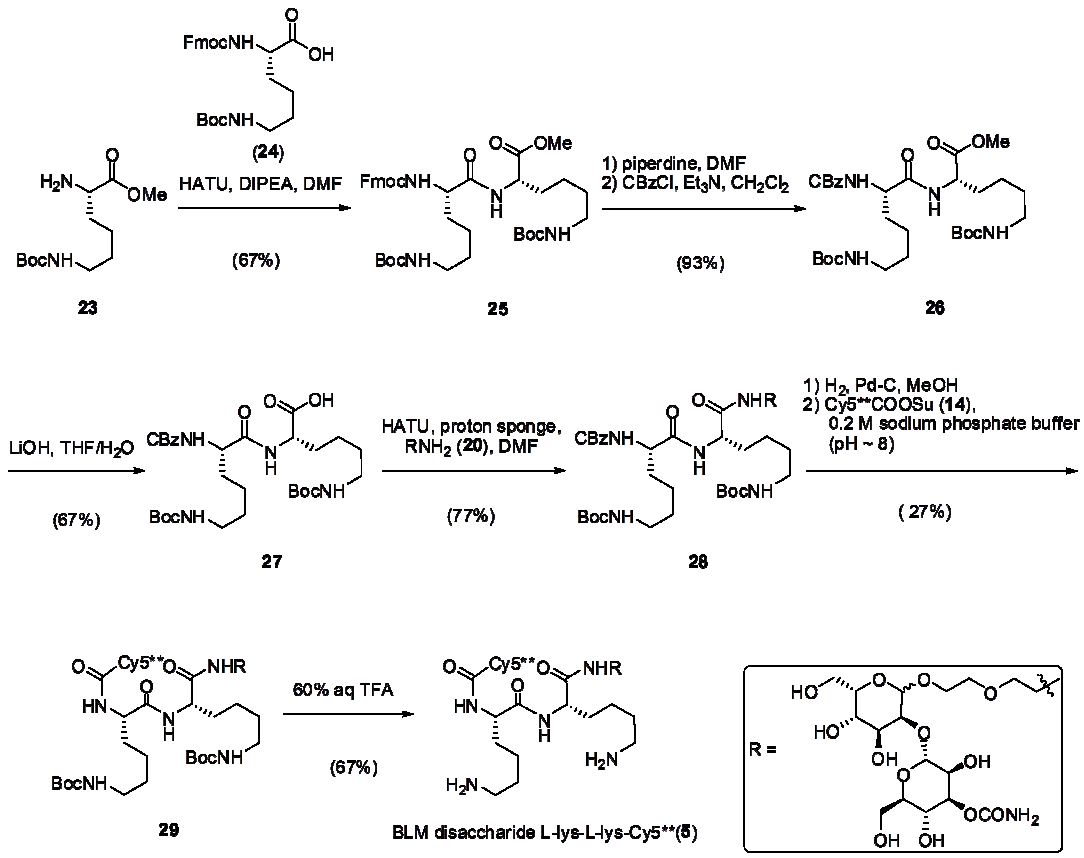
Supporting Information

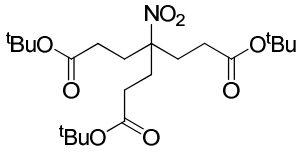
for

Structural Features Facilitating Tumor Cell Targeting and Internalization by Bleomycin and its Disaccharide

Zhiqiang Yu, Rakesh Paul, Chandrabali Bhattacharya, Trevor C. Bozeman, Michael J. Rishel, and Sidney M. Hecht

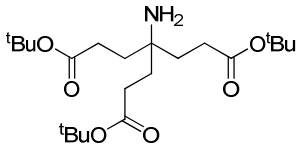
Scheme S1. Synthetic Route Utilized for the Preparation of BLM disaccharide-L-Lys-L-Lys-Cy5 (5)**





4-(2-*tert*-Butoxycarbonylethyl)-4-nitro-heptanedioic Acid Di-*tert*-butyl Ester (7).[1]

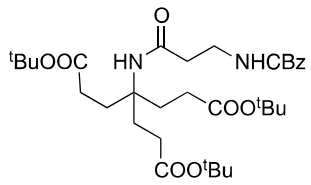
To a solution of 2.14 mL (2.43 g; 39.8 mmol) of nitromethane in 10 mL of dimethoxyethane at 65 °C was added 0.40 mL of 40% aq tetrabutylammonium hydroxide solution and the reaction mixture was heated to 75 °C. To the reaction mixture was added dropwise 18.2 mL (125 mmol) of *tert*-butyl acrylate (**6**). To this mixture was added 0.8 mL of 40% aq tetrabutylammonium hydroxide solution in portions over a period of 1 h. The reaction mixture was stirred at 75 °C for 2 h, and was then concentrated under diminished pressure. The residue was dissolved in 100 mL of diethyl ether. The ether layer was washed successively with two 30-mL portions of 10% aq citric acid solution, two 30-mL portions of sat aq $NaHCO_3$ solution, and 20 mL of brine. The solution was dried over anhydrous Na_2SO_4, then filtered and concentrated under diminished pressure. The residue was crystallized from absolute ethanol to afford compound **7** as colorless needles: yield 16.1 g (91%); mp 92-94 °C, lit[1] mp 98-100 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 27H) and 2.19 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 28.2, 29.9, 30.5, 81.3, 92.3 and 171.2.



