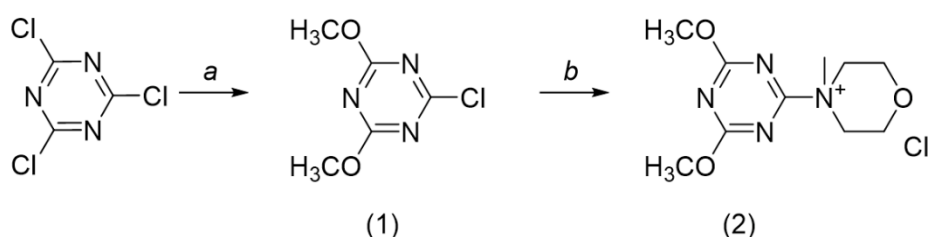
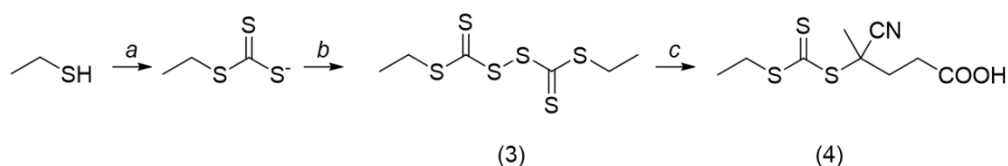


Supplementary Materials: Polymer Coated Oncolytic Adenovirus to Selectively Target Hepatocellular Carcinoma Cells

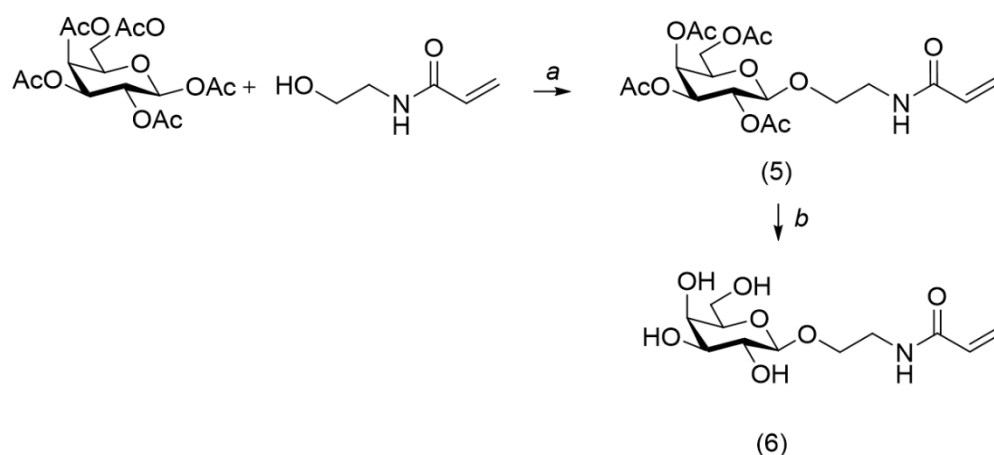
Mariangela Garofalo, Federica Bellato, Salvatore Magliocca, Alessio Malfanti, Lukasz Kuryk, Beate Rinner, Samuele Negro, Stefano Salmaso, Paolo Caliceti, Francesca Mastrotto



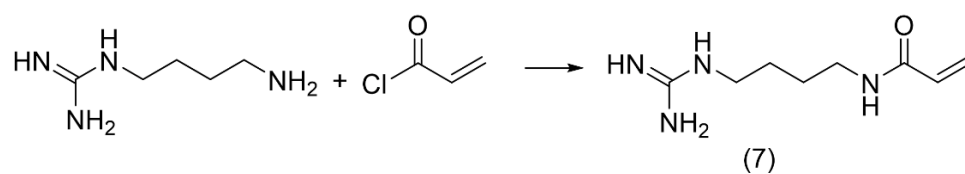
Scheme S1. Synthesis of CDMT (1) intermediate and DMTMM (2). *Reagent and conditions:* (a) MeOH, milliQ water, Na₂CO₃, from 0 to 35 °C, 12 h; (b) N-methylmorpholine, THF, room temperature, 30 min.



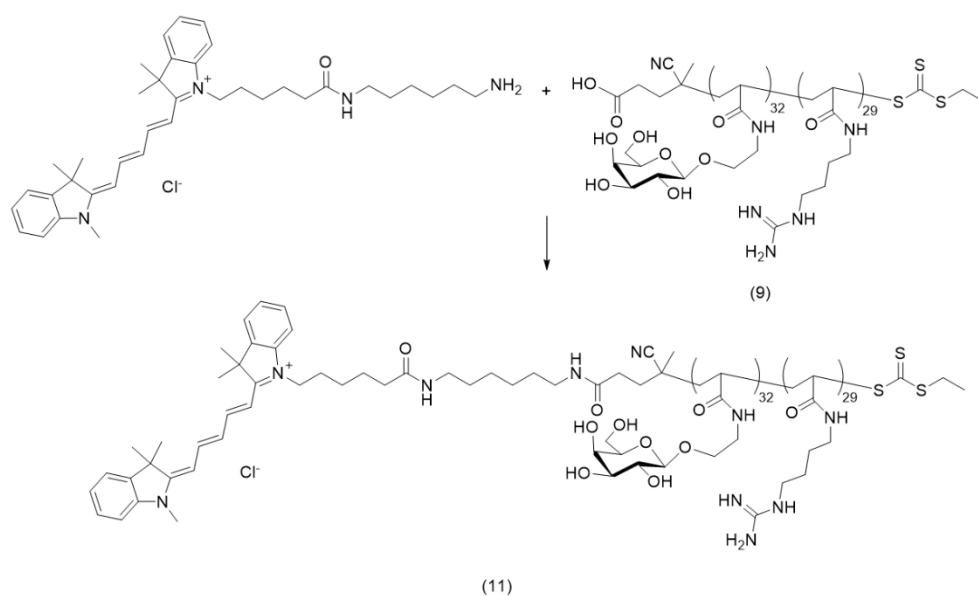
Scheme S2. Synthesis of bis(ethylsulfanylthiocarbonyl)disulfide (3) intermediate and 4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid (4). *Reagents and conditions:* (a) CS₂, NaOH, water:acetone 4:1, 0 °C, 1 h; (b) K₃[Fe(CN)₆]; (c) 4,4'-azobis(cyanopentanoic acid), EtOH, 60 °C, 16 h.



