# Supporting Information for

# Carbohydrate Dependent Targeting of Cancer Cells by Bleomycin-Microbubble Conjugates

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**Synthesis of Biotinylated BLM $A_5$**

**Biotin *N*-Hydroxysuccinimide Ester.** To a solution of 101 mg (0.41 mmol) of biotin in 2 mL of DMF was added 67 mg (0.41 mmol) of 1,1'-carbonyldiimidazole at 78 °C with further heating until $CO_2$ evolution ceased. The solution was cooled to room temperature and stirred for 3 h. To the reaction mixture was added a solution of 47 mg (0.41 mmol) of *N*-hydroxysuccinimide in 2 mL of DMF. The solution was stirred overnight at room temperature. The solvent was concentrated under diminished pressure and the product was crystallized from 2-propanol and then DMF-ether to afford a fine white powder. yield 95 mg (68%). $^1$H NMR ($CDCl_3$) δ 1.43-1.66 (m, 7H), 2.58 (m, 1H), 2.66 (t, 2H), 2.75 (s, 4H), 3.05 (m, 1H), 4.11 (m, 1H), 4.27 (m, 1H), 6.36 (d, 2H, $J$ =18.4 Hz); mass spectrum, *m/z* 342.1 ($M^+$).

**Biotinylated BLM $A_5$ (2a)**. To a solution of 10 mg (7.0 μmol) of Cu(II)•BLM $A_5$ in 0.75 mL of aq. 0.1 M NaOAc at 0 °C was added 7.0 mg (21.0 μmol) of biotin *N*-hydroxysuccinimde ester. The solution was stirred at 0-4 °C for 48 h, then treated with three 5-mL aliquots of $CHCl_3$ to extract unreacted biotin *N*-hydroxysuccinimde ester. The aqueous solution was lyophilized to afford a blue powder. The residue was dissolved in 15% aq. EDTA and stirred overnight at room temperature. The crude product was applied to an Alltima $C_{18}$ column for purification by reversed phase HPLC; yield 3.2 mg (30%); mass spectrum (TOF $ES^+$), *m/z* 833.8400 $(M+Na)^{++}$ ($C_{67}H_{103}N_{21}O_{23}S_3$ requires 833.8427).

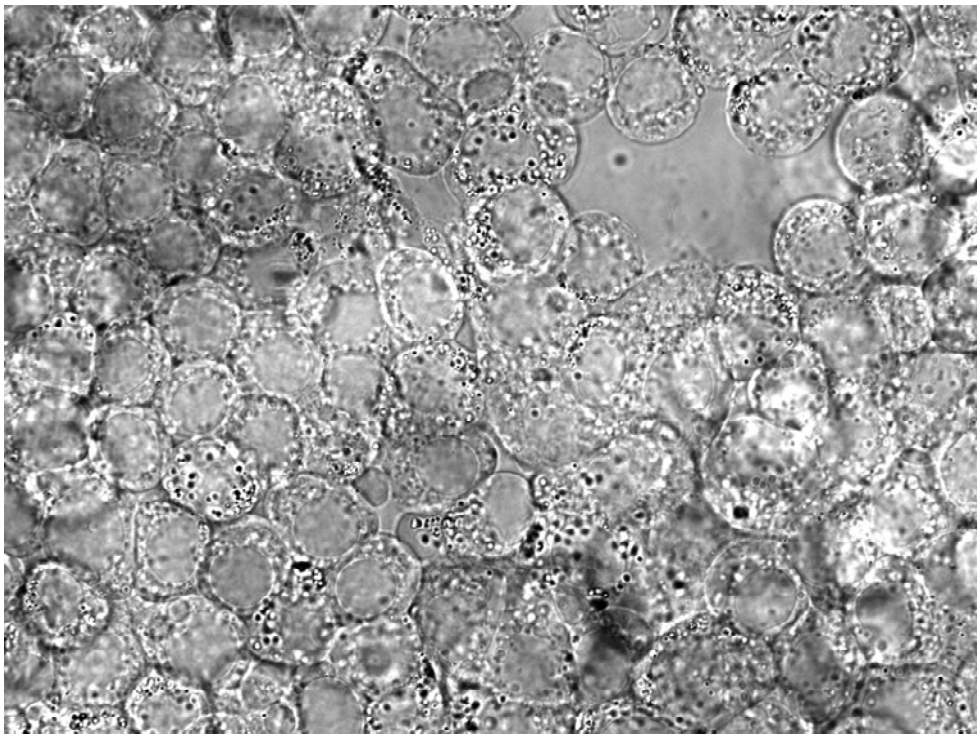
**Biotinylated Deglyco BLM $A_5$ (2b)**. Prepared in the same fashion as **2a** starting from deglyco BLM $A_5$; mass spectrum (TOF $ES^+$), *m/z* 650.2861 $(M+Na)^{++}$ ($C_{54}H_{82}N_{20}O_{12}S_3$ requires 650.2870).

