

Supporting Information
for DOI: 10.1055/a-2093-3528

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Supplementary Information

CD44 and EGFR Dual-Targeted Antibody-Recruiting Complex Based on Hyaluronic Acid Grafted with β -Cyclodextrin and Multivalent Rhamnose for Cancer Immunotherapy

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Supporting figures

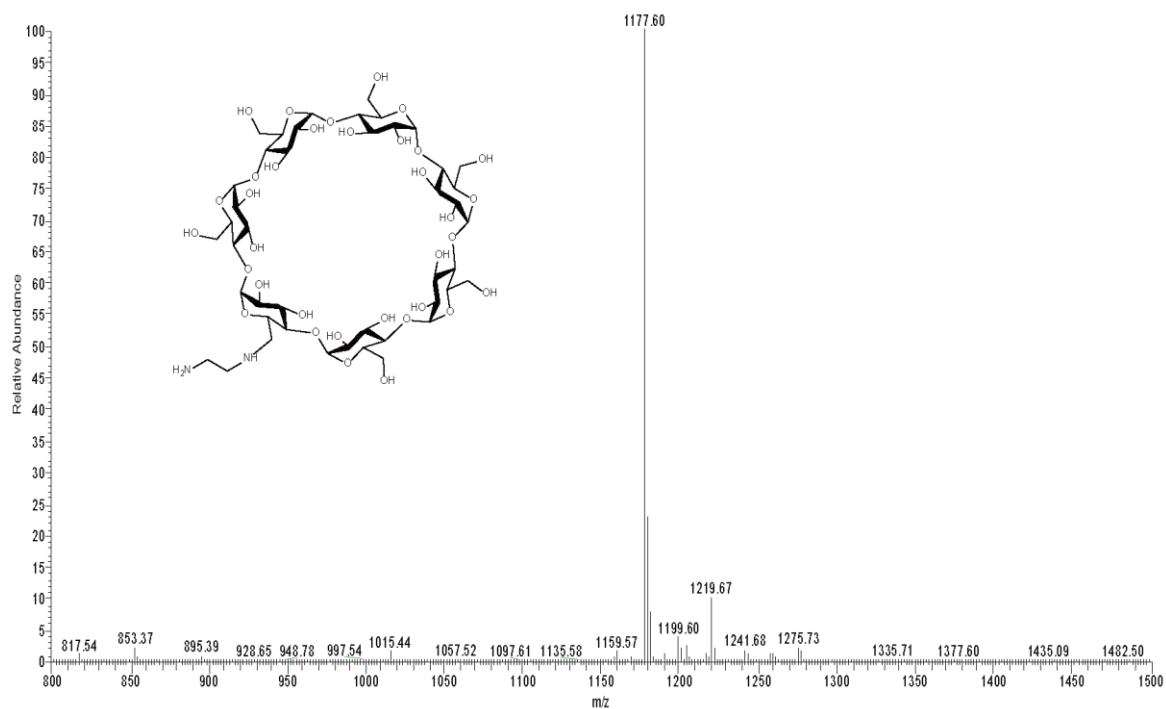


Figure S1. ESI-MS of β -CD-EDA. m/z $[M+H]^+$ calcd for $C_{44}H_{76}N_2O_{34}$: 1177.43; found: 1177.60.