Scheme S3. Synthesis of 2,3,4,6-O-tetraacetyl-D-galactopyranosyloxyethyl acrylamide (5) and its deprotection to yield D-galactopyranosyloxyethyl acrylamide (6). *Reagents and conditions:* (i) BF₃Et₂O, CH₂Cl₂, 0 °C; (ii) KOH, CH₃OH.



Scheme S4. Synthesis of agmatine acrylamide (7). *Reagents and conditions:* K_2CO_3 , NaOH, 0 °C, 16 h.



Scheme S5. Synthesis of Cy5-Gal₃₂-b-Agm₂₉ (11) by coupling reaction of Cy5-NH₂ with Gal₃₂-b-Agm₂₉ copolymer. *Reagent and conditions:* DMTMM, 50 mM MES pH 6.8, room temperature, 16 h.

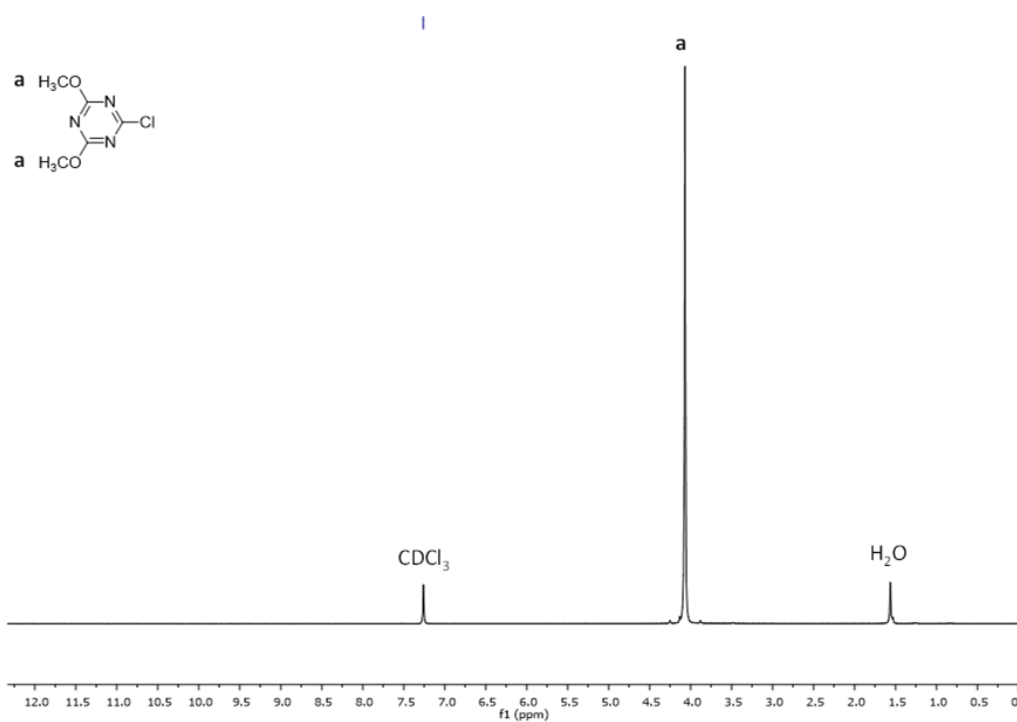


Figure S1. 1H NMR of CDMT (1) in $CDCl_3$.

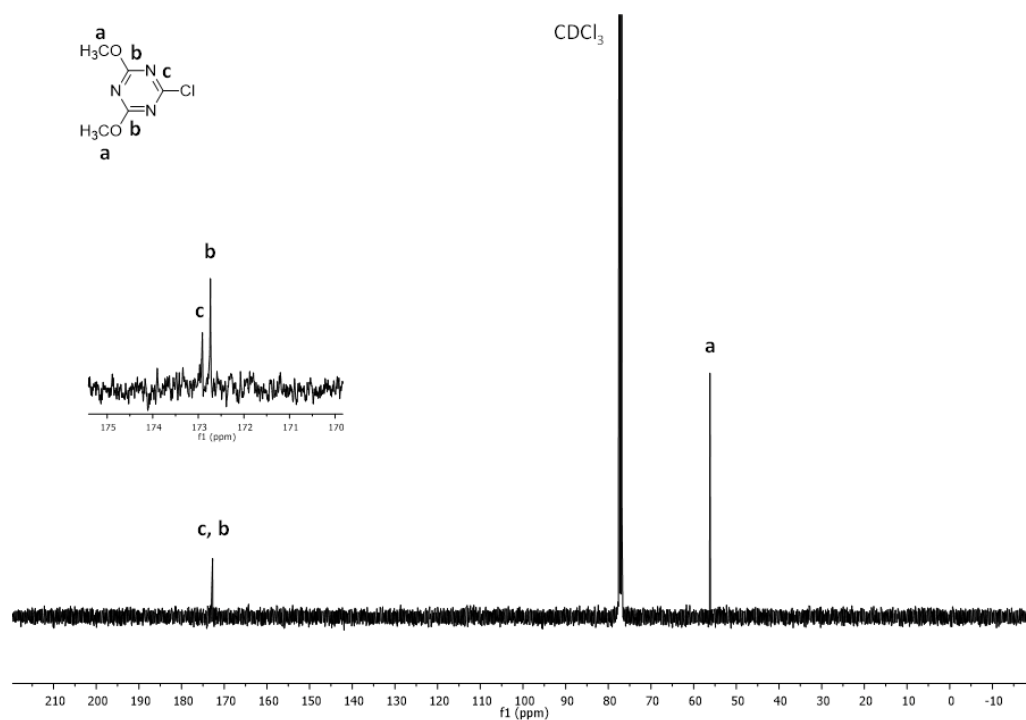


Figure S2. ¹³C NMR of CDMT (2) in CDCl₃.

