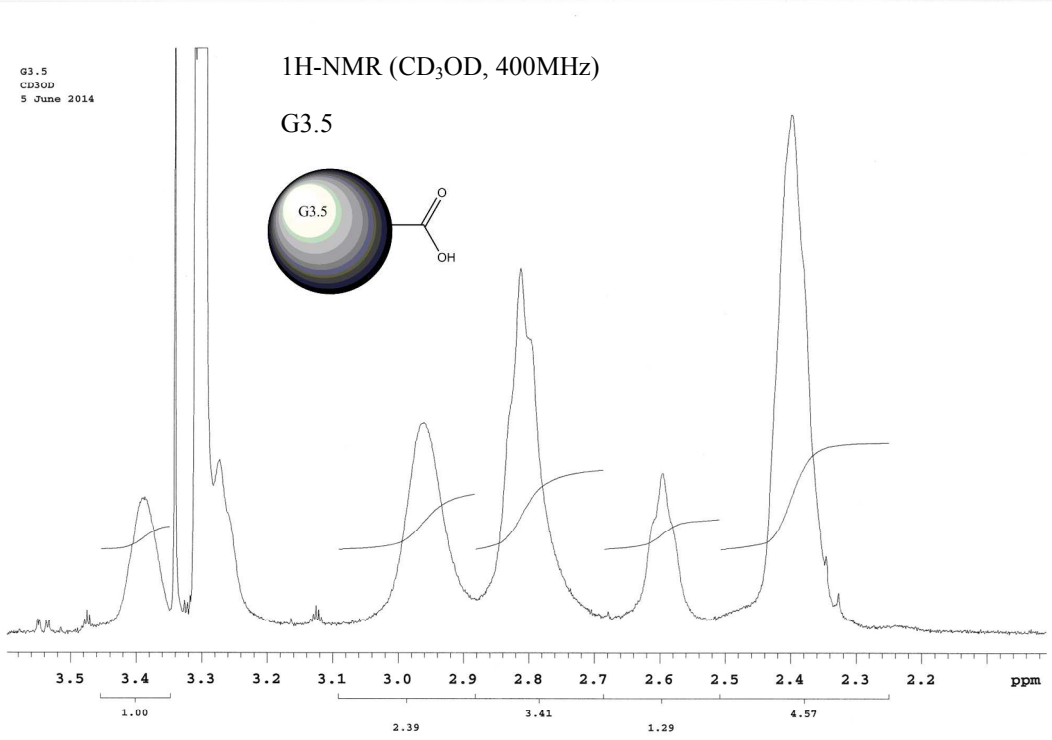


Figure S9. H1-NMR Spectra of G3.5 PAMAM dendrimer (zoom with integration). Solvent was CD_3OD.

