Supporting Information

for

Modified Bleomycin Disaccharides Exhibiting Improved Tumor Cell Targeting

Manikandadas M. Madathil, Chandrabali Bhattacharya, Zhiqiang Yu, Rakesh Paul, Michael J. Rishel, and Sidney M. Hecht

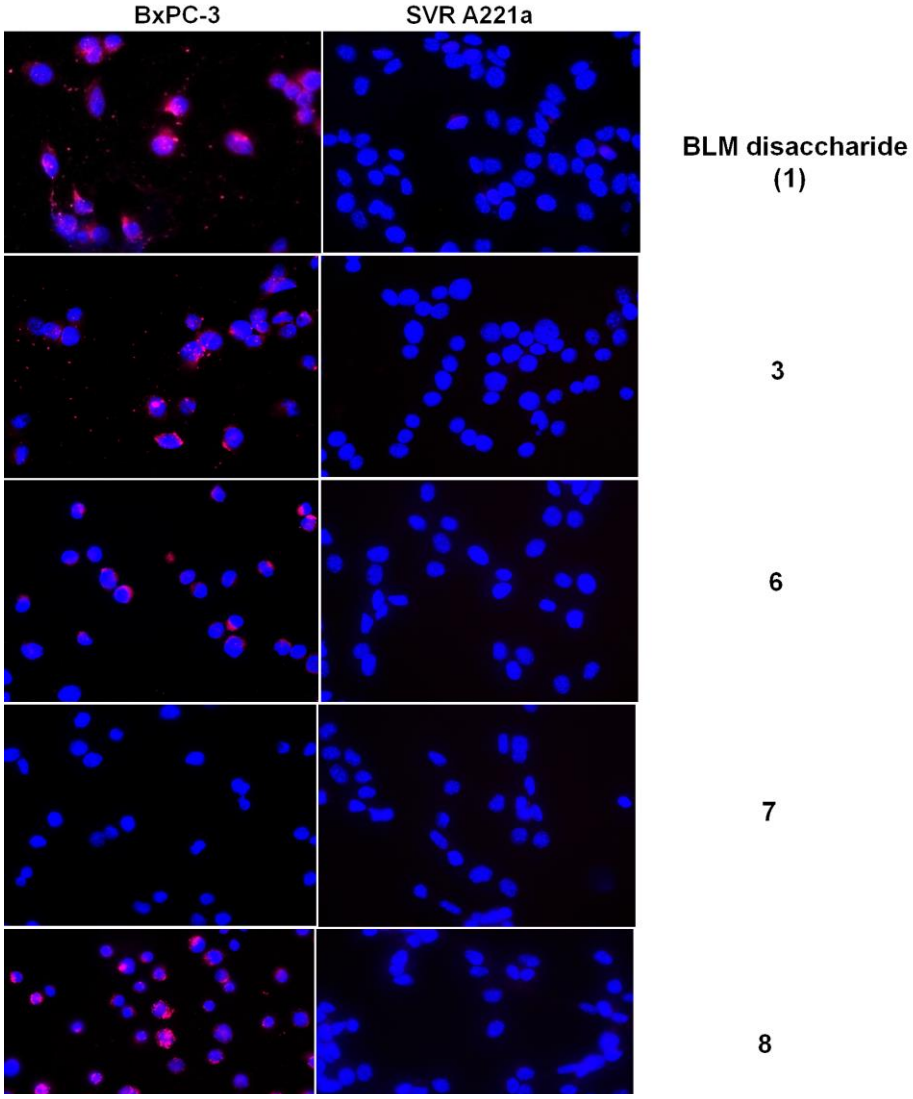


Figure S1. Comparison of binding/uptake of disaccharide-Cy5** conjugates **1**, **3**, **6**, **7** and **8** in BxPC-3 and SVR A221a cell lines. The cells were treated with 25 μM disaccharide-Cy5** conjugates at 37 °C for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. The cell nuclei were stained with 2-(4-amidinophenyl)-6-indolecarbamidine (DAPI). Fluorescence imaging was carried out with a 2 s exposure time.

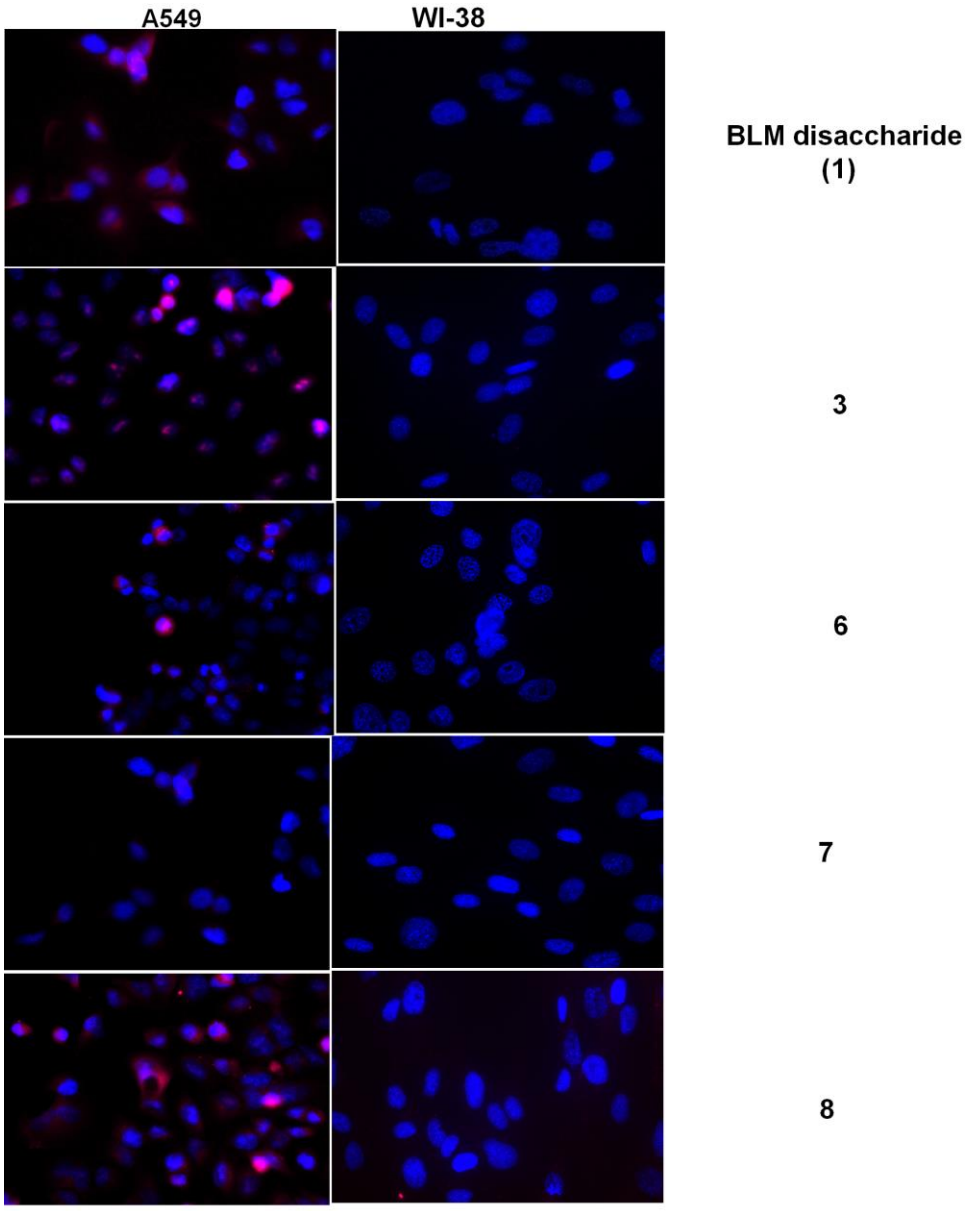


Figure S2. Comparison of binding/uptake of disaccharide-Cy5** conjugates **1**, **3**, **6**, **7** and **8** in A549 and WI-38 cell lines. The cells were treated with 25 μM disaccharide-Cy5** conjugates at 37 °C for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. The cell nuclei were stained with 2-(4-amidinophenyl)-6-indolecarbamidine (DAPI). Fluorescence imaging was carried out with a 2 s exposure time.

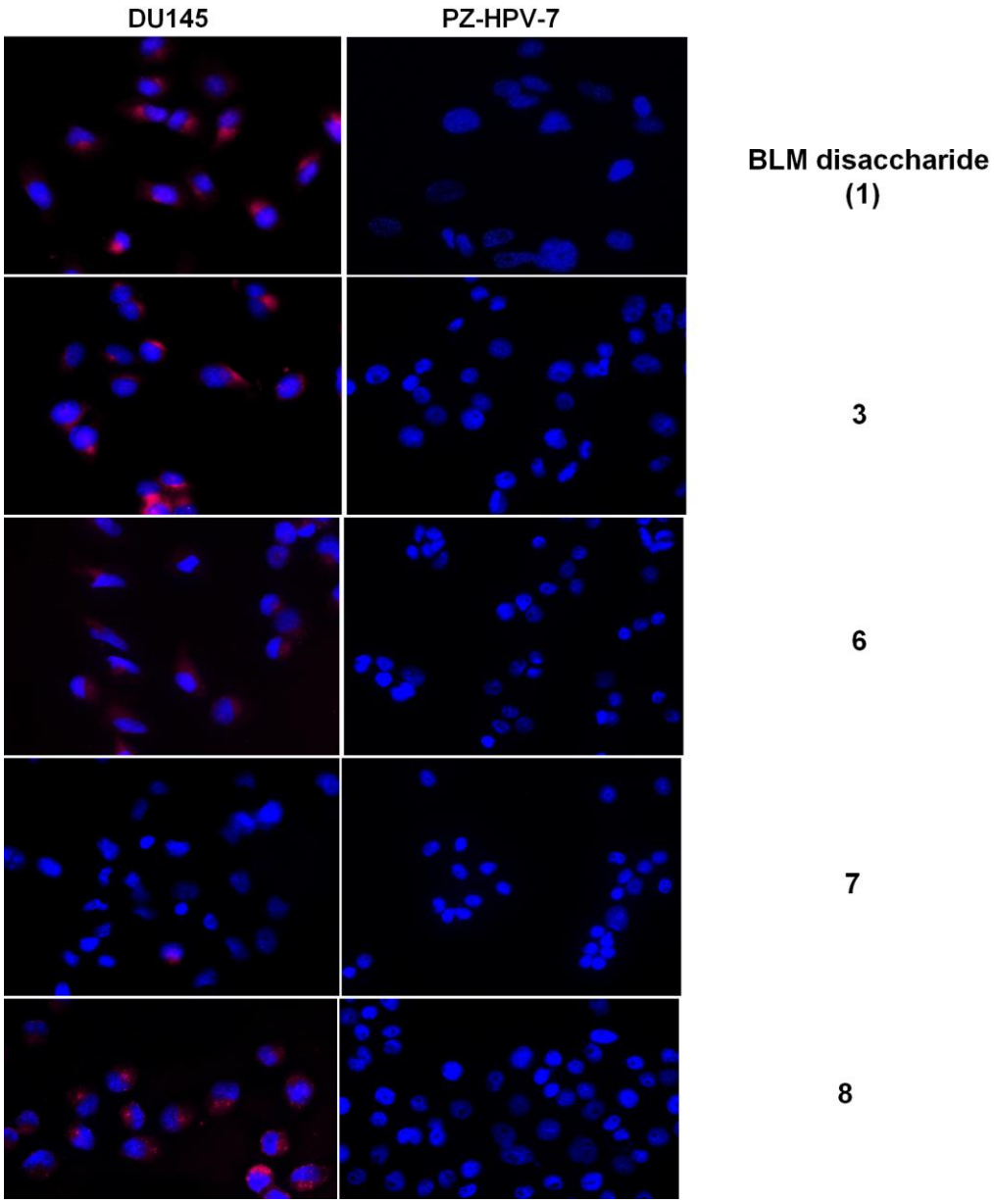


Figure S3. Comparison of binding/uptake of disaccharide-Cy5** conjugates **1**, **3**, **6**, **7** and **8** in DU-145 and PZ-HPV-7 cell lines. The cells were treated with 25 μM disaccharide-Cy5** conjugates at 37 °C for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. The cell nuclei were stained with 2-(4-amidinophenyl)-6-indolecarbamidine (DAPI). Fluorescence imaging was carried out with a 2 s exposure time.