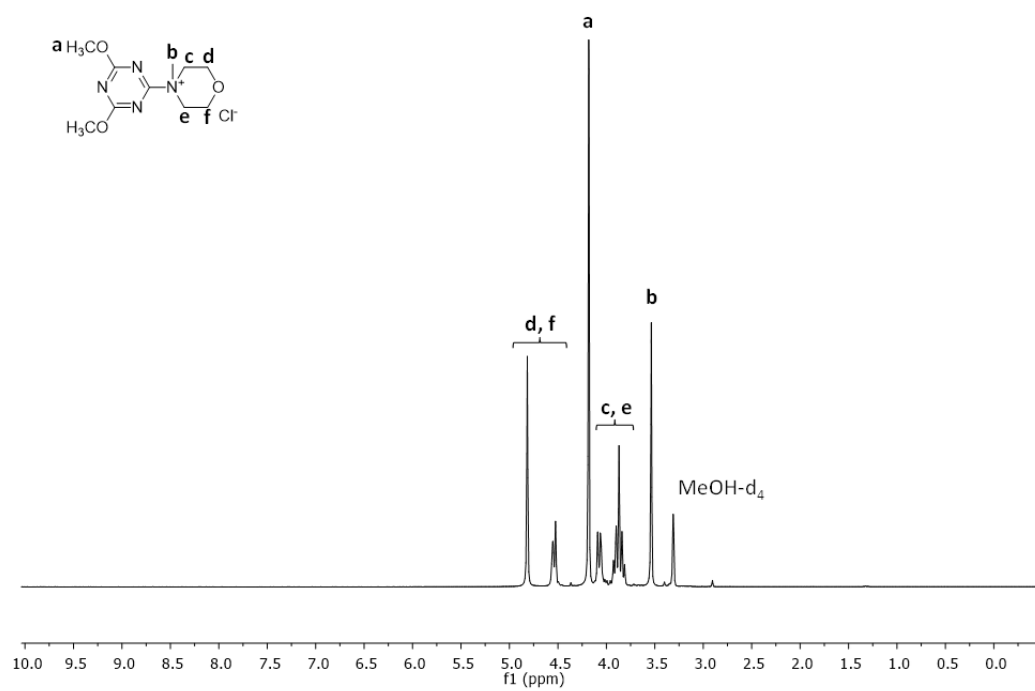


Figure S3. ¹H NMR of DMTMM (2) in MeOH-*d*₄.

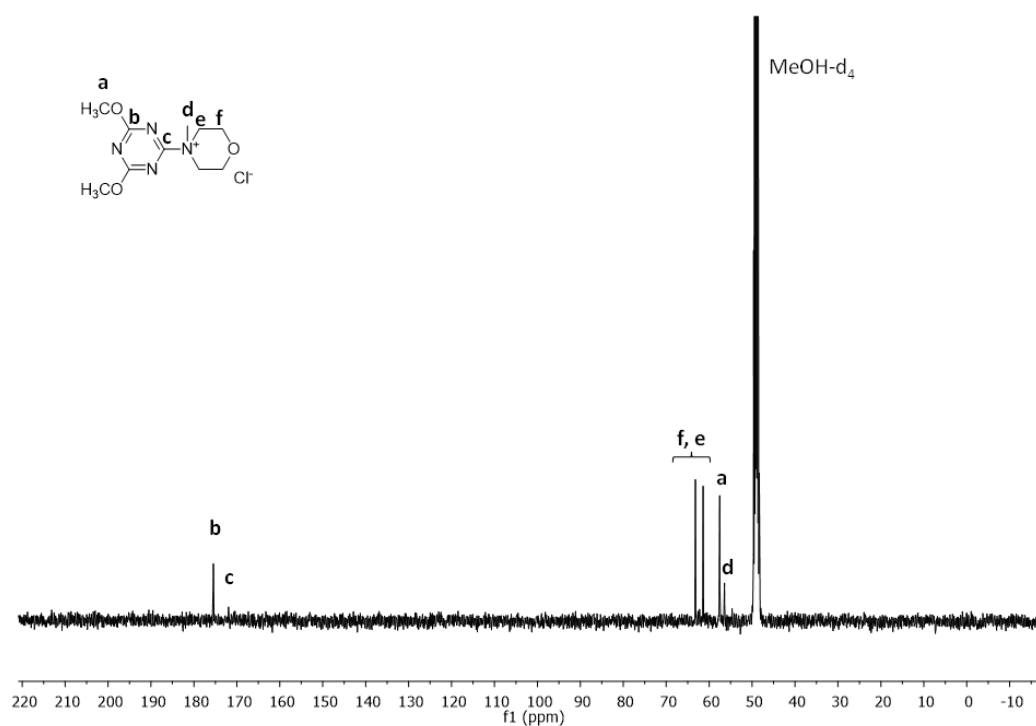


Figure S4. ¹³C NMR of DMTMM (2) in MeOH-*d*₄.