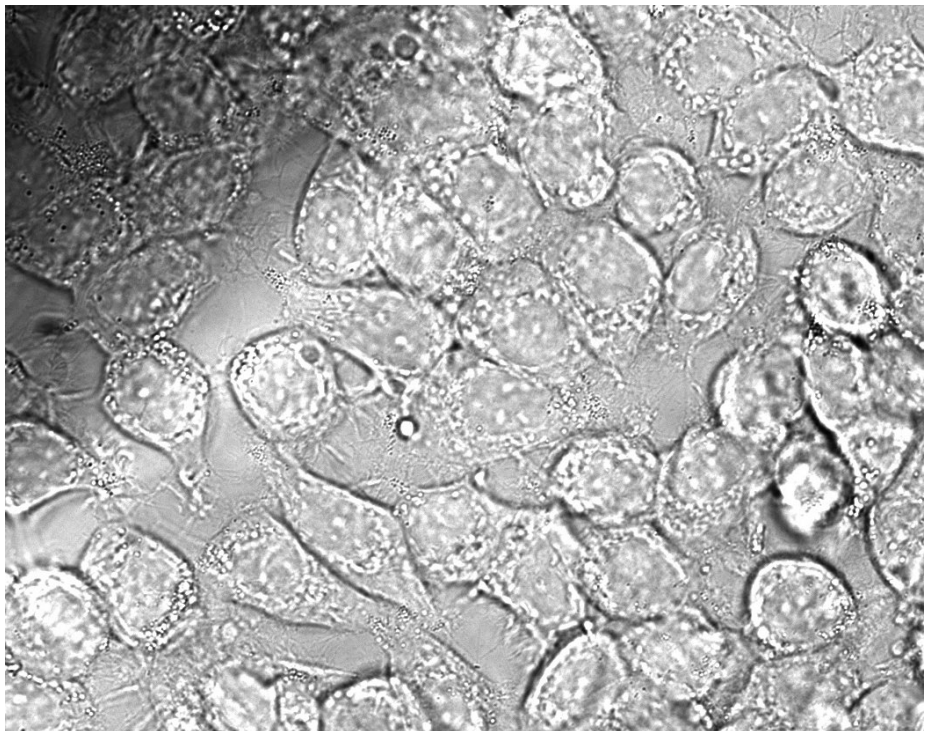
**Bleomycin $A_5$-Derivatized Microbubbles.** To create BLM $A_5$-derivatized microbubbles, the bleomycin $A_5$ derivative **2a** was conjugated to Targestar$^B$ Targeted Ultrasound Contrast Agent (Targeson). Four hundred μL of Coupling Reagent (Targeson) was added to 1.5 mL of conjugated Targestar$^B$ microbubbles and incubated for 20 min at room temperature, with gentle agitation every 5 min. The product was divided into two syringes, rinsed with 1.75 mL of Infusion Buffer, and then centrifuged for 3 min (400 x g, 10 °C). The infranatant was then drained to 1 mL. Fifty μL of 500 μM biotinylated bleomycin $A_5$ (**2a**) was added to one of the

vials and both were incubated at room temperature for 20 min with gentle agitation every 5 min. To each sample was added 1.75 mL of Infusion Buffer before centrifugation for 3 min at 400 x g (10 °C). This solution was then drained to 1.0 mL before recovery of the supernatant and repetition of the previous step. Finally, the supernatant was resuspended in Infusion Buffer to a final volume of 2.0 mL.

**Monitoring of Microbubble Attachment to Cultured Cells.** Attachment of the (BLM-conjugated) microbubbles to the cultured MCF-7 breast cancer cells was imaged using an inverted microscope Zeiss Axiovert 200M fitted with an AxioCam MRm camera. Adherent cancer cells on glass cover slips were assembled into a parallel plate flow chamber (Bioptechs FCS2, Micro-Environmental Systems) with a constant temperature maintained at 37 $^{o}$C. The prepared solution of microbubbles was introduced into the parallel plate flow chamber via 1/8-inch diameter tubing (Silastic) at a controlled rate of 0.01 mL/min using an adjustable infusion-withdrawal syringe pump (Harvard Apparatus, Holliston, MA). A negative control experiment was performed using a preparation of Targestar$^{B}$ microbubbles without any added biotinylated bleomycin $A_5$ ligand. 'Normal' human breast MCF-10A cells were used in parallel experiments for comparison purposes.

Larger version of Figure 3:





Larger version of Figure 4: