Supplementary Information for

Azido-Galactose Outperforms Azido-Mannose for Metabolic Labeling and Targeting of Hepatocellular Carcinoma

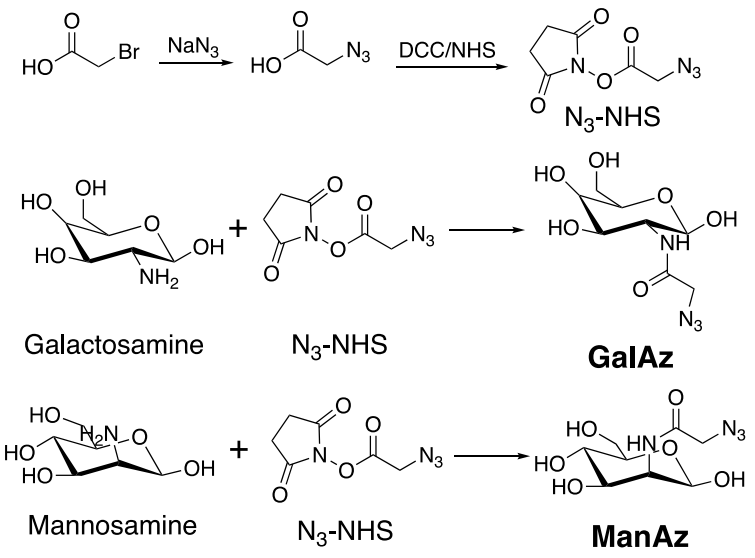
Hua Wang[1], Yang Liu[1], Ming Xu[1], Jianjun Cheng*[1]

[1]Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States

Materials. D-Mannosamine hydrochloride and other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise noted. *N*-hydroxysuccinimide (NHS) was purchased from Acros Organics (Renningen, Germany). DBCO-Cy5 was purchased from KeraFAST, Inc (Boston, MA, USA). 1-^{14}C acetic acid was purchased from Perkin Elmer (Waltham, MA, USA). Anhydrous hexane, ethyl acetate, and dimethylformamide (DMF) were purified by passing them through alumina columns and kept anhydrous by storing them in the presence of molecular sieves. Ultima Gold™ liquid scintillation cocktail was purchase from Perkin Elmer (Waltham, MA, USA). Phosphate-buffered saline (PBS) and Dulbecco's Modified Eagle Medium (DMEM) were obtained from Invitrogen (Carlsbad, CA, USA). Fetal Bovine Serum (FBS) was obtained from Lonza Walkersville Inc (Walkersville, MD, USA). BD Falcon culture plates were purchased from Fisher Scientific (Hampton, NH, USA). ProLong Gold antifade reagent was purchased from Life Technologies (Carlsbad, CA, USA).

Instrumentation. Nuclear magnetic resonance (NMR) analyses were conducted on a Varian U500 (500 MHz) or a VXR500 (500 MHz) spectrometer. Infrared spectra were recorded on a PerkinElmer 100 serial FTIR spectrophotometer (PerkinElmer, Waltham, MA, USA). Confocal laser scanning microscopy (CLSM) images were taken on a Zeiss LSM 700 Confocal Microscope (Carl Zeiss, Thornwood, NY, USA). Flow cytometry analyses of cells were conducted with a BD FACS Canto 6-color flow cytometry analyzer (BD, Franklin Lakes, NJ, USA). Fluorescence

intensity of cells was measured on an IN Cell Analyzer 2200 system (GE Healthcare Life Sciences, Pittsburgh, PA, USA). ^{14}C radioactivity was measured on a Packard Tri-Carb 2200CA liquid scintillation counter (Perkin Elmer, Waltham, MA, USA). In vivo and ex vivo images of animals were taken on the Bruker In-Vivo Imaging System (Bruker, Billerica, MA, USA). Frozen tissues were embedded with optimum cutting temperature (O.C.T.) compound (Sakura Finetek USA, Torrance, CA, USA), sectioned by a Leica CM3050S Cryostat, and mounted onto glass slides.



Scheme S1. Synthetic route of GalAz and ManAz.