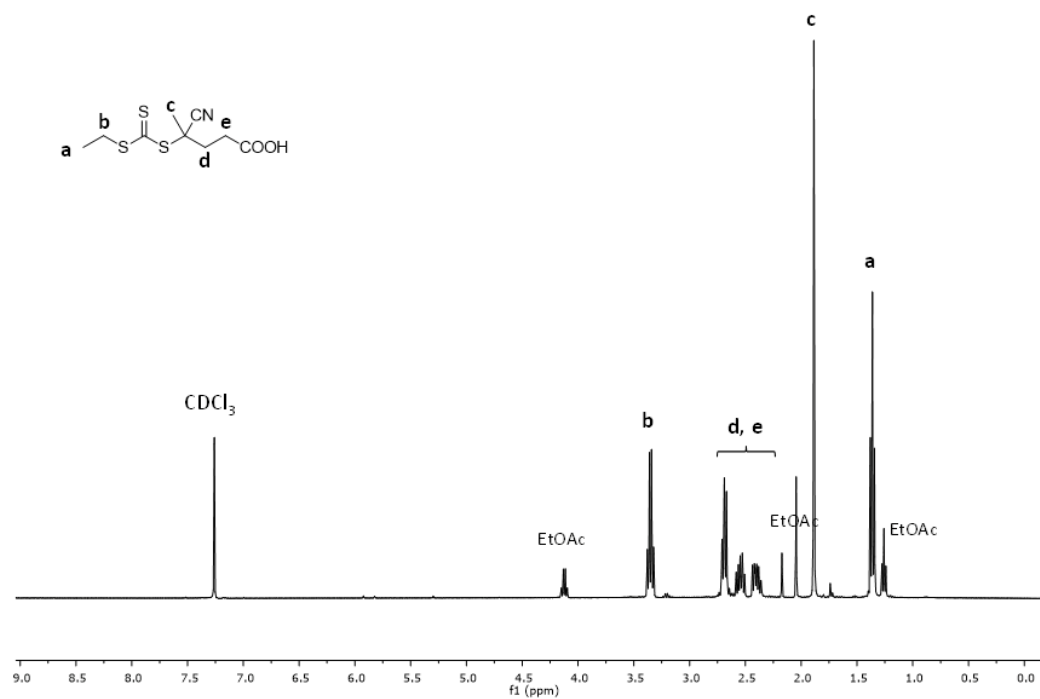


Figure S5. ¹H NMR in CDCl₃ of purified 4-cyano-4(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid (4).

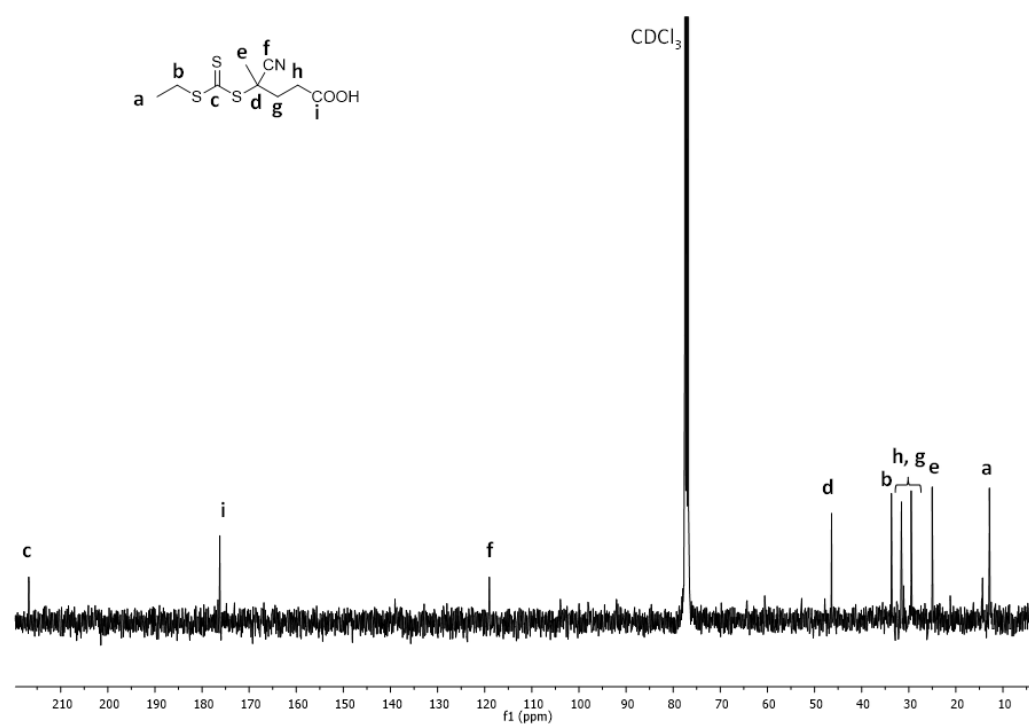


Figure S6. ¹³C NMR in CDCl₃ of purified 4-cyano-4(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid (4). *Signals at 14.34, 21.19, 60.58 ppm were due to EtOAc traces.

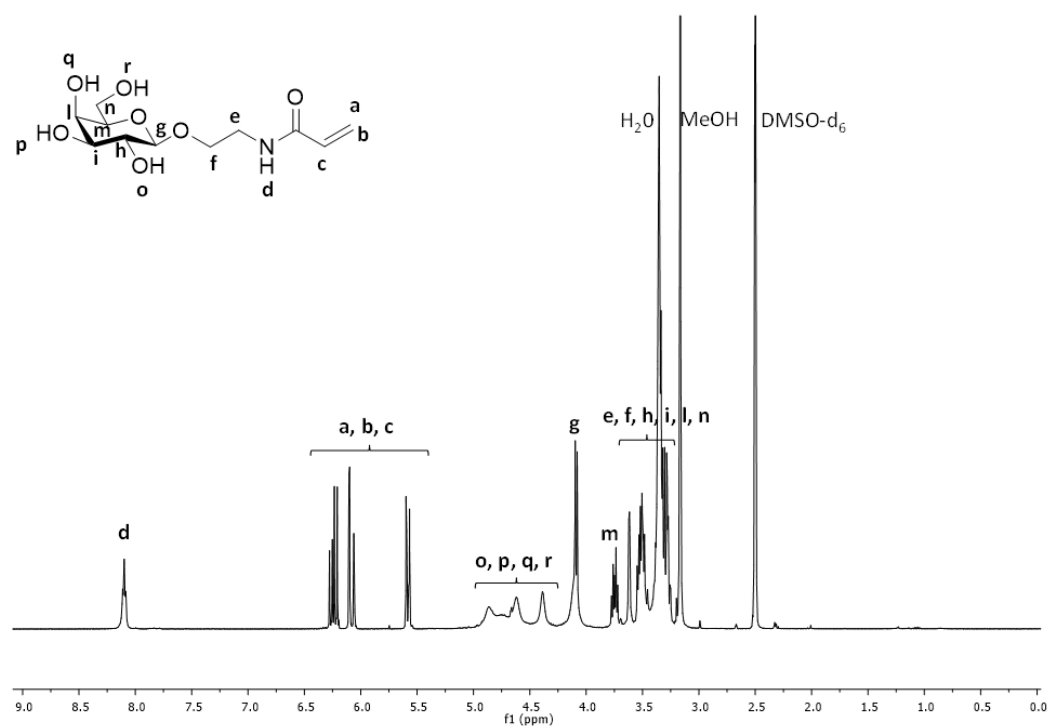


Figure S7. ¹H NMR of β-D-galactopyranosyloxyethyl acrylamide (6) in DMSO-*d*₆ after purification.

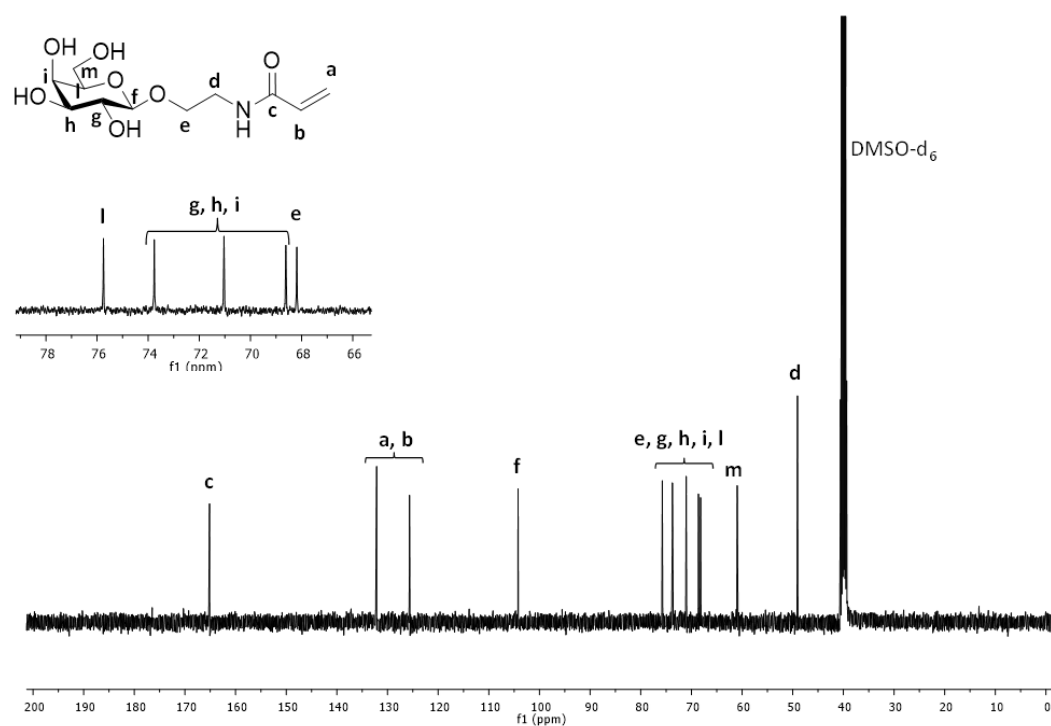


Figure S8. ^{13}C NMR of β -D-galactopyranosyloxyethyl acrylamide (6) in $\text{DMSO}-d_6$ after purification.

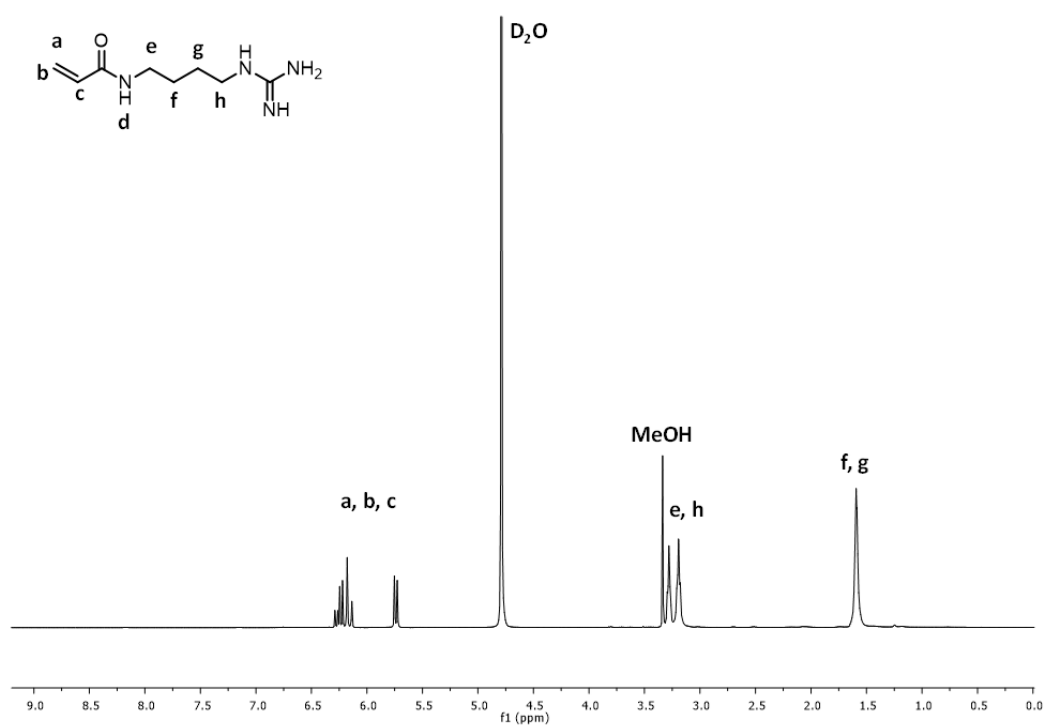


Figure S9. ^1H NMR of agmatine acrylamide (7) in D_2O after purification.