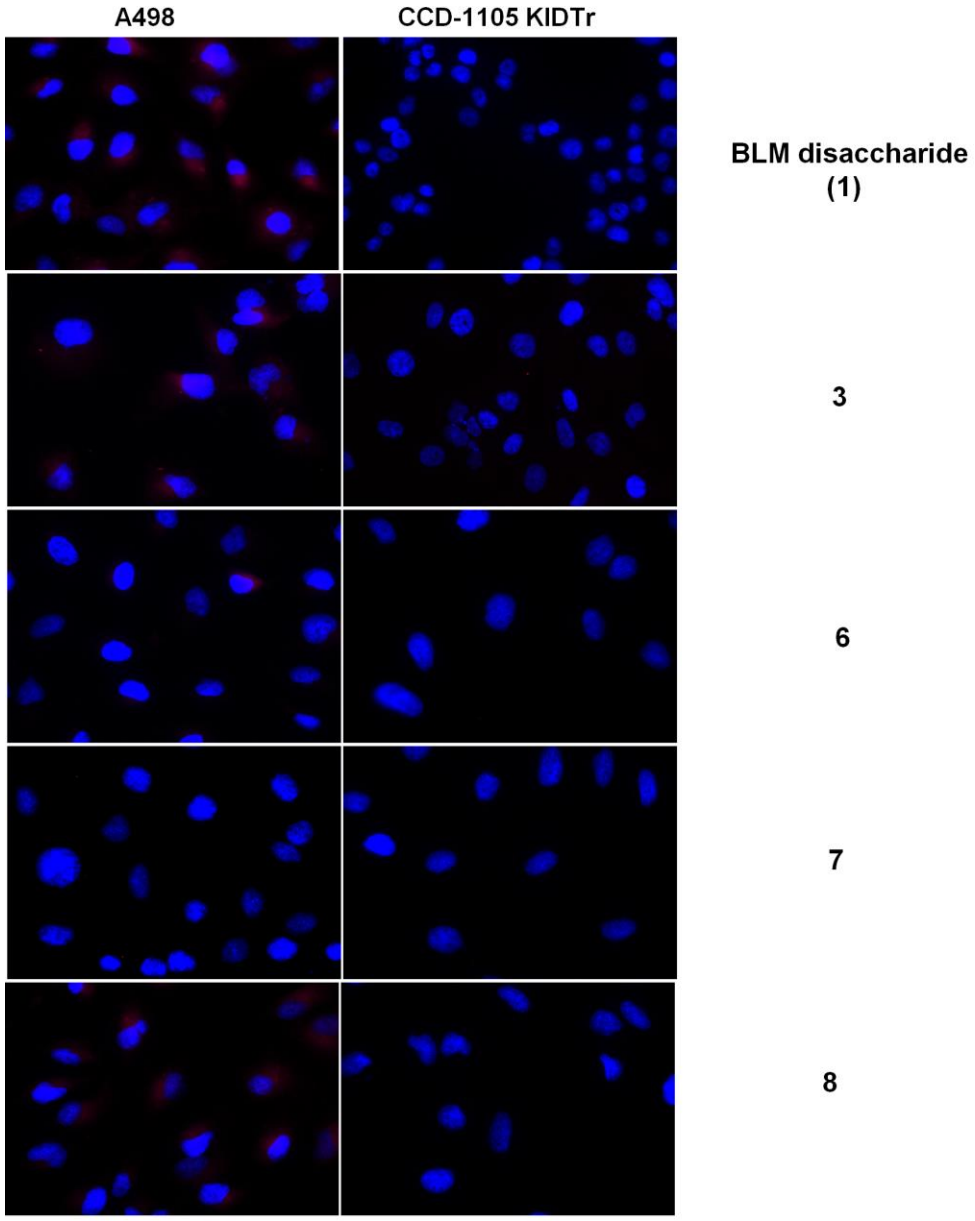
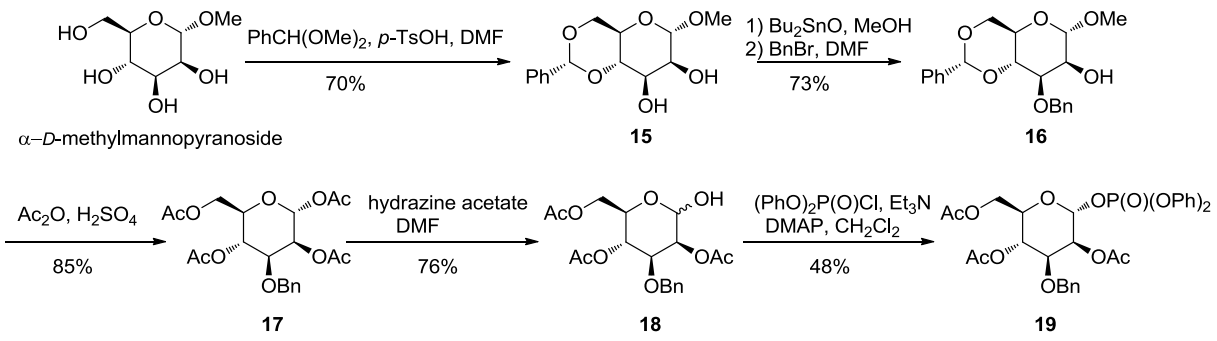


Figure S4. Comparison of binding/uptake of disaccharide-Cy5** conjugates **1**, **3**, **6**, **7** and **8** in A498 and CCD 1105 KIDTr cell lines. The cells were treated with 25 μM disaccharide-Cy5** library conjugates at 37 °C for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. The cell nuclei were stained with 2-(4-amidinophenyl)-6-indolecarbamidine (DAPI). Fluorescence imaging was carried out with a 2 s exposure time.

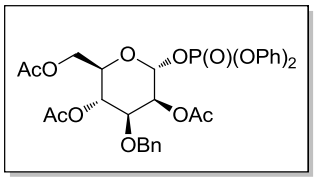
EXPERIMENTAL PROCEDURES

General Methods. The chemicals were all ACS reagent grade and were used without further purification. Reactions were carried out under an argon atmosphere unless otherwise specified. Flash column chromatography was carried out using silica gel (Silicycle R10030B, 60 particle size, 230-400 mesh), applying a low pressure stream of nitrogen. Analytical thin layer chromatographic separations were carried out on glass plates coated with silica gel (60 particle size F254, SiliCycle TLG-R10011B-323). TLC chromatograms were developed by immersing the plates in ceric ammonium molybdate stain, or else visualized by UV irradiation (254 nm). Melting points were recorded on a MelTemp apparatus and are uncorrected. Tetrahydrofuran was distilled from sodium/benzophenone ketyl and dichloromethane was distilled from calcium hydride. ^{1}H and ^{13}C NMR spectra were recorded on a Gemini 300 or Varian Inova 400, or on a Varian Inova 500 spectrometer, using $CDCl_3$ as solvent and internal standard, unless otherwise indicated. ^{1}H NMR chemical shifts were reported relative to residual $CDCl_3$ 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; quin, quintet. Cyanine dyes were prepared at General Electric. High resolution mass spectrometric data was obtained at the Michigan State Mass Spectrometry Facility or at the Arizona State University CLAS High Resolution Mass Spectrometry Facility.

Scheme S1. Synthesis of C3 *O*-Benzyl Mannose Glycosyl Donor 19



Compounds **15**, **16** and **17** were synthesized according to published procedures.[1]

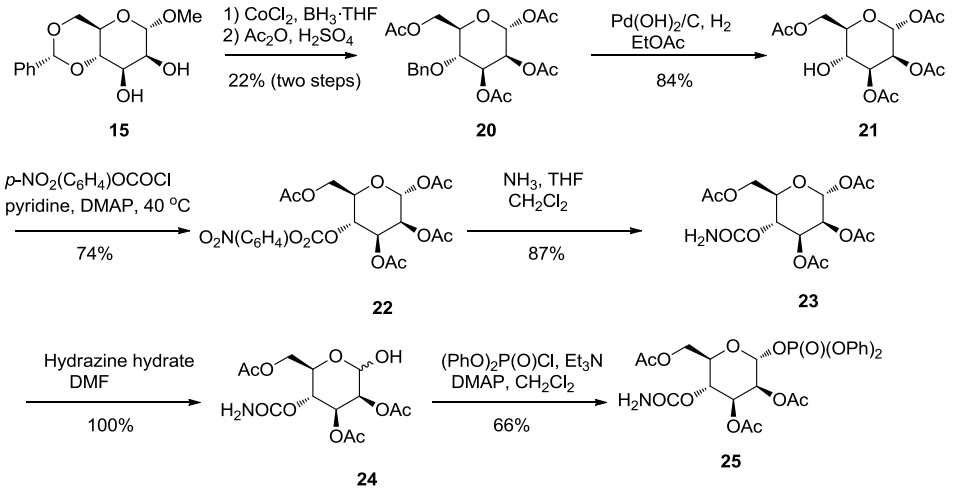


2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl Diphenyl Phosphate (19). To a solution containing 1.40 g (3.19 mmol) of acetate **17** in 25 mL of DMF was added 353 mg (3.83 mmol) of hydrazine acetate. The solution was stirred at room temperature for 1.5 h and quenched by the addition of 100 mL of ethyl acetate. The organic phase was washed with three 50-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded monosaccharide **18** as a colorless oil. This material was used immediately in the next reaction: yield 968 mg (76%); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.02 (s, 3H), 2.10 (s, 3H), 3.90 (dd, 1H, *J* = 9.70 and 3.30 Hz), 4.00-4.11 (m, 2H), 4.16 (ddd, 1H, *J* = 12.3, 7.7 and 4.6 Hz), 4.33 (s, 1H), 4.38 (dd, 1H, *J* = 12.3 and 4.3 Hz), 4.60 (d, 1H, *J* = 12.2 Hz), 5.13-5.23 (m, 2H), 5.28-5.33 (m, 1H) and 7.18-7.31 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.2,

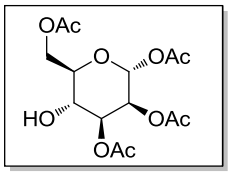
20.78, 20.85, 21.0, 60.6, 62.9, 67.5, 68.5, 68.8, 71.4, 74.0, 92.3, 127.78, 127.83, 128.4, 137.7, 169.9, 170.6 and 171.1.

To a stirred solution containing 968 mg (2.44 mmol) of pyranoside **18** in 144 mL of anh dichloromethane was added 372 mg (3.05 mmol) of DMAP, 3.67 mL (2.66 g, 26.3 mmol) of Et_3N and 4.83 mL (6.26 g, 23.4 mmol) of diphenylphosphoryl chloride. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 300 mL of ethyl acetate and 150 mL of satd aq $NaHCO_3$. The organic layer was washed with three 50-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded **19** as a colorless oil: yield 737 mg (48%); silica gel TLC R_f 0.38 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.93 (s, 3H), 1.97 (s, 3H), 2.10 (s, 3H), 3.84 (dd, 1H, *J* = 9.70 and 3.30 Hz,), 3.89-4.03 (m, 2H), 4.10-4.20 (m, 1H), 4.33 (d, 1H, *J* = 12.1 Hz), 4.57 (d, 1H, *J* = 12.1 Hz), 5.27 (t, 1H, *J* = 10 Hz), 5.38 (dd, 1H, *J* = 8.6 and 6.2 Hz), 5.91 (dd, 1H, *J* = 6.4 and 1.6 Hz) and 7.16-7.38 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.62, 20.67, 61.8, 66.2, 67.2, 67.3, 70.9, 71.5, 73.4, 77.4, 96.47, 96.53, 119.90, 119.95, 125.66, 125.67, 125.71, 125.72, 127.7, 127.9, 128.3, 129.85, 129.89, 137.2, 150.09, 150.13, 150.16, 169.3, 169.6 and 170.4; mass spectrum (APCI), *m/z* 629.1770 $(M + H)^+$ ($C_{31}H_{34}O_{12}P$ requires 629.1788).

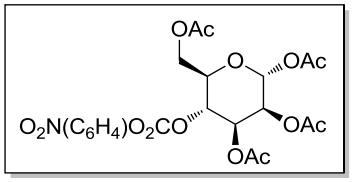
Scheme S2. Synthesis of C4 Mannose Glycosyl Donor 25



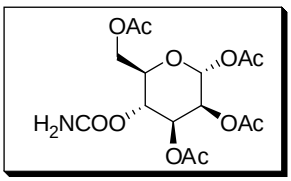
Compound **20** was synthesized according to published procedures.[2,3]



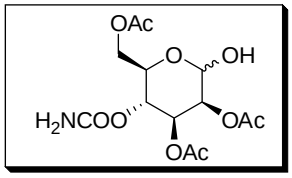
1,2,3,6-Tetra-*O*-acetyl-α-D-mannopyranoside (21).[4] To a solution of 1.63 g (3.72 mmol) of **20** in 33 mL of ethyl acetate was added 308 mg of $Pd(OH)_2/C$ and the reaction was stirred under 1 atm of H_2 overnight. The catalyst was removed by filtration through a pad of Celite and the filtrate was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 × 5 cm). Elution with 2:1 hexanes–ethyl acetate afforded **21** as a colorless oil: yield 1.09 g (84%); silica gel TLC R_f 0.25 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H), 2.12 (s, 6H), 2.14 (s, 3H), 2.94 (br s, 1H), 3.83-3.92 (m, 2H), 4.24-4.27 (m, 1H), 4.50-4.54 (m, 1H), 5.18-5.23 (m, 2H) and 6.04 (d, 1H, J = 1.6 Hz).



1,2,3,6-Tetra-*O*-acetyl-4-*O*-(*p*-nitrophenyloxy)carbonyloxy-α-D-mannopyranoside (22). To a solution of 1.74 g (5.00 mmol) of **21** in 17.8 mL of pyridine was added 2.44 g (20.0 mmol) of DMAP and 4.03 g (20.0 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 ^{o}C for 2.5 h at which time it was poured into a two-phase mixture of 50 mL of ethyl acetate and 19 mL of water. The organic layer was washed successively with three 25-mL portions of 1 N HCl, 25 mL of satd aq $NaHCO_3$ and 25 mL of brine. The solution was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (28 × 5 cm). Elution with 1:2 ethyl acetate–hexanes afforded **22** as a colorless foam: yield 1.91 g (74%); silica gel TLC R_f 0.21 (2:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3H), 2.11 (s, 3H), 2.18 (s, 3H), 2.19 (s, 3H), 4.15-4.20 (m, 2H), 4.53-4.58 (m, 1H), 5.23 (t, 1H, *J* = 9.9 Hz), 5.32-5.33 (m, 1H), 5.45 (dd, 1H, *J* = 10.1, 3.5 Hz), 6.12 (d, 1H, *J* = 1.9 Hz), 7.38 (d, 2H, *J* = 9.2 Hz) and 8.29 (d, 2H, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 20.66, 20.69, 20.8, 61.6, 68.3, 68.6, 70.1, 70.9, 90.4, 121.6, 125.4, 145.7, 151.7, 155.1, 167.9, 169.5, 169.8 and 170.6; mass spectrum (ESI), *m*/*z* 531.1472 $(M + NH_4)^+$ ($C_{21}H_{27}N_2O_{14}$ requires *m*/*z* 531.1462).

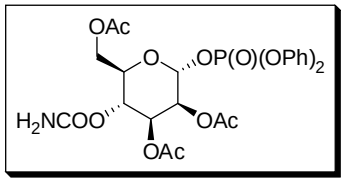


1,2,3,6-Tetra-*O*-acetyl-4-*O*-(carbamoyloxy)-α-D-mannopyranoside (23). To a solution of 2.02 g (3.93 mmol) of **22** in 107 mL of dichloromethane was added a solution of 37.0 mL of anh THF that had been saturated with NH_3 at 0 °C. The reaction mixture was allowed to warm to room temperature and then stirred at room temperature overnight. The solution was concentrated under diminished pressure and the residue was purified by flash chromatography on a silica gel column (15 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded **23** as a colorless foam: yield 1.22 g (87%); silica gel TLC R_f 0.12 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.09 (s, 3H), 2.16 (s, 3H), 2.17 (s, 3H), 4.00-4.05 (m, 1H), 4.15-4.19 (m, 1H), 4.26-4.31 (m, 1H), 4.73 (br s, 2H), 5.19 (t, 1H, J = 10.1 Hz), 5.24-5.25 (m, 1H), 5.34-5.37 (m, 1H) and 6.07 (d, 1H, J = 1.9 Hz); ^{13}C NMR ($CDCl_3$) δ 20.68, 20.72, 20.76, 20.85, 62.3, 66.7, 68.4, 68.6, 70.7, 90.6, 154.9, 168.0, 169.8, 170.1 and 170.7; mass spectrum (APCI), m/z 392.1203 $(M + H)^+$ ($C_{15}H_{22}NO_{11}$ requires m/z 392.1193).



2,3,6-Tri-*O*-acetyl-4-*O*-(carbamoyloxy)-α-D-mannopyranoside (24). To a solution containing 553 mg (1.41 mmol) of **23** in 9.20 mL of anh DMF was added 182 mg (1.98 mmol) of hydrazine acetate. The solution was stirred at 25 °C for 2 h and then treated with 120 mL of ethyl acetate. The organic solution was washed successively with 120 mL of water, 120 mL of satd aq. $NaHCO_3$, 120 mL of brine, and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford **24** as a colorless foam:

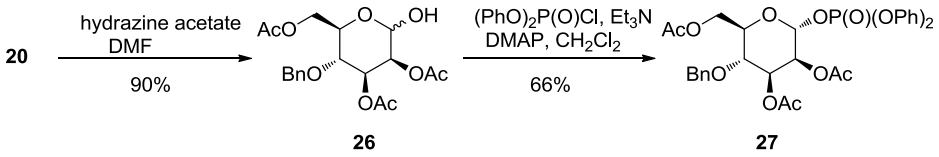
yield 501 mg (quant.); silica gel TLC R_f 0.28 (1:3 hexanes–ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H), 2.08 (s, 3H), 2.14 (s, 3H), 4.17-4.24 (m, 3H), 4.58-4.64 (br s, 1H), 5.10 (t, 1H, *J* = 9.6 Hz), 5.07-5.15 (br s, 2H), 5.20-5.22 (m, 2H) and 5.37-5.41 (m, 1H); mass spectrum (ESI), m/z 348.0932 $(M - H)^-$ ($C_{13}H_{18}NO_{10}$ requires *m*/*z* 348.0931).

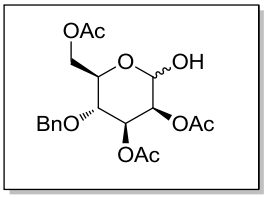


2,3,6-Tri-*O*-acetyl-4-*O*-(carbamoyloxy)-α-D-mannopyranosyl Diphenyl Phosphate (25). To a solution of 496 mg (1.42 mmol) of **24** in 29.0 mL of dichloromethane at 0 °C was added 217 mg (1.78 mmol) of DMAP, 2.10 mL (15.0 mmol) of Et_3N, and 2.80 mL (13.6 mmol) of diphenylphosphoryl chloride under an argon atmosphere. The reaction mixture was stirred for 1.5 h and the solution was poured into a two-phase mixture of 43 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The organic layer was washed with two 20-mL portions of brine, dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (20 × 3 cm). Elution with 2:3 hexanes–ethyl acetate afforded **25** as a colorless oil: yield 460 mg (56%); silica gel TLC R_f 0.33 (1:3 hexanes–ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H), 2.12 (s, 3H), 2.24 (s, 3H), 4.15-4.19 (m, 1H), 4.28-4.32 (m, 1H), 4.37-4.41 (m, 1H), 4.80 (s, 1H), 4.82-4.90 (br s, 2H), 5.21-5.30 (m, 1H), 5.41-5.50 (m, 1H), 5.95-5.97 (m, 1H), 7.24-7.36 (m, 6H) and 7.44-7.48 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 20.56, 20.62, 20.7, 61.9, 66.3, 68.0, 68.7, 68.8, 70.8, 96.0, 120.01, 120.05, 120.16,

120.21, 125.7, 125.85, 125.86, 129.93, 129.99, 169.6, 169.9, 170.6; mass spectrum (APCI), *m*/*z* 582.1387 $(M + H)^+$ ($C_{25}H_{29}NO_{13}P$ requires *m*/*z* 582.1377).

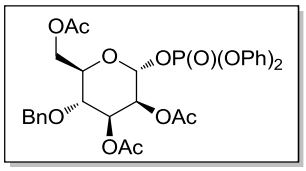
Scheme S3. Synthesis of C4 *O*-Benzyl Mannose Glycosyl Donor 27





2,3,6-Tri-*O*-acetyl-4-*O*-benzyl-α,β-D-mannopyranose (26). To a stirred solution containing 1.09 g (2.49 mmol) of acetate **18** in 20 mL of anh DMF was added 274 mg (2.98 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 100 mL of ethyl acetate. The organic layer was washed with three 50-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded pyranoside **26** as a colorless oil: yield 884 mg (90%); silica gel TLC R_f 0.36 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 2.01 (s, 3H), 2.08 (s, 3H), 3.77 (t, 1H, *J* = 10 Hz), 4.11 (ddd, 1H, *J* = 9.7, 4.1 and 2.1 Hz), 4.17-4.34 (m, 2H), 4.69-4.48 (m, 3H), 5.09 (s, 1H), 5.17-5.23 (m, 1H), 5.33-5.38 (m, 1H) and 7.18-7.32 (m, 5H); ^{13}C

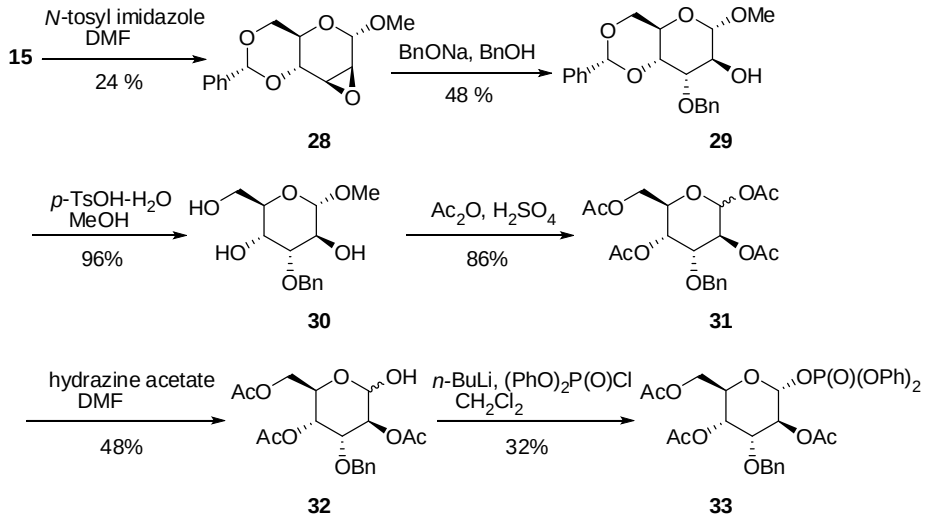
NMR ($CDCl_3$) δ 20.69, 20.73, 63.1, 69.2, 70.5, 71.5, 72.8, 74.6, 77.4, 91.8, 127.6, 127.8, 128.3, 137.5, 170.0, 170.2 and 171.0; mass spectrum (APCI), *m/z* 397.1483 $(M + H)^+$ ($C_{19}H_{25}O_9$ requires *m/z* 397.1498).



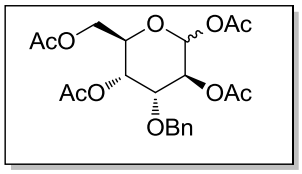
2,3,6-Tri-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl Diphenyl Phosphate (27). To a stirred solution containing 812 mg (2.05 mmol) of **26** in 80 mL of anh dichloromethane was added 313 mg (2.56 mmol) of DMAP and 3.10 mL (2.25 g, 22.1 mmol) of Et_3N, as well as 4.10 mL (5.33 g, 19.7 mmol) of diphenylphosphoryl chloride. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 300 mL of ethyl acetate and 150 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 50-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded **27** as a colorless oil: yield 857mg (66%); silica gel TLC R_f 0.29 (1:1 ethyl acetate–hexanes); ^1H NMR ($CDCl_3$) δ 1.93 (s, 3H), 1.96 (s, 3H), 2.09 (s, 3H), 3.80 (t, 1H, *J* = 9.6 Hz), 3.91-4.12 (m, 2H), 4.18 (dd, 1H, *J* = 12.2 and 4.2 Hz), 4.50-4.68 (m, 2H), 5.27-5.38 (m, 2H), 5.80 (d, 1H, *J* = 6.1 Hz) and 7.11-7.38 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 62.1, 68.9, 70.7, 71.6, 71.9, 74.8, 77.4, 96.1, 119.9, 120.1, 125.6, 127.7, 128.0, 128.4, 129.8, 137.2, 149.9, 150.1, 169.3, 169.4

and 170.3; mass spectrum (APCI), *m/z* 629.1794 $(M + H)^+$ ($C_{31}H_{34}O_{12}P$ requires *m/z* 629.1788).

Scheme S4. Synthesis of C3 *O*-Benzyl Altrose Glycosyl Donor 33

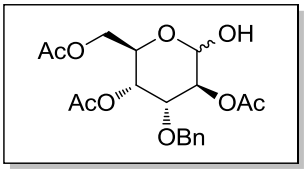


Compounds **28**[5,6], **29**[7] and **30**[7] were synthesized according to literature procedures.



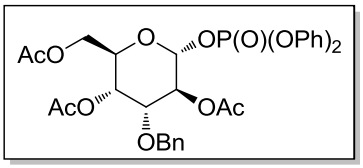
1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-α,β-D-altropyranoside (31). To a solution containing 532 mg (1.87 mmol) of methyl pyranoside **30** in 13 mL of Ac_2O was added a catalytic amount of H_2SO_4. The solution was stirred overnight at room temperature. The reaction mixture was then poured into a stirred mixture of 120 mL of ethyl acetate and 80 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic

layer was washed with brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded product **31** as a 3:2 mixture of α and β anomers as determined by ^{1}H NMR: yield 705 mg (86%); silica gel TLC R_f 0.55 (1:1 ethyl acetate–hexanes); α anomer: ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H), 2.06-2.09 (m, 6H), 2.14 (s, 3H), 3.96 (t, 1H, *J* = 3.2 Hz), 4.11-4.16 (m, 1H), 4.24-4.37 (m, 2H), 4.55-4.75 (m, 2H), 5.03-5.09 (m, 1H), 5.29 (s, 1H), 5.99 (d, 1H, *J* = 11.3 Hz) and 7.27-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.91, 20.92, 21.04, 21.05, 62.6, 66.3, 66.6, 68.0, 72.46, 72.49, 91.4, 127.8, 128.1, 128.5, 137.5, 169.0, 169.7, 169.8 and 170.9; mass spectrum (APCI), *m/z* 379.1387 $(M - CH_3COO)^+$ ($C_{19}H_{23}O_8$ requires *m/z* 379.1393).



2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α,β-D-altropyranoside (32). To a solution containing 1.93 g (4.40 mmol) of monosaccharide **31** in 35 mL of anh DMF was added 486 mg (5.28 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and then quenched by the addition of 100 mL of ethyl acetate. The organic layer was washed with three 50-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded **32** as a colorless oil: yield 837 mg (48%); silica gel TLC R_f 0.31 (1:1 ethyl acetate–hexanes). The product, isolated as a mixture of anomers, was analyzed by ^{1}H

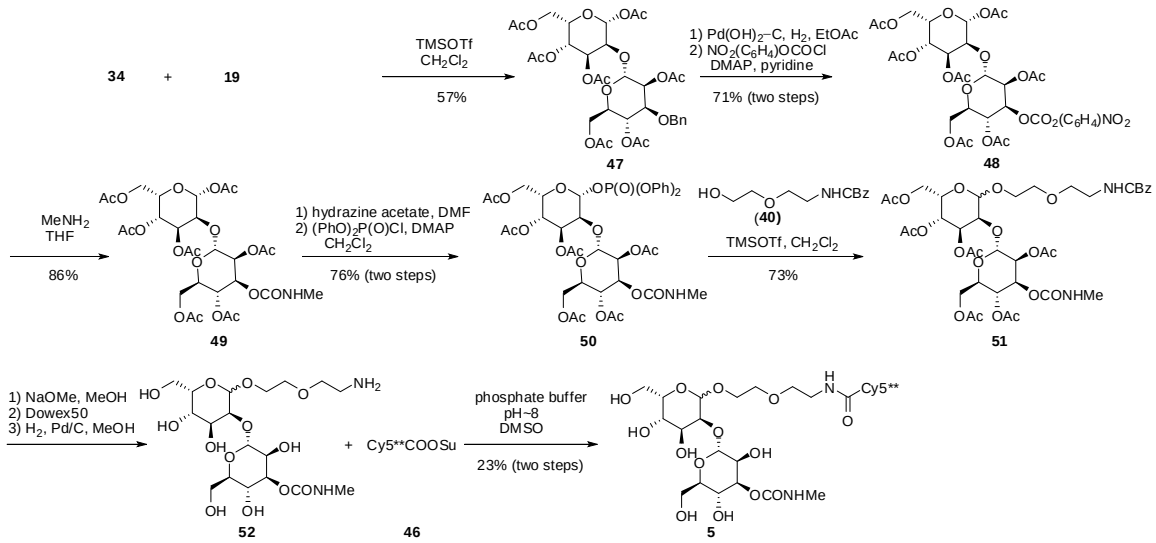
NMR; ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 1.96 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 3.73-3.95 (br s, 1H), 3.98-4.05 (m, 1H), 4.09 (d, 1H, *J* = 8.6 Hz), 4.12-4.27 (m, 4H), 4.32 (dt, 1H, *J* = 14.2 and 7.1 Hz), 4.36-4.46 (m, 1H), 4.54-4.75 (m, 4H), 4.89-4.94 (m, 2H), 4.96-5.08 (m, 4H), 5.24 (t, 1H, *J* = 12.1 Hz) and 7.41-7.27 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.80, 20.82, 20.86, 20.98, 21.02, 62.9, 63.2, 64.1, 66.2, 66.9, 68.3, 70.0, 70.3, 72.9, 73.3, 73.8, 74.2, 91.6, 92.8, 128.1, 128.2, 128.4, 128.5, 128.7, 128.8, 136.2, 137.3, 169.73, 169.78, 169.83, 170.4, 170.95 and 170.96; mass spectrum (APCI), *m/z* 379.1394 $(M - OH)^+$ ($C_{19}H_{23}O_8$ requires *m/z* 379.1393).