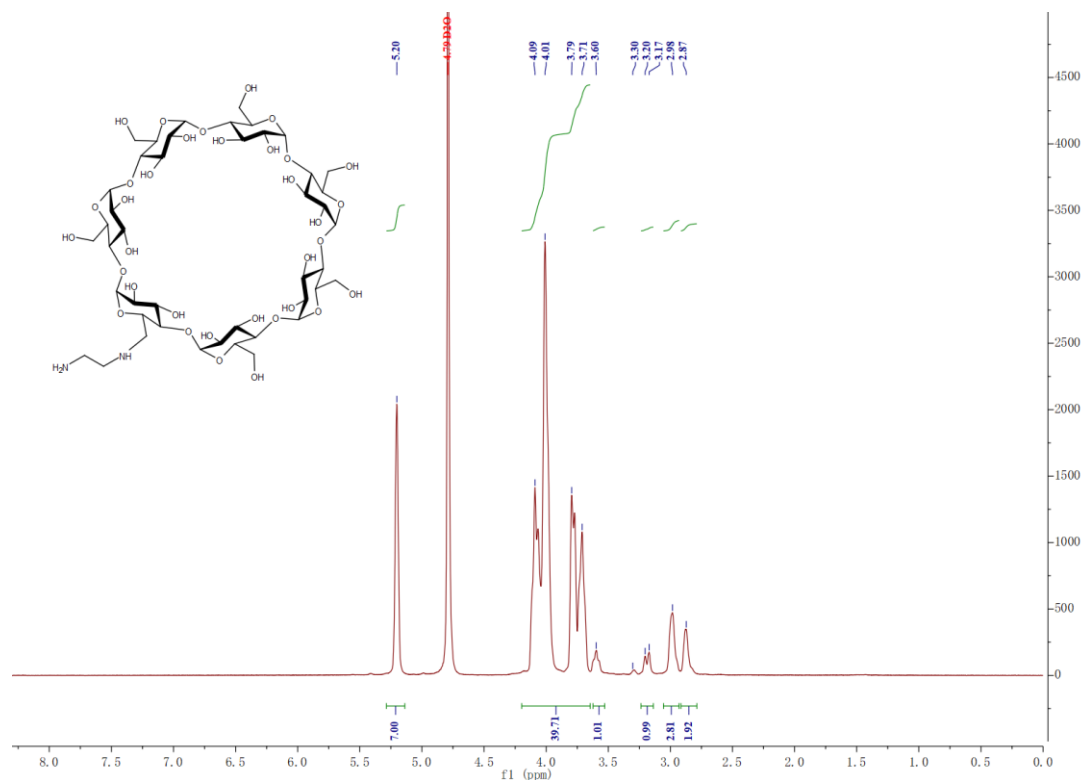


Figure S2. ¹H NMR (400 MHz) spectra and peaks assignments of β-CD-EDA in D₂O. ¹H NMR (400 MHz, D₂O) δ 5.20 (s, 7H), 4.05-3.75 (m, 40H), 3.60 (s, 1H), 3.17 (d, *J* = 12.6 Hz, 1H), 2.98 (s, 3H), 2.87 (s, 2H). The peak at 5.20 ppm is apparent singlet because the anomeric peaks overlap.

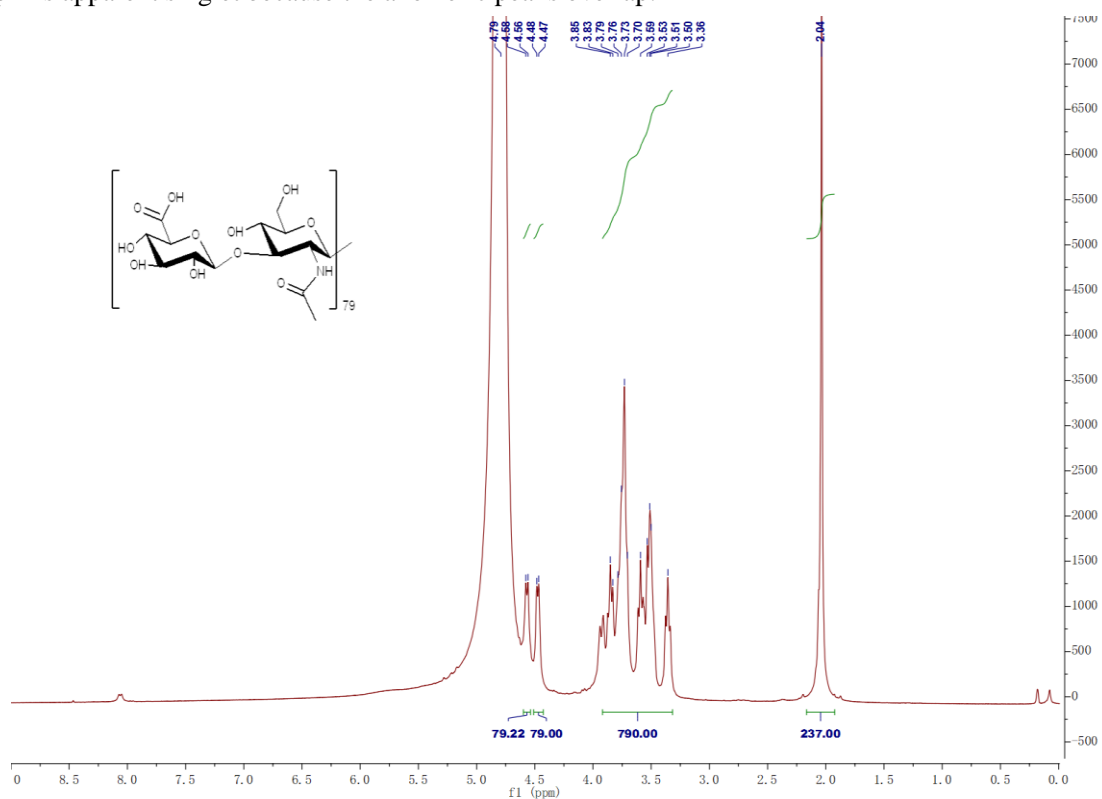


Figure S3. ¹H NMR (400 MHz) spectra and peaks assignments of HA in D₂O. ¹H NMR (400 MHz, D₂O) δ 4.57 (d, *J*=8.2 Hz, 79H), δ 4.47 (d, *J*=7.7Hz, 79H), 3.92-3.32 (m, 790H), 2.04 (s, 237H).

