Supporting Information

for

The Carbamoylmannose Moiety of Bleomycin Mediates Selective Tumor Cell Targeting

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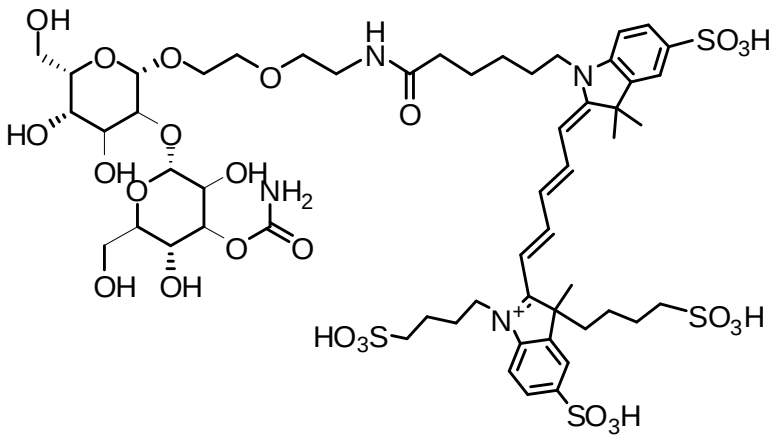


Figure S1. Chemical structure of BLM disaccharide-Cy5**.

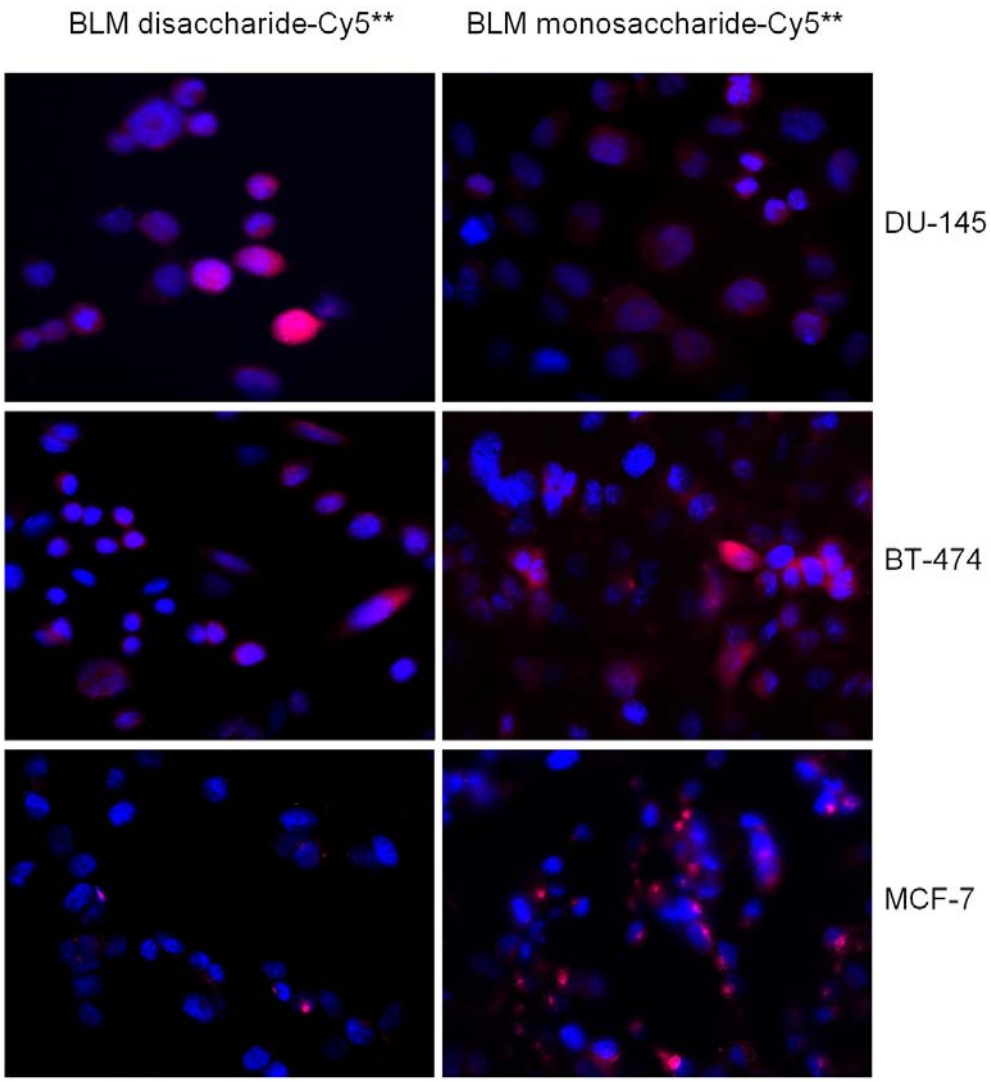


Figure S2. Comparison of binding/uptake of BLM disaccharide-Cy5** and BLM monosaccharide-Cy5** conjugates in DU-145, BT-474 and MCF-7 cell lines. The cells were treated with 25 μM BLM disaccharide-Cy5** or BLM monosaccharide-Cy5** at 37 °C for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. The nuclei were stained with DAPI. Fluorescence imaging was carried out with a 3 s exposure time.