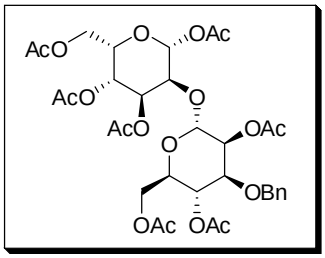


2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α-D-altropyranosyl Diphenyl Phosphate (33). To a stirred solution containing 637 mg (1.61 mmol) of pyranoside **32** in 2.7 mL of anh dichloromethane was added 1.21 mL (1.6 M, 1.93 mmol) of *n*-BuLi solution at −78 °C. The reaction mixture was stirred at this temperature for 10 min and 400 μL (520 mg, 1.93 mmol) of diphenylphosphoryl chloride was added dropwise. The reaction mixture was stirred at −78 °C for an additional 10 min and poured into a mixture of 20 mL of ethyl acetate and 10 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded phosphate ester **33** as a colorless oil: yield 324 mg

(32%); 121 mg of unreacted starting material was also recovered; silica gel TLC R_f 0.40 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 1.98 (s, 3H), 2.00 (d, 3H, *J* = 2.1 Hz), 3.99 (dd, 1H, *J* = 6.3 and 3.1 Hz), 4.05-4.28 (m, 3H), 4.50-4.62 (m, 2H), 5.13 (dd, 1H, *J* = 7.0 and 3.2 Hz), 5.19 (dd, 1H, *J* = 6.4 and 2.2 Hz), 5.96 (dd, 1H, *J* = 7.1 and 2.2 Hz) and 7.12-7.36 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.74, 20.76, 20.9, 62.8, 66.9, 68.20, 68.28, 71.6, 72.94, 72.97, 95.5, 120.30, 120.35, 125.7, 128.0, 128.2, 128.5, 129.8, 129.9, 137.1, 150.2, 150.4, 169.9 and 170.6; mass spectrum (APCI), *m/z* 569.1598 (M – $CH_3COO)^+$ ($C_{29}H_{30}O_{10}P$ requires *m/z* 569.1576).

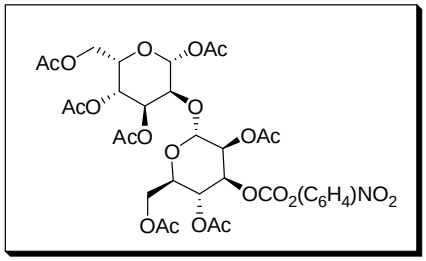
Scheme S5. Synthesis of C3 Modified Mannose Disaccharide–Dye Conjugate 5





1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl)-β-L-gulopyranose (47). To a stirred solution containing 340 mg (0.98 mmol) of gulose acceptor **34** and 737 mg (1.17 mmol) of mannose donor **19** in 7.0 mL of anh dichloromethane cooled to 0 °C was added 352 µL (526 mg, 1.95 mmol) of TMSOTf at 0 °C. The reaction mixture was stirred for 10 min at which time it was poured into a mixture of 30 mL of ethyl acetate and 30 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with two 20-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded disaccharide **47** as a colorless oil: yield 407 mg (57%); silica gel TLC R_f 0.31 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 2.00-2.01 (m, 6H, *J* = 2.8 Hz,), 2.04 (s, 3H, *J* = 5.3 Hz), 2.08 (d, 6H, *J* = 1.9 Hz), 2.12 (s, 3H), 3.61 (ddd, 1H, *J* = 12.7, 9.6 and 3.3 Hz), 3.84-3.95 (m, 2H), 3.96-4.20 (m, 4H), 4.26-4.37 (m, 2H), 4.59 (t, 1H, *J* = 10.4 Hz,), 4.90-5.18 (m, 4H), 5.39 (dd, 1H, *J* = 11.1 and 3.3 Hz), 5.86 (d, 1H, *J* = 8.3 Hz) and 7.24 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.56, 20.59, 20.61, 20.64, 20.65, 20.8, 61.4, 62.3, 65.5, 66.9, 67.2, 67.5, 69.4, 71.2, 73.8, 90.5, 95.1, 127.6, 127.7, 127.9, 128.27, 137.4, 168.7, 168.8, 169.1, 169.4,

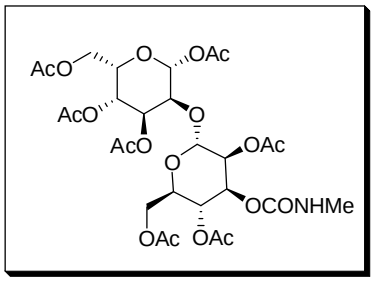
169.6, 170.3 and 170.4; mass spectrum (APCI), *m/z* 727.2444 $(M + H)^+$ ($C_{33}H_{43}O_{18}$ requires 727.2450).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-((*p*-nitrophenyloxy)carbonyloxy)-α-D-mannopyranosyl)-β-L-gulopyranose (48). To a solution containing 470 mg (0.56 mmol) of disaccharide **47** in 40 mL of ethyl acetate was added a catalytic amount of $Pd(OH)_2/C$ and the reaction mixture was stirred overnight under 1 atm of H_2. The solvent was filtered through a pad of Celite and the filtrate was concentrated under diminished pressure to afford a crude residue. The residue was used directly in the next reaction; silica gel TLC R_f 0.16 (1:2 ethyl acetate–hexanes); mass spectrum (APCI), *m/z* 637.1993 $(M + H)^+$ ($C_{26}H_{37}O_{18}$ requires 637.1980).

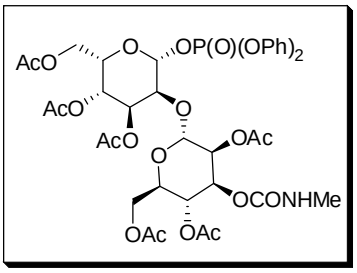
To a solution containing 338 mg (0.53 mmol) of the crude residue in 2 mL of pyridine was added 259 mg (2.12 mmol) of DMAP and 471 mg (2.12 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 °C overnight at which time it was poured into a mixture of 30 mL of ethyl acetate and 10 mL of distilled water. The organic and aqueous layers were separated and the organic layer was washed successively with three 10-mL portions of 1 N HCl and 10 mL of satd aq $NaHCO_3$. The organic layer was then washed with brine and dried ($MgSO_4$). The solvent was concentrated under

diminished pressure to afford a crude residue. The residue applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded the ester **48** as a colorless foam: yield 320 mg (71% over two steps); silica gel TLC R_f 0.24 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.05 (s, 3H), 2.06-2.14 (m, 15H), 3.95 (dd, 1H, *J* = 8.4 and 3.0 Hz), 3.99-4.16 (m, 4H), 4.16-4.27 (m, 2H), 4.30 (dd, 1H, *J* = 15.0 and 8.7 Hz,), 5.21-5.35 (m, 2H), 5.39 (dd, 1H, *J* = 14.8 and 11.5 Hz), 4.91-5.08 (m, 2H), 5.84 (d, 1H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 9.0 Hz) and 8.21 (d, 2H, *J* = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 20.57, 20.63, 20.64, 20.70, 20.71, 20.8, 61.3, 61.9, 65.3, 65.5, 67.6, 67.7, 69.2, 69.8, 71.3, 74.3, 90.5, 94.9, 122.0, 125.3, 145.6, 151.4, 155.2, 168.6, 169.2, 169.37, 169.41, 169.7, 170.36 and 170.43; mass spectrum (APCI), *m/z* 742.1841 (M-AcOH)$^+$ ($C_{31}H_{36}NO_{20}$ requires 742.1831).



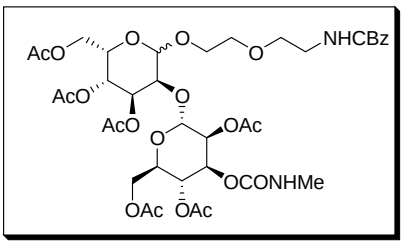
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(*N*-methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (49). To a solution containing 320 mg (0.40 mmol) of disaccharide **48** in 12 mL of THF was added 200 μL (0.40 mmol) of 2 M methylamine in THF at 0 °C. The reaction mixture was stirred at room temperature for 15 h, at which time silica gel TLC analysis indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl

acetate–hexanes afforded disaccharide **49** as a colorless oil: yield 239 mg (86%); silica gel TLC R_f 0.17 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.98 (d, 6H, *J* = 7.5 Hz), 2.03-2.11 (m, 12H), 2.13 (d, 3H, *J* = 8.8 Hz), 2.69 (d, 3H, *J* = 4.2 Hz), 3.88-4.22 (m, 6H), 4.31 (t, 1H, *J* = 6.0 Hz), 4.67 (d, 1H, *J* = 4.1 Hz), 4.89-5.01 (m, 2H), 5.00-5.10 (m, 2H), 5.12-5.20 (m, 1H), 5.38 (s, 1H) and 5.82 (d, 1H, *J* = 8.3 Hz,); ^{13}C NMR ($CDCl_3$) δ 20.66, 20.69, 20.71, 20.79, 27. 6, 61.4, 62.1, 65.4, 66.0, 67.7, 69.17, 69.27, 69.33, 69.38, 71.31, 77.36, 90.6, 94.8, 155.4, 168.6, 169.2, 169.4, 169.8, 170.42 and 170.49; mass spectrum (APCI), *m/z* 694.2206 $(M + H)^+$ ($C_{28}H_{40}NO_{19}$ requires 694.2195).



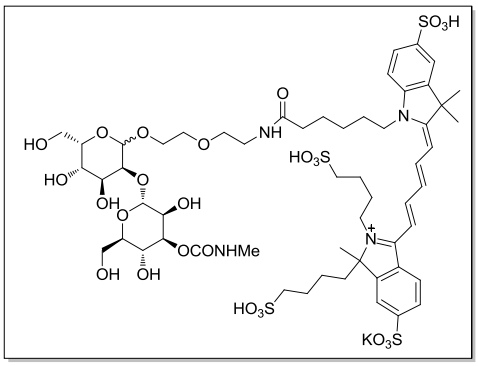
3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(*N*-methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (50). To a solution containing 65.0 mg (0.09 mmol) of disaccharide **49** in 0.8 mL of anh DMF was added 11.0 mg (0.11 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 20 mL of ethyl acetate. The organic layer was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue which was used directly in the next reaction; mass spectrum (APCI), *m/z* 652.2086 $(M + H)^+$ ($C_{26}H_{38}NO_{18}$ requires 652.2089).

To a stirred solution containing 43.0 mg (0.07 mmol) of the crude residue in 4.0 mL of anh dichloromethane was added 10.0 mg (0.08 mmol) of DMAP and 100 μL (72.0 mg, 0.71 mmol) of Et_3N and 131 μL (170 mg, 0.06 mmol) of diphenylphosphoryl chloride. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed successively with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded phosphate ester **50** as a colorless oil: yield 44 mg (76% over two steps); silica gel TLC R_f 0.25 (3:1 ethyl acetate–hexanes); ^1H NMR ($CDCl_3$) δ 1.70 (s, 3H), 1.98 (s, 3H), 2.06 (s, 3H), 2.12 (d, 6H, *J* = 11.4 Hz), 2.21 (s, 3H), 2.75 (d, 3H, *J* = 4.5 Hz), 3.93-4.22 (m, 5H), 4.25-4.40 (m, 2H), 4.56 (d, 1H, *J* = 4.6 Hz), 4.93-5.05 (m, 2H), 5.12-5.24 (m, 2H), 5.29 (s, 1H), 5.44 (s, 1H), 5.65-5.73 (m, 1H) and 7.13-7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.9, 27.7, 36.7, 61.3, 62.0, 65.7, 67.5, 69.2, 69.4, 69.7, 71.2, 71.3, 71.7, 95.6, 96.29, 96.34, 120.36, 120.41, 125.7, 125.8, 129.7, 130.0, 150.2, 150.3, 150.4, 150.5, 155.3, 169.36, 169.42, 169.49, 169.9, 170.5 and 170.7; mass spectrum (APCI), *m/z* 884.2369 $(M + H)^+$ ($C_{38}H_{47}O_{21}PN$ requires 884.2378).