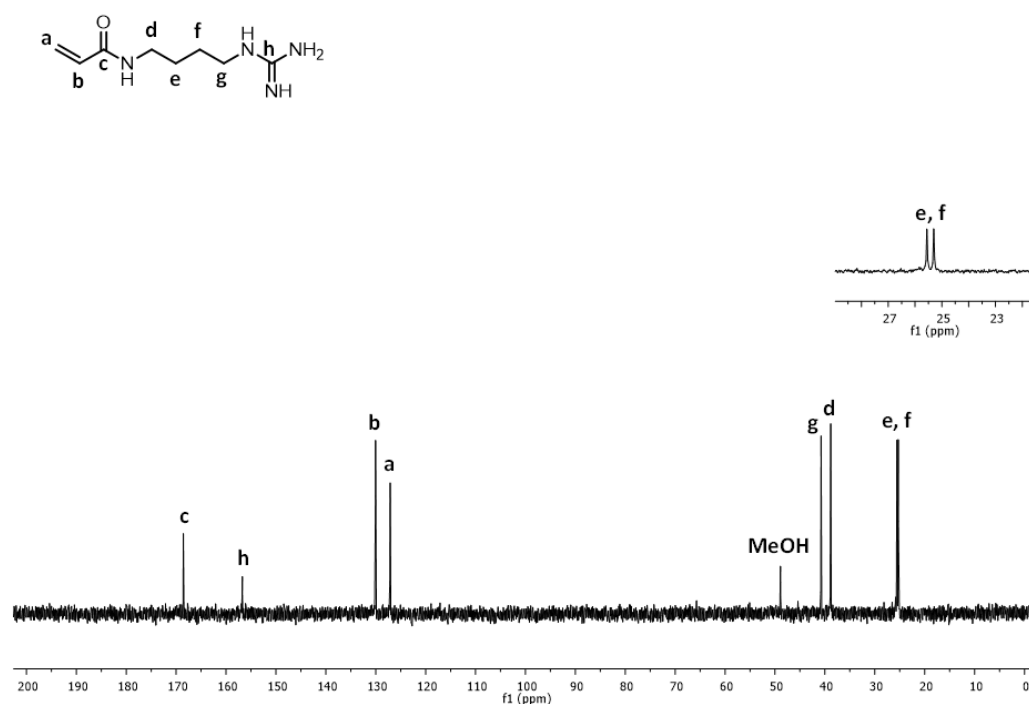


Figure S10. ^{13}C NMR of agmatine acrylamide (7) in D_2O after purification.

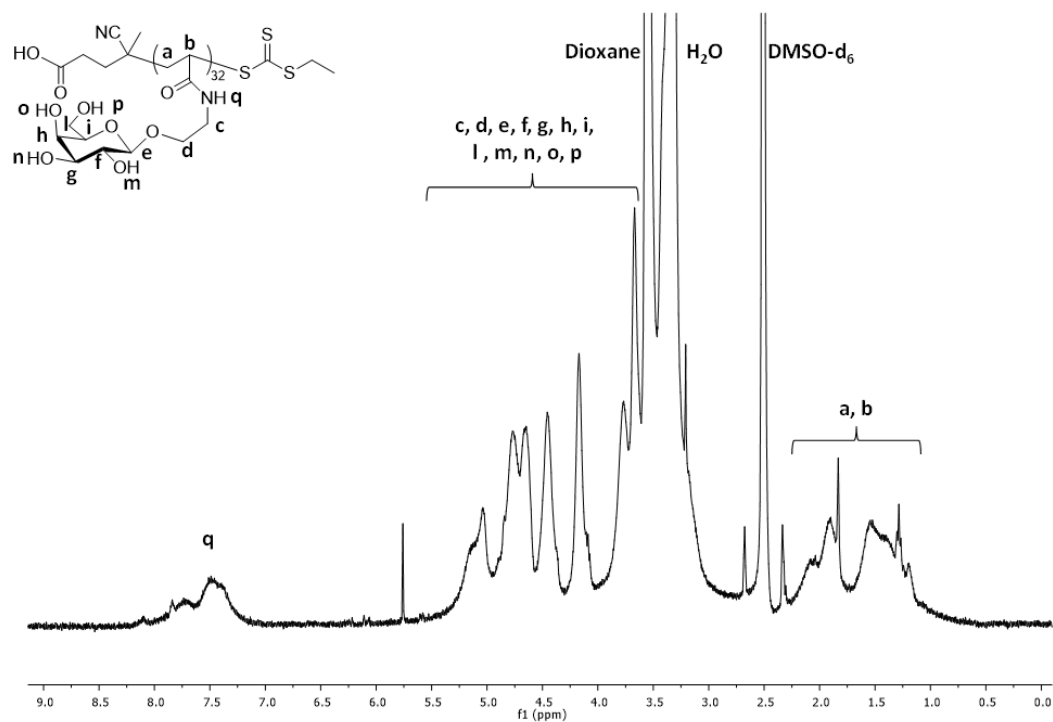


Figure S11. ^1H NMR in $\text{DMSO-}d_6$ of macro-CTA Gal_{32} (8) performed on an aliquot of the polymerisation mixture withdrawn at the end of the first polymerisation step. The intermediate was used for the next polymerisation step without isolation or purification.