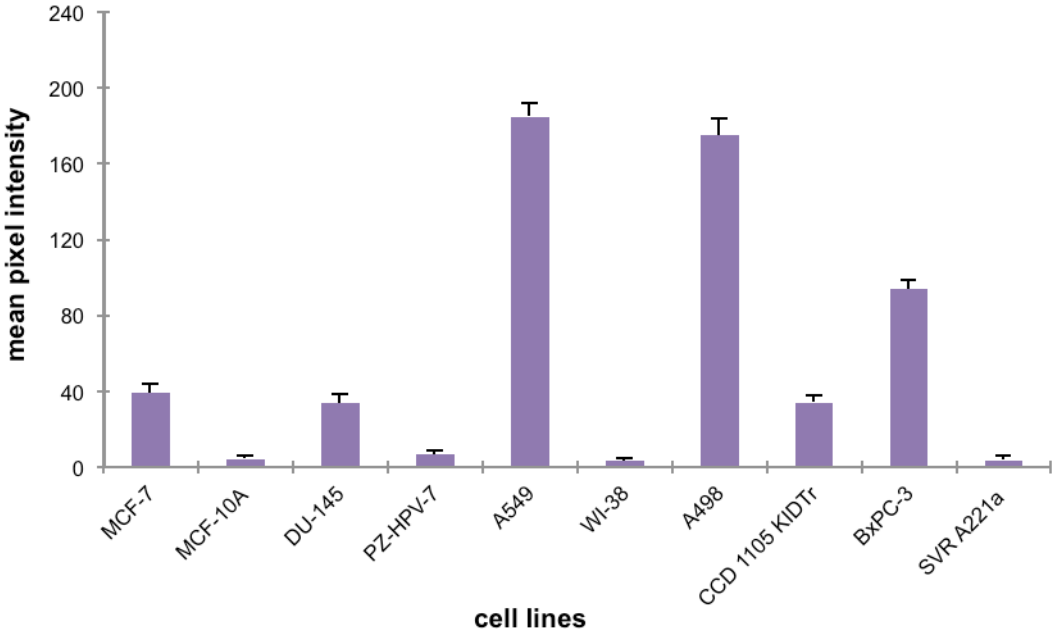


Figure S3. Quantification of the binding/uptake of BLM monosaccharide-Cy5** in five cancer and matched normal cells. The cells were treated with 25 μM dye conjugates and irradiated for 3 s prior to imaging.

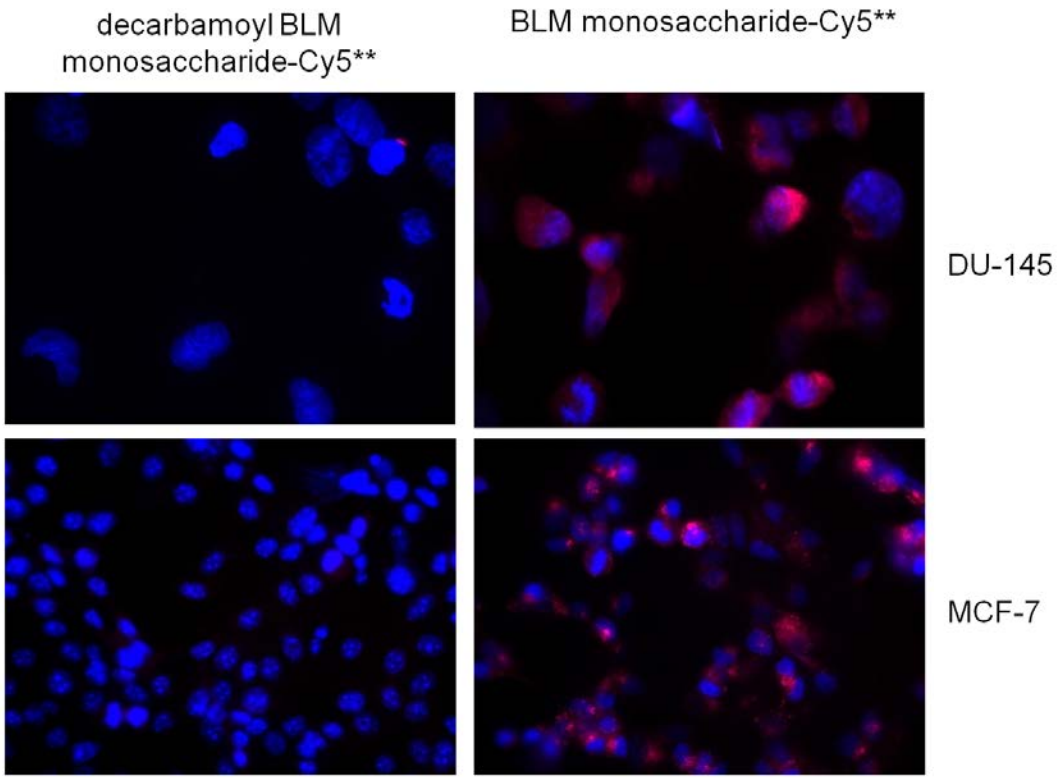


Figure S4. Comparison of binding/uptake of decarbamoyl BLM monosaccharide-Cy5** and BLM monosaccharide-Cy5** conjugates in DU-145 and MCF-7 cell lines. The cells were treated with 25 μM decarbamoyl BLM monosaccharide-Cy5** or BLM monosaccharide-Cy5** at 37 °C for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. The nuclei were stained with DAPI. Fluorescence imaging was carried out with a 3 s exposure time.