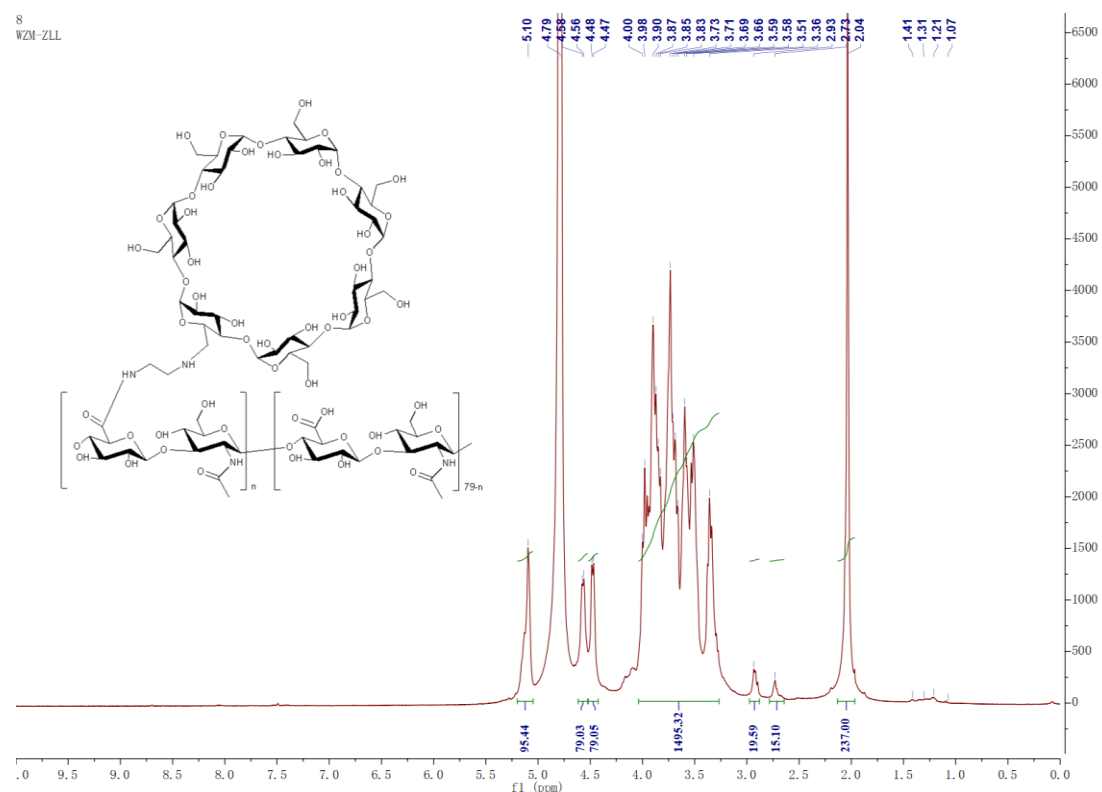


Figure S4. ^1H NMR (400 MHz) spectra and peaks assignments of HACD in D_2O . ^1H NMR (400 MHz, D_2O) δ 5.10 (s, 95H, anomeric of β -CD), 4.57 (d, $J = 7.1$ Hz, 79H, anomeric of HA), 4.48 (d, $J = 7.6$ Hz, 79H, anomeric of HA), 4.04 – 3.27 (m, 1495H), 2.93 (s, 20H), 2.73 (s, 15H), 2.04 (s, 237H).

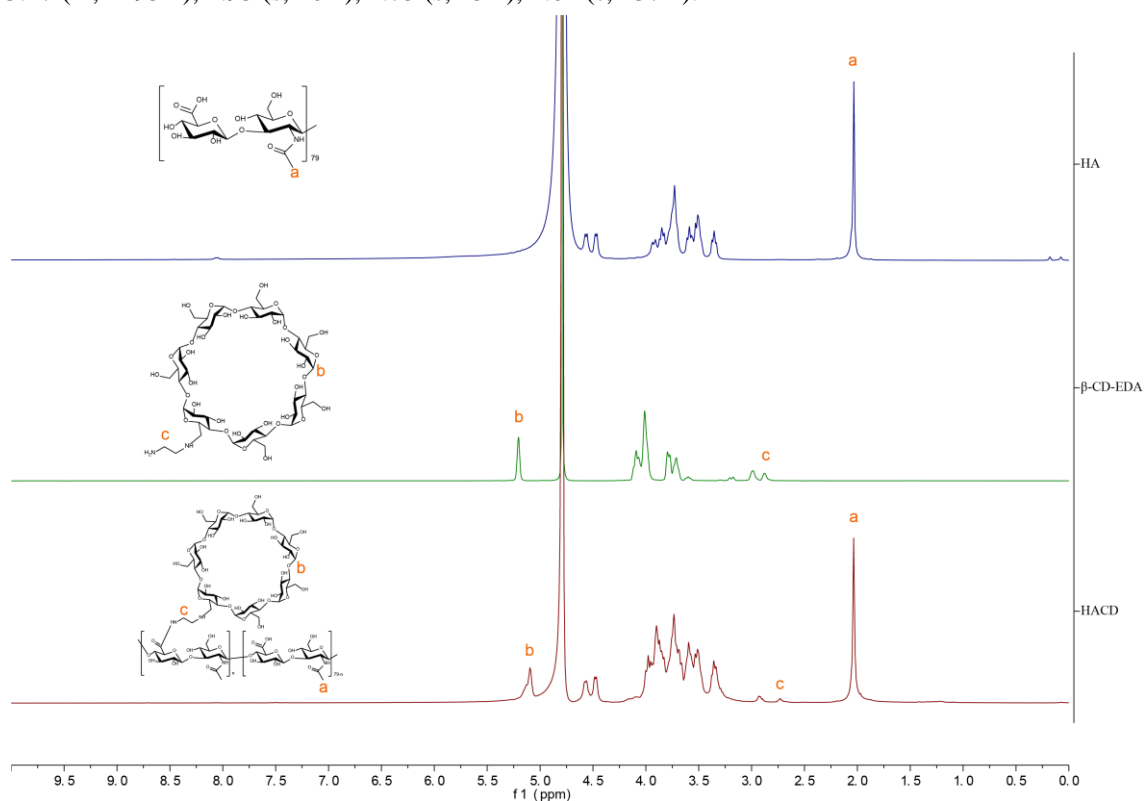


Figure S5. Characterization comparison of HA, β -CD-EDA and HACD.

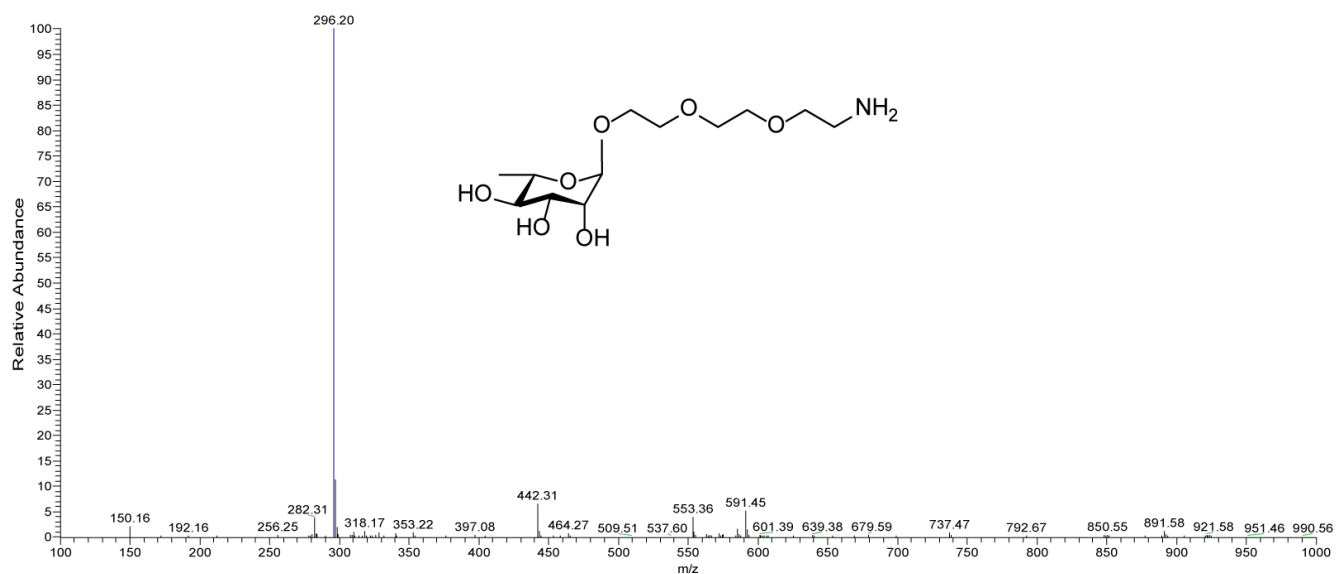


Figure S6. ESI-MS of Rha-PEG₃-NH₂. *m/z* [M+H]⁺ calcd for C₁₂H₂₅NO₇: 296.16; found: 296.20.

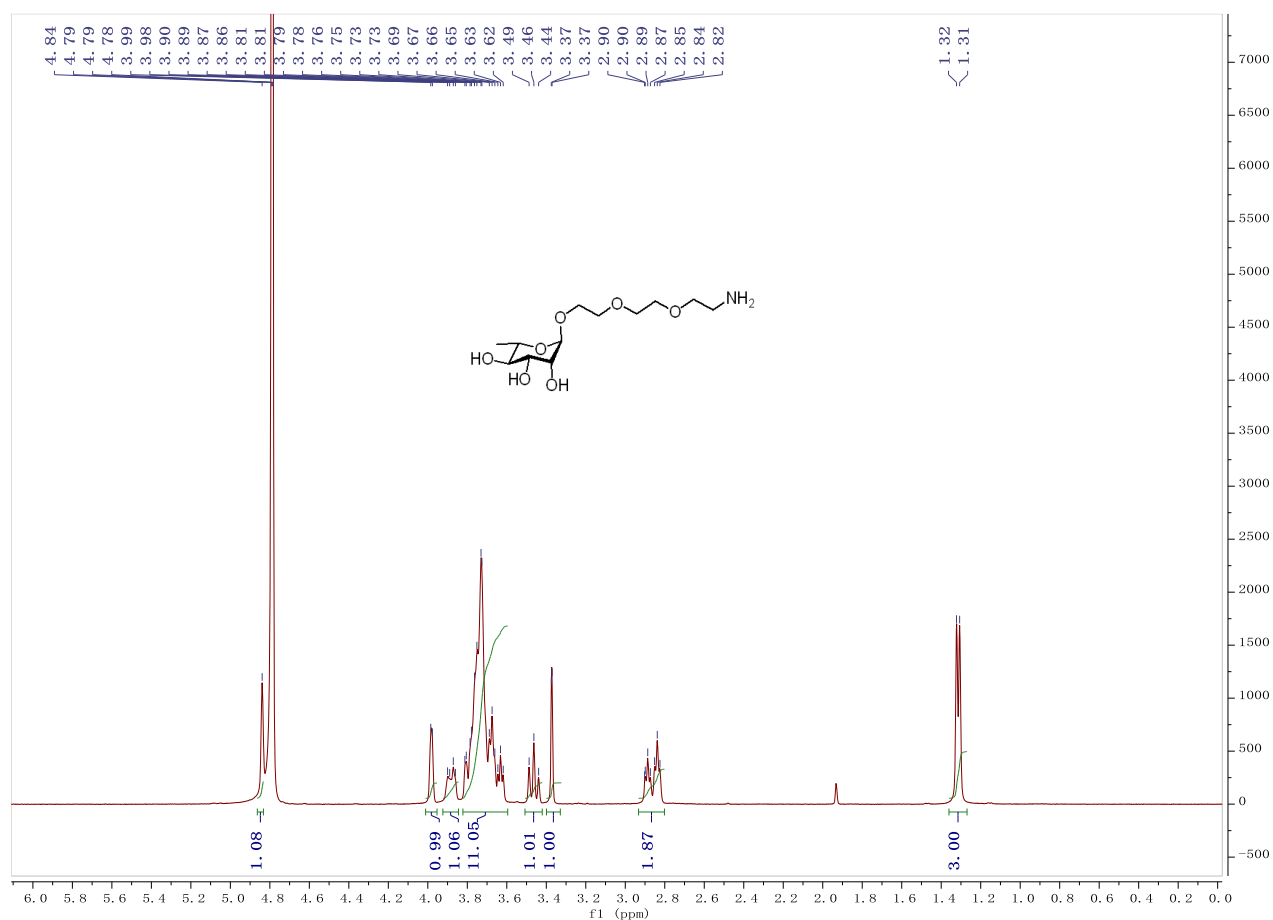


Figure S7. ¹H NMR (400 MHz) spectra and peaks assignments of Rha-PEG₃-NH₂ in D₂O. ¹H NMR (400 MHz, D₂O) δ 4.84 (s, 1H), 3.98 (d, *J* = 3.3 Hz, 1H), 3.88 (dd, *J* = 11.6, 4.3 Hz, 1H), 3.81 – 3.62 (m, 11H), 3.46 (t, *J* = 9.6 Hz, 1H), 3.37 (d, *J* = 1.5 Hz, 1H), 2.87 (dt, *J* = 19.4, 5.3 Hz, 2H), 1.31 (d, *J* = 6.5 Hz, 3H).