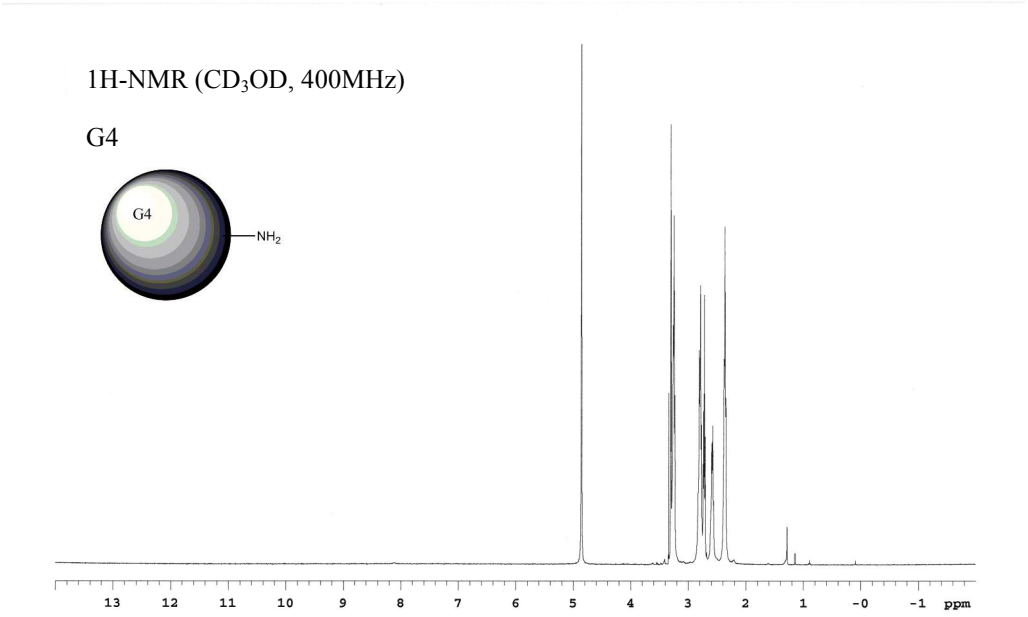


Figure S10. H1-NMR Spectra of G4 PAMAM dendrimers. Solvent was CD_3OD.

1H-NMR (CD_3OD, 400MHz)

G4

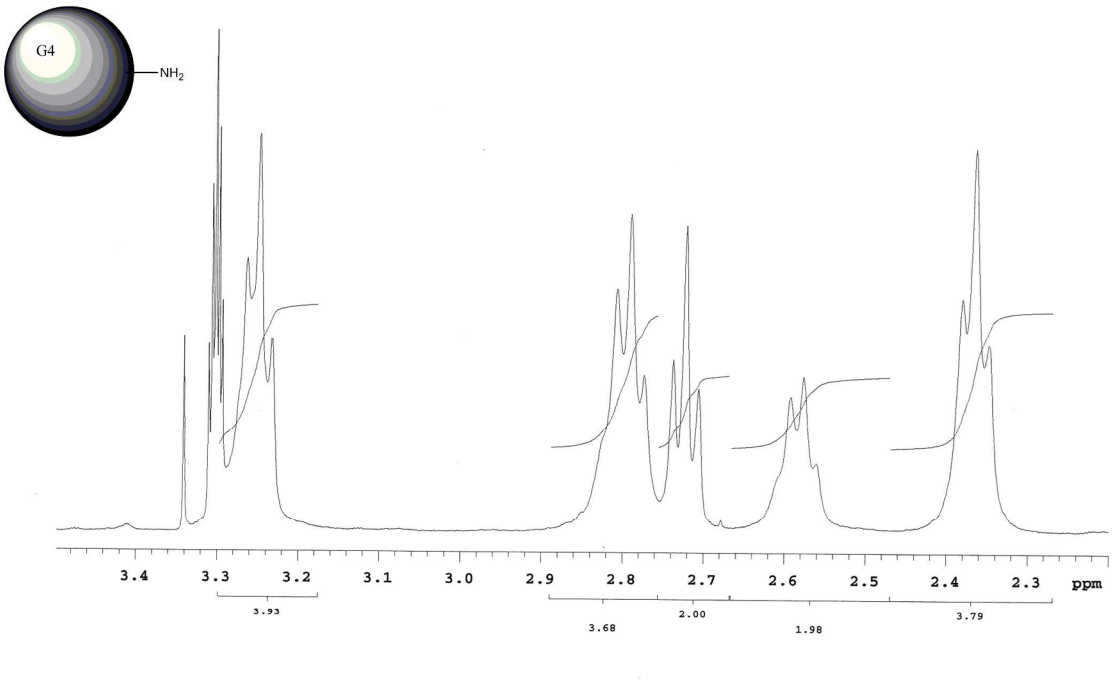


Figure S11. H1-NMR Spectra of G4 PAMAM dendrimers (zoom with integration). Solvent was CD_3OD.

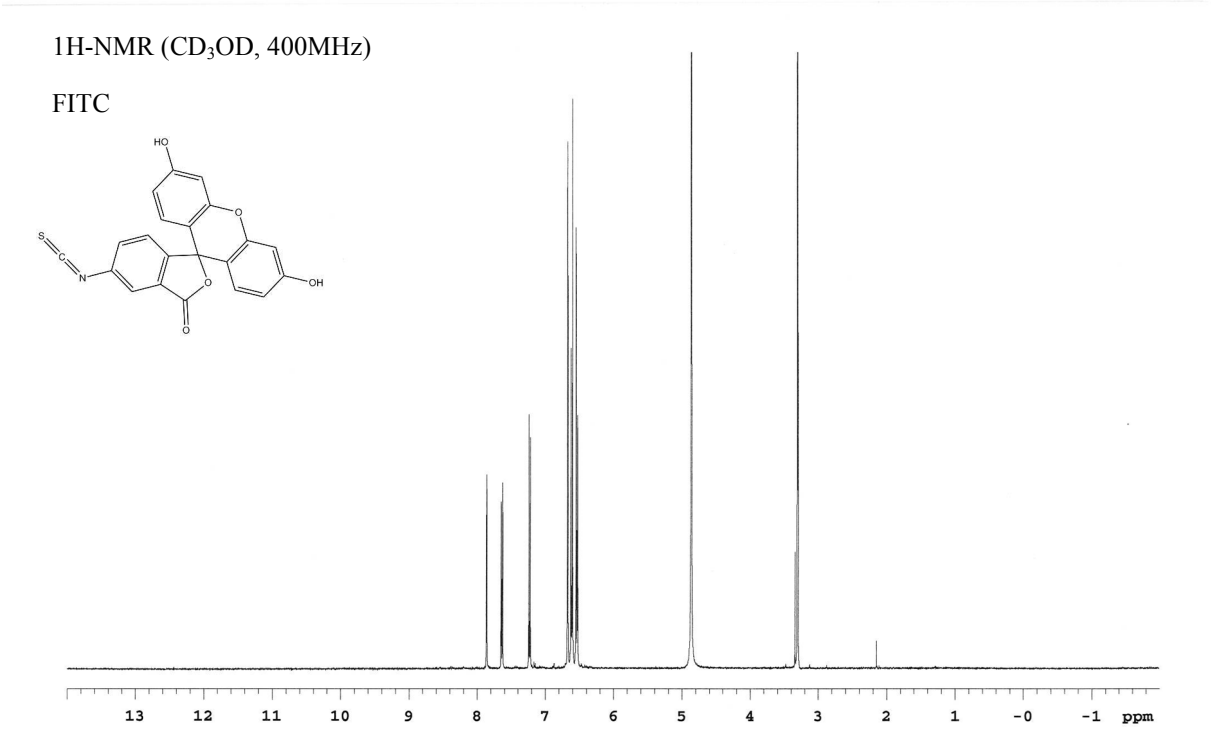


Figure S12. H1-NMR Spectra of FITC as control. Solvent was CD_3OD.