4-Amino-4-(2-*tert*-butoxycarbonylethyl)heptanedioic Acid Di-*tert*-butyl Ester (8).[1]

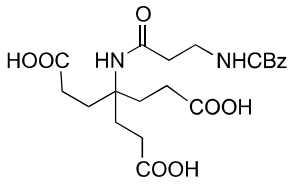
A mixture of 1.02 g (2.29 mmol) of compound **7**, ~6 mL of Raney Ni (suspension in ethanol) and 18 mL of absolute ethanol was shaken in a Parr shaker at room temperature under 52 psi H_2 for 72 h. The reaction mixture was filtered through a pad of Celite and the filtrate was

concentrated under diminished pressure to afford amine **8** as a waxy solid, which was used directly in the next step: yield 0.88 g (92%); silica gel TLC R_f 0.14 (1:3 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 27H), 1.58 (t, 6H, J = 8.4 Hz) and 2.22 (t, 6H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 28.0, 29.9, 34.4, 52.3, 80.3 and 173.0.

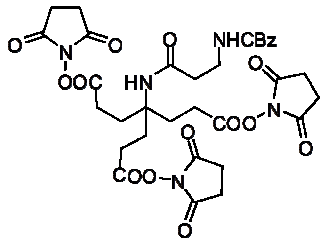


4-(3-Benzyloxycarbonylaminopropionylamino)-4-(2-*tert*-butoxycarbonylethyl)heptanedioic Acid Di-*tert*-butyl Ester (9).

To a solution of 0.84 g (2.02 mmol) of compound **8** and 0.43 g (1.91 mmol) of CBz-*β*-alanine in 15 mL of dry DMF were added 0.74 g (1.95 mmol) of HATU and 0.82 g (3.82 mmol) of proton sponge. The resulting yellow reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under diminished pressure and the residue was dissolved in 80 mL of ethyl acetate. The ethyl acetate layer was washed with two 40-mL portions of 2 M aq HCl, two 30-mL portions of H_2O, and 20 mL of brine, then dried over anhydrous Na_2SO_4, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (12 × 3 cm). Elution with 1:1 hexanes–ethyl acetate gave compound **9** as a colorless solid: yield 1.17 g (98%); silica gel TLC R_f 0.40 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 27H), 1.94 (t, 6H, J = 8.0 Hz), 2.19 (t, 6H, J = 8.4 Hz), 2.34 (m, 2H), 3.44 (m, 2H), 5.09 (s, 2H), 5.57 (br s, 1H), 5.99 (br s, 1H) and 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 28.1, 29.8, 30.0, 36.8, 37.3, 57.8, 66.6, 80.8, 128.0, 128.5, 136.7, 156.6, 170.9 and 172.9; mass spectrum (ESI), *m/z* 621.3753 $(M + H)^+$ ($C_{33}H_{53}N_2O_9$ requires *m/z* 621.3746).

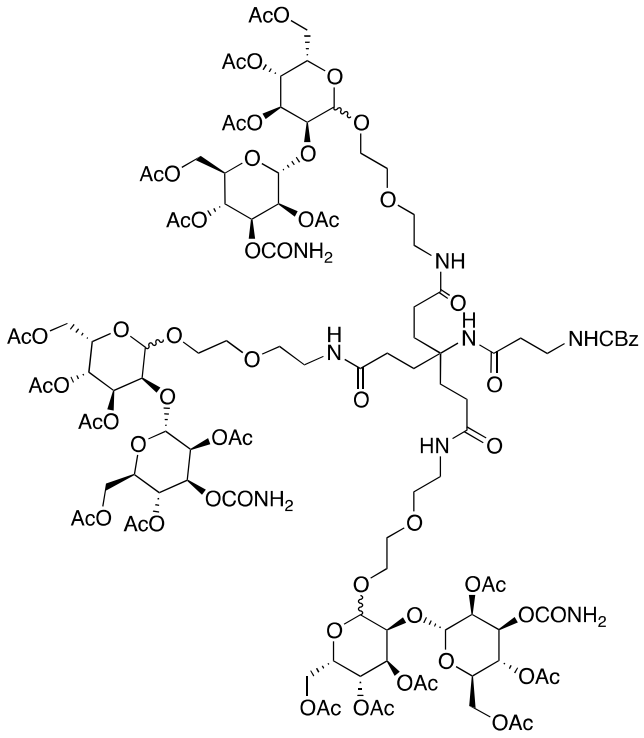


4-(3-Benzyloxycarbonylaminopropionylamino)-4-(2-carboxyethyl)heptanedioic Acid (10). A solution of 1.21 g (1.93 mmol) of **9** in 25 mL of formic acid was stirred at room temperature for 12 h. The reaction mixture was then concentrated under diminished pressure. The residue was co-evaporated with six 10-mL portions of toluene to afford tri-acid **10** as a colorless oil: yield 0.91 g (100%); ^{1}H NMR (CD_3OD) δ 2.01 (m, 6H), 2.26 (m, 6H), 2.40 (m, 2H), 3.36 (m, 2H), 5.07 (s, 2H) and 7.31 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 28.1, 29.0, 36.2, 37.3, 56.4, 65.2, 127.71, 127.75, 137.2, 156.0, 170.0 and 174.5; mass spectrum (ESI), *m/z* 453.1886 $(M + H)^+$ ($C_{21}H_{29}N_2O_9$ requires *m/z* 453.1868).



4-(3-Benzyloxycarbonylaminopropionylamino)-4-[2-(2,5-dioxopyrrolidin-1-yloxycarbonyl)ethyl]heptanedioic Acid Bis-(*N*-hydroxysuccinimidyl) Ester (11). To a solution of 0.48 g (1.06 mmol) of compound **10** and 0.44 g (3.82 mmol) of *N*-hydroxysuccinimide in 9 mL dry THF at 0 °C was added dropwise a solution of 0.83 g (4.03 mmol) of DCC in 2 mL of dry THF. The reaction mixture was stirred at 5 °C for 16 h. The reaction mixture was then concentrated under diminished pressure and the residue was suspended in 10 mL of acetonitrile. The suspension was filtered and the filtrate was concentrated under diminished pressure. The residue was the purified by crystallization from absolute ethanol

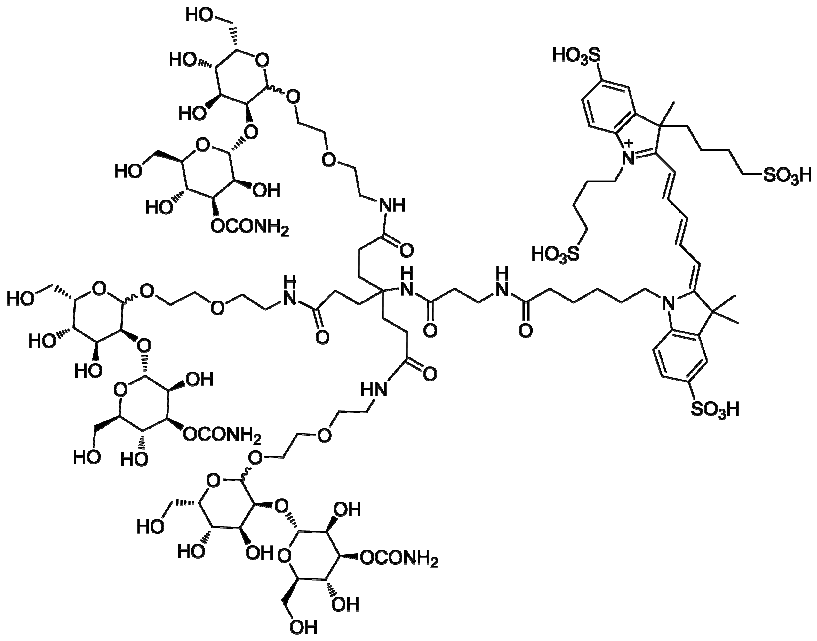
to afford **11** as colorless crystals: yield 366 mg (46%); ^{1}H NMR (CD_3CN) δ 2.08 (m, 6H), 2.31 (m, 2H), 2.58 (m, 6H), 2.74 (s, 12H), 3.28 (m, 2H), 5.02 (s, 2H), 5.73 (br s, 1H), 6.10 (br s, 1H) and 7.32 (m, 5H); ^{13}C NMR (CD_3CN) δ 25.9, 26.3, 29.5, 37.0, 37.9, 58.0, 66.7, 128.6, 128.7, 129.3, 138.3, 157.2, 169.8, 171.0 and 172.1; mass spectrum (ESI), *m/z* 744.2342 $(M + H)^+$ ($C_{33}H_{38}N_5O_{15}$ requires *m/z* 744.2359).



Peracetylated BLM-disaccharide Trimer (13).

Hydrogen was bubbled through a mixture containing 18 mg (21 μmol) of CBz-protected peracetylated disaccharide amine **12**[2] and a catalytic amount of Pd/C in 5 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 the crude amine as a colorless oil, which was used immediately in the next step: crude yield 14 mg; mass spectrum (MALDI) *m/z* 725.28 $(M + H)^+$ (theoretical *m/z* 725.26).

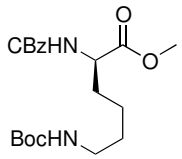
To a solution containing 14.0 mg (19.0 μmol) of the free amine derived from **12** and 20 μL (15.0 mg, 0.14 mmol) of triethylamine in 1.5 mL of dry DMF was added 1.60 mg (2.20 μmol) of **11** and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was then concentrated under diminished pressure, and the residue was purified by flash chromatography on a silica gel column (14 × 1 cm). Elution with 32:15:1 → 11:10:1 chloroform–acetone–methanol afforded peracetylated BLM-disaccharide trimer **13** as a colorless oil: yield 4.50 mg (81%); silica gel TLC R_f 0.60 (4:4:1 chloroform–acetone–methanol); mass spectrum (MALDI), *m/z* 2595.11 $(M + Na)^+$ (theoretical m/z 2594.90); mass spectrum (ESI), *m/z* 1297.4575 $(M + H + Na)^{2+}$ ($C_{108}H_{155}N_8O_{63}Na$ requires *m/z* 1297.4529).



BLM disaccharide-Cy5 Trimer (3).**

To a solution of 5.00 mg (1.94 μmol) of **13** in 2 mL of dry MeOH was added 0.3 mL of a 25% solution (w/w) of NaOMe in MeOH. The reaction mixture was shaken at room temperature for 2 h. One hundred mg of Dowex 50W resin was added and the mixture was shaken at room temperature for 30 min. The mixture was filtered, diluted to 5 mL with methanol and a catalytic

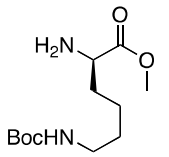
amount of Pd/C was added. Hydrogen was bubbled through the mixture for 30 min and the reaction mixture was filtered. The filtrate was concentrated to obtain the fully deprotected BLM disaccharide trimer as a colorless solid: crude yield 2.60 mg (80%). To 110 μg (0.11 μmol) of Cy5**COOSu (**14**) was added 100 μL of 0.2 M aq sodium phosphate buffer (pH ~ 8). This solution was added to a vial containing 0.56 mg (0.34 μmol) of the deprotected BLM-disaccharide trimer in 40 μL of DMSO and the reaction mixture was shaken at room temperature overnight in the dark. The crude reaction mixture was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using 0.1% aq CF_3COOH and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq CF_3COOH–CH_3CN → 45:55 0.1% aq CF_3COOH–CH_3CN) over a period of 30 min at a flow rate of 3 mL/min. Fractions containing the desired product eluted at 14.8 min (monitoring at 651 nm) and were collected, frozen, and lyophilized to give the BLM-disaccharide-Cy5** trimer **3** as a blue solid: yield 43 μg (15%); mass spectrum (MALDI), *m/z* 2588.4 $(M)^+$ (theoretical *m/z* 2587.8); mass spectrum (ESI), *m/z* 848.6223 $(M - 4H)^{3-}$ ($C_{102}H_{167}N_{10}O_{56}S_4$ requires *m/z* 848.6215).



N^{α}-CBz-N^{ε}-Boc-D-lysine Methyl Ester (16).[3]

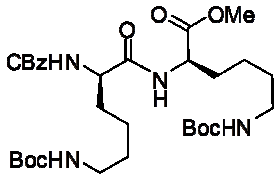
To a mixture of 149 mg (0.39 mmol) of N^{α}-CBz-N^{ε}-Boc-D-lysine (**15**) and 108 mg (0.78 mmol) of K_2CO_3 in 4 mL of anhydrous DMF was added 73.0 μL (0.17 g; 1.20 mmol) of CH_3I at room temperature. The reaction mixture was stirred at 50 °C for 2 h. The cooled reaction mixture was diluted with 50 mL of diethyl ether. The organic layer was washed successively with 20 mL of H_2O, 20 mL of 0.1 N aq HCl and 15 mL of brine. The ether layer was dried (Na_2SO_4) and concentrated under diminished pressure. The residue was purified by flash chromatography on a

silica gel column (12 × 3 cm). Elution with 3:1 → 1:1 hexanes–ethyl acetate afforded compound **16** as a colorless oil: yield 134 mg (87%); silica gel TLC R_f 0.31 (3:1 hexanes–ethyl acetate); ^{1}H NMR (DMSO-d_6) δ 1.31–1.67 (m, 15H), 2.87 (m, 2H), 3.62 (m, 1H), 3.99 (s, 3H), 5.03 (s, 2H), 6.78 (m, 1H), 7.71 (m, 1H) and 7.36 (m, 5H).



N^{ε}-Boc-D-lysine Methyl Ester (17).[3]

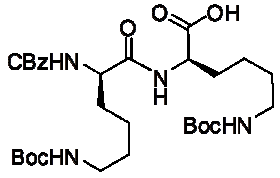
Hydrogen was bubbled through a mixture of 134 mg (0.34 mmol) of compound **16** and a catalytic amount of Pd/C in 8 mL of methanol for 10 min. The reaction mixture was stirred under an atmosphere of H_2 for 30 min and then filtered through a pad of Celite. The filtrate was concentrated under diminished pressure to obtain compound **17** as a colorless oil: yield 79.0 mg (89%); silica gel TLC R_f 0.46 (20:1 chloroform–methanol); ^{1}H NMR ($CDCl_3$) δ 1.32–1.77 (m, 15H), 3.10 (d, 2H, J = 6.1 Hz), 3.43 (dd, 1H, J = 9.6 and 3.7 Hz), 3.70 (s, 3H) and 4.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.8, 28.4, 29.8, 34.4, 40.3, 51.9, 54.2, 79.0, 155.9 and 176.4.



N^{α}-CBz-N^{ε}-Boc-D-lysyl-N^{ε}-Boc-D-lysine Methyl Ester (18).

To a solution of 79.0 mg (0.30 mmol) of **17**, 109 mg (0.29 mmol) of **15** and 123 mg (0.57 mmol) of 1,8-bis(dimethylamino)naphthalene (proton sponge) in 4 mL of anhydrous DMF was added 163 mg (0.43 mmol) of HATU. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under diminished pressure and the residue was dissolved