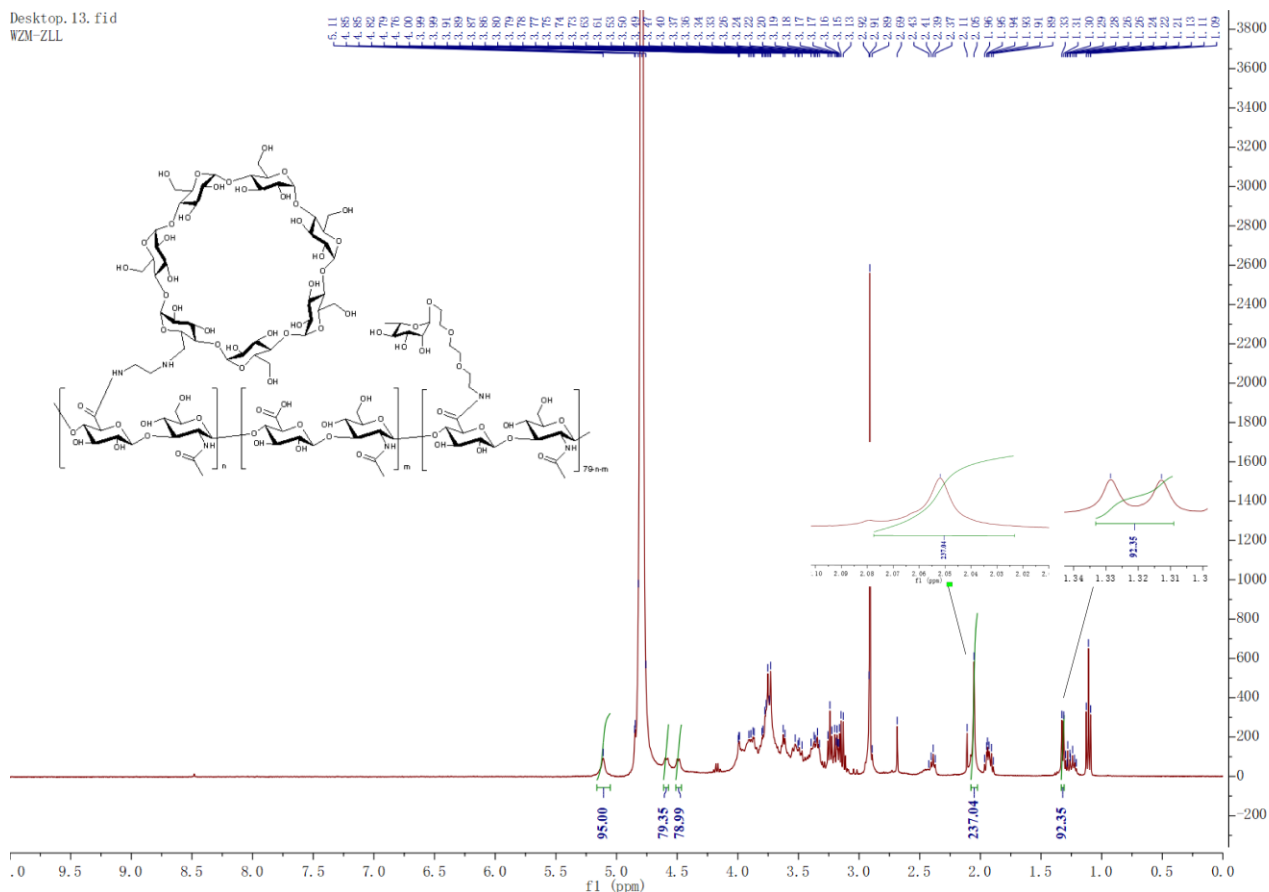


Figure S8. ^1H NMR (400 MHz) spectra and peaks assignments of Rha-HACD in D_2O . ^1H NMR (400 MHz, D_2O) δ 5.11 (s, 95H, anomeric of β -CD), 4.59 (d, $J = 8.1$ Hz, 79H, anomeric of HA), 4.49 (d, $J = 7.6$ Hz, 79H, anomeric of HA), 2.05 (s, 237H), 1.32 (d, $J = 6.3$ Hz, 92H, CH_3 of Rha).

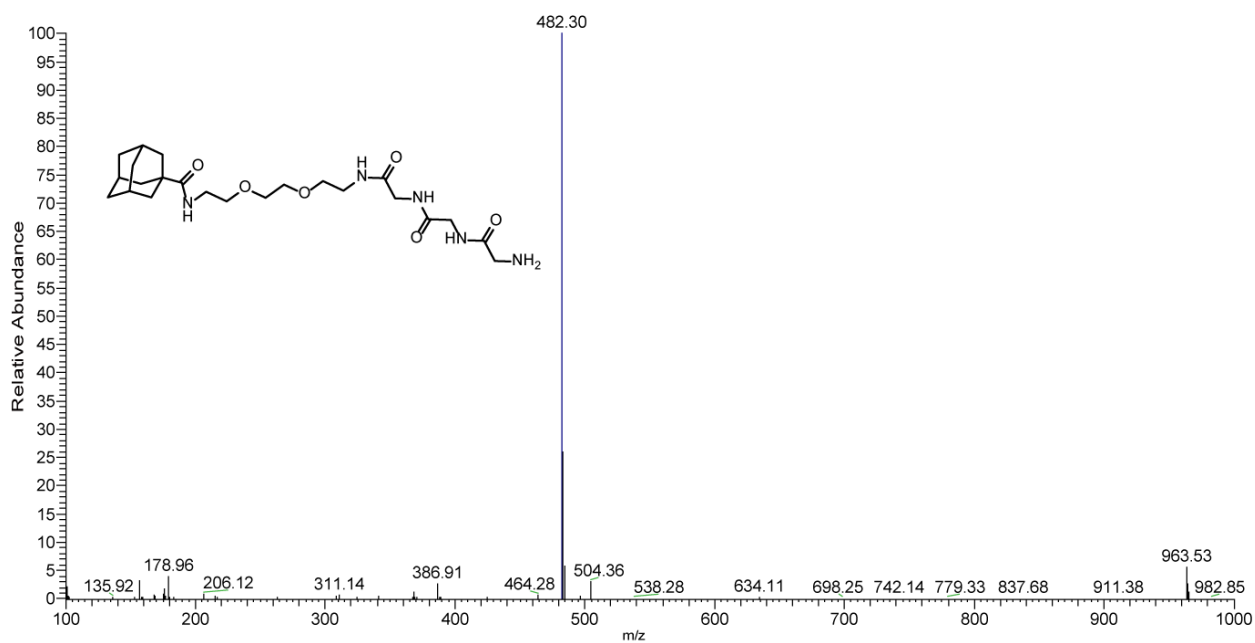


Figure S9. ESI-MS of GGG-PEG₂-ada. m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{39}\text{N}_5\text{O}_6$: 482.29; found: 482.30.