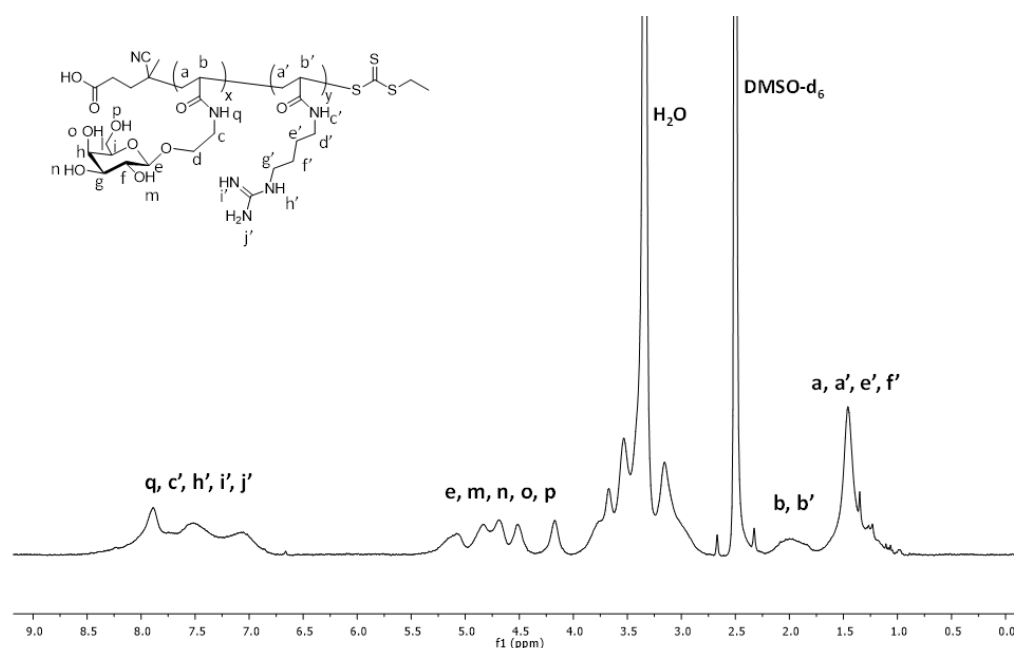


Figure S12. ^1H NMR in $\text{DMSO-}d_6$ of $\text{Gal}_{32}\text{-}b\text{-Agm}_{29}$ (9) block copolymer after purification by dialysis.

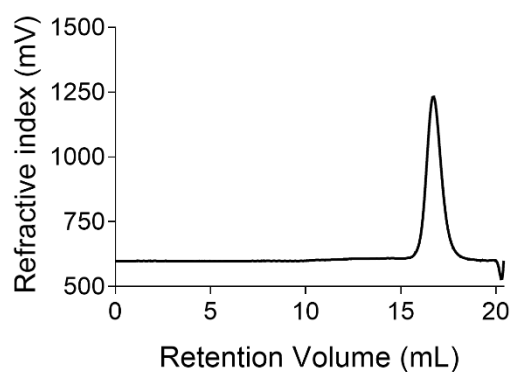


Figure S13. Gel permeation chromatography (GPC) profile of $\text{Gal}_{32}\text{-}b\text{-Agm}_{29}$ (9) block copolymer after purification by dialysis and freeze-drying.

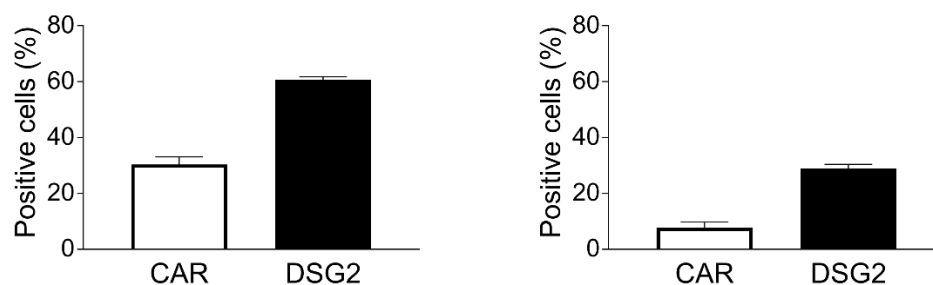


Figure S14. HepG2 (left panels) and A549 (right panels): positive cells for CAR and DSG2 were measured via flow cytometry by specific antibody staining.

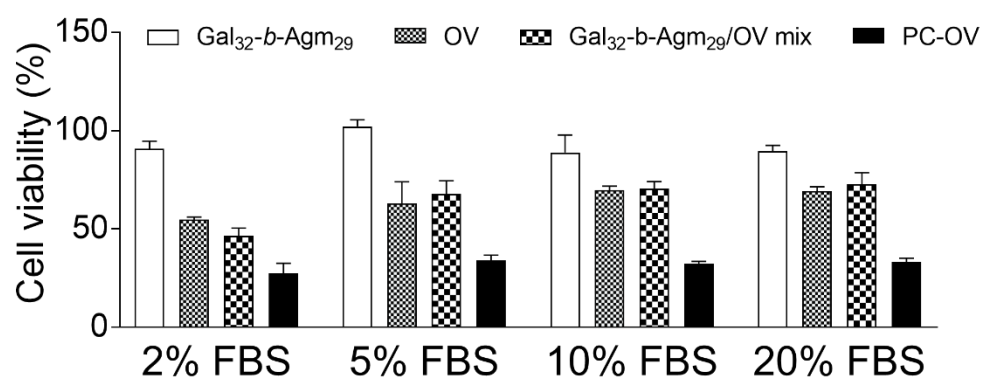


Figure S15. In vitro cytotoxicity on HepG2 cells of naked OV, Gal₃₂-b-Agm₂₉ alone, Gal₃₂-b-Agm₂₉/OV mix and PC-OV measured by MTS cell-viability assay at day 3 post-treatment using FBS increasing % in the culture medium. Control untreated cells were used as reference (100% cell viability).

