Cytosolic Uptake of Large Monofunctionalized Dextrans

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Table S1. Pearson's correlation coefficients (*r*) for localization of a fluorogenic dextran conjugate and either Hoechst 33342 nuclear stain or Lysotracker acidic vesicle stain

Dextran Conjugate	*r* (Hoechst 33342)	*r* (Lysotracker)
2	0.19 ± 0.04	0.02 ± 0.05
3a	–0.63 ± 0.03	0.43 ± 0.02
3b	–0.42 ± 0.02	0.46 ± 0.05
TAMRA–dextran	–0.55 ± 0.04	0.31 ± 0.03

Table S2. Dextran branching as determined by ^{1}H-NMR spectroscopy

Dextran	α(1,6):α(1,4)	α(1,6):α(1,3)
D1 (100-kDa from Fina Biosciences)	30:1	230:1
D2 (70-kDa from Thermo Fisher Scientific)	29:1	101:1
D4 (100-kDa from Sigma–Aldrich)	46:1	166:1

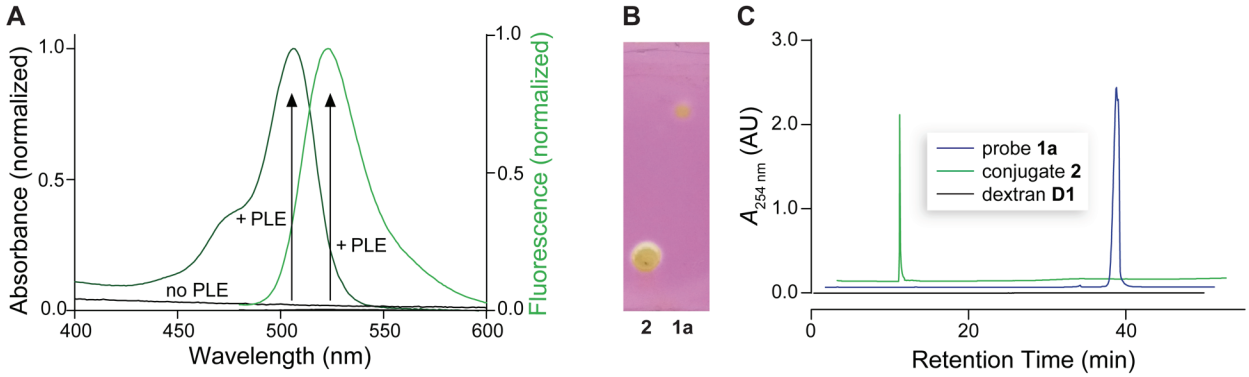


Figure S1. Characterization of dextran conjugates. (A) Spectra showing the response of conjugate **2** (10 μM) to treatment with pig liver esterase (9 nM) for 30 min. (B) Eluted TLC plate showing that purified conjugate **2** has no detectable contamination from residual small-molecule probe **1a**. (C) C4 HPLC traces showing that conjugate **2** has no detectable contamination from small-molecule probe **1a**.

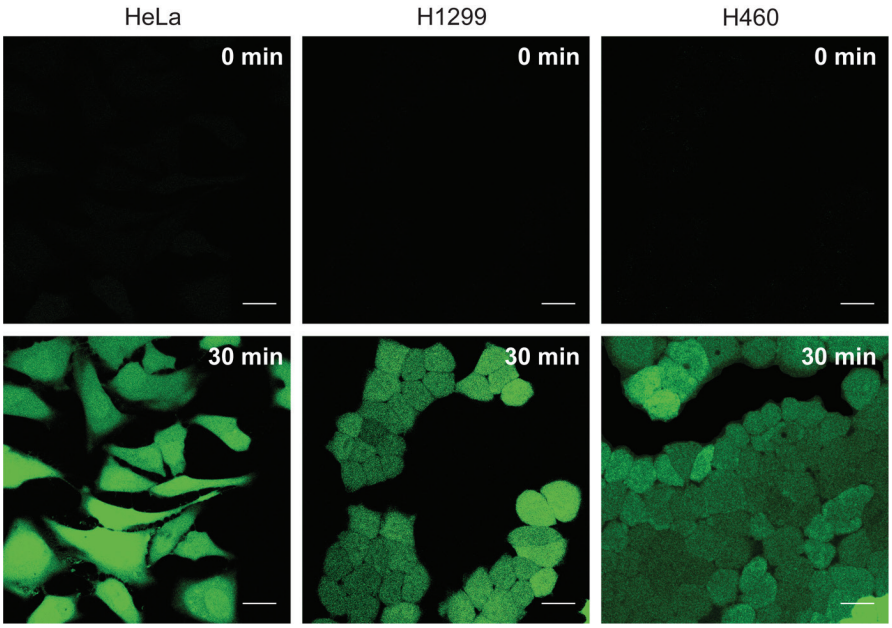


Figure S2. Confocal microcsopy of HeLa, H1299, and H460 cells at 0 or 30 min after incubation with conjugate **2** (10 μM). Scale bars: 25 μm.

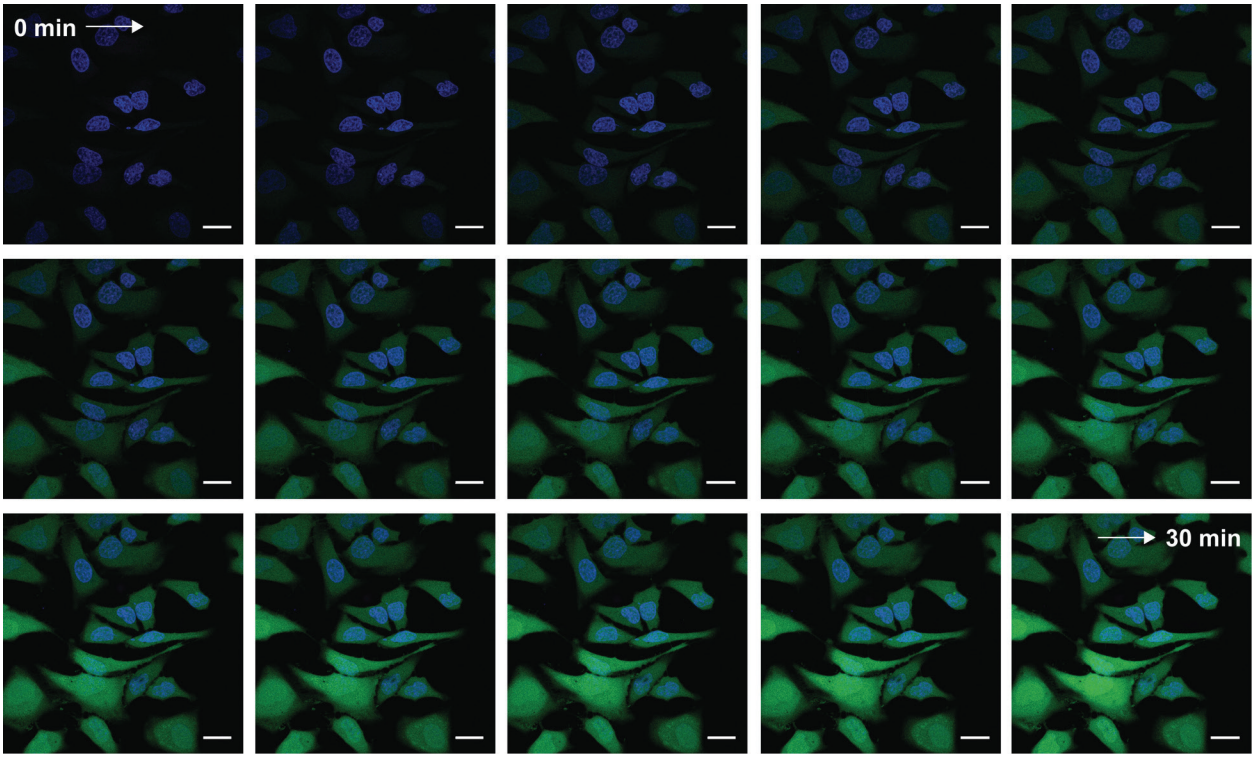


Figure S3. Confocal microscopy images showing the time-course for the uptake of conjugate **2** (10 μM) by live HeLa cells. Image-acquisition began immediately after the on-stage addition of conjugate **2** in 300 μL of OptiMEM. Cells were stained with Hoechst 33342. Scale bars: 25 μm.

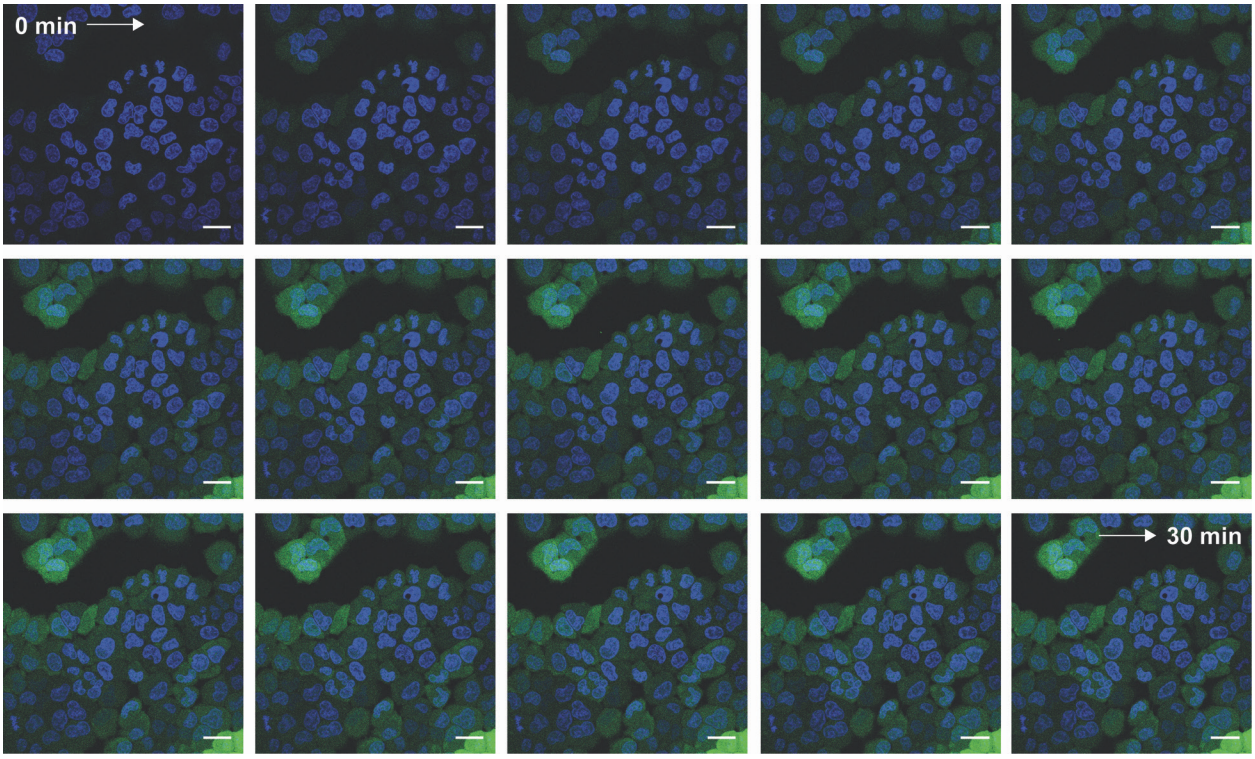


Figure S4. Confocal microscopy images showing the time-course for the uptake of conjugate **2** (10 μM) by live H460 cells. Image-acquisition began immediately after the on-stage addition of conjugate **2** in 300 μL of OptiMEM. Cells were stained with Hoechst 33342. Scale bars: 25 μm.

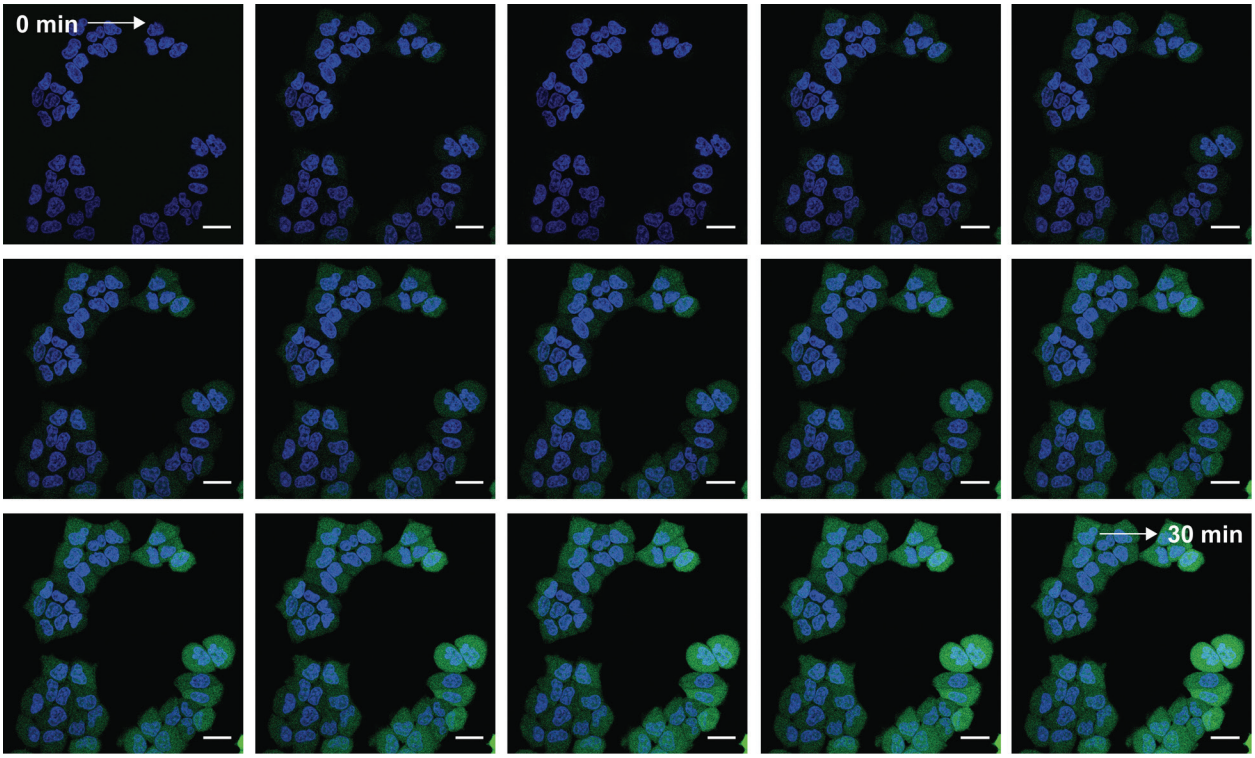


Figure S5. Confocal microscopy images showing the time-course for the uptake of conjugate **2** (10 μM) by live H1299 cells. Image-acquisition began immediately after the on-stage addition of conjugate **2** in 300 μL of OptiMEM. Cells were stained with Hoechst 33342. Scale bars: 25 μm.

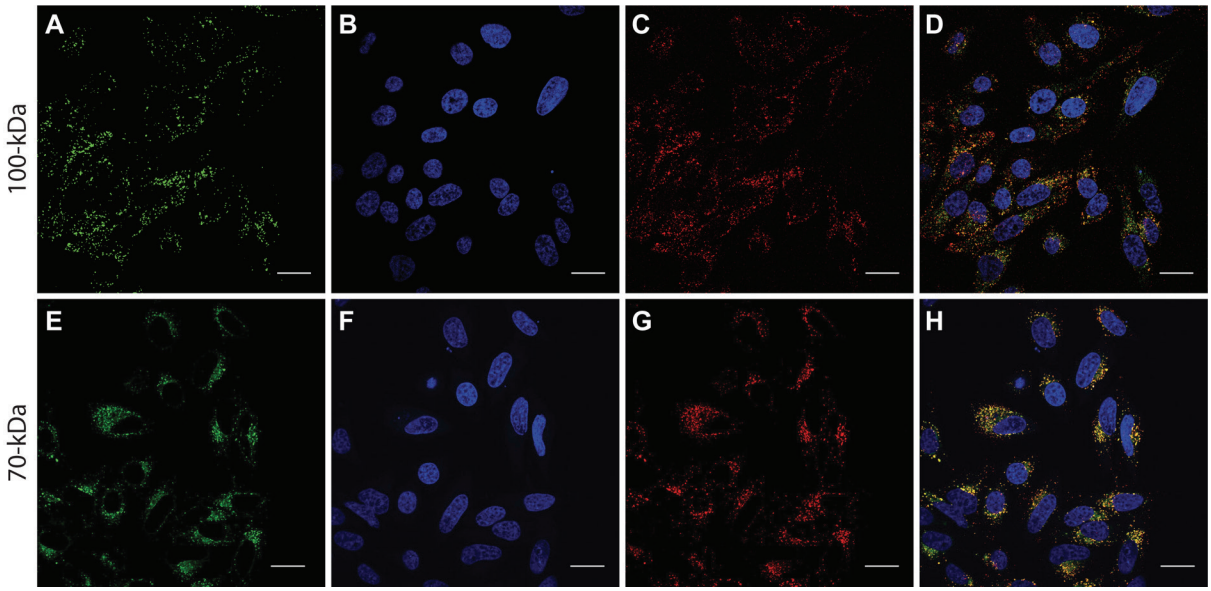


Figure S6. Confocal microscopy images of live HeLa cells incubated with 100-kDa TAMRA–dextran (A–D) or 70-kDa TAMRA–dextran (E–H) at 10 μM for 30 min at 37 °C. Images correspond to fluorescence of TAMRA–dextran (A,E), Hoechst 33342 (B,F), Lysotracker (C,G), and overlay (D,H). Scale bars: 25 μm.

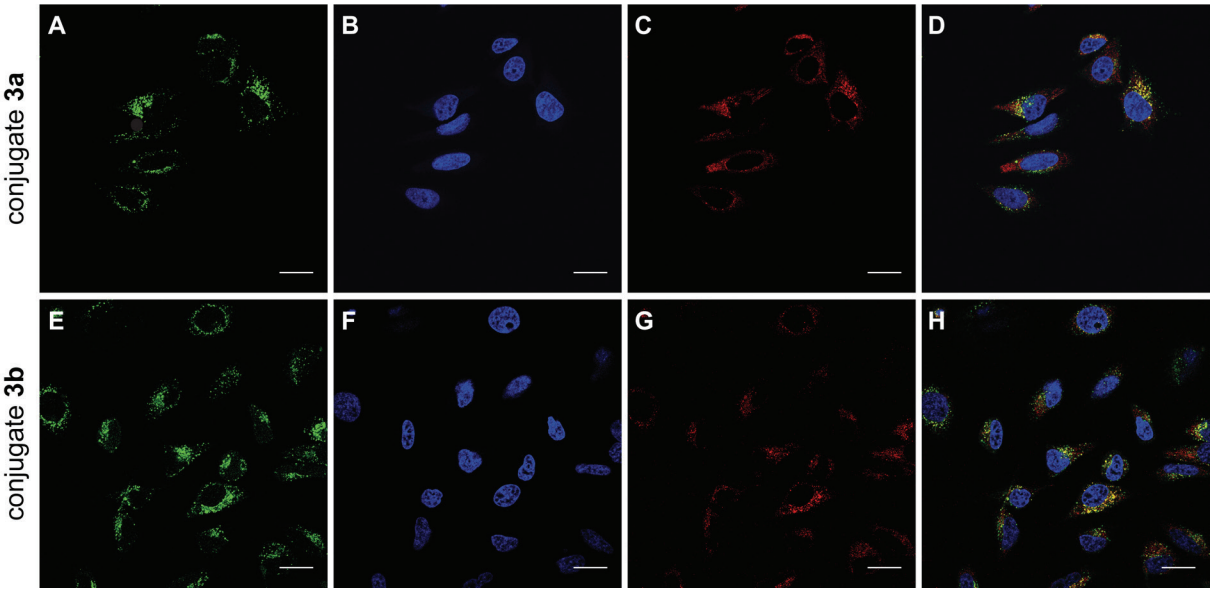


Figure S7. Confocal microscopy images of live HeLa cells incubated for 30 min with conjugate **3a** (A–D) or conjugate **3b** (E–H) at 10 μM for 30 min at 37 °C. Images correspond to fluorescence of conjugates (A,E), Hoechst 33342 (B,F), Lysotracker (C,G), and overlay (D,H). Scale bars: 25 μm.

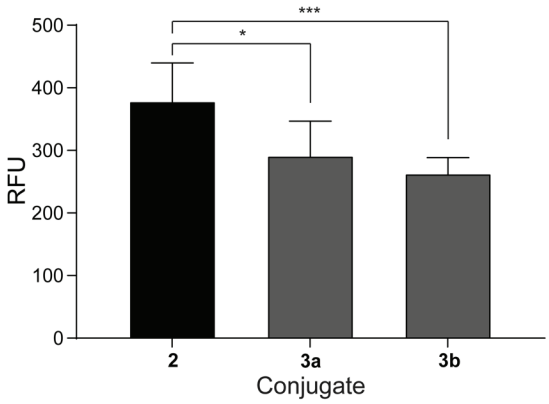


Figure S8. Graph showing the average fluorescence signal per cell measured in live HeLa cells incubated with dextran conjugates. Cells were incubated with conjugates **2**, **3a**, or **3b** (10 μM), washed, and imaged by confocal microscopy. *, $p < 0.05$; ***, $p < 0.001$.

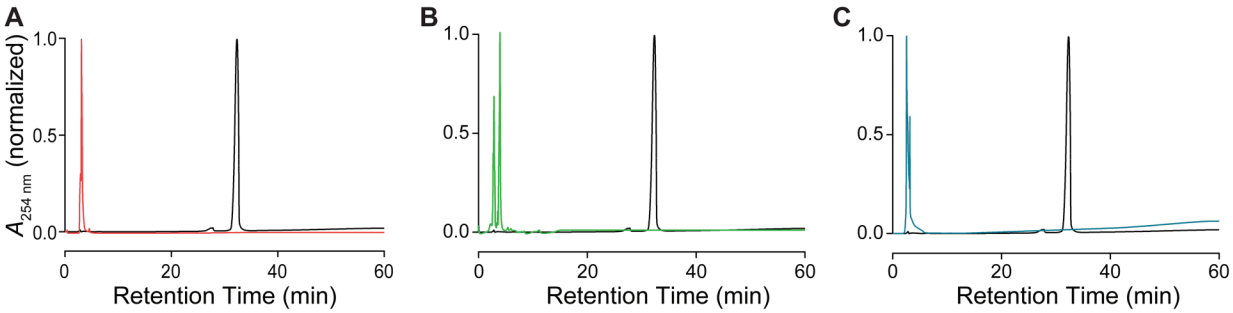


Figure S9. C4 HPLC traces of probe **1a** (black) and conjugate **2** (color) after incubation for 1 h in 1.0 M HCl (A), DMEM containing FBS (B), or HeLa cell lysate (C).

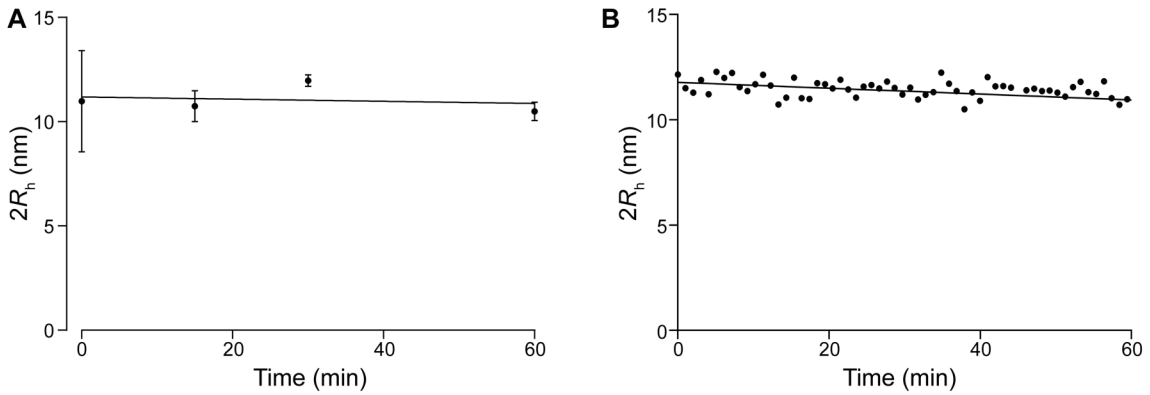


Figure S10. Graphs showing the time-course of the hydrodynamic radius (R_h) of dextran **D1** upon treatment with 1.0 M HCl at 25 °C (A) or 60 °C (B).

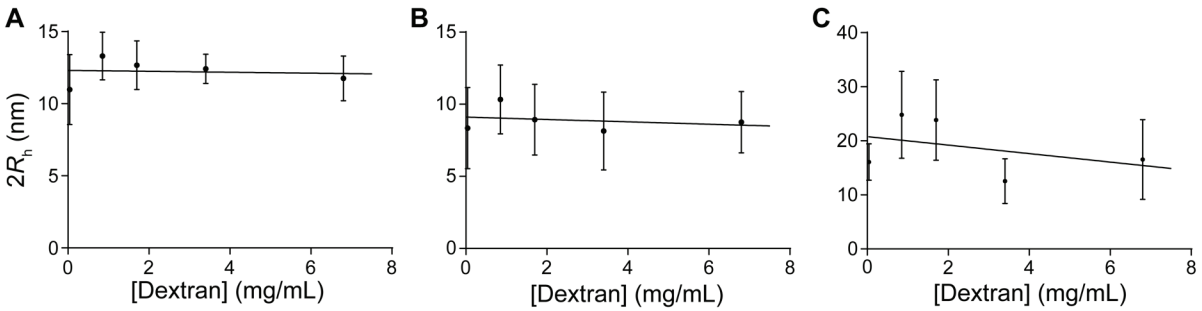


Figure S11. Graphs showing the effects of concentration on the hydrodynamic radius (R_h) of dextran **D1** (A), dextran **D2** (B), and dextran **D3** (C).

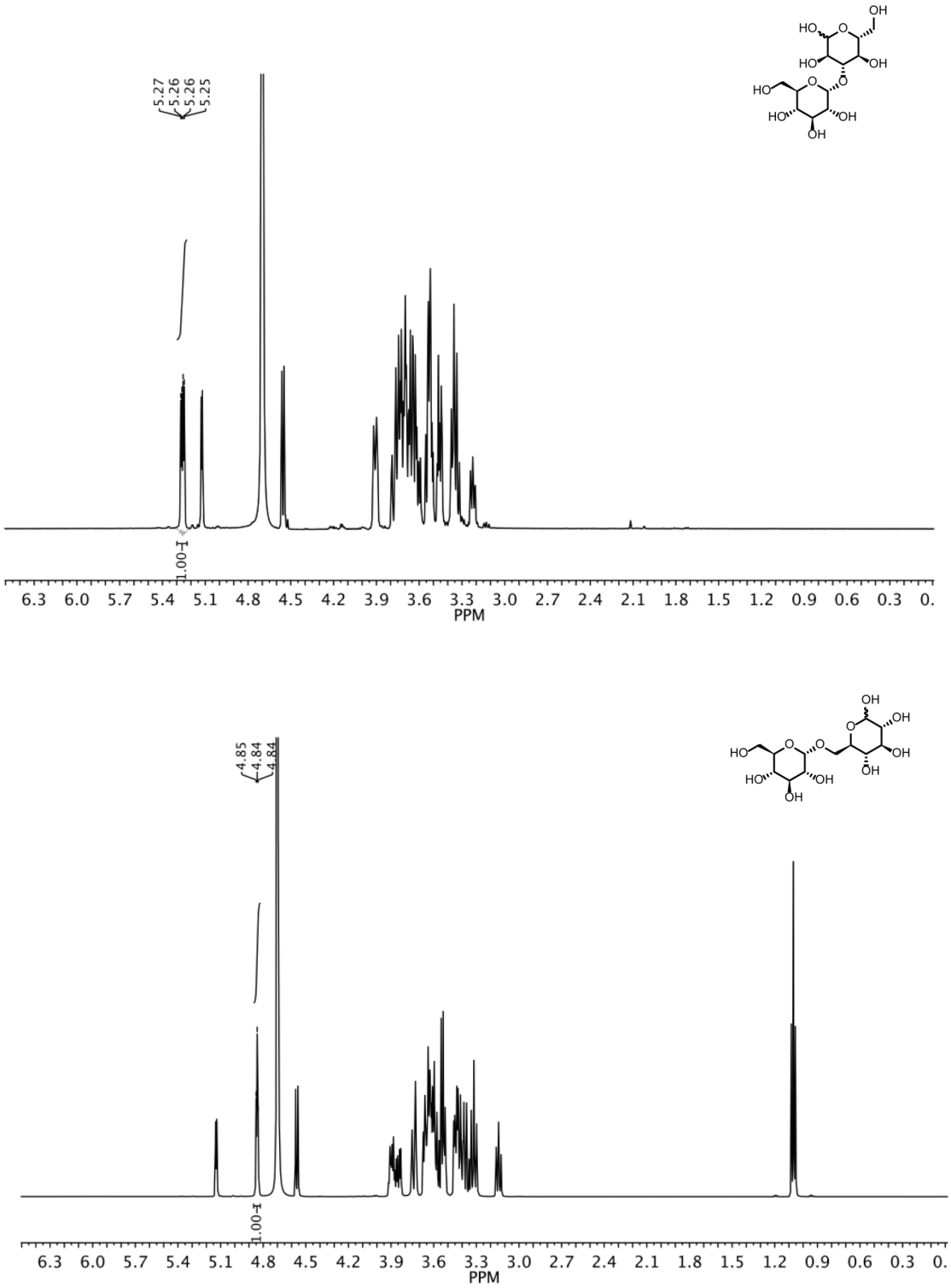
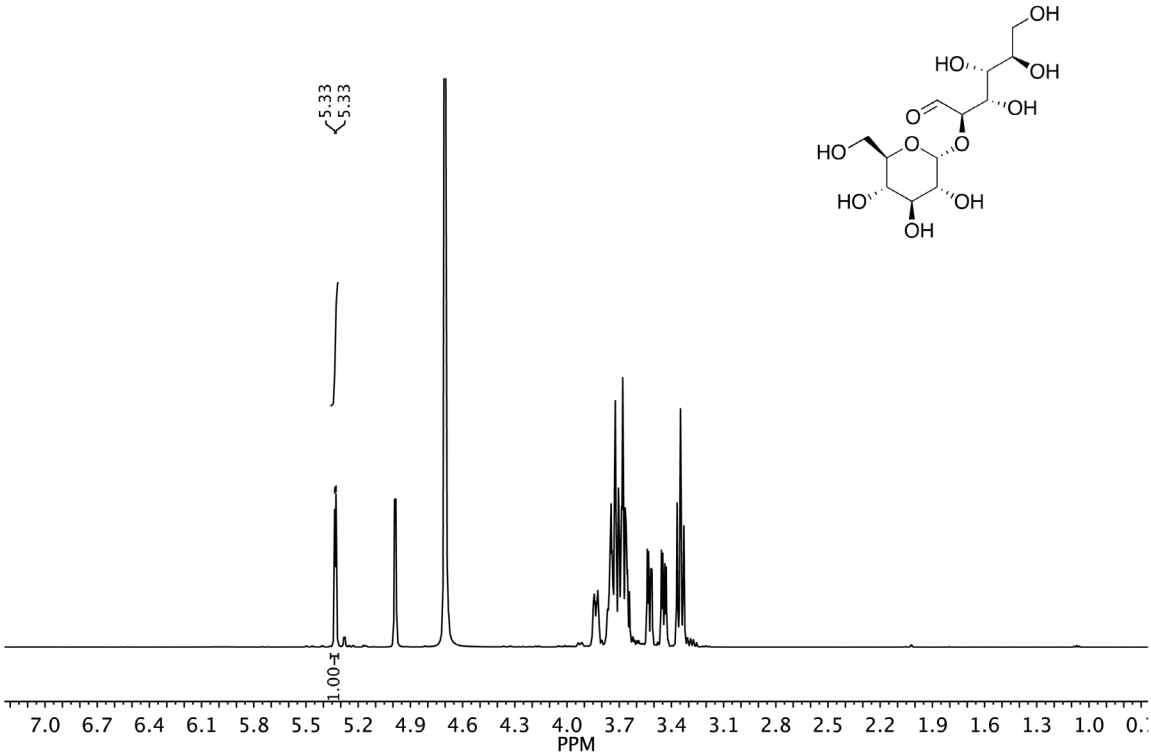