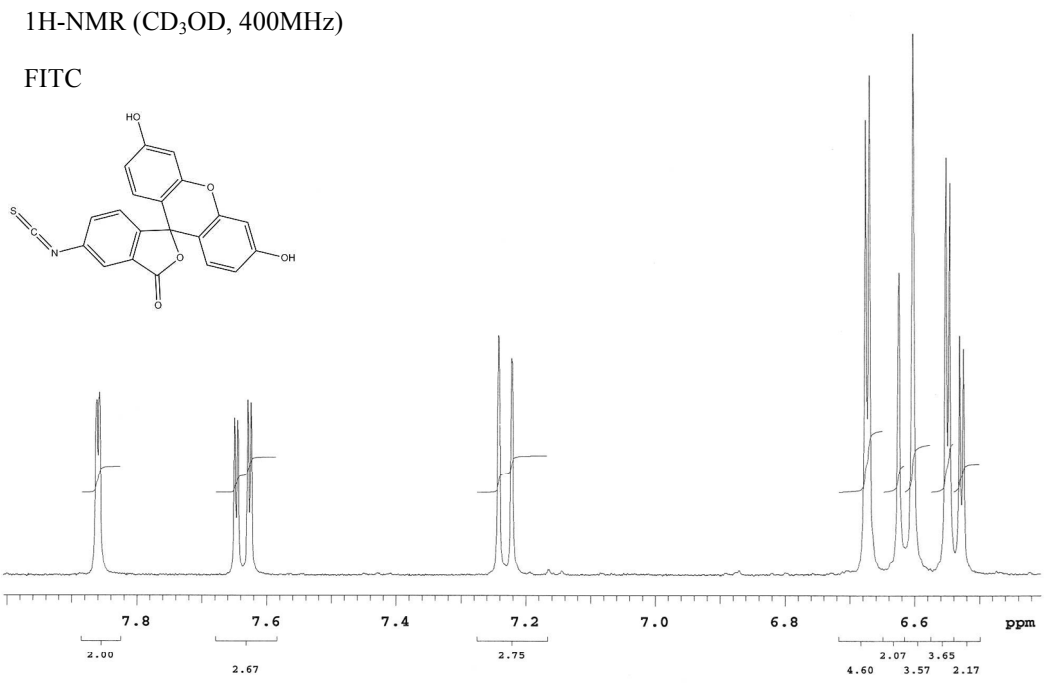


Figure S13. H1-NMR Spectra of FITC (zoom with integration). Solvent was CD_3OD.