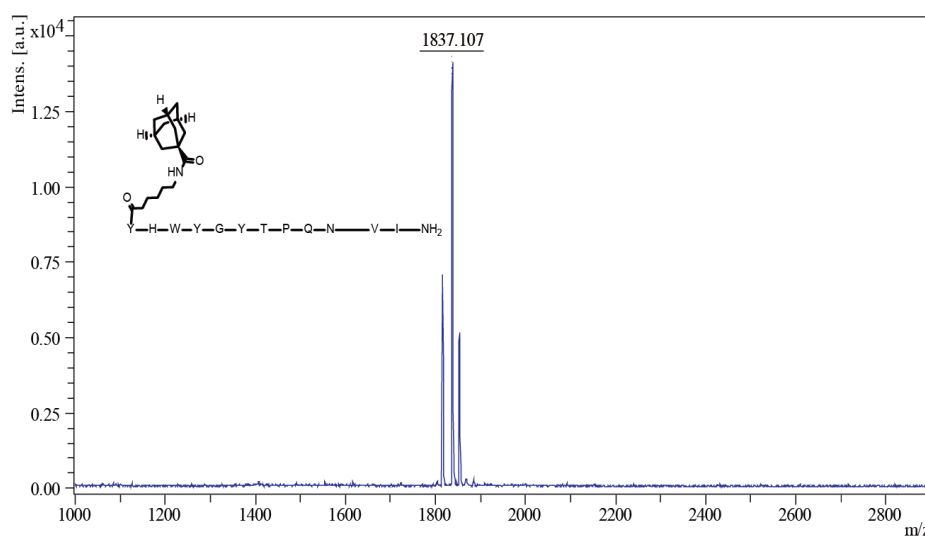


Figure S10. MALDI-TOF MS of ada-GE11. m/z [M+Na]⁺ calcd for C₉₂H₁₂₂N₁₈O₂₁: 1837.88; found: 1837.11.

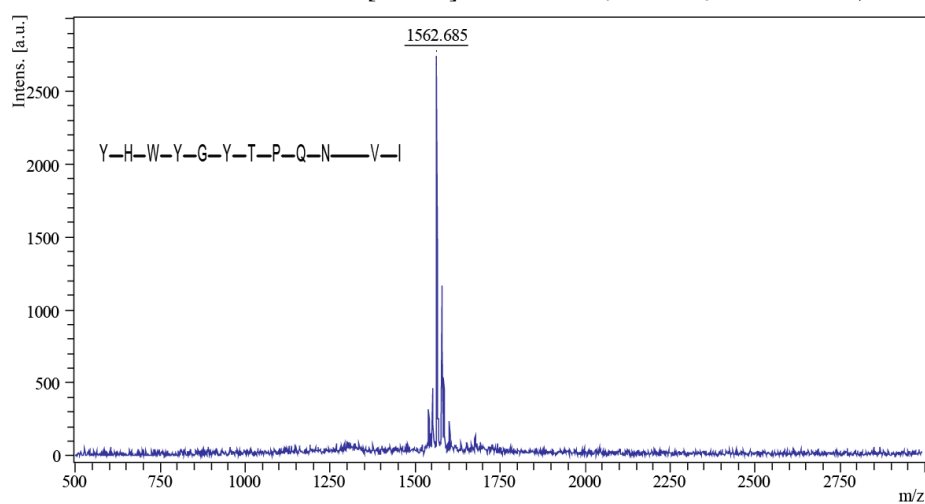


Figure S11. MALDI-TOF MS of GE11. m/z [M+Na]⁺ calcd for C₇₅H₉₇N₁₇O₁₉: 1562.71; found: 1562.69.

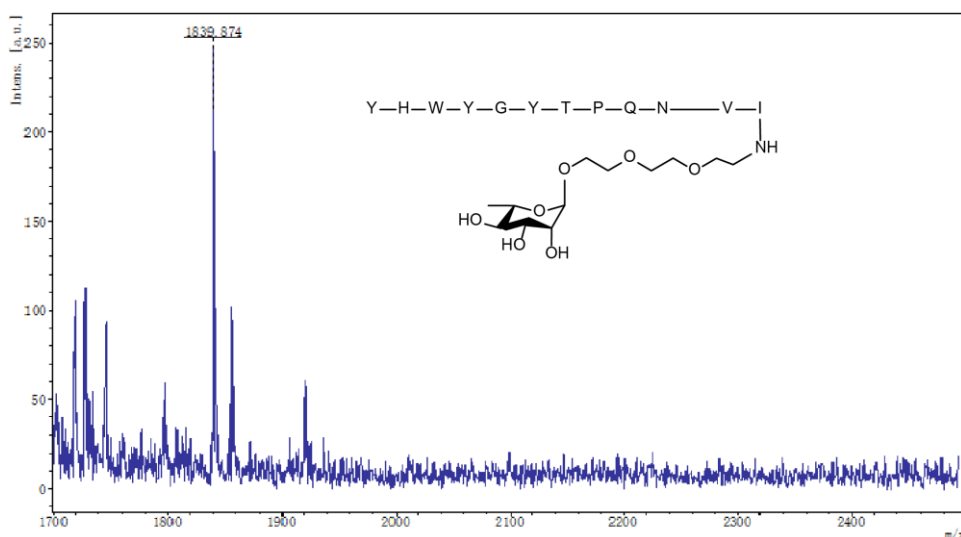


Figure S12. MALDI-TOF MS of Rha-GE11. m/z [M+Na]⁺ calcd for C₈₇H₁₂₀N₁₈O₂₅: 1839.87; found: 1839.87.