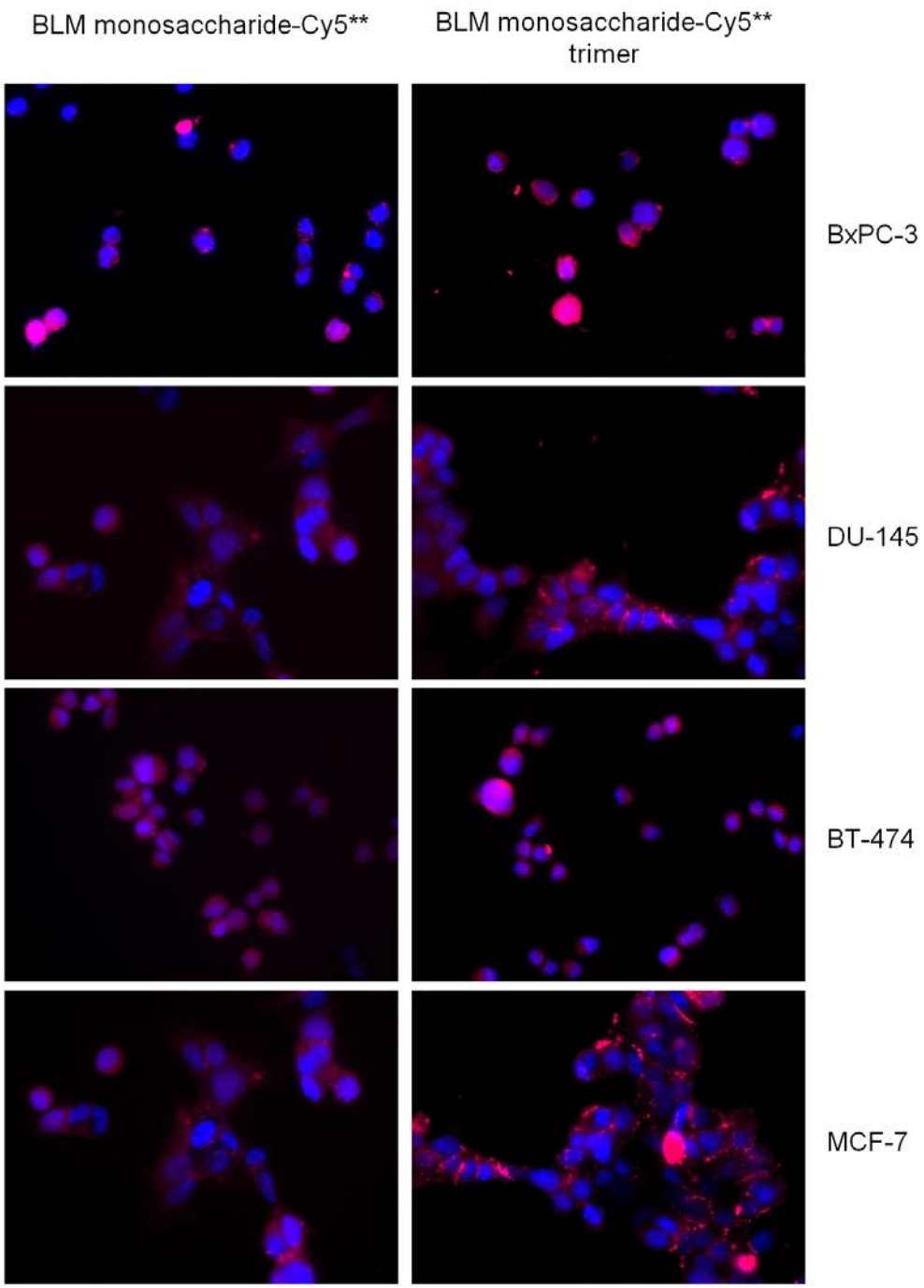
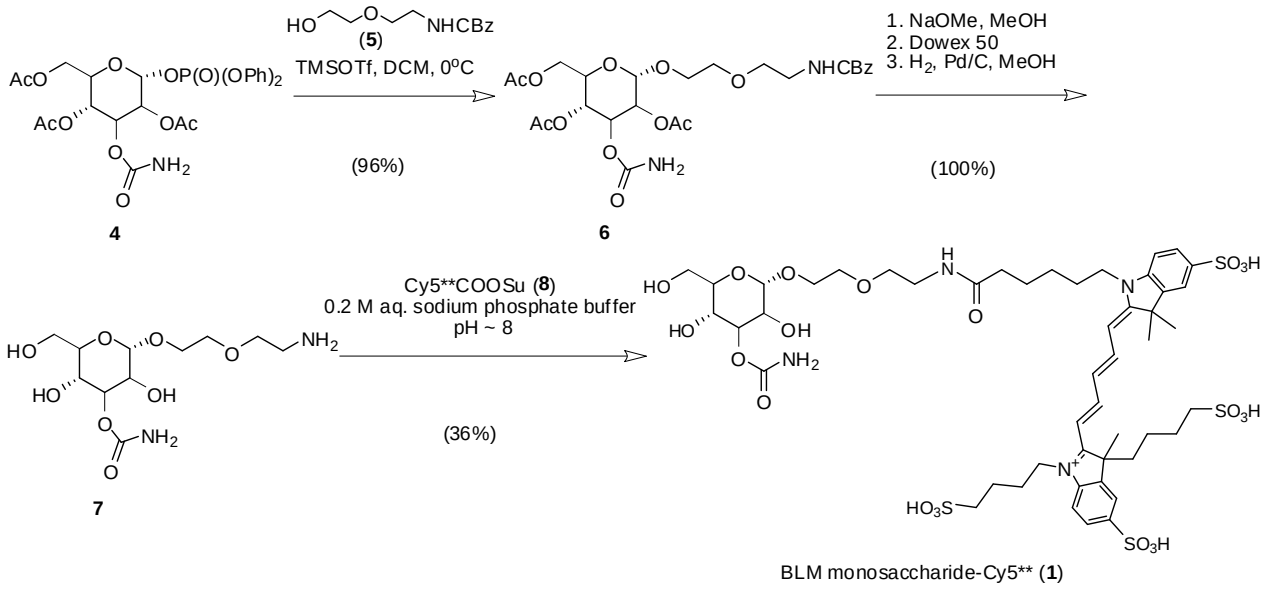
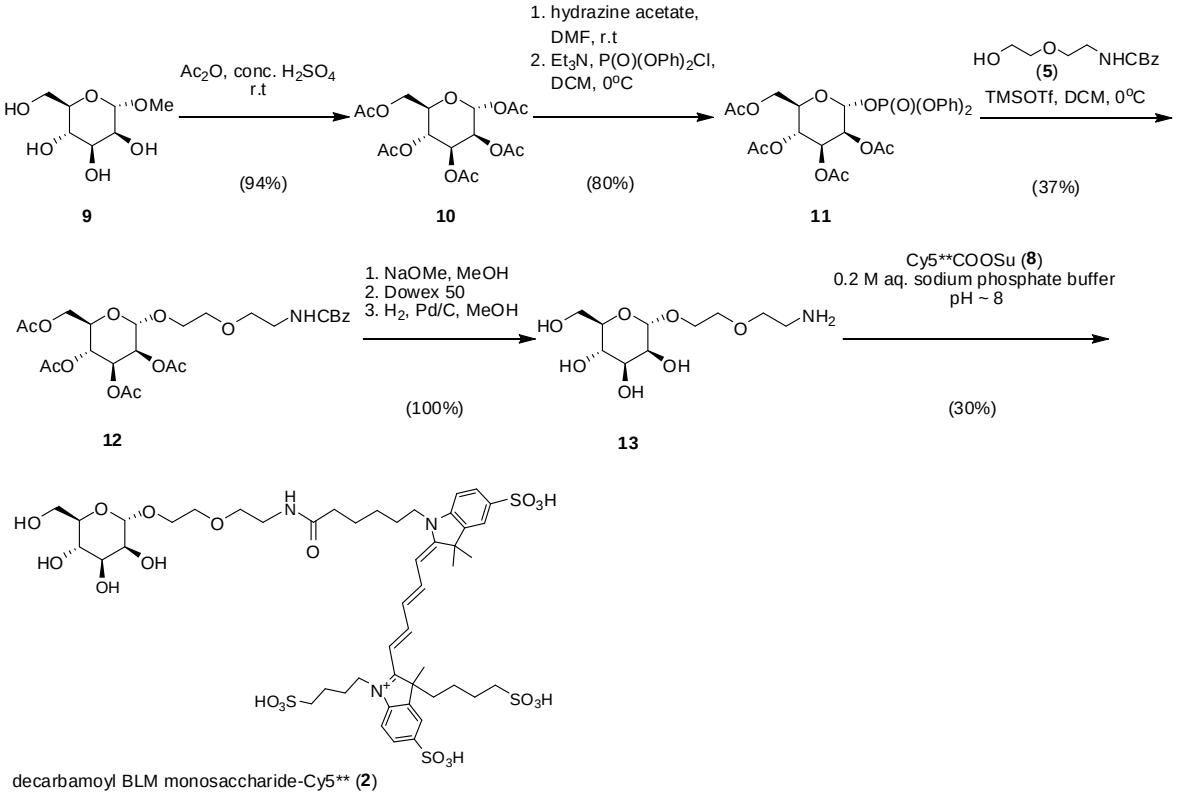


Figure S5. Comparison of binding/uptake of BLM monosaccharide-Cy5** and BLM monosaccharide-Cy5** trimer conjugates in BxPC-3, DU-145, BT-474 and MCF-7 cell lines. The cells were treated with 25 μM BLM monosaccharide-Cy5** or BLM monosaccharide-Cy5** trimer at 37 °C for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. The nuclei were stained with DAPI. Fluorescence imaging was carried out with a 3 s exposure time.

Scheme S1. Synthetic Route Utilized for the Preparation of BLM Monosaccharide-Cy5**

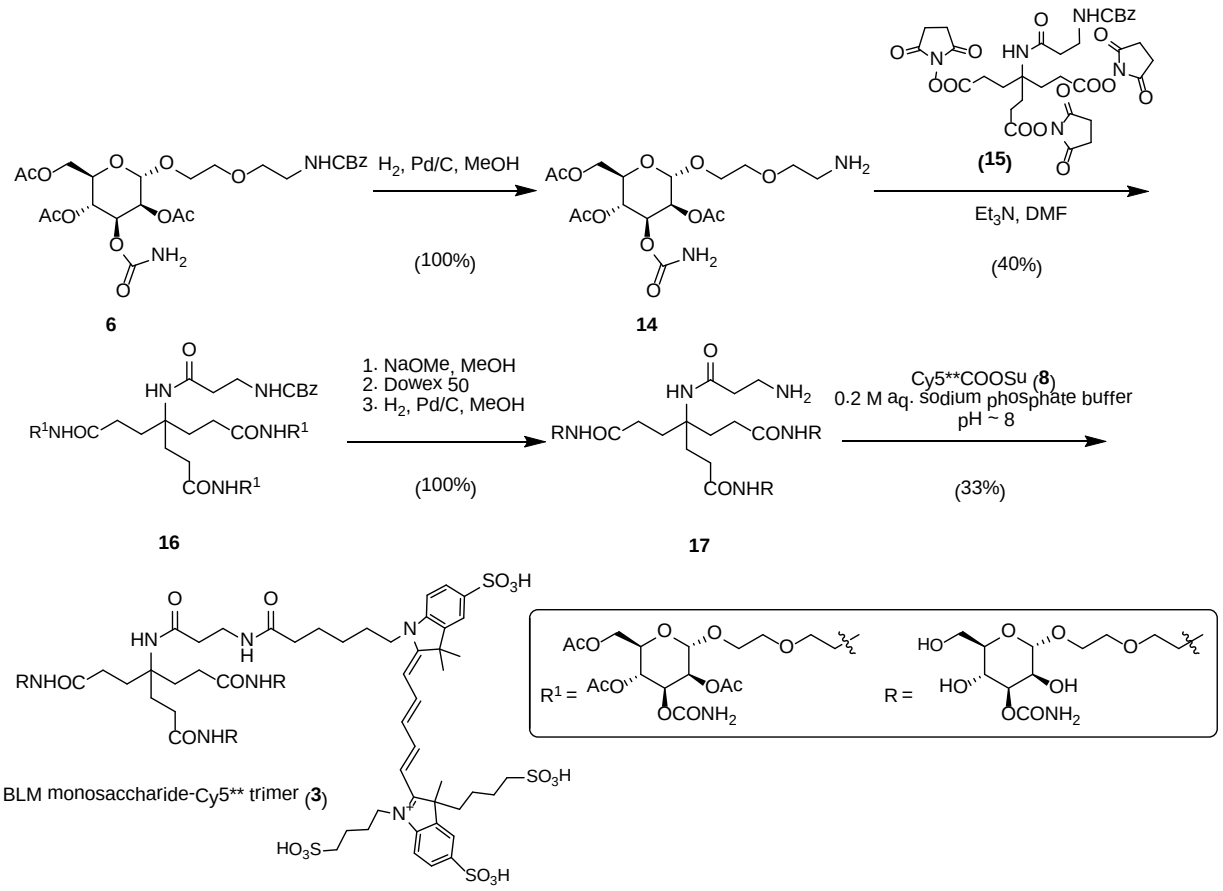


Scheme S2. Synthetic Route Utilized for the Preparation of Decarbamoyl BLM monosaccharide-Cy5**



decarbamoyl BLM monosaccharide-Cy5** (**2**)

Scheme S3. Synthetic Route Utilized for the Preparation of BLM Monosaccharide-Cy5 Trimer**



EXPERIMENTAL PROCEDURES

Materials. The chemicals employed were all ACS reagent grade and were used without further purification. Dichloromethane was distilled from calcium hydride and tetrahydrofuran from sodium/benzophenone ketyl. Anhydrous methanol was used as purchased. The reactions were carried out under an argon atmosphere in flame-dried glassware. Flash column chromatography was carried out using silica gel (Silicycle R10030B, 60 particle size, 230-400 mesh), applying a low-pressure stream of nitrogen and also with dry air. Analytical thin layer chromatographic separations were carried out on glass plates coated with silica gel (60 particle size F254, Silicycle TLG-R10011B-323). The TLC chromatograms were developed by immersing the plates in a solution of Hanessian's stain followed by heating (heat gun or hot plates). ^{1}H and ^{13}C NMR spectra were recorded on Varian Inova 400 MHz and 100 MHz spectrometers, respectively, using $CDCl_3$ as solvent and internal standard. ^{1}H NMR chemical shifts were reported relative to residual $CHCl_3$ at 7.26 ppm; ^{13}C NMR shifts were reported relative to the central line of $CDCl_3$ at 77.16 ppm. Splitting patterns are designated as s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; q, quartet. High-resolution mass spectrometric data were obtained at the Michigan State Mass Spectrometry Facility or at the Arizona State University CLAS High Resolution Mass Spectrometry Facility.