Figure S12. ^{1}H-NMR spectra of nigerose (top) and isomaltose (bottom) in D_2O.



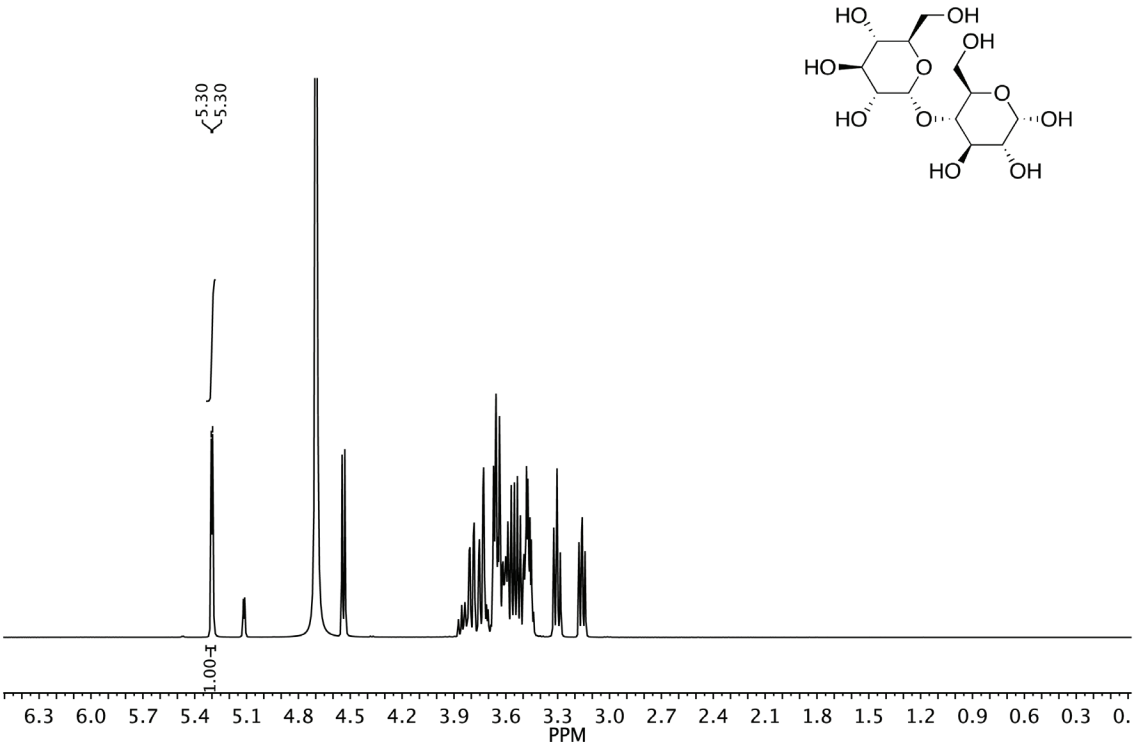


Figure S13. ^{1}H-NMR spectra of kojibiose (top) and maltose (bottom) in D_2O.