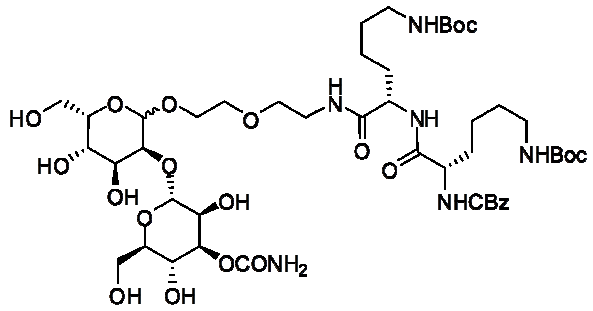
in 80 mL of diethyl ether. The ether layer was washed successively with three 25-mL portions of 1 N aq HCl, 30 mL of satd aq $NaHCO_3$ solution and 20 mL of brine. The organic layer was dried (Na_2SO_4) and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (15 × 3 cm). Elution with 2:1 → 1:1 hexanes–ethyl acetate afforded methyl ester **18** as a colorless oil: yield 150 mg (84%); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.30–1.53 (m, 26H), 1.67 (m, 2H), 1.83 (m, 2H), 3.06 (s, 4H), 3.72 (s, 3H), 4.21 (d, 1H, J = 5.8 Hz), 4.54 (m, 1H), 4.77 (m, 2H), 5.10 (s, 2H), 5.64 (s, 1H), 6.75 (d, 1H, J = 6.9 Hz) and 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 22.4, 22.5, 27.8, 28.5, 29.3, 29.5, 29.6, 31.7, 39.9, 40.1, 52.1, 54.8, 67.1, 79.2, 128.2, 128.3, 128.6, 136.3, 156.2, 156.3, 156.4,171.9 and 172.7; mass spectrum (ESI), *m/z* 623.3652 $(M + H)^+$ ($C_{31}H_{51}N_4O_9$ requires *m/z* 623.3650).



N^{α}-CBz-N^{ε}-Boc-D-lysyl-N^{ε}-Boc-D-lysine (19).

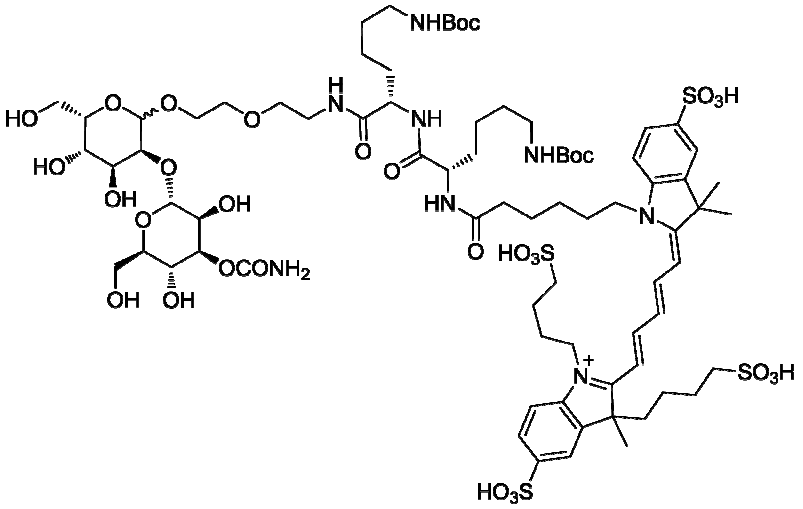
To a solution containing 145 mg (0.37 mmol) of methyl ester **18** in 3.2 mL of THF was added a solution of 35.0 mg (1.47 mmol) of LiOH in 1.6 mL of water. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then diluted with 20 mL of Et_2O and 35 mL of water and the phases were separated. The cooled aqueous phase (ice bath) was acidified to pH ~ 3 with 5% aq $NaHSO_4$ and extracted with three 30-mL portions of ethyl acetate. The combined organic layer was washed with 20 mL of brine, dried over anhydrous Na_2SO_4, filtered and concentrated under diminished pressure to afford the free acid **19** as a colorless oil: yield 129 mg (91%); silica gel TLC R_f 0.27 (1:3 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.24–1.96 (m,

30H), 3.04 (d, 4H, J = 5.1 Hz), 4.25 (m, 2H), 4.89 (s, 1H), 5.06 (d, 2H, J = 12.7 Hz), 6.05 (m, 1H), 7.25 (m, 5H) and 10.55 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.5, 22.6, 28.5, 29.5, 31.5, 32.4, 40.2, 52.3, 54.8, 67.0, 79.4, 128.1, 128.2, 128.6, 136.4, 156.4, 156.5, 158.0, 172.4 and 174.9; mass spectrum (ESI), *m/z* 609.3495 $(M + H)^+$ ($C_{30}H_{49}N_4O_9$ requires *m/z* 609.3494).



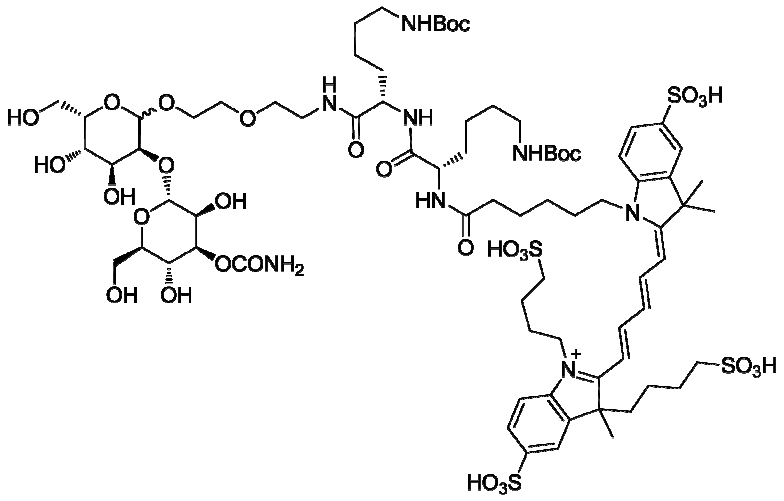
N^{α}-CBz-N^{ε}-Boc-D-lysyl-N^{ε}-Boc-D-lysyl-BLM disaccharide (21).