2,4,6-Tri-*O*-acetyl-3-*O*-(carbamoyl)-α-D-mannopyranosyl Benzyl 2-(Ethoxy)ethylcarbamate (6). To a solution of 1.87 g (3.22 mmol) of **4** and 692 mg (2.89 mmol) of **5** in 50 mL of anhydrous CH_2Cl_2, was added 1.10 mL (1.29 g, 5.79 mmol) of TMSOTf at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, at which time it was poured into a two-phase solution of 70 mL of EtOAc and 28 mL of satd aq $NaHCO_3$.The organic layer was washed with two 30-mL portions of brine, dried (Na_2SO_4), filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (25 × 5 cm). Elution with 1:1→1:2→1:3 hexanes–ethyl acetate afforded compound **6** as a colorless oil: yield 1.58 g (96%); silica gel TLC R_f 0.26 (1:3 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 3.39 (m, 2H), 3.54 (m, 2H), 3.64 (m, 3H), 3.79 (m, 1H), 4.08 (m, 2H), 4.26 (m, 1H), 4.71 (br s, 2H), 4.91 (s, 1H), 5.10 (s, 2H), 5.25 (m, 3H), 5.37 (br s, 1H) and

7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.0, 41.1, 62.7, 66.5, 66.8, 67.3, 68.5, 70.0, 70.1, 70.3, 70.4, 77.4, 97.6, 128.1, 128.2, 128.6, 136.8, 155.3, 170.1, 170.2, 170.8; mass spectrum (APCI), *m/z* 571.2141 $(M + H)^+$ ($C_{25}H_{35}N_2O_{13}$ requires *m/z* 571.2139).

BLM Monosaccharide-Cy5 (1).** To a solution of 4.60 mg (8.06 μmol) of compound **6** in 2 mL of anh methanol, was added 0.20 mL of a freshly prepared 25% w/w solution of sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 2.5 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered; mass spectrum (MALDI), *m/z* 467.27 $(M + Na)^+$; mass spectrum (APCI), *m/z* 445.1815 $(M + H)^+$ ($C_{19}H_{29}N_2O_{10}$ requires *m/z* 445.1822). To the solution of the crude product in anh methanol was added Pd/C and H_2 gas was bubbled through for 45 min. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite and the filtrate was concentrated under diminished pressure to afford **7**, which was used directly for the next reaction; mass spectrum (MALDI), *m/z* 333.22 $(M + Na)^+$; mass spectrum (APCI), *m/z* 311.1455 $(M + H)^+$ ($C_{11}H_{23}N_2O_8$ requires *m/z* 311.1454).

To 122 μg (0.39 μmol) of **7** was added a solution of 110 μg (0.11 μmol) of Cy5**COOSu (**8**) in 100 μL of 0.2 M phosphate buffer and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4.5 mL/min. The fractions containing the desired product eluted at 22.5 min and were collected, frozen and lyophilized to give **1** as a blue solid: yield 50 μg (36%); mass spectrum (MALDI), *m/z* 1201.47 $(M – H + Na)^+$, 1223.47 $(M – 2H + 2Na)^+$, 1245.45 $(M – 3H + 3Na)^+$, 1267.43 $(M – 4H + 4Na)^+$, ($C_{49}H_{71}N_4O_{21}S_4$ requires m/z 1179.35); mass spectrum (TOF), *m/z* 588.1614 $(M – 3H)^{2-}$ ($C_{49}H_{68}N_4O_{21}S_4^{2-}$ requires *m/z* 588.1629).

Penta-*O*-acetyl-*α*-D-mannopyranose (10). To a solution containing 1.00 g (5.15 mmol) of methyl-*α*-D-mannopyranose (**9**) in 19.0 mL of Ac_2O was added a catalytic amount of H_2SO_4, and

the solution was stirred at room temperature for 12 h. The reaction mixture was poured into a stirred mixture of 150 mL of ethyl acetate and 80 mL of satd aq $NaHCO_3$. The organic phase was separated and washed with 40 mL of satd aq $NaHCO_3$, 30 mL of brine, then dried (Na_2SO_4) and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (18 × 5 cm). Elution with 5:1→3:1 hexanes–ethyl acetate afforded **10** as a colorless oil: yield 1.97 g (98%); silica gel TLC R_f 0.60 (1:2 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.86 (s, 3H), 1.91 (s, 3H), 1.95 (m, 3H), 2.04 (m, 6H), 3.94 (m, 2H), 4.13 (m, 1H), 5.12 (s, 1H), 5.20 (m, 2H) and 5.94 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.40, 20.43, 20.47, 20.53, 20.6, 61.9, 65.3, 68.1, 68.6, 70.4, 76.8, 77.2, 77.5, 167.8, 169.3, 169.5, 169.7 and 170.3.

2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl Diphenyl Phosphate (11). To a solution of 525 mg (1.34 mmol) **10** in 8.10 mL of dry DMF, was added 170 mg (1.88 mmol) of hydrazine acetate. The reaction was stirred at room temperature for 2 h until analysis by silica gel TLC indicated it was complete. The reaction mixture was diluted with 50 mL of ethyl acetate and washed with three 20-mL portions of brine. The aqueous layer was re-extracted with three 30-mL portions of ethyl acetate. The combined organic layer was dried (Na_2SO_4) and concentrated under diminished pressure and dried to afford compound as a colorless oil; silica gel TLC R_f 0.39 (3:1 hexanes–ethyl acetate). To the solution of 397 mg (1.14 mmol) of the above residue alcohol in 16.5 mL of dry CH_2Cl_2, was added 180 mg (1.47 mmol) of DMAP and 1.60 mL (11.4 mmol) of Et_3N. The reaction mixture was stirred for 10 min, followed by the addition of 2.30 mL (10.9 mmol) of diphenyl chlorophosphate dropwise at 0 °C. The solution was stirred at 0 °C for 1.5 h and was poured into a mixture of 200 mL of EtOAc and 80 mL of saturated aq $NaHCO_3$ soln. The organic layer was washed with two 50-mL portions of brine, dried over Na_2SO_4, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (18 × 5 cm). Elution with 3:1→2:1 hexanes–ethyl acetate afforded compound **11** as a colorless oil: yield 424 mg (54% over two steps); silica gel TLC R_f 0.54 (3:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.22 (s, 3H), 3.98 (dd, 1H, J = 12.4 and 2.0 Hz), 4.14 (m, 1H), 4.25 (dd, 1H, J = 12.4 and 4.8 Hz), 5.40 (m, 3H), 5.92 (dd, 1H, J = 6.8 and 1.6 Hz), 7.28-7.33 (m, 6H) and 7.40-7.45 (m, 4H); ^{13}C NMR

($CDCl_3$) δ 20.70, 20.72, 20.78, 20.84, 61.8, 65.2, 68.3, 68.7, 68.8, 70.9, 96.17, 96.22, 120.18, 120.22, 120.3, 120.4, 125.90, 125.91, 126.0, 130.1, 130.2, 169.6, 169.9 and 170.7.

2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl Benzyl 2-(2-Ethoxy)ethylcarbamate (12). To a solution of 300 mg (0.52 mmol) of phosphate ester **11** and 111 mg (0.46 mmol) of the alcohol **5** in 5.5 mL of anhydrous CH_2Cl_2, was added 168 μL (207 mg, 0.93 mmol) of TMSOTf at 0 °C. The reaction was stirred at 0 °C for 18 min and was then poured into a mixture of 100 mL of EtOAc and 40 mL of saturated aq $NaHCO_3$. The organic layer was washed with two 40-mL portions of brine, dried (Na_2SO_4), filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (25 × 2.5 cm). Elution with 2:1→1:2 hexanes–ethyl acetate afforded compound **12** as a colorless oil: yield 110 mg (37%); silica gel TLC R_f 0.35 (1:3 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 2.00 (s, 3H), 2.08 (s, 3H), 2.13 (s, 3H), 3.40 (m, 2H), 3.53 (m, 2H), 3.62 (m, 3H), 3.77 (m, 1H), 4.06 (m, 1H), 4.11 (m, 1H), 4.24 (m, 1H), 4.87 (br s, 1H), 5.08 (s, 2H), 5.24 (m, 2H), 5.35 (m, 2H), 7.26-7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.80, 20.82, 20.9, 21.0, 39.4, 41.1, 62.7, 66.4, 66.8, 67.2, 68.6, 69.1, 69.8, 70.1, 70.4, 97.7, 128.2, 128.6, 136.8, 169.9, 170.0, 170.3, 170.8 and 170.9; mass spectrum (MALDI), *m/z* 592.34 $(M + Na)^+$; mass spectrum (APCI), *m/z* 570.2182 $(M + H)^+$ ($C_{26}H_{36}NO_{13}$ requires *m/z* 570.2187).

Decarbamoyl BLM Monosaccharide-Cy5 (2).** To a solution of 8.90 mg (15.6 μmol) of compound **12** in 2.00 mL of anh methanol was added 0.2 mL of a freshly prepared 25% w/w solution of sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 2.5 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered; mass spectrum (MALDI) *m/z* 424.24 $(M + Na)^+$; mass spectrum (APCI), *m/z* 402.1759 $(M + H)^+$ ($C_{18}H_{28}NO_9$ requires *m/z* 402.1764). To a solution of the crude product in 5 mL methanol was added Pd/C and H_2 gas was bubbled through for 45 min. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite and then concentrated under diminished pressure to afford **13**, which was used for the next reaction; mass

spectrum (MALDI), *m/z* 268.25 $(M + H)^+$, 290.25 $(M + Na)^+$; mass spectrum (APCI), *m/z* 268.1391 $(M + H)^+$ ($C_{10}H_{22}NO_7$ requires *m/z* 268.1396).

To 152 μg (0.57 μmol) of **13** was added a solution of 110 μg (0.11 μmol) of Cy5**COOSu (**8**) in 100 μL of 0.2 M phosphate buffer and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4.5 mL/min. The fractions containing the desired product eluted at 19.5 min and were collected, frozen and lyophilized to give **2** as a blue solid: yield 39 μg (30%); mass spectrum (MALDI), *m/z* 1158.40 $(M – H + Na)^+$, 1180.47 $(M – 2H + 2Na)^+$; mass spectrum (TOF), *m/z* 1136.3409 $(M)^+$ ($C_{48}H_{70}N_3O_{20}S_4$ requires *m/z* 1136.3435).

BLM Monosaccharide Trimer (16). H_2 gas was bubbled through a mixture containing 36 mg (21 μmol) of **6** and a catalytic amount of Pd/C in 6 mL of dry THF for 45 min. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated under diminished pressure to obtain crude **14** as a colorless oil, which was used immediately in the next step: crude yield 27 mg (99%); silica gel TLC R_f 0.29 (1:3 hexanes–ethyl acetate); mass spectrum (MALDI), *m/z* 459.26 $(M + Na)^+$; mass spectrum (APCI), *m/z* 437.1768 $(M + H)^+$ ($C_{17}H_{29}N_2O_{11}$ requires *m/z* 437.1772).