2-[2-(Benzyloxycarbonylamino)ethyloxy]ethyl 3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(*N*-methylcarbamoyl)-α-D-mannopyranosyl)-α,β-L-gulopyranose (51). To a stirred solution containing 44 mg (50 μmol) of the phosphate ester **50** in 0.6 mL of anh dichloromethane was added a solution of 11 mg (40 μmol) of the CBz-protected linker **41** in 0.6 mL of anh dichloromethane at 0 °C. To the cooled reaction mixture was added 16 μL (20 mg, 90 μmol) of TMSOTf and the reaction mixture was stirred at 0 °C for 15 min. The reaction mixture was then poured into a mixture of 10 mL ethyl acetate and 10 mL satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed successively with three 10-mL portions of water and brine, and then dried ($MgSO_4$). The organic layer was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded linker conjugate **51** as a colorless oil. The product was isolated as a (5:3) mixture of anomers: yield 32 mg (73%); silica gel TLC R_f 0.11 (12:12:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) (major anomer) δ 2.03 (s, 3H), 2.05 (s, 3H), 2.06-2.15 (m, 12H), 2.71 (d, 3H, J = 4.8 Hz), 3.40 (s, 1H), 3.51-3.74 (m, 6H), 3.79-3.89 (m, 1H), 3.92-4.01 (m, 1H), 3.99-4.21 (m, 4H), 4.21-4.41 (m, 2H), 4.55-4.63 (m, 2H), 4.89-5.04 (m, 2H), 5.09 (d, 2H, J = 5.6 Hz), 5.12-5.30 (m, 3H), 5.32-5.41 (m, 1H), 5.65-5.73 (m, 1H) and 7.27-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) (mixture of anomers) δ 20.78, 20.83, 20.87, 20.91, 20.93, 20.98, 21.0, 27.67, 27.69, 40.9, 41.1, 53.6, 61.8, 61.9, 62.3, 62.7, 63.9, 65.6, 65.7, 66.1, 66.4, 66.7, 67.9, 68.0, 68.6, 68.8, 69.0, 69.3, 69.5, 69.72, 69.76, 70.0, 70.1, 70.3, 70.4, 70.52, 70.55, 70.7, 72.3, 97.1, 97.2, 120.38, 120.43, 128.2, 128.3, 128.60, 128.65, 129.8, 130.0, 136.8, 155.7, 156.7, 169.33, 169.37, 169.39, 169.47, 169.54, 169.6, 170.0, 170.5, 170.6, 170.7, 170.8

and 170.9; mass spectrum (APCI), *m/z* 873.3150 $(M + H)^+$ ($C_{38}H_{53}N_2O_{21}$ requires 873.3141).

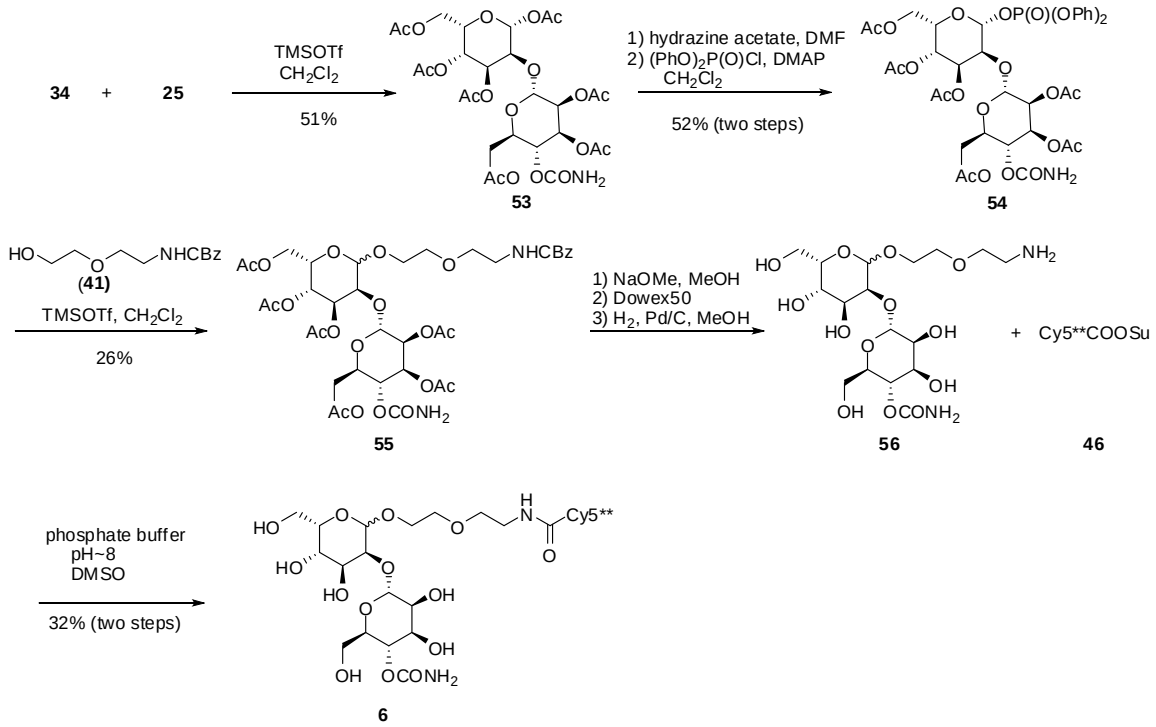


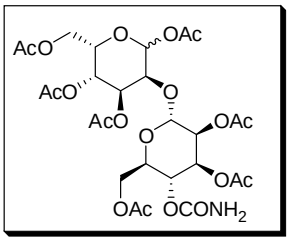
Disaccharide-dye Conjugate 5. To a solution of 5.80 mg (6.60 mmol) of compound **51** in 2 mL of anh methanol was added a freshly prepared solution of 1.0 mL of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectrometric analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was added a catalytic amount of Pd/C and H_2 gas was bubbled through for 1 h. 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 then concentrated under diminished pressure to afford **52**, which was used directly in the next reaction; mass spectrum (APCI), *m/z* 487.2133 $(M + H)^+$ ($C_{18}H_{35}N_2O_{13}$ requires *m/z* 487.2139).

To 87.0 μg (0.18 μmol) of **52** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (**46**) in 150 μL of 0.2 M phosphate buffer, pH 8.0, and the reaction mixture

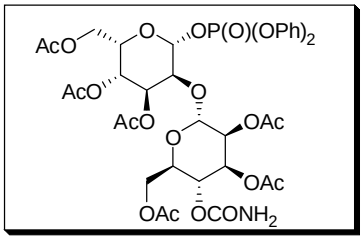
was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 23.9 min and were collected, frozen and lyophilized to give **5** as a blue solid: yield 27 μg (23% over two steps); mass spectrum (APCI), *m/z* 676.1984 $(M - K - 2H)^{2-}$ ($C_{56}H_{80}N_4O_{26}S_4^{2-}$ requires *m/z* 676.1977).

Scheme S6. Synthesis of C4 Mannose Disaccharide–Dye Conjugate 6





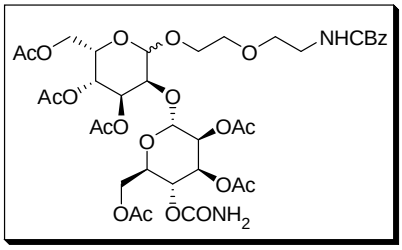
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(carbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (53). To a solution of 460 mg (0.79 mmol) of **34** in 5.1 mL of dichloromethane containing 4Å molecular sieves was added 191 mg (0.33 mmol) of **25** in 4.8 mL of dichloromethane. The solution was cooled to 0 ^{o}C and was then treated with 220 μL (1.22 mmol) of TMSOTf. The reaction mixture was stirred for 20 min at which time it was poured into a two-phase mixture of 70 mL of ethyl acetate and 43 mL of satd aq $NaHCO_3$. The organic layer was separated and washed with two 50-mL portions of brine, then dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (25 × 3 cm). Elution with 3:1 ethyl acetate–hexanes afforded **52** as a colorless oil. The product was isolated as a (5:1) mixture of anomers: yield 275 mg (51%); silica gel TLC R_f 0.26 (3:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) (major anomer) δ 1.99 (s, 3H), 2.05 (s, 3H), 2.12 (s, 3H), 2.13 (s, 6H), 2.14 (s, 3H), 2.18 (s, 3H), 3.97-4.00 (m, 1H), 4.03-4.16 (m, 2H), 4.26-4.28 (m, 1H), 4.33-4.37 (m, 1H), 4.73 (br s, 2H), 4.94-4.97 (m, 1H), 4.99-5.01 (m, 1H), 5.06-5.09 (m, 2H), 5.13-5.15 (m, 2H), 5.14-5.15 (m, 1H), 5.43 (t, 1H, J = 3.6 Hz) and 5.88 (d, 1H, J = 8.3 Hz); ^{13}C NMR ($CDCl_3$) (mixture of anomers) δ 20.56, 20.63, 20.68, 20.69, 20.7, 20.8, 20.9, 61.3, 62.2, 65.5, 65.89, 66.9, 67.0, 67.57, 67.60, 68.66, 68.71, 69.4, 69.8, 71.3, 90.6, 95.1, 155.0, 155.2, 168.7, 169.24, 169.26, 169.5, 170.0, 170.4 and 170.6; mass spectrum (FAB), m/z 680.2045 $(M + H)^+$ ($C_{27}H_{38}NO_{19}$ requires m/z 680.2038).



3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(carbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (54). To a solution containing 62.0 mg (0.09 mmol) of disaccharide **53** in 1.0 mL of anh DMF was added 12.0 mg (0.13 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 2.5 h and quenched by the addition of 15 mL of ethyl acetate. The organic solution was washed successively with 10-mL portions of water, satd aq $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford the product as a yellow oil: yield 51 mg (88%); silica gel TLC R_f 0.1 (1:3 hexanes–ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.07 (s, 3H), 2.12 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 3.74-3.77 (m, 1H), 4.11-4.19 (m, 2H), 4.23-4.26 (m, 2H), 4.33-4.38 (m, 1H), 4.53-4.56 (br s, 2H), 4.94-4.95 (m, 1H), 4.97-5.01 (m, 2H), 5.09-5.15 (m, 3H), 5.26-5.30 (m, 1H) and 5.39 (t, 1H, J = 3.6 Hz). The crude residue was used directly in the next reaction.

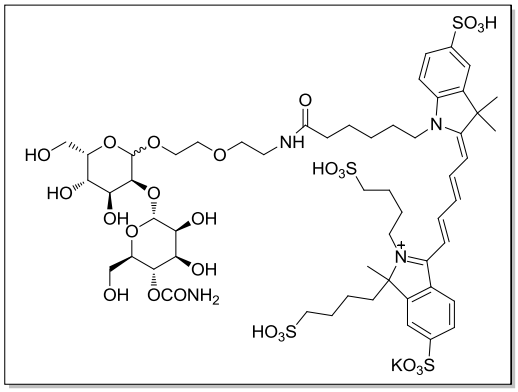
To a stirred solution containing 51.0 mg (0.10 mmol) of the crude residue in 3 mL of anh dichloromethane was added 15.0 mg (0.12 mmol) of DMAP, 147 μL (106 mg, 1.04 mmol) of Et_3N and 194 μL (252 mg, 0.94 mmol) of diphenylphosphoryl chloride. The reaction mixture was stirred at 0 °C for 2 h and was then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of

water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 2 cm). Elution with 2:1 ethyl acetate–hexanes afforded the phosphate ester **54** as a colorless oil: yield 41 mg (52% over two steps); silica gel TLC R_f 0.23 (3:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 1.97 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 2.20 (s, 3H), 3.95-4.06 (m, 2H), 4.08-4.15 (m, 1H), 4.17-4.21 (m, 2H), 4.25-4.35 (m, 2H), 4.94-5.00 (m, 2H), 5.05-5.13 (m, 3H), 5.20-5.23 (m, 1H), 5.40-5.45 (br s, 2H), 5.70 (t, 1H, J = 8.0 Hz), 7.15-7.21 (m, 4H), 7.28-7.38 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 20.62, 20.68, 20.76, 61.13, 61.98, 65.42, 66.41, 67.39, 68.60, 68.92, 69.21, 71.58, 95.06, 96.19, 120.18, 120.23, 120.44, 120.49, 125.59, 125.66, 129.66, 129.93, 154.86, 169.28, 169.48, 169.80, 170.40 and 170.63; mass spectrum (APCI), m/z 870.2230 $(M + H)^+$ ($C_{37}H_{45}NO_{21}P$ requires m/z 870.2222).



2-[2-(Benzyloxycarbonylamino)ethyloxy]ethyl 3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(carbamoyl)-α-D-mannopyranosyl)-α,β-L-gulopyranose (55). To a stirred solution containing 27.0 mg (0.03 mmol) of phosphate ester **54** in 3.9 mL of anh dichloromethane was added a solution of 8.20 mg (0.03 mmol) of CBz-protected linker **41** in 3.9 mL of anh dichloromethane at 0 °C. To the cooled solution was added 8.20 μL (10.1 mg, 0.04 mmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 15 min

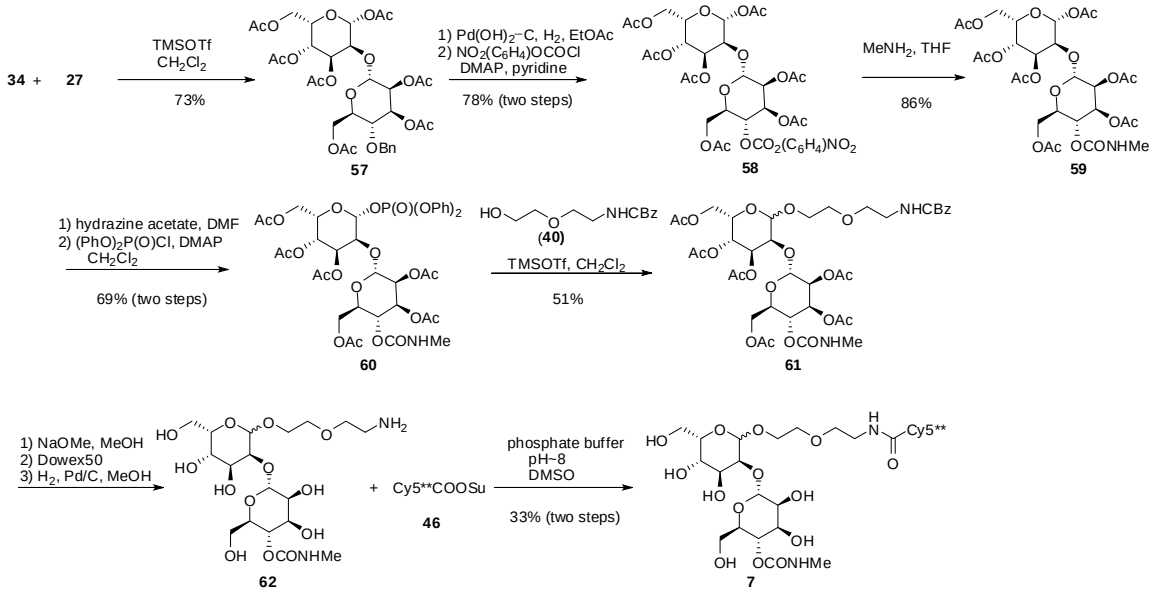
and then poured into a mixture of 20 mL of ethyl acetate and 4 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of water and brine, and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (12 × 2 cm). Elution with 3:1 ethyl acetate–hexanes afforded **55** as a colorless oil. The product was isolated as a mixture of anomers: yield 7 mg (26%); silica gel TLC R_f 0.11 (4:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) (major anomer) δ 1.99 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.13 (s, 3H), 3.33-3.45 (br s, 2H), 3.56-3.65 (m, 2H), 3.67-3.73 (m, 2H), 3.82-3.88 (m, 1H), 3.96 (t, 1H, *J* = 4.0 Hz), 4.03-4.11 (m, 3H), 4.12-4.19 (m, 2H), 4.30 (dd, 1H, *J* = 12.0 and 5.7 Hz), 4.42 (t, 1H, *J* = 6.5 Hz), 4.93-4.98 (m, 3H), 5.00-5.03 (m, 1H), 5.07 (s, 2H), 5.12-5.17 (m, 2H), 5.24-5.30 (m, 3H) and 7.30-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.81, 20.89, 20.90, 20.93, 21.0, 29.8, 41.2, 62.3, 62.9, 63.8, 65.7, 67.0, 67.1, 68.1, 68.69, 68.72, 69.6, 70.1, 71.1, 77.5, 97.2, 97.6, 128.32, 128.38, 128.7, 136.5, 155.5, 156.9, 169.5, 169.8, 169.9, 170.2, 170.7 and 170.8; mass spectrum (APCI), *m*/*z* 859.2975 $(M + H)^+$ ($C_{37}H_{51}N_2O_{21}$ requires *m*/*z* 859.2984).

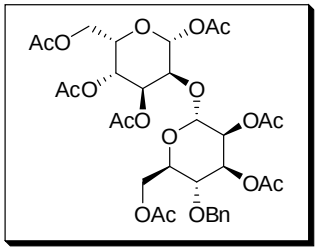


Disaccharide-dye Conjugate 6. To a solution containing 2.20 mg (2.56 mmol) of compound **55** in 1 mL of anh methanol was added a freshly prepared solution of 500 μL of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectrometric analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was then added a catalytic amount of Pd/C and H_2 gas was bubbled through for 1 h. 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 **55**, which was used directly in the next reaction; mass spectrum (APCI), *m/z* 473.1972 $(M + H)^+$ ($C_{17}H_{33}N_2O_{13}$ requires *m/z* 473.1983).

To 101 μg (0.21 μmol) of **55** was added a solution of 106 μg (0.11 μmol) of Cy5**COOSu (**46**) in 100 μL of 0.2 M phosphate buffer, pH 8.0, and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 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 mL/min. The fractions containing the desired product eluted at 23.5 min and were collected, frozen and lyophilized to give **6** as a blue solid: yield 44 μg (32% over two steps); mass spectrum (APCI), *m/z* 669.1880 $(M – K – 2H)^{2–}$ ($C_{55}H_{78}N_4O_{26}S_4^{2–}$ requires *m/z* 669.1899).

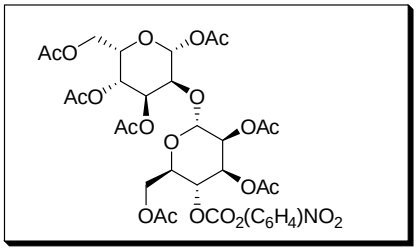
Scheme S7. Synthesis of C4 Modified Mannose Disaccharide–Dye Conjugate 7





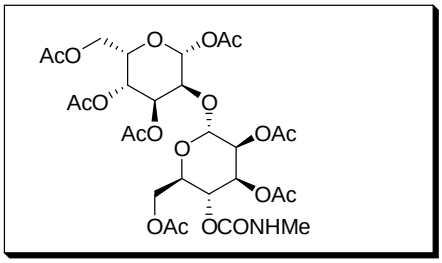
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl)-β-L-gulopyranose (57). To a stirred solution containing 217 mg (0.62 mmol) of gulose acceptor **34** and 471 mg (0.75 mmol) of mannose donor **27** in 4.5 mL of anh dichloromethane cooled to 0 °C was added 230 µL (283 mg, 1.25 mmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 10 min and then poured into a mixture of 30 mL of ethyl acetate and 30 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with two 20-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude

residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded **56** as a colorless oil: yield 330 mg (73%); silica gel TLC R_f 0.25 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 2.02 (s, 3H), 2.07 (t, 6H, *J* = 3.2 Hz), 2.08-2.11 (m, 6H), 2.15 (d, 3H, *J* = 3.7 Hz), 3.70-3.83 (m, 1H), 3.92-4.18 (m, 4H), 4.23-4.40 (m, 2H), 4.50-4.71 (m, 2H), 4.89 (dd, 1H, *J* = 7.2 and 1.7 Hz), 4.96-4.99 (m, 1H), 5.01-5.10 (m, 2H), 5.10-5.16 (m, 1H), 5.35-5.45 (m, 1H), 5.85 (d, 1H, *J* = 8.4 Hz) and 7.18-7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.68, 20.71, 20.73, 20.79, 20.84, 20.88, 20.9, 61.4, 65.6, 67.7, 69.1, 69.5, 70.3, 71.3, 71.7, 72.4, 74.8, 90.7, 95.0, 127.6, 127.89, 127.99, 128.46, 128.49, 137.6, 168.8, 169.32, 169.36, 169.4, 169.7, 170.5 and 170.6; mass spectrum (APCI), *m/z* 727.2439 $(M + H)^+$ ($C_{33}H_{43}O_{18}$ requires *m/z* 727.2450).

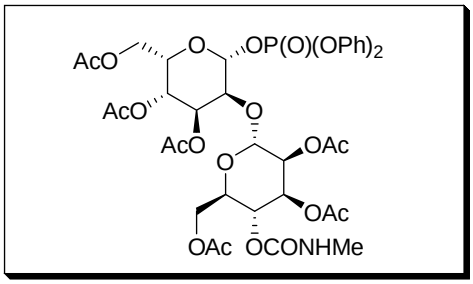


1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-((*p*-nitrophenyloxy)carbonyloxy)-α-D-mannopyranosyl)-β-L-gulopyranose (58). To a solution containing 140 mg (0.19 mmol) of disaccharide **57** in 13 mL of ethyl acetate was added a catalytic amount of $Pd(OH)_2$/C and the reaction mixture was stirred overnight under 1 atm of H_2. The solution was filtered through a pad of Celite and the filtrate was concentrated under diminished pressure to afford a crude residue. The residue was used directly in the next reaction; silica gel TLC R_f 0.08 (1:1 ethyl acetate–hexanes).

To a solution containing 120 mg (0.19 mmol) of the crude residue in 2.0 mL of anh pyridine was added 92.0 mg (0.76 mmol) of DMAP and 168 mg (0.76 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 °C overnight and then poured into a mixture of 30 mL of ethyl acetate and 10 mL of H_2O. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of 1 N HCl and then with 10-mL portions of satd aq $NaHCO_3$ and brine. The organic solution was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded ester **58** as colorless foam: yield 121 mg (78% over two steps); silica gel TLC R_f 0.30 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 2.03 (s, 3H), 2.11 (d, 6H, *J* = 5.0 Hz), 2.14 (s, 3H), 2.19 (d, 3H, *J* = 5.4 Hz), 3.99 (dd, 1H, *J* = 8.4 and 3.3 Hz), 4.02-4.25 (m, 4H), 4.27 (d, 1H, *J* = 2.4 Hz), 4.35 (t, 1H, *J* = 6.0 Hz), 4.46-4.55 (m, 2H), 4.93-5.01 (m, 2H), 5.11-5.18 (m, 2H), 5.24 (dd, 1H, *J* = 10.1 and 3.3 Hz), 5.32 (dd, 1H, *J* = 7.7 and 4.3 Hz), 5.43 (t, 1H, *J* = 3.5 Hz), 5.89 (d, 1H, *J* = 8.5 Hz), 7.29-7.39 (m, 2H) and 8.25 (t, 2H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$) δ 20.69, 20.71, 21.0, 61.3, 61.7, 65.6, 67.7, 68.6, 68.8, 70.0, 71.3, 71.4, 90.6, 95.1, 121.7, 125.4, 145.7, 151.8, 155.2, 168.7, 169.29, 169.33, 169.38, 169.58, 169.65, 169.7, 169.8, 170.44, 170.46 and 170.58; mass spectrum (APCI), *m/z* 802.2035 $(M + H)^+$ ($C_{33}H_{40}NO_{22}$ requires *m/z* 802.2042).



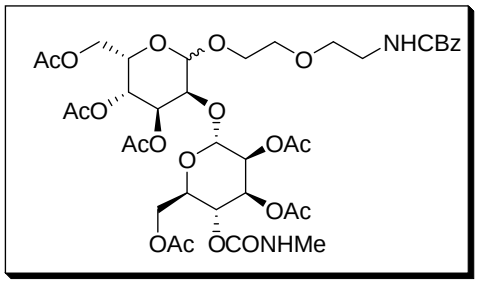
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(*N*-methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (59). To a solution containing 121 mg (0.15 mmol) of **58** in 3.2 mL of anh THF was added 76.0 μL (0.15 mmol) of a 2 M solution of CH_3NH_2 in THF at 0 °C. The reaction mixture was stirred at room temperature for 15 h at which time silica gel TLC analysis indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded disaccharide **59** as a colorless oil: yield 90 mg (86%); silica gel TLC R_f 0.14 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.96 (t, 3H, *J* = 3.4 Hz), 2.04 (d, 3H, *J* = 6.4 Hz), 2.11 (dd, 12H, *J* = 5.4 and 2.8 Hz), 2.17 (d, 3H, *J* = 2.5 Hz), 2.76 (d, 3H, *J* = 4.8 Hz), 3.97 (dd, 1H, *J* = 8.4 and 3.2 Hz), 4.00-4.39 (m, 3H), 4.48-4.80 (m, 1H), 4.93 (d, 1H, *J* = 7.2 Hz), 4.99 (dd, 1H, *J* = 7.0 and 4.4 Hz), 5.04-5.10 (m, 2H), 5.08-5.17 (m, 2H), 5.29 (dd, 1H, *J* = 13.2 and 9.8 Hz), 5.42 (t, 1H, *J* = 3.5 Hz), 5.87 (d, 1H, *J* = 8.4 Hz) and 6.28 (d, 1H, *J* = 4.2 Hz); ^{13}C NMR ($CDCl_3$) δ 20.68, 20.75, 20.76, 20.80, 20.82, 20.84, 27.8, 61.5, 61.8, 62.5, 62.7, 65.6, 66.0, 66.3, 66.8, 67.8, 68.9, 69.75, 69.79, 71.4, 90.7, 169.3, 169.59, 169.61, 169.65, 170.53, 170.55 and 170.7; mass spectrum (APCI), *m/z* 694.2199 $(M + H)^+$ ($C_{28}H_{40}NO_{19}$ requires *m/z* 694.2195).



3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(*N*-methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (60). To a solution containing 44.0 mg (0.06 mmol) of disaccharide **59** in 0.5 mL of anh DMF was added 7.00 mg (0.08 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 20 mL of ethyl acetate. The organic solution was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was used directly in the next reaction.

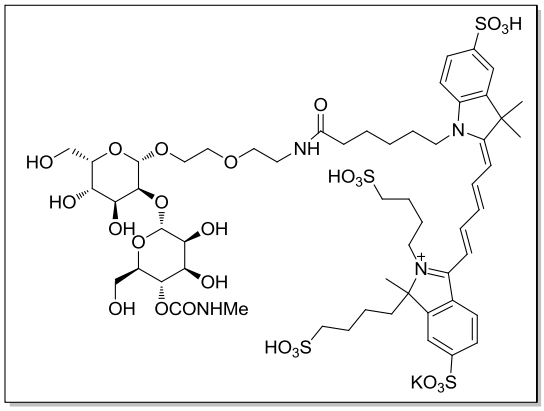
To a stirred solution containing 43.0 mg (0.07 mmol) of the crude residue in 4 mL of anh dichloromethane was added 10.0 mg (0.08 mmol) of DMAP, 100 μL (72.0 mg, 0.71 mmol) of Et_3N and 130 μL (160 mg, 0.63 mmol) of diphenylphosphoryl chloride. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solution was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded the phosphate ester **60** as a colorless oil: yield 38 mg (69% over two steps); silica gel TLC R_f 0.48 (2:1 ethyl

acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.00 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 2.21 (s, 3H), 2.57 (d, 3H, *J* = 4.0 Hz), 3.70 (s, 1H), 4.03 (s, 2H), 4.15 (d, 2H, *J* = 9.6 Hz), 4.24 (d, 2H, *J* = 12.2 Hz), 4.32-4.38 (m, 1H), 4.99 (d, 2H, *J* = 12.6 Hz), 5.05-5.25 (m, 2H), 5.30 (s, 1H), 5.45 (s, 1H), 5.71 (d, 1H, *J* = 7.4 Hz) and 7.19-7.41 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.77, 20.83, 20.89, 20.93, 27.6, 61.3, 62.3, 65.6, 66.3, 67.5, 68.8, 69.2, 69.5, 70.7, 70.8, 71.7, 95.1, 96.4, 120.4, 125.7, 129.8, 130.0, 150.4, 155.4, 169.4, 169.6, 169.9, 170.5 and 170.8; mass spectrum (APCI), *m/z* 884.2381 $(M + H)^+$ ($C_{38}H_{47}NO_{21}P$ requires *m/z* 884.2378).