To a solution containing 3.0 mg (6.3 μmol) of disaccharide amine **20** (obtained by hydrogenolysis and deacetylation of **12**[2]), 3.8 mg (6.2 μmol) of **19** and 4.5 μL (3.2 mg; 25 μmol) of DIPEA in 0.15 mL of anhydrous DMF was added 4.8 mg (13 μmol) of HATU. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was separated on an Econosil C_{18} reversed phase semi-preparative HPLC column (250 × 10 mm, 10 μm) using 0.1% aq CF_3COOH and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq CF_3COOH–CH_3CN → 45:55 0.1% aq CF_3COOH–CH_3CN) over a period of 30 min at a flow rate of 3 mL/min. Fractions containing the desired product eluted at 29.8 min (monitoring at 220 nm) and were collected, frozen, and lyophilized to give **21** as a colorless solid: yield 2.5 mg (37%); mass spectrum (ESI), *m/z* 1063.5280 $(M + H)^+$ ($C_{47}H_{79}N_6O_{21}$ requires *m/z* 1063.5293).



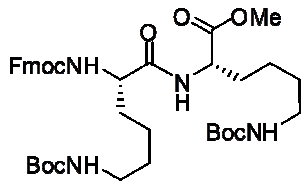
N^{α}-Cy5-N^{ε}-Boc-D-lysyl-N^{ε}-Boc-D-lysyl-BLM disaccharide (22).**

Hydrogen was bubbled through a solution of 1.50 mg (4.20 μmol) of compound **21** in 2 mL of methanol for 10 min, and the reaction mixture was stirred for 30 min under a hydrogen atmosphere. The reaction mixture was filtered and the filtrate was concentrated under diminished pressure to afford the deprotected amine as a colorless solid: yield 1.1 mg. To 1.10 mg (1.20 μmol) of the amine was added 0.44 mL of 0.2 M aq sodium phosphate buffer (pH ~ 8). Two hundred μL (0.50 mg; 0.54 μmol) of this solution was added to a vial containing 110 μg (0.11 μmol) of Cy5** carboxylic acid succinimidyl ester (**14**) and the reaction mixture was shaken at room temperature overnight in the dark. The crude reaction mixture was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using 0.1% aq CF_3COOH and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq CF_3COOH–CH_3CN → 45:55 0.1% aq CF_3COOH–CH_3CN) over a period of 30 min at a flow rate of 3 mL/min. Fractions containing the desired product eluted at 20.1 min (monitoring at 651 nm) and were collected, frozen, and lyophilized to give the dye conjugate **22** as a blue solid: yield 52.0 μg (26%); mass spectrum (ESI), *m/z* 897.3370 $(M - 3H)^{2-}$ ($C_{77}H_{118}N_8O_{32}S_4$ requires *m/z* 897.3373).



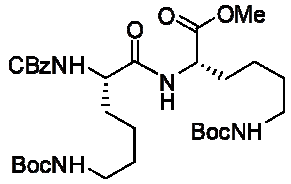
N^{α}-Cy5-D-lysyl-D-lysyl-BLM disaccharide (4).**

A solution of 45 μg (25 nmol) of **22** in 0.2 mL of 60% aq CF_3COOH was shaken at room temperature for 40 min and was then purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using 0.1% aq CF_3COOH and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq CF_3COOH–CH_3CN → 45:55 0.1% aq CF_3COOH–CH_3CN) over a period of 30 min at a flow rate of 3 mL/min. Fractions containing the desired product eluted at 16.2 min (monitoring at 651 nm) and were collected, frozen, and lyophilized to give **4** as a blue solid: yield 25 μg (62%); mass spectrum (ESI), *m/z* 797.2845 $(M - 3H)^{2-}$ ($C_{67}H_{102}N_8O_{28}S_4$ requires *m/z* 797.2850).



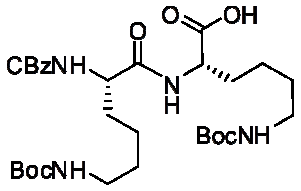
N^{α}-Fmoc-N^{ε}-Boc-L-lysyl-N^{ε}-Boc-L-lysine Methyl Ester (25). To a solution of 60.0 mg (0.23 mmol) of compound **23**[4], 108 mg (0.23 mmol) of compound **24** and 99.0 mg (0.46 mmol) of 1,8-bis(dimethylamino)naphthalene (proton sponge) in 4 mL of anhydrous DMF, was added 131 mg (0.35 mmol) of HATU. The reaction mixture was stirred at room temperature for 16 h. The