To a solution containing 27.0 mg (61.8 μmol) of **14** in 0.53 mL of dry DMF, were added 13.0 μL (0.09 mmol) of triethylamine and 15.2 mg (204 μmol) of **15** and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (15 × 1.5 cm). Elution with 16:12:1→11:12:1 chloroform–acetone–methanol afforded BLM monosaccharide trimer **16** as a colorless oil: yield 15 mg (43%); silica gel TLC R_f 0.56 (4:4:1 chloroform–acetone–methanol); mass spectrum (MALDI), *m/z* 1730.76 $(M + Na)^+$; mass spectrum (TOF), *m/z* 854.3351 $(M + 2H)^{2+}$ ($C_{72}H_{108}N_8O_{39}$ requires *m/z* 854.3357).

BLM Monosaccharide-Cy5 Trimer (3).** To a solution of 4.20 mg (2.46 μmol) of **16** in 2 mL of anh methanol was added 200 μL of a freshly prepared 25% w/w solution of sodium

methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 2.5 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered; mass spectrum (MALDI), *m/z* 1351.40 $(M + Na)^+$ (theoretical m/z 1328.56). To the solution of the crude product in anh methanol was added Pd/C and H_2 gas was bubbled through for 45 min. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite and then concentrated under diminished pressure to afford 2.9 mg of **17** (100%), which was used directly for the next reaction; mass spectrum (MALDI), *m/z* 1217.62 $(M + Na)^+$; mass spectrum (TOF), *m/z* 1229.4961 $(M + Cl)^-$ ($C_{46}H_{82}N_8O_{28}Cl$ requires *m/z* 1229.4927).

To 540 μg (0.448 μmol) of **17**, was added a solution of 110 μg (0.11 μmol) of Cy5**COOSu (**8**) in 100 μL of 0.2 M phosphate buffer and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 28 min at a flow rate of 4.5 mL/min. The fractions containing the desired product eluted at 21.0 min (monitoring at 651 nm) and were collected, frozen and lyophilized to give **3** as a blue solid: yield 77 μg (33%); mass spectrum (MALDI), *m/z* 2085.85 $(M + Na - H)^+$, 2107.85 $(M + 2Na - 2H)^+$; mass spectrum (TOF), *m/z* 686.6584 $(M - 4H)^{3-}$ ($C_{84}H_{127}N_{10}O_{41}S_4$ requires *m/z* 686.5679).

Cell Growth Conditions. MCF-7 cells (ATCC HBT-22), A498 cells (ATCC HTB-41), A549 cells (ATCC CCL-185) and BT474 cells (ATCC HTB-20) were grown in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone, South Logan, UT) and 1% penicillin–streptomycin mix antibiotic supplement (Cellgro, Manassas, VA). MCF-10A cells (ATCC CRL-10317) were grown in MEGM (Invitrogen, Grand Island, NY) supplemented with 100 ng/mL cholera toxin (Sigma-Aldrich) and 1% penicillin-streptomycin mix antibiotic supplement. DU-145 (ATCC HTB-81) prostate cells, BxPC-3 (ATCC CRL-1687) pancreas cells, SVR A221a (CRL-2386) pancreatic normal cells, PZ-HPV-7 (ATCC CRL-2221)

prostate normal cells, WI-38 (ATCC CCL-75) lung normal cells and CCD-1105 KIDTr (CRL-2305) kidney normal cells were grown in MEM (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone) and 1% penicillin–streptomycin mix antibiotic supplement. Cell lines were maintained at 37 °C under a humidified atmosphere of 5% CO_2 and 95% air.

Fluorescence microscopy. The fluorescent images were obtained using a Zeiss Axiovert 200M inverted fluorescence microscope fitted with an AxioCam MRm camera equipped with a 300-w xenon lamp (Sutter, Novato, CA) and a Cy5 cyanine filter (Chroma, Bellows Falls, VT). The cells were grown on 16-well Lab-Tek glass chamber slides at a cell density of 5000 cells/well (Thermo Scientific, Waltham, MA) at 37 °C for 48 h. When the cell confluency reached about 70%, the cells were rinsed twice with phosphate buffered saline (PBS), and the old medium was replaced with RPMI 1640 (no phenol red). Subsequently, the dye-labeled conjugates were added to the final desired concentrations and incubation of the cells was carried out at 37 °C for 1 h. Thereafter, the cells were washed with PBS, and fixed with 4% paraformaldehyde at 37 °C for 5 min. Finally, the slide was mounted with Prolong Antifade Gold reagent with DAPI (Invitrogen). The cells were imaged using a Zeiss EC Plan Neofluor 40x/1.3 DIC M27 oil objective and the target cells were counted for quantification. For comparative studies, the exposure time and source intensity were kept identical for accurate measurements. Three different viewing fields each containing at least 10 individual cells were analyzed from each experiment. The mean pixel intensities [per unit area of the cells] in the viewing fields were measured to give the normalized fluorescence and quantification values were generated by using AxioVision 4 v 4.7.1.0 software in conjunction with the interactive measurement tool.