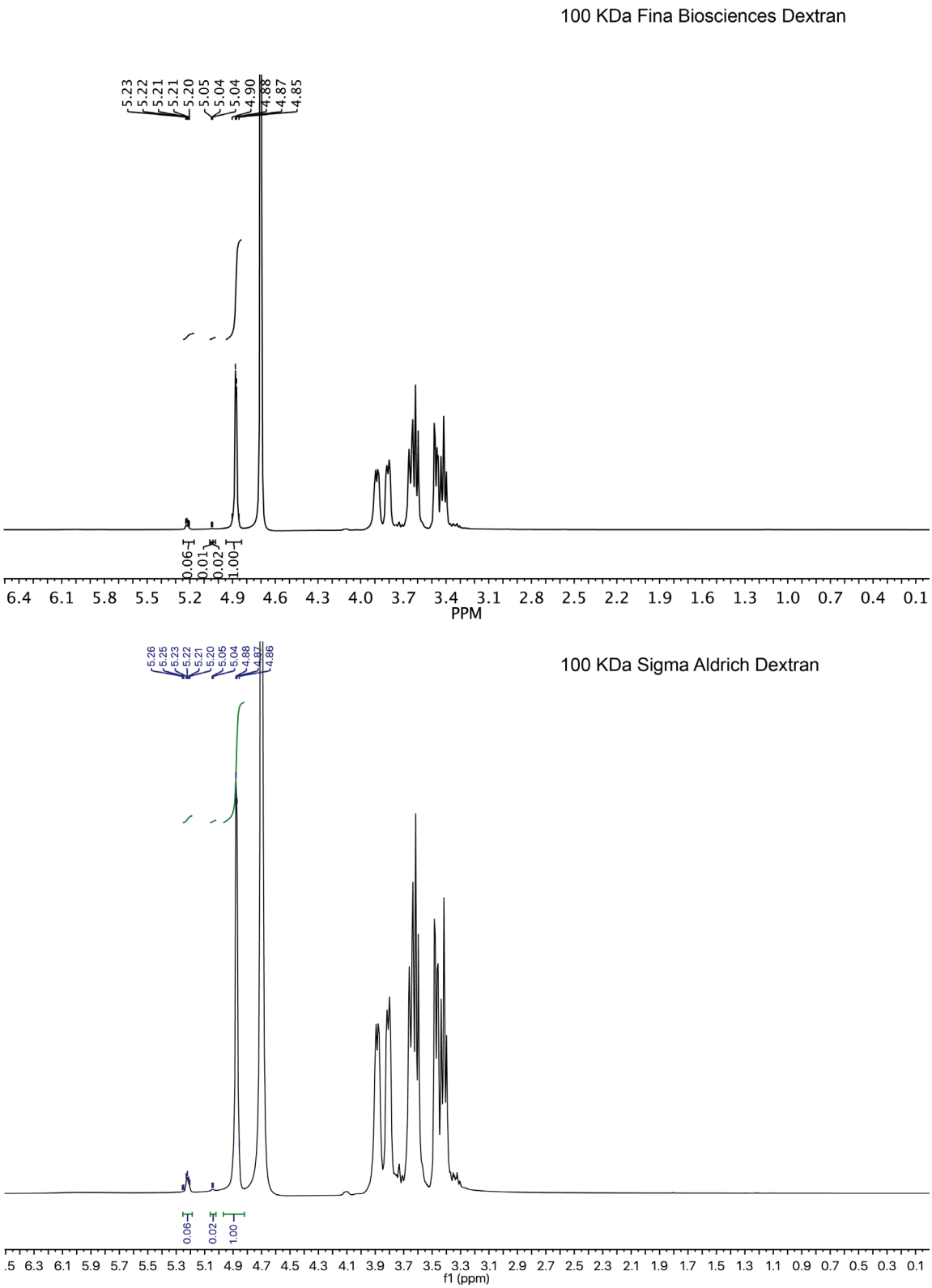


Figure S14. ^{1}H-NMR spectra of dextran **D1** (top) and dextran **D4** (bottom) in D_2O.

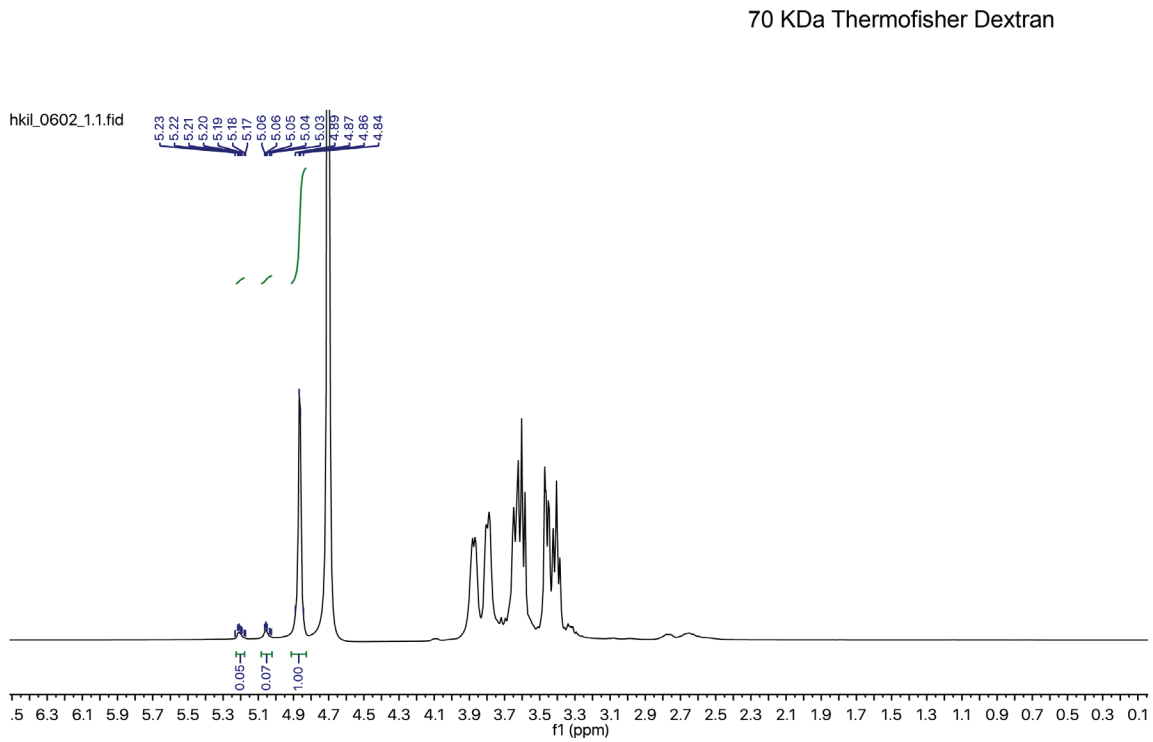
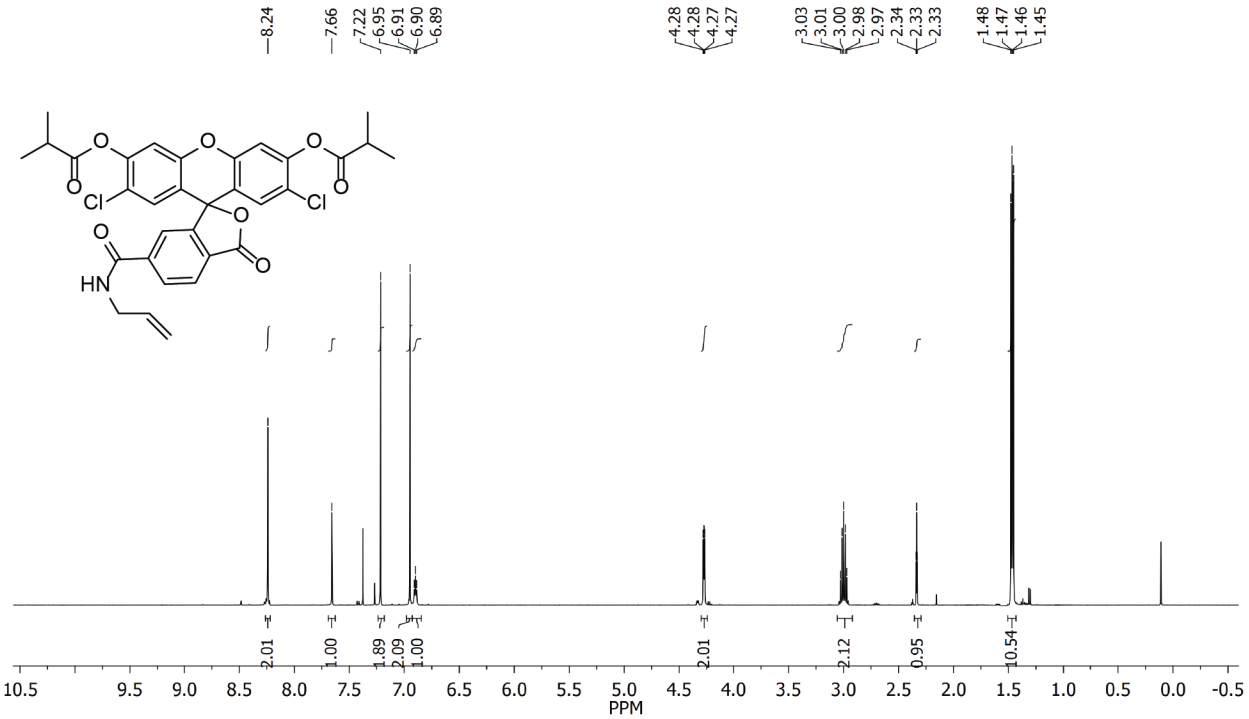
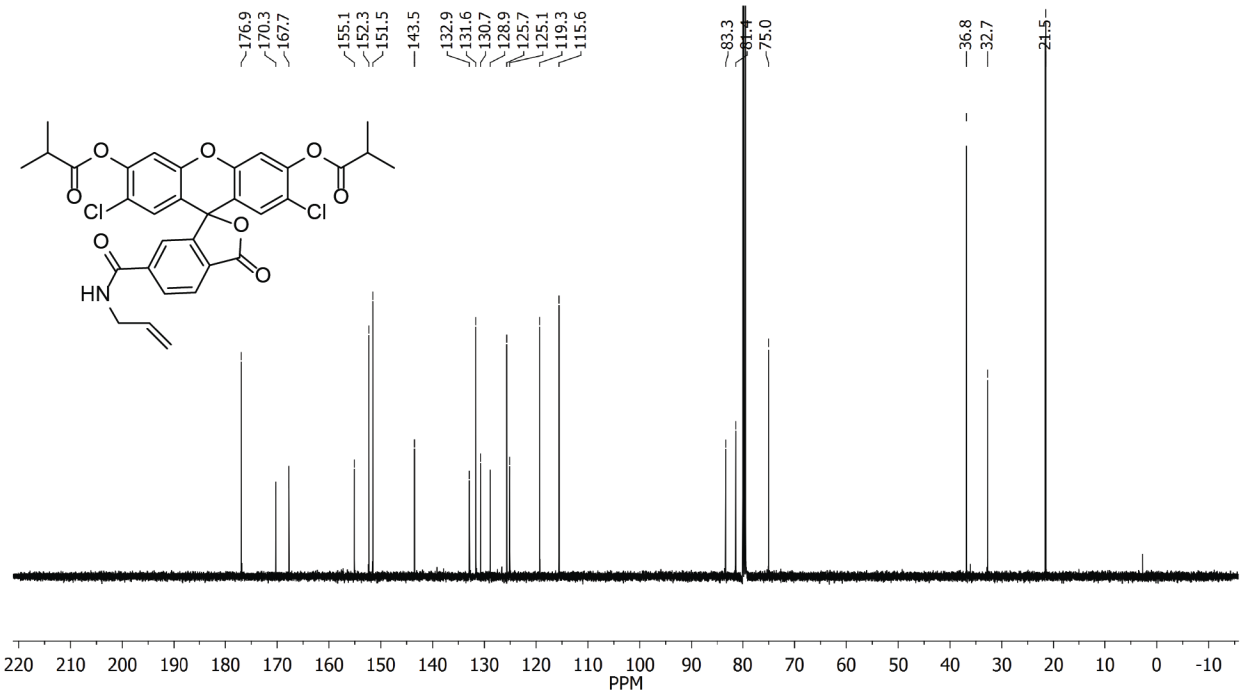


Figure S15. ^{1}H-NMR spectrum of dextran **D2** in D_2O.

NMR Spectra of Compounds 1a and 1b

^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra of compound **1a**





^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra of compound **1b**