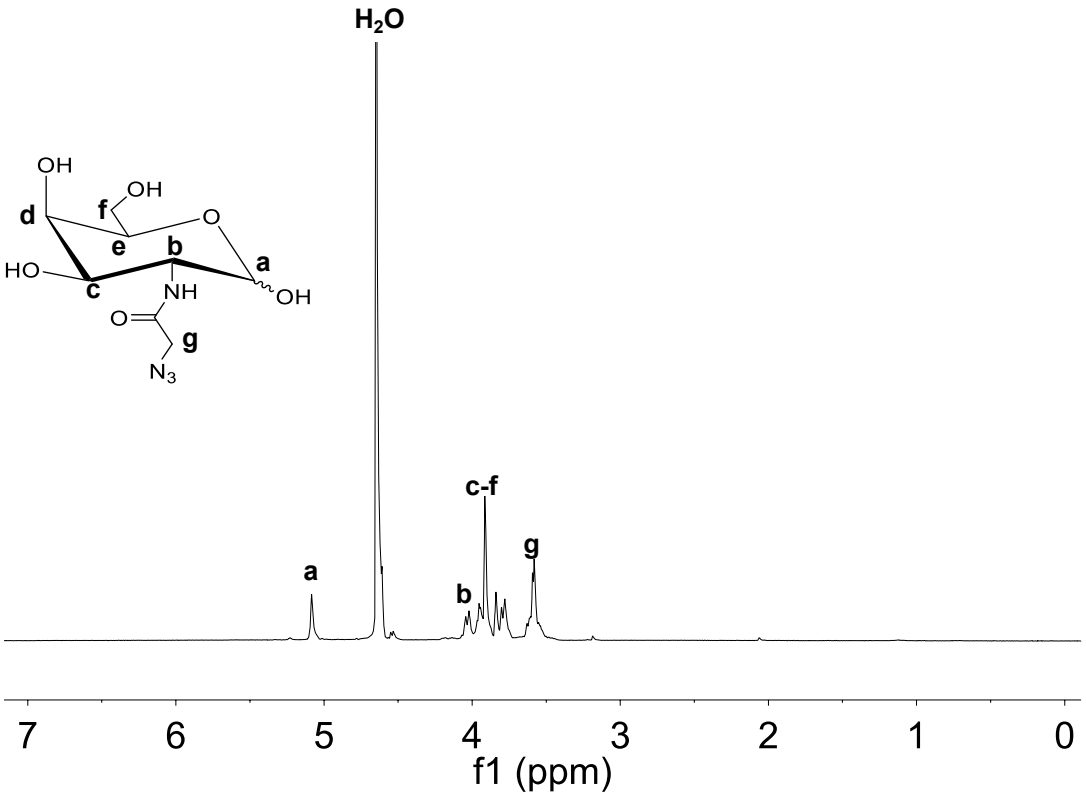


Figure S1. ^{1}H NMR spectrum of GalAz in D_2O. All peaks have been assigned to corresponding protons (a-g).

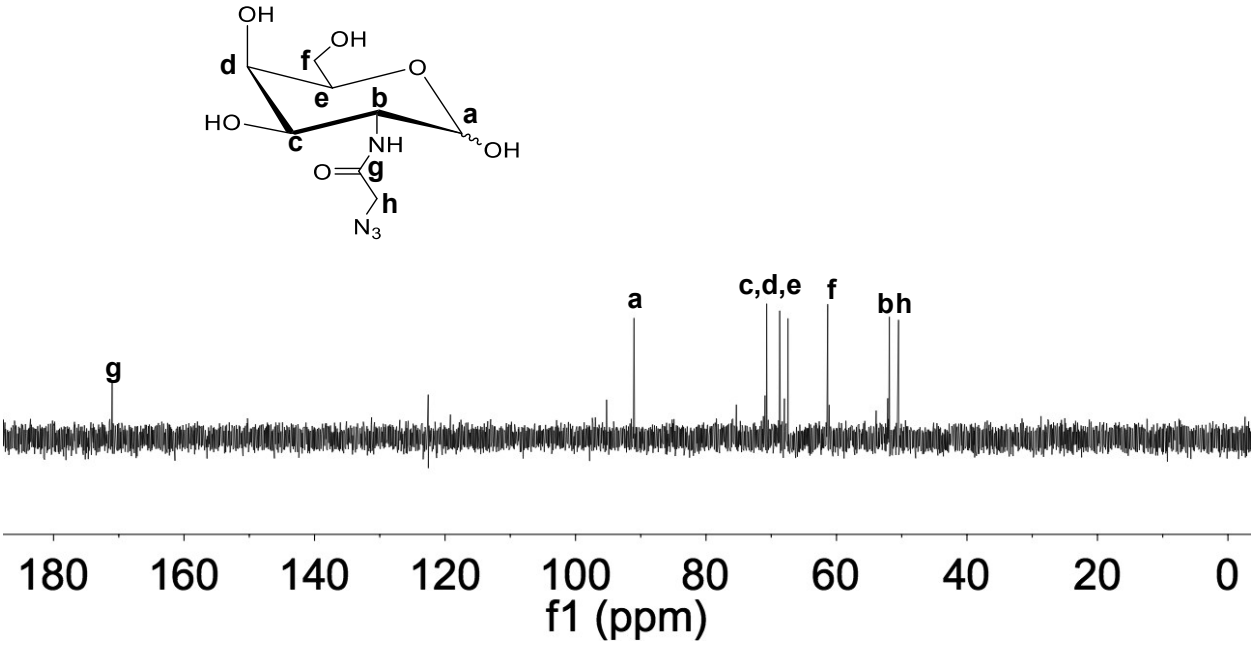


Figure S2. ^{13}C NMR spectrum of GalAz in D_2O. All peaks have been assigned to corresponding carbons (a-h).

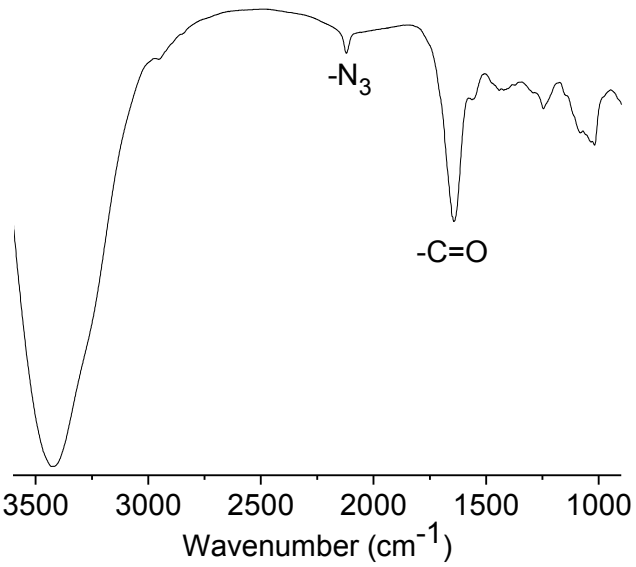


Figure S3. FTIR spectrum of GalAz. The peak at ~2106 cm^{-1} indicates the existence of azido groups (N_3).

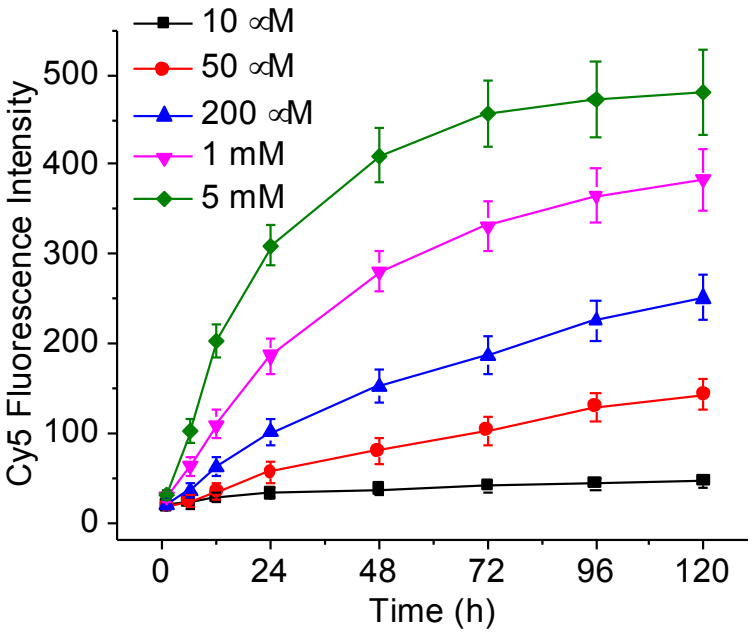


Fig. S4. In vitro labeling kinetics of ManAz in HepG2 cells. HepG2 cells were incubated with different concentrations of ManAz (10 μM, 50 μM, 200 μM, 1 mM, and 5 mM) for different time (1, 6, 12, 24, 48, 72, 96, and 120 h), and stained with DBCO-Cy5 (20 μM) for 40 min. Average Cy5 fluorescence intensity was measured on a GE-analyzer.