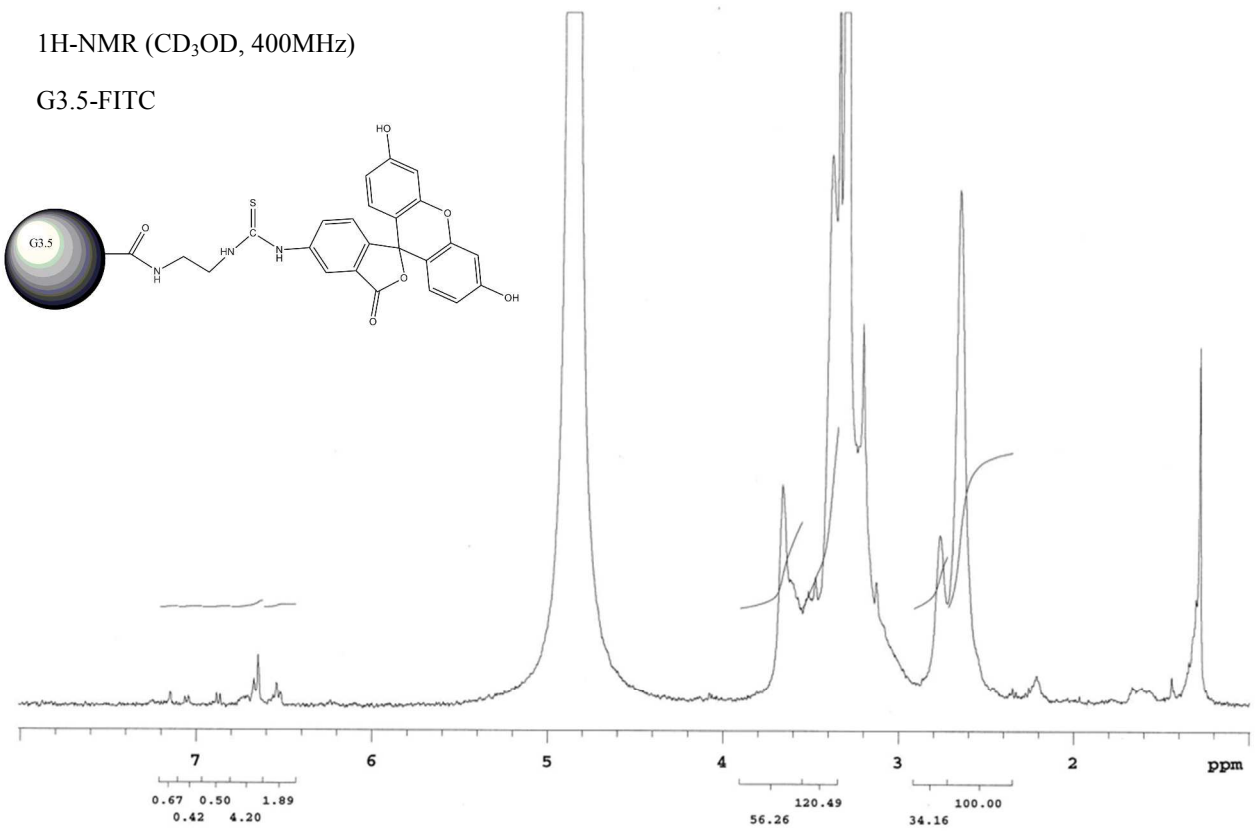


Figure S14. NMR Spectra of G3.5-FITC as control. The broad triplet at 2.54 ppm arises from the methyl groups in the outer ethylenediamines and thus is estimated to represent 34 methyl groupss based on previous work[1]. The FITC peaks appear in the 6-7ppm range and the peak at 6.55 is estimated to represent 1 mole. The calculated ratio is thus 2.9 moles G3.5 per mole FITC. Solvent was CD_3OD.

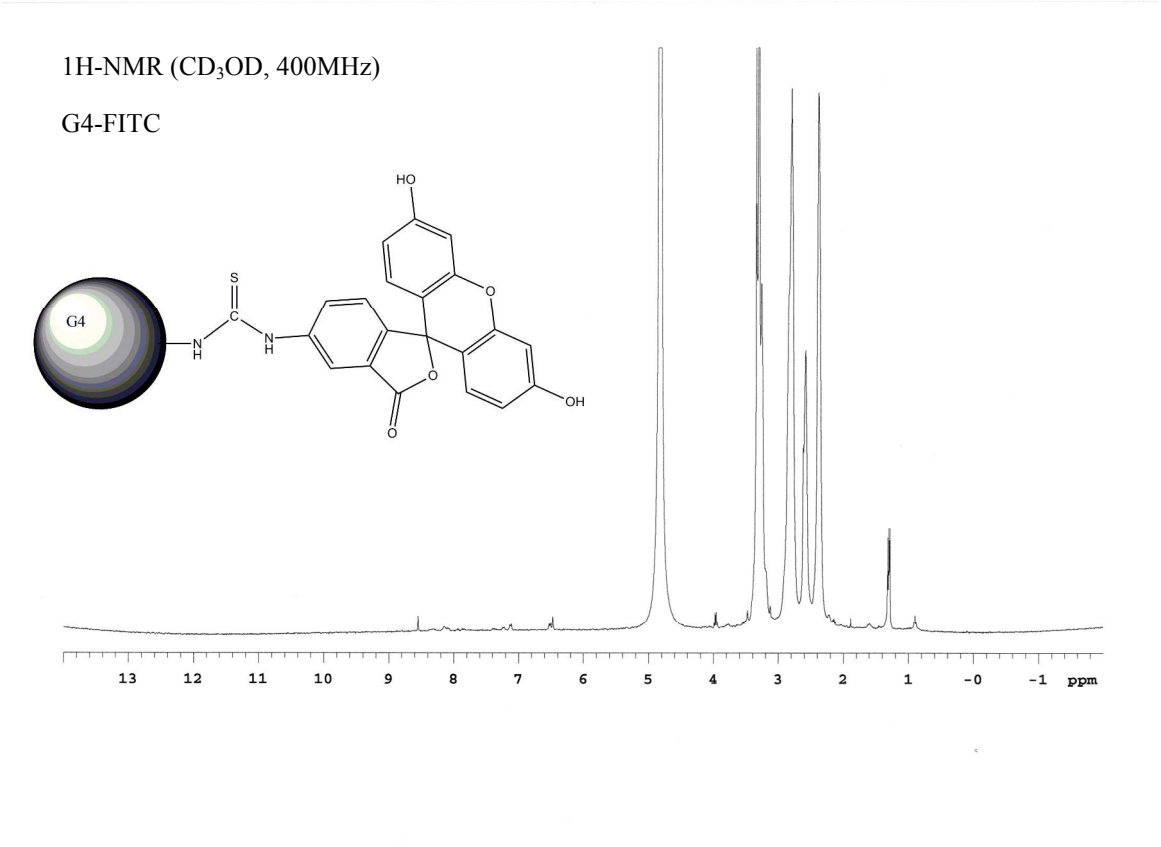


Figure S15. NMR Spectra G4-FITC. Solvent was CD_3OD.