2-[2-(Benzyloxycarbonylamino)ethyloxy]ethyl 3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(*N*-methylcarbamoyl)-α-D-mannopyranosyl)-α,β-L-gulopyranose (61). To a stirred solution containing 38.0 mg (0.04 mmole) of phosphate ester **60** in 0.5 mL of anh dichloromethane was added a solution of 10.0 mg (0.04 mmole) of CBz-protected linker **41** in 0.5 mL of anh dichloromethane at 0 °C. To the cooled reaction mixture was then added 14.0 μL (17.0 mg, 0.08 mmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 15 min and then poured into a mixture of 20 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude

residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded **61** as a colorless oil. The product was isolated as a mixture of anomers: yield 19 mg (51%); silica gel TLC R_f 0.14 (12:12:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) (major anomer) δ 1.92-2.14 (m, 18H), 2.71 (t, 3H, *J* = 4.1 Hz), 3.40 (d, 3H, *J* = 4.9 Hz), 3.52-3.77 (m, 8H), 3.85 (dd, 1H, *J* = 8.4 and 3.2 Hz), 3.95 (t, 1H, *J* = 3.9 Hz), 4.27 (dd, 2H, *J* = 13.4 and 7.3 Hz), 4.40 (t, 1H, *J* = 6.4 Hz), 4.88-5.04 (m, 3H), 5.05-5.22 (m, 6H), 5.25 (dd, 1H, *J* = 7.3 and 3.6 Hz) and 7.28-7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.78, 20.83 20.85, 20.87, 20.92, 20.95, 27.7, 61.9, 62.3, 63.1, 63.8, 65.7, 66.8, 66.9, 68.1, 68.7, 68.8, 69.6, 69.8, 70.2, 71.0, 72.3, 97.2, 97.5, 128.27, 128.33, 128.65, 128.67, 169.5, 169.7, 169.8, 169.9, 170.57, 170.63 and 170.7; mass spectrum (APCI), *m/z* 873.3142 $(M + H)^+$ ($C_{38}H_{53}N_2O_{21}$ requires *m/z* 873.3141).

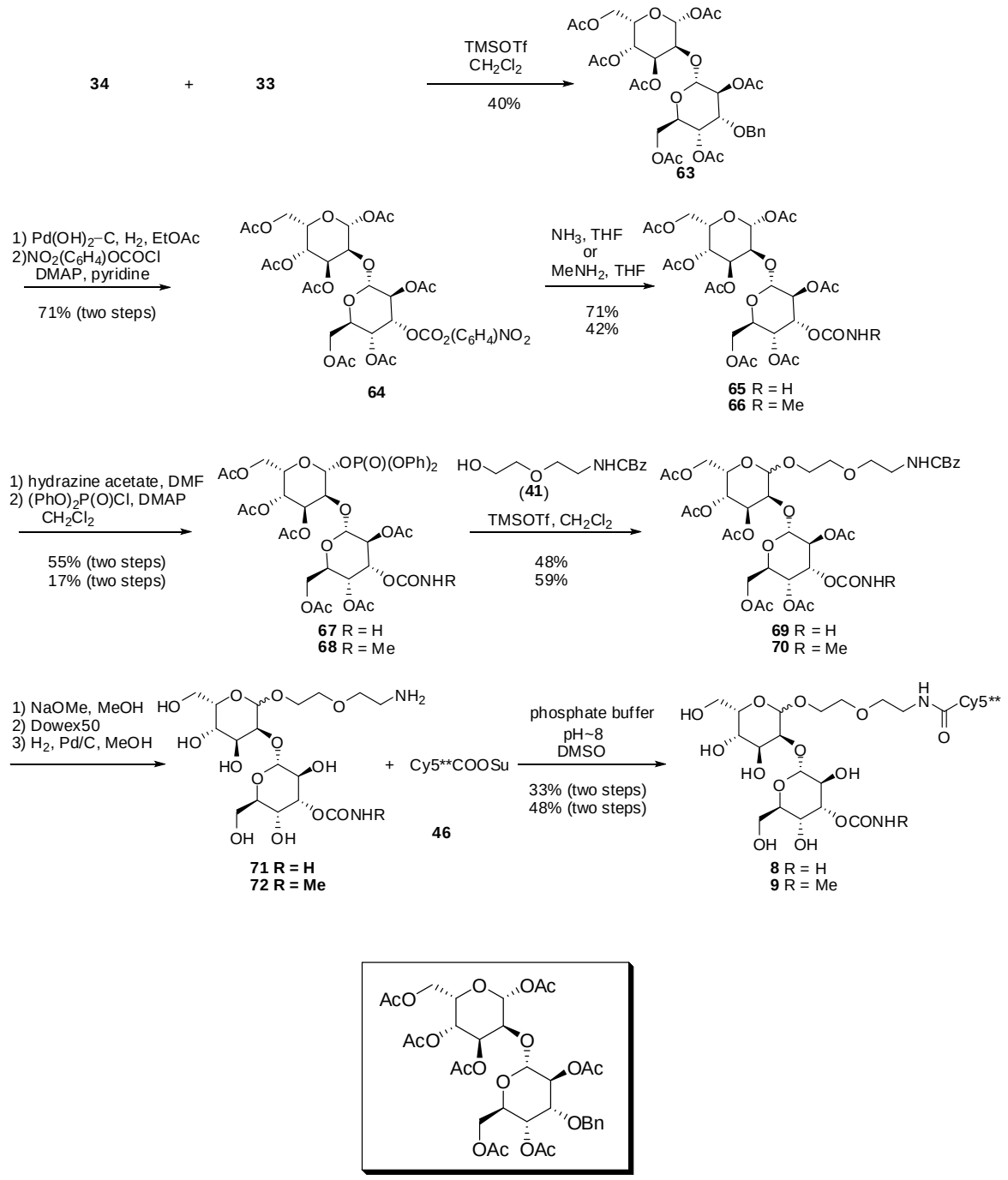


Disaccharide-dye Conjugate 7. To a solution containing 2.70 mg (3.10 mmol) of **60** in 2 mL of anh methanol was added a freshly prepared solution of 1.0 mL of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF

mass spectrometric analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was added a catalytic amount of Pd/C and H_2 gas was bubbled through for 1 h. 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 **62**, which was used directly in the next reaction; mass spectrum (APCI), *m/z* 487.2153 $(M + H)^+$ ($C_{18}H_{35}N_2O_{13}$ requires *m/z* 487.2139).

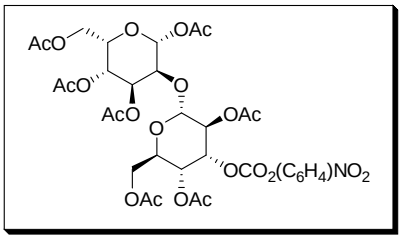
To 134 μg (0.27 μmol) of **62** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (**46**) in 150 μL of 0.2 M phosphate buffer, pH 8.0, and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 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 mL/min. The fractions containing the desired product eluted at 24.8 min and were collected, frozen and lyophilized to give **7** as a blue solid: yield 60 μg (33% over two steps); mass spectrum (APCI), *m/z* 676.1995 $(M – K – 2H)^{2–}$ ($C_{56}H_{80}N_4O_{26}S_4^{2–}$ requires *m/z* 676.1977).

Scheme S8. Synthesis of C3 Modified Altrose Disaccharide–Dye Conjugates 8 and 9



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-altropyranosyl)-β-L-gulopyranose (63). To a stirred solution containing 180 mg (0.52 mmol) of gulose acceptor **34** and 324 mg (0.52 mmol) of altrose donor **33** in 3.7 mL of anh dichloromethane at 0 °C was added 190 µL (234 mg, 1.03 mmol) of TMSOTf. The

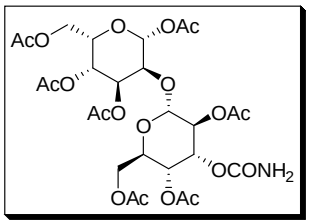
reaction mixture was stirred at 0 °C for 10 min at which time it was poured into a mixture of 30 mL of ethyl acetate and 30 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with two 20-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded disaccharide **62** as a colorless oil: yield 149 mg (40%); silica gel TLC R_f 0.24 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.93 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.04-2.06 (m, 6H), 2.08 (s, 3H), 3.72-3.83 (m, 1H), 3.94-4.16 (m, 2H), 4.16-4.35 (m, 3H), 4.35-4.62 (m, 3H), 4.79-5.01 (m, 4H), 5.24 (d, 1H, J = 0.4 Hz), 5.35-5.42 (m, 1H), 5.90 (d, 1H, J = 8.4 Hz) and 7.15-7.30 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.80, 20.81, 20.86, 20.89, 21.0, 61.6, 62.6, 65.3, 65.5, 66.4, 67.8, 68.4, 68.8, 72.0, 72.7, 90.6, 95.4, 127.4, 127.6, 127.9, 128.5, 137.7, 169.0, 169.2, 169.4, 169.5, 169.9, 170.5 and 170.7; mass spectrum (APCI), *m/z* 667.2230 (M – $CH_3COO)^+$ ($C_{31}H_{39}O_{16}$ requires *m/z* 667.2238).



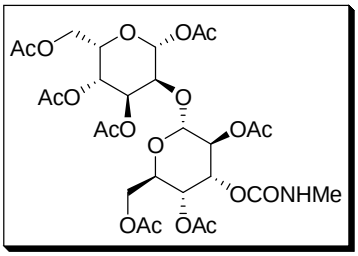
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-((*p*-nitrophenyloxy)carbonyloxy)-α-D-altropyranosyl)-β-L-gulopyranose (64). To a solution containing 190 mg (0.26 mmol) of disaccharide **63** in 18 mL of ethyl acetate was added a catalytic amount of $Pd(OH)_2$/C and the reaction mixture was stirred overnight under 1 atm of H_2. The solvent

was filtered through a pad of Celite and the filtrate was concentrated under diminished pressure to afford a crude residue. The crude product was used directly in the next reaction; silica gel TLC R_f 0.12 (1:1 ethyl acetate–hexanes).

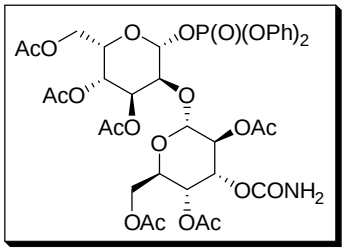
To a solution containing 198 mg (0.31 mmol) of the crude residue in 1.1 mL of anh pyridine was added 151 mg (1.24 mmol) of DMAP and 280 mg (1.24 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 °C overnight and then poured into a mixture of 30 mL of ethyl acetate and 10 mL of H_2O. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of 1 N HCl and 10-mL portions of satd aq $NaHCO_3$ and brine. The solvent was dried ($MgSO_4$) and then concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded ester **64** as a colorless foam: yield 177 mg (71% over two steps); silica gel TLC R_f 0.28 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 3.99-4.17 (m, 3H), 4.23-4.38 (m, 2H), 4.41-4.50 (m, 1H), 4.89-5.02 (m, 2H), 5.02-5.13 (m, 2H), 5.20 (dt, 1H, *J* = 10.4 and 5.2 Hz), 5.25-5.34 (m, 1H), 5.43 (t, 1H, *J* = 3.5 Hz), 5.94 (d, 1H, *J* = 8.4 Hz), 7.42 (t, 2H, *J* = 7.1 Hz) and 8.22-8.30 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.66, 20.71, 20.72, 20.76, 20.9, 61.5, 62.2, 64.7, 65.1, 65.4, 67.6, 68.1, 68.6, 71.3, 72.1, 90.5, 94.5, 121.4, 125.4, 136.0, 145.6, 149.8, 151.6, 155.2, 168.8, 168.9, 169.1, 169.3, 169.5, 170.4 and 170.6; mass spectrum (APCI), *m/z* 742.1851 (M – $CH_3COO)^+$ ($C_{31}H_{36}NO_{20}$ requires *m/z* 742.1831).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-altropyranosyl)-β-L-gulopyranoside (65). To a solution containing 73.0 mg (0.09 mmol) of ester **64** in 2 mL of anh THF was added a solution of 0.7 mL of anh THF saturated with NH_3 at 0 °C. The reaction mixture was allowed to warm to room temperature and was then stirred for 2.5 h, at which time silica gel TLC analysis indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 3:1 ethyl acetate–hexanes afforded disaccharide **65** as a colorless oil: yield 44 mg (71%); silica gel TLC R_f 0.38 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H), 2.05 (s, 3H), 2.11 (s, 6H), 2.13 (s, 3H), 2.16 (s, 3H), 2.17 (s, 3H), 3.98 (dd, 1H, *J* = 8.1 and 3.3 Hz), 4.02-4.38 (m, 7H), 4.75 (d, 1H, *J* = 3.3 Hz), 4.82-4.96 (m, 2H), 4.99-5.12 (m, 2H), 5.13 (dd, 1H, *J* = 7.8 and 4.4 Hz), 5.44 (t, 1H, *J* = 3.7 Hz) and 6.11 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 20.72, 20.75, 20.79, 20.82, 20.83, 20.87, 21.2, 61.8, 62.4, 64.6, 64.9, 65.5, 66.8, 67.6, 69.0, 69.5, 71.7, 91.0, 94.4, 155.6, 168.9, 169.3, 169.4, 169.6, 170.2, 170.5 and 170.7; mass spectrum (APCI), *m/z* 680.2039 $(M + H)^+$ ($C_{27}H_{38}NO_{19}$ requires *m/z* 680.2038).