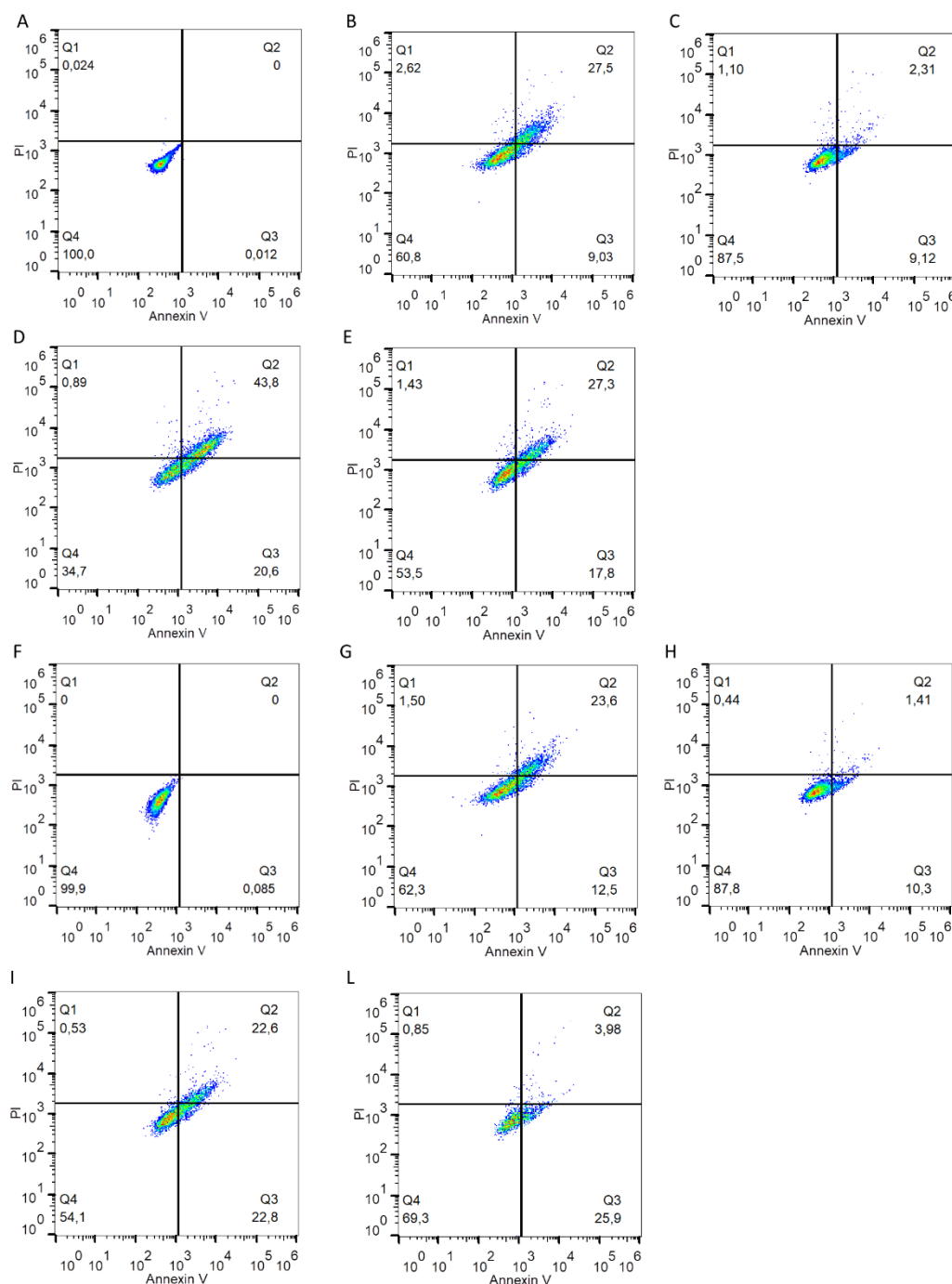


Figure S16. Dot plot with percentage of Annexin V/PI positive HepG2 (A–E) or A549 (F–L) cells, after 48 h treatment. Data shown is representative of at least three independent experiments. (A) and (F): control cells; (B) and (G): virus treated cells; (C) and (H): Gal32-b-Agm29 treated cells; (D) and (I): PC-OV treated cells; (E) and (L): Gal32-b-Agm29/OV mix treated cells.

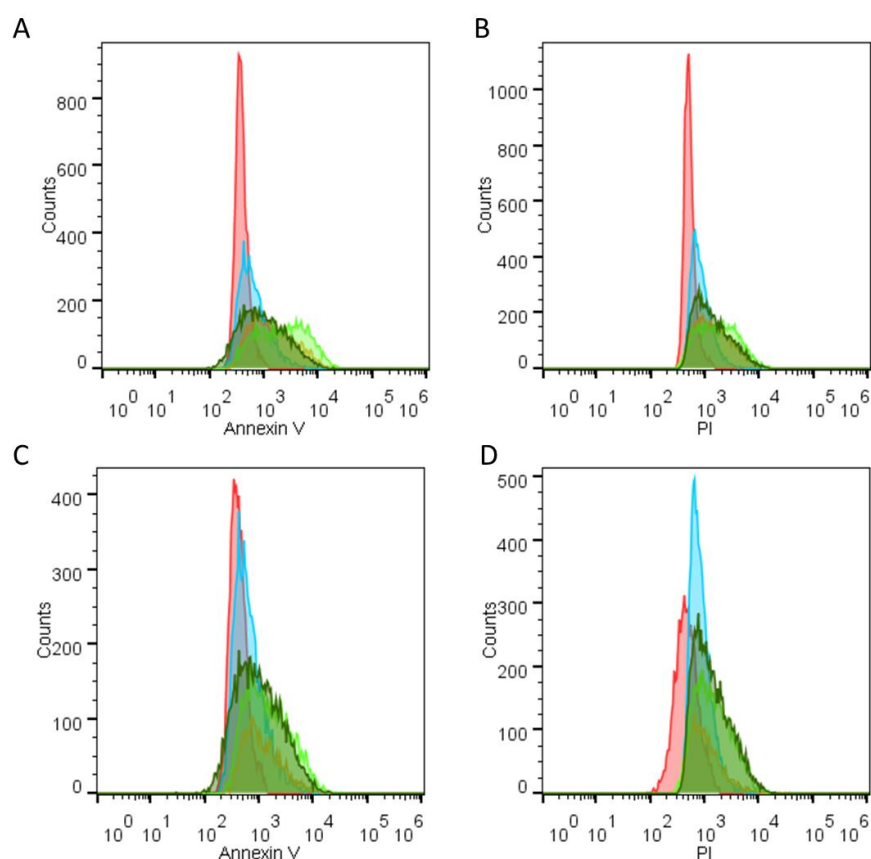


Figure S17. Representative flow cytometry analysis data from Annexin V (A,C) / PI (B,D) assay performed on HepG2 (A,B) and A549 (C,D) cells incubated for 48 h with medium only (control cells, red), Gal_{32-b}-Agm₂₉ polymer (light blue), OV alone (dark green), PC-OV (light green), and Gal_{32-b}-Agm₂₉/OV mix (orange).