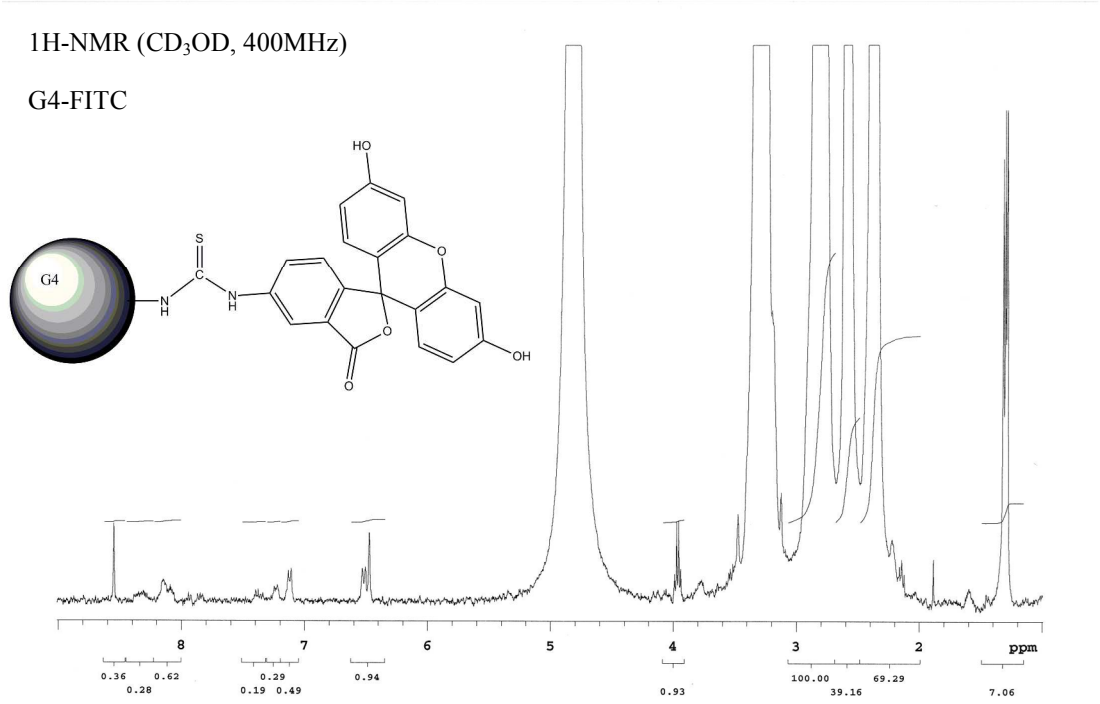


Figure S16. NMR Spectra of G4-FITC (zoom with integration). The spectra of the G4 showed the presence of FITC peaks in the 6-7ppm range. The mole ratio of FITC dendrimer based on the dendrimer peak at 2.54ppm (representing 56 methyls) was estimated to be 0.7 moles G4 per mole FITC. The FITC peak at 6.55 is set to 1 mole. The solvent was CD_3OD.

Fluorescent images of preserved tissues after fixation in formaldhyde were taken by confocal microscopy to create color images overlayed with fluorescence (Olympus Fluoview 1000). Tissues were stained with alcian blue/neutral red to avoid overt autofluoresence. Three laser scans (488nm, 543nm, 633nm wavelength) were taken and transmitted light was combined to create a single color image, while fluorescence was recorded occurring from the 488nm scan (detected emissions: 500-525nm) to provide overlay images. Images obtained had minor bubbles occurring throughout due to drying, but overall showed no significant staining pattern for free FITC versus G3.5 and G4 FITC (Fig. S17)

Figure S17. Fluorescent overlay of Alcian Blue/Neutral Red Stained Jejunal Tissue. Fluorescent images showed significant deep penetration of FITC labeled compounds in all three treatments (G4-FITC Top, G3.5-FITC Middle, FITC bottom). The images proceed from right to left: color image of stained tissue, fluorescent image, and overlay of color and fluorescence.

Bibliography

(1) Jenkins, A. SYNTHESIS AND FUNCTIONALISATION OF PAMAM DENDRIMERS AS HISTONE REPLACEMENT MOLECULES, University of Southampton: England, 2009.