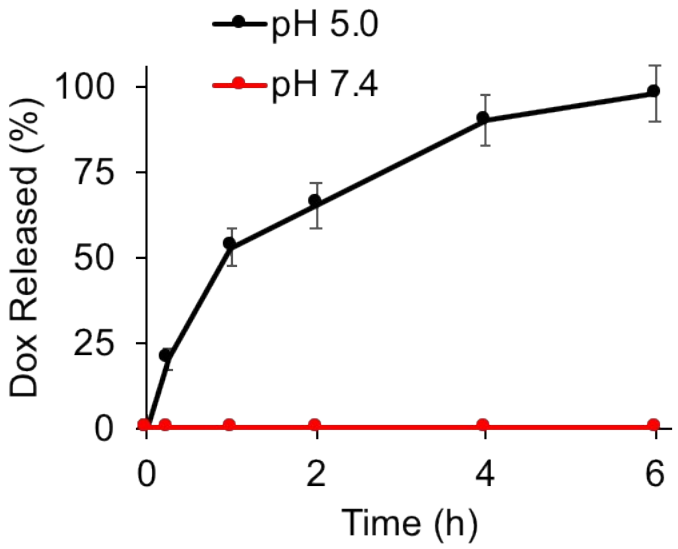


Fig. S5. Degradation kinetics of DBCO-hz-Dox in pH 7.4 and 5.0. respectively. Data are Data were presented as mean ± SD (n=3).

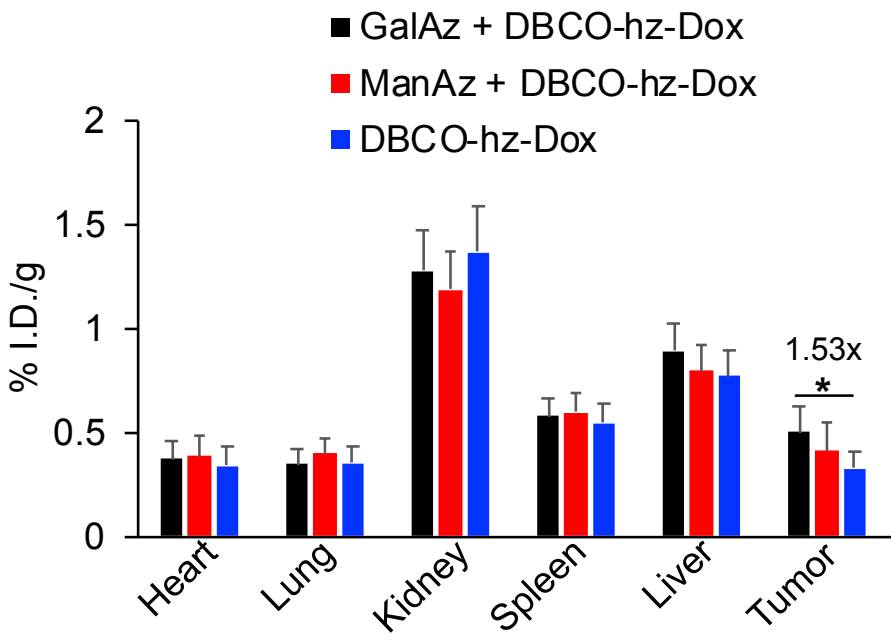


Figure S6. Retained drug in tumors and major organs from mice treated with GalAz + DBCO-hz-Dox, ManAz + DBCO-hz-Dox, or DBCO-hz-Dox alone, respectively at 48 h post injection of DBCO-hz-Dox. GalAz or ManAz (200 mg/kg) was i.v. injected once daily for three days, followed by i.v. injection of DBCO-hz-Dox (8.0 mg/kg in Dox equivalent) on day 4. Retained drug was quantified using HPLC method and normalized to % I.D./g. Data were presented as mean ± SD (n=5) and analyzed by Student's t-test (two-tailed, $0.01 < *P \leq 0.05$, $0.001 < **P \leq 0.01$, and $***P \leq 0.001$).