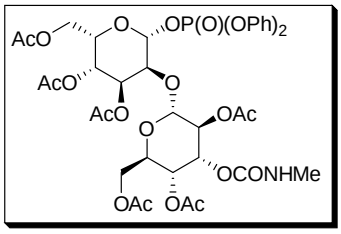
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(*N*-methylcarbamoyl)-α-D-altropyranosyl)-β-L-gulopyranose (66). To a solution containing 86.0 mg (0.11 mmol) of ester **64** in 2.4 mL of anh THF was added 54.0 μL (0.11 mmol) of a 2 M solution of CH_3NH_2 in THF at 0 °C. The reaction mixture was stirred at room temperature for 15 h at which time analysis by silica gel TLC indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (35 × 2 cm). Elution with 2:1 ethyl acetate–hexanes afforded disaccharide **66** as a colorless oil: yield 31 mg (42%); silica gel TLC R_f 0.13 (3:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H), 2.05 (s, 3H), 2.11 (s, 6H), 2.13 (s, 3H), 2.15 (s, 3H), 2.16 (s, 3H), 2.79 (d, 3H, *J* = 4.7 Hz), 3.98 (dd, 1H, *J* = 8.0 and 3.3 Hz), 4.04-4.30 (m, 4H), 4.33 (dt, 1H, *J* = 12.1 and 6.1 Hz), 4.71-4.77 (m, 1H), 4.84-4.95 (m, 1H), 5.06 (dd, 2H, *J* = 10.1 and 6.6 Hz), 5.11-5.19 (m, 1H), 5.21-5.41 (m, 2H), 5.43 (dd, 1H, *J* = 10.0 and 6.3 Hz) and 6.10 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 20.77, 20.81, 20.82, 20.85, 20.88, 20.9, 21.3, 27.8, 61.8, 62.5, 64.8, 65.0, 65.5, 66.4, 66.7, 67.6, 69.2, 71.6, 91.1, 94.7, 155.9, 169.0, 169.3, 169.4, 169.6, 170.1, 170.5 and 170.8; mass spectrum (APCI), *m/z* 694.2204 $(M + H)^+$ ($C_{28}H_{40}NO_{19}$ requires *m/z* 694.2195).



3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-altropyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (67). To a solution containing 44.0 mg (60.0 μmol) of disaccharide **65** in 0.5 mL of anh DMF was added 7.00 mg (80.0 μmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and then quenched by the addition of 20 mL of ethyl acetate. The organic layer was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was used directly in the next reaction.

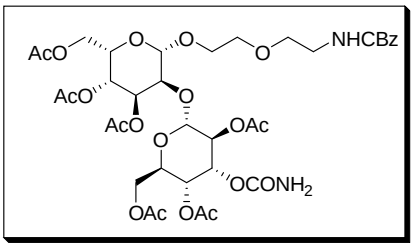
To a stirred solution containing 41.0 mg (60.0 μmol) of the crude residue in 4 mL of anh dichloromethane was added 10.0 mg (80.0 μmol) of DMAP, 100 μL (72.0 mg, 0.68 mmol) of Et_3N and 125 μL (162 mg, 0.61 mmol) of diphenylphosphoryl chloride at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 2:1 ethyl acetate–hexanes afforded phosphate ester **67** as a colorless oil: yield 31 mg (55% over two steps); silica gel TLC R_f 0.30 (2:1 ethyl

acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.12 (d, 3H, *J* = 2.8 Hz), 2.15 (d, 6H, *J* = 3.9 Hz), 3.98-4.09 (m, 2H), 4.09-4.25 (m, 4H), 4.26-4.36 (m, 2H), 4.66 (d, 1H, *J* = 9.8 Hz), 4.83 (d, 1H, *J* = 2.1 Hz), 4.91 (d, 1H, *J* = 6.4 Hz), 5.03 (t, 1H, *J* = 5.7 Hz), 5.09-5.19 (m, 2H), 5.45 (d, 1H, *J* = 3.2 Hz), 5.74 (t, 1H, *J* = 8.0 Hz) and 7.09-7.41 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.62, 20.66, 20.77, 20.83, 20.88, 61.6, 62.2, 64.5, 64.7, 65.1, 67.1, 67.3, 68.9, 71.7, 94.1, 120.28, 120.32, 120.37, 125.98, 125.99, 126.23, 126.24, 129.93, 129.94, 130.1, 155.9, 168.8, 169.0, 169.3, 169.5, 170.4, and 170.8; mass spectrum (APCI), *m/z* 870.2230 $(M + H)^+$ ($C_{37}H_{45}NO_{21}P$ requires *m/z* 870.2222).

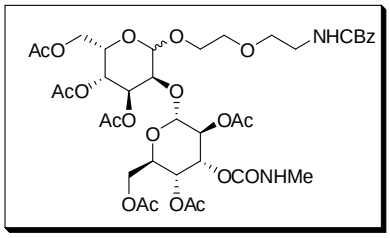


3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(*N*-methylcarbamoyl)-α-D-altropyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (68). To a solution containing 31.0 mg (40.0 μmol) of disaccharide **66** in 0.5 mL of anh DMF was added 5.00 mg (50.0 μmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and then quenched by the addition of 20 mL of ethyl acetate. The organic solution was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was used directly in the next reaction.

To a stirred solution containing 22.0 mg (30.0 μmol) of the residue in 2 mL of anh dichloromethane was added 6.00 mg (40.0 μmol) of DMAP, 52.0 μL (38.0 mg, 370 μmol) of Et_3N and 70.0 μL (91.0 mg, 330 μmol) of diphenylphosphoryl chloride at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 2:1 ethyl acetate–hexanes afforded phosphate ester **68** as a colorless oil: yield 7.0 mg (17% over two steps); silica gel TLC R_f 0.28 (3:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.85 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.12 (s, 3H), 2.15 (d, 6H, *J* = 2.5 Hz), 2.63 (d, 3H, *J* = 4.7 Hz), 3.98-4.08 (m, 2H), 4.09-4.26 (m, 3H), 4.30 (t, 1H, *J* = 6.1 Hz), 4.63 (d, 1H, *J* = 10.5 Hz), 4.80 (d, 1H, *J* = 3.0 Hz), 4.89 (s, 1H), 5.00-5.06 (m, 1H), 5.13 (dd, 1H, *J* = 10.5 and 3.1 Hz), 5.18 (d, 1H, *J* = 3.0 Hz), 5.45 (d,1H, *J* = 2.9 Hz), 5.73 (t, 1H, *J* = 8.0 Hz), 6.46 (d, 1H, *J* = 4.8 Hz) and 7.12-7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.67, 20.72, 20.77, 20.8, 20.9, 27.4, 61.6, 62.3, 64.67, 64.72, 65.1, 66.7, 67.2, 69.1, 71.7, 94.2, 96.52, 96.56, 120.1, 120.2, 120.32, 120.37, 126.0, 126.1, 129.9, 130.1, 156.1, 168.8, 169.0, 169.4, 169.5, 170.5 and 170.8; mass spectrum (APCI), *m/z* 884.2403 $(M + H)^+$ ($C_{38}H_{47}NO_{21}P$ requires *m/z* 884.2378).

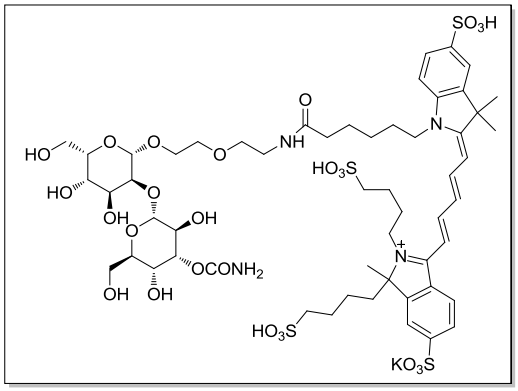


2-[2-(Benzyloxycarbonylamino)ethyloxy]ethyl 3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-Tri-*O*-acetyl-3-*O*-carbamoyl-α-D-altropyranosyl)-β-L-gulopyranose (69). To a stirred solution containing 31 mg (40 μmol) of phosphate ester **67** in 0.45 mL of anh dichloromethane was added a solution of 8.0 mg (30 μmol) of CBz-protected linker **41** in 0.45 mL of anh dichloromethane at 0 °C. To the reaction mixture was added 12 μL (15 mg, 80 μmol) of TMSOTf and the reaction mixture was stirred at 0 °C for 15 min. The reaction mixture was poured into a mixture of 10 mL of ethyl acetate and 10 mL satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed successively with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded **69** as a colorless oil: yield 15 mg (48%); silica gel TLC R_f 0.17 (11:11:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) δ 1.95-2.07 (m, 6H), 2.07-2.15 (m, 12H), 3.41 (t, 2H, *J* = 9.5 Hz), 3.59 (d, 2H, *J* = 5.0 Hz), 3.61-3.71 (m, 3H), 3.87 (dt, 1H, *J* = 12.8 and 6.5 Hz), 3.94-4.04 (m, 1H), 4.04-4.20 (m, 3H), 4.21-4.26 (m, 1H), 4.36-4.48 (m, 1H), 4.49-4.60 (m, 1H), 4.75 (d, 1H, *J* = 7.5 Hz), 4.84-5.05 (m, 4H), 5.05-5.20 (m, 4H), 5.21-5.29 (m, 1H), 5.32-5.49 (m, 2H) and 7.27-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.54, 20.62, 20.66, 20.8, 20.9, 61.6, 62.2, 64.5, 64.7, 65.1, 67.1, 67.3, 68.9, 71.7, 94.1, 96.52, 96.55, 120.28, 120.32, 120.37, 126.0, 126.2, 129.9, 130.1, 156.0, 168.8, 169.0, 169.3, 169.5, 170.4 and 170.8; mass spectrum (APCI), *m/z* 859.2973 $(M + H)^+$ ($C_{37}H_{51}N_2O_{21}$ requires *m/z* 859.2984).



2-[2-(Benzyloxycarbonylamino)ethyloxy]ethyl 3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(*N*-methylcarbamoyl)-α-D-altropyranosyl)-α,β-L-gulopyranose (70). To a stirred solution containing 17 mg (19 μmole) of phosphate ester **68** in 0.25 mL of anh dichloromethane was added a solution of 5.0 mg (17 μmole) of CBz-protected linker **41** in 0.25 mL of anh dichloromethane at 0 °C. To the reaction mixture was added 7.0 μL (8.6 mg, 34 μmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 15 min and then poured into a mixture of 10 mL ethyl acetate and 10 mL satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded **70** as a colorless oil: yield 10 mg (59%); silica gel TLC R_f 0.14 (11:11:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) δ 1.97 (d, 3H, *J* = 8.6 Hz), 2.04 (d, 3H, *J* = 4.2 Hz), 2.07-2.15 (m, 12H), 2.75 (d, 3H, *J* = 4.7 Hz), 3.34-3.44 (m, 2H), 3.51-3.70 (m, 8H), 3.72 (dd, 1H, *J* = 10.3 and 5.6 Hz), 3.82-3.93 (m, 1H), 3.95-4.25 (m, 3H), 4.26-4.56 (m, 1H), 4.63 (d, 1H, *J* = 7.2 Hz), 4.86-5.02 (m, 1H), 4.96-5.28 (m, 6H), 5.33-5.51 (m, 1H), 5.83 (d, 1H, *J* = 4.7 Hz) and 7.27-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.79, 20.84, 20.86, 20.89, 20.93, 21.0, 29.8, 41.0, 61.9, 62.2, 62.3, 62.7, 62.9, 65.26, 65.33, 66.9, 67.1, 70.2, 70.4, 70.5, 72.3, 128.3, 128.4, 128.66, 128.67,

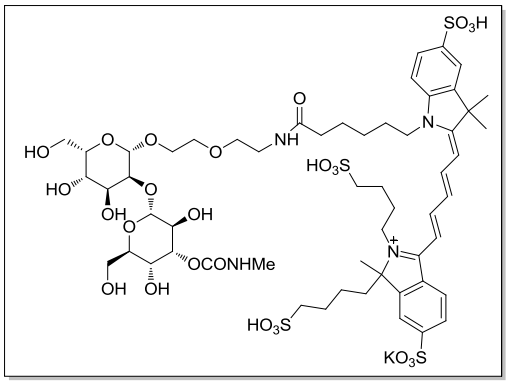
136.6, 169.61, 169.65, 169.68, 170.6, 170.7, 170.8 and 170.9; mass spectrum (APCI), *m/z* 873.3150 $(M + H)^+$ ($C_{38}H_{53}N_2O_{21}$ requires *m/z* 873.3141).



Disaccharide-dye Conjugate 8. To a solution containing 2.40 mg (2.80 mmol) of compound **68** in 2 mL of anh methanol was added a freshly prepared solution of 1.0 mL of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectrometric analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was then added a catalytic amount of Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction was filtered through Celite and the filtrate was then concentrated under diminished pressure to afford **70**, which was used for the next reaction. Mass spectrum (APCI), *m/z* 473.1978 $(M + H)^+$ ($C_{17}H_{33}N_2O_{13}$ requires *m/z* 473.1983).

To 87.0 μg (0.18 μmol) of **70** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (**46**) in 150 μL of 0.2 M phosphate buffer, pH 8.0, and the reaction mixture

was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 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 mL/min. The fractions containing the desired product eluted at 23.5 min and were collected, frozen and lyophilized to give **8** as a blue solid: yield 39 μg (33% over two steps); mass spectrum (APCI), *m/z* 669.1916 $(M - K - 2H)^{2-}$ ($C_{55}H_{78}N_4O_{26}S_4^{2-}$ requires *m/z* 669.1899).



Disaccharide-Dye Conjugate 9. To a solution containing 1.00 mg (1.10 mmol) of compound **69** in 2 mL of anh methanol was added a freshly prepared solution of 1.0 mL of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectrometric analysis. The reaction mixture was then quenched by the addition of 300 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was added a catalytic amount of Pd/C and H_2 gas was bubbled through for 1 h. 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 then concentrated under diminished pressure to afford **71**, which was used directly for the next reaction; mass spectrum (APCI), *m/z* 487.2143 $(M + H)^+$ ($C_{18}H_{35}N_2O_{13}$ requires *m/z* 487.2139).

To 87.0 μg (0.18 μmol) of **71** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (**46**) in 150 μL of 0.2 M phosphate buffer, pH 8.0, and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 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 mL/min. The fractions containing the desired product eluted at 24.7 and were collected, frozen and lyophilized to give **9** as a blue solid: yield 57 μg (48% over two steps); mass spectrum (APCI), *m/z* 676.1967 $(M - K - 2H)^{2-}$ ($C_{56}H_{80}N_4O_{26}S_4^{2-}$ requires *m/z* 676.1977).

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