reaction mixture was concentrated under diminished pressure and the residue was dissolved in 50 mL of diethyl ether. The ether layer was washed successively with three 15-mL portions of 1 N aq HCl, 20 mL of satd aq $NaHCO_3$ soln and 20 mL of brine. The organic layer was then dried (Na_2SO_4) and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (15 × 3 cm). Elution with 2:1 → 1:1 hexanes–ethyl acetate afforded compound **25** as a colorless oil: yield 110 mg (67%); silica gel TLC R_f 0.42 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.41 (m, 26H), 1.66 (m, 2H), 1.84 (m, 2H), 3.06 (m, 4H), 3.66 (s, 3H), 4.40 (m, 4H), 4.54 (dd, 1H, *J* = 12.4 and 7.4 Hz), 4.82 (s, 2H), 5.81 (s, 1H), 6.92 (d, 1H, *J* = 6.1 Hz), 7.27 (m, 2H), 7.37 (t, 2H, *J* = 7.5 Hz), 7.58 (d, 2H, *J* = 7.3 Hz) and 7.73 (d, 2H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 22.3, 22.5, 28.47, 28.48, 29.4, 29.6, 31.5, 32.3, 39.9, 40.0, 47.1, 52.2, 52.4, 54.6, 67.2, 79.1, 119.98, 120.01, 125.16, 125.21, 127.1, 127.7, 141.28, 141.29, 143.8, 143.9, 156.20, 156.24, 156.3, 171.9 and 172.6; mass spectrum (ESI), *m/z* 711.3976 $(M + H)^+$ ($C_{38}H_{55}N_4O_9$ requires *m/z* 711.3964).



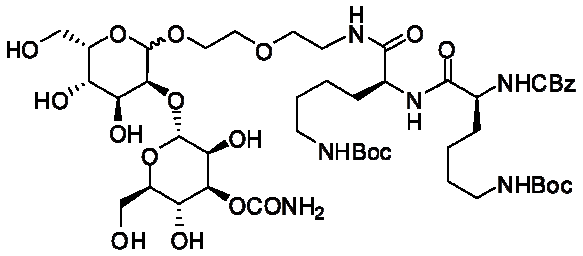
***N*$^{\alpha}$-CBz-*N*$^{\varepsilon}$-Boc-L-lysyl-*N*$^{\varepsilon}$-Boc-L-lysine Methyl Ester (26).** To a solution of 110 mg (0.15 mmol) of compound **25** in 2 mL of anhydrous DMF, was added 0.4 mL of piperidine and the solution was stirred at room temperature for 1 h. The reaction mixture was concentrated under diminished pressure and the residue was co-evaporated with three 10-mL portions of toluene. To a solution of the residue in 3 mL of dry THF, were added 0.22 mL (0.16 mg; 1.60 mmol) of anhydrous triethylamine and 0.11 mL (0.13 g, 0.77 mmol) of benzyl chloroformate. The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was then diluted with 60

mL of diethyl ether, then washed with two 20-mL portions of water and 20 mL of brine. The ether layer was dried over Na_2SO_4 and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (15 × 3 cm). Elution with 2:1 → 1:2 hexanes–ethyl acetate afforded the methyl ester **26** as a colorless oil: yield 82.0 mg (85%); silica gel TLC R_f 0.50 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.30–1.53 (m, 26H), 1.67 (dd, 3H, J = 13.7 and 7.1 Hz), 1.83 (m, 2H), 3.06 (s, 4H), 3.72 (s, 3H), 4.21 (d, 1H, J = 5.8 Hz), 4.67 (m, 3H), 5.10 (s, 2H), 5.64 (s, 1H), 6.75 (d, 1H, J = 6.9 Hz) and 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 22.4, 22.5, 27.8, 28.5, 29.3, 29.5, 29.6, 31.7, 39.9, 40.1, 52.1, 54.8, 67.1, 79.2, 128.2, 128.3, 128.6, 136.3, 156.2, 156.3, 156.4,171.9 and 172.7.

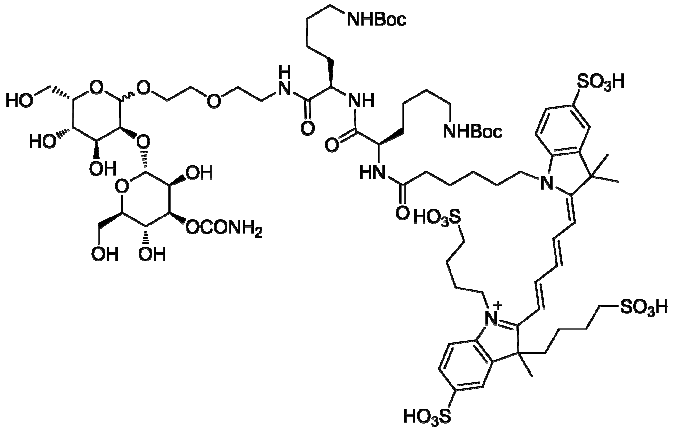


***N*$^{\alpha}$-CBz-*N*$^{\varepsilon}$-Boc-L-lysyl-*N*$^{\varepsilon}$-Boc-L-lysine (27).** To a solution containing 82.0 mg (0.13 mmol) of compound **26** in 1.2 mL of THF, was added a solution of 13.0 mg of LiOH (0.53 mmol) in 0.6 mL of water. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with 10 mL of diethyl ether and 25 mL of water. The aqueous phase was separated, cooled, acidified to pH ~ 3 with 5% aq $NaHSO_4$ solution and extracted with three 20-mL portions of ethyl acetate. The combined organic layer was then washed with 10 mL of brine, dried over Na_2SO_4, filtered and concentrated under diminished pressure to afford the acid **27** as a colorless oil: yield 62 mg (78%); silica gel TLC R_f 0.27 (1:3 hexanes–ethyl acetate); ^{1}H NMR (CD_3OD) δ 1.44 (s, 26H), 1.68 (m, 2H), 1.86 (m, 2H), 3.01 (m, 4H), 4.14 (m, 1H), 4.38 (m, 1H), 5.09 (s, 2H) and 7.33 (m, 5H); ^{13}C NMR (CD_3OD) δ 24.0, 24.1, 28.8, 30.4, 30.5, 32.3, 32.9,

41.0, 41.1, 53.4, 53.5, 56.2, 67.6, 79.8, 128.8, 129.0, 129.4, 138.2, 158.4, 158.49, 158.53, 174.9 and 175.1.