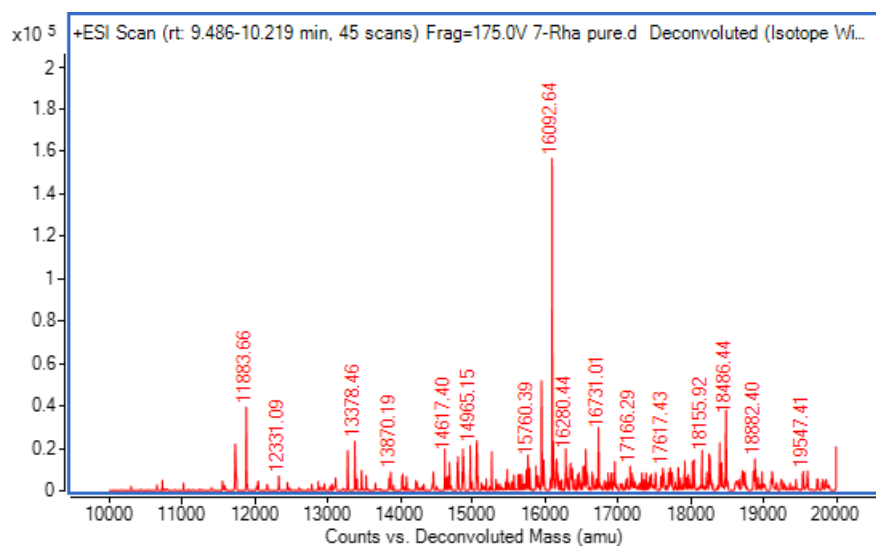


Figure S13. MS of Rha-7D12. Observed: 16092.6 Da, calculated: 16092.4 Da.

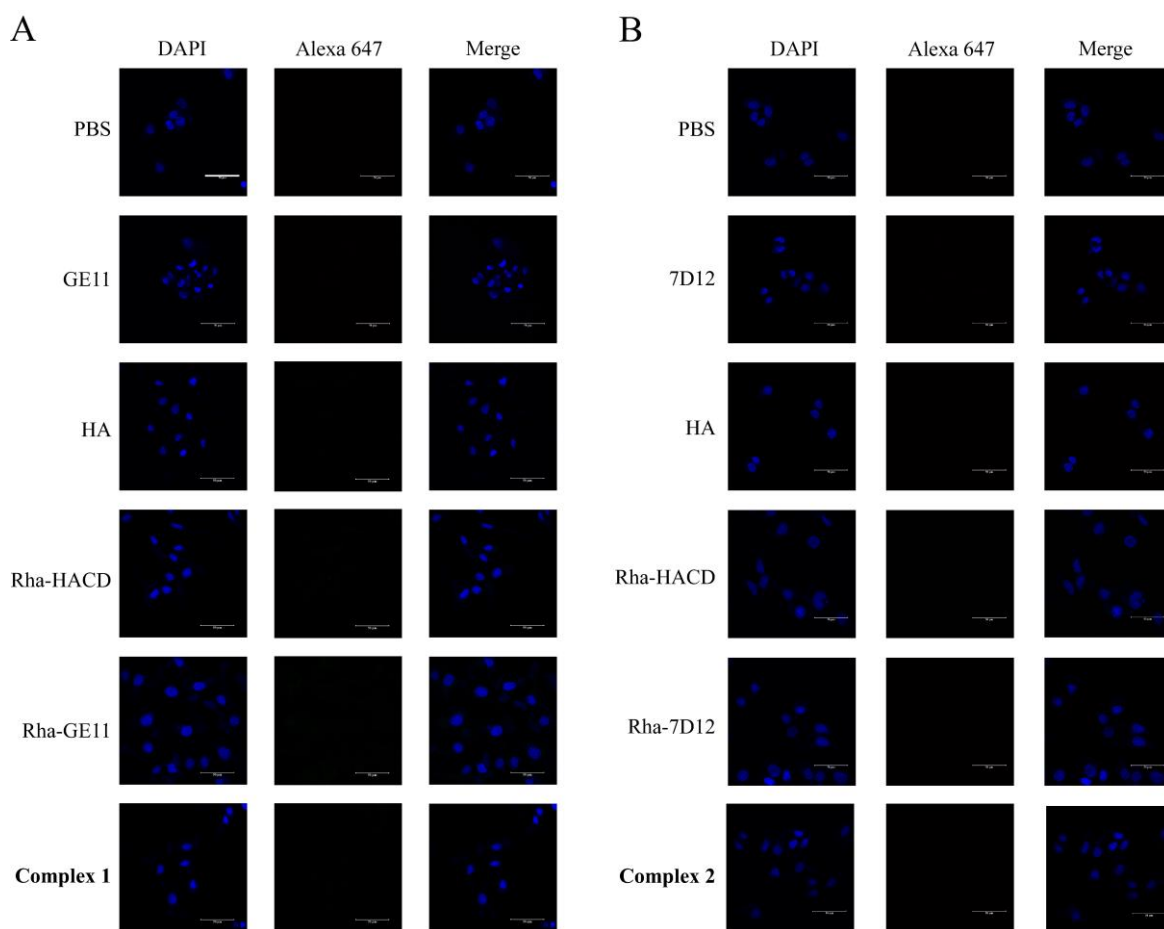


Figure S14. Evaluation of the anti-Rha antibody recruiting capacities of conjugates. (A) and (B): Immunofluorescence of MCF-7 cells treated with relevant compounds in the presence of Alexa Fluor 647-conjugated anti-Rha IgGs. Scale bar: 50 μ m.