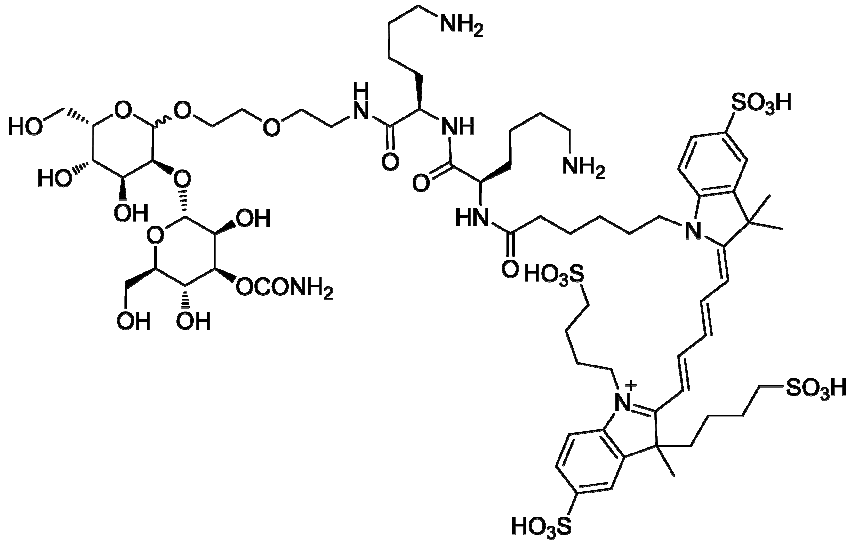


***N*$^{\alpha}$-CBz-*N*$^{\varepsilon}$-Boc-L-lysyl-*N*$^{\varepsilon}$-Boc-L-lysyl-BLM-disaccharide (28).** To a solution of 5.0 mg (10 μmol) of compound **20**[2], 4.5 mg (7.4 μmol) of dipeptide **27** and 6.4 mg (30 μmol) of 1,8-bis(dimethylamino)naphthalene (proton sponge) in 0.2 mL of anhydrous DMF was added 5.6 mg (15 μmol) of HATU. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using 0.1% aq CF_3COOH and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq CF_3COOH–CH_3CN → 45:55 0.1% aq CF_3COOH–CH_3CN) over a period of 30 min at a flow rate of 3 mL/min. The fractions containing the desired product eluted at 29.8 min (monitoring at 220 nm) and were collected, frozen, and lyophilized to afford compound **28** as a colorless solid: yield 6 mg (76%); mass spectrum (ESI), *m/z* 1063.5287 $(M + H)^+$ ($C_{47}H_{79}N_6O_{21}$ requires *m/z* 1063.5293).



***N*$^{\alpha}$-Cy5**-*N*$^{\varepsilon}$-Boc-L-lysyl-*N*$^{\varepsilon}$-Boc-L-lysyl-BLM-disaccharide (29).** Hydrogen was bubbled through a solution of 4.50 mg (4.20 μmol) of compound **28** in 2 mL of methanol for 10 min, and the reaction mixture was stirred for 30 min under a hydrogen atmosphere. The reaction mixture was filtered and the filtrate was concentrated under diminished pressure to obtain the free amine as a colorless solid: crude yield 4.2 mg.

To 0.50 mg (0.54 μmol) of the above crude solid was added a solution of 110 μg (0.11 μmol) of Cy5**COOSu (**14**) in 200 μL of 0.2 M phosphate buffer and the reaction mixture was stirred overnight in the dark. The crude reaction mixture was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using 0.1% aq CF_3COOH and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq CF_3COOH–CH_3CN → 45:55 0.1% aq CF_3COOH–CH_3CN) over a period of 30 min at a flow rate of 3 mL/min. The fractions containing the desired product eluted at 20.2 min (monitoring at 651 nm) and were collected, combined, frozen and lyophilized to give the dye conjugate **29** as a blue solid: yield 50.0 μg (27%); mass spectrum (ESI), *m/z* 897.3375 $(M - 3H)^{2-}$ ($C_{77}H_{118}N_8O_{32}S_4$ requires *m/z* 897.3373).



***N*$^{\alpha}$-Cy5**-L-lysyl-L-lysyl-BLM disaccharide (5).** A solution containing 32 μg (18 nmol) of **29** in 200 μL of 60% aq CF_3COOH, was shaken at room temperature for 40 min and was purified on an Econosil C_{18} reversed phase semi-preparative (250 × 10 mm, 10 μm) HPLC column using 0.1% aq CF_3COOH and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq CF_3COOH–CH_3CN → 45:55 0.1% aq CF_3COOH–CH_3CN) over a period of 30 min at a flow rate of 3 mL/min. The fractions containing the desired product eluted at 16.1 min (monitoring at 651 nm) and were collected, frozen, and lyophilized to afford the dye conjugate **5** as a blue solid: yield 19 μg (67%); mass spectrum (ESI), *m/z* 797.2850 $(M - 3H)^{2-}$ ($C_{67}H_{102}N_8O_{28}S_4$ requires *m/z* 797.2850).

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