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C-Glycosyl compounds bind to receptors on the surface of *Escherichia coli* and can target proteins to the organism

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ABSTRACT

A series of *C*-mannopyranosyl derivatives have been synthesized and their inhibitory activity towards the receptor-mediated adhesion of *E. coli* to yeast cells has been tested. Total inhibition of yeast-cell agglutination by *C*-glycosyl derivatives **4** and **9** is achieved at a concentration approximately one order of magnitude lower than that of methyl α-D-mannopyranoside, indicating that the binding affinity to the receptor is related to the hydrophobicity of the carbon-linked side chain. A biotin-linked *C*-glycosyl derivative of mannose (compound **9**) has been synthesized and used to target avidin and streptavidin to the bacterial cell surface. Of the *C*-glycosyl derivatives tested in our study, the conjugate of compound **9** with avidin had the highest avidity for the bacterial receptors, inhibiting agglutination at a concentration three orders of magnitude lower than methyl α-D-mannopyranoside. The use of such bifunctional compounds as the mannose–biotin conjugate **9** is a general strategy to target molecules to pathogenic organisms via their cell-surface carbohydrate receptors and to change the antigenicity of the bacterial cell surface.

INTRODUCTION

Pathogenic organisms bind to the surface of cells using carbohydrate receptors called lectins[1–3]. Several strains of enterobacteria have proteinaceous appendages termed pili (or fimbriae) that are located on the surface of the organism[4]. Type 1 pili are found on the surface of pathogens such as *Escherichia, Klebsiella, Shigella* and *Salmonella* and contain receptors specific for terminal α-linked mannosides[5–7]. The mannose-specific receptor, a 28 kD lectin found on the tip of the pilus, mediates the adhesion of pathogenic bacteria to host cells, a process that is essential for infectivity[8–11]. We describe herein the synthesis of carbon-linked glycosyl derivatives which bind to the mannose receptor of a strain of *E. coli* with capsular serotype K1 that is responsible for sepsis and meningitis in human infants[11]. We also report the synthesis of a biotinylated *C*-glycosyl derivatives that can be used in conjunction with avidin or streptavidin to target proteins to the surface of the pathogen as shown schematically in Fig. 1. Our results demonstrate that *C*-glycosyl derivatives can specifically inhibit the binding of *E. coli* K1 to yeast cells and can be used to alter the surface properties of the bacterial cell.

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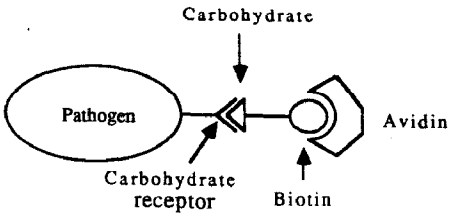


Fig. 1. Schematic model for targeting proteins to the cell surface.

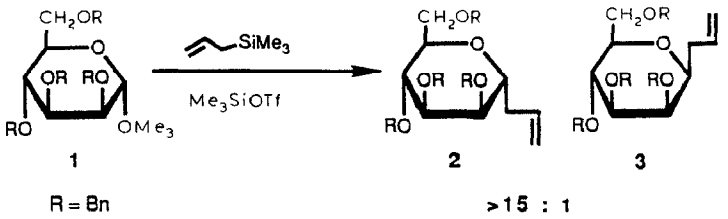
Since carbon-linked glycoside analogs are stable to enzymic hydrolysis, these molecules may be used to prevent bacterial adhesion *in vivo* and serve to target molecules to the pathogen*.

RESULTS AND DISCUSSION

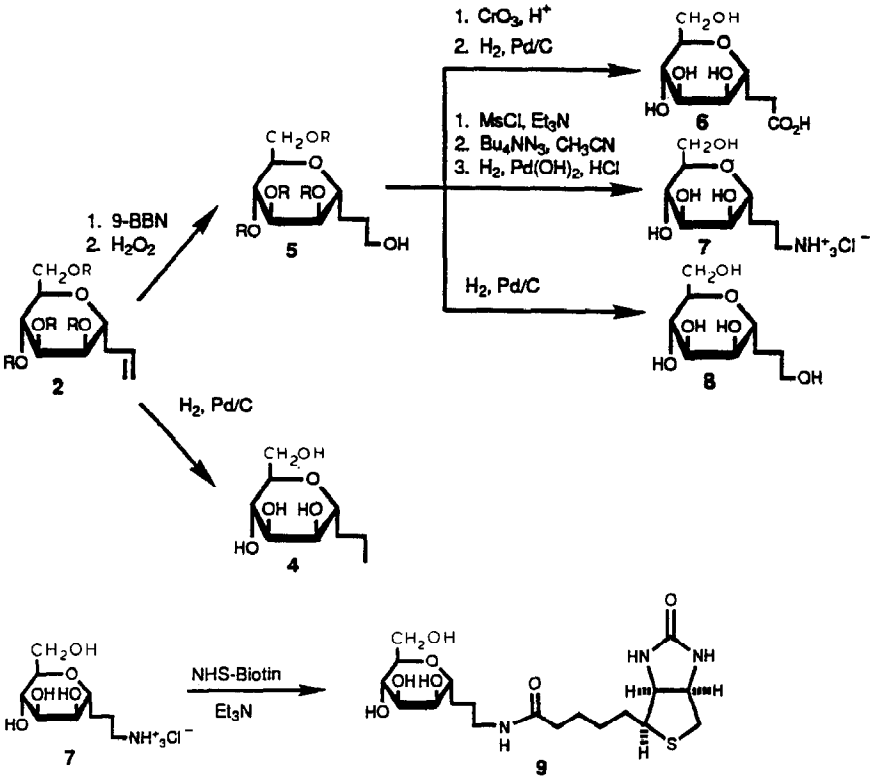
The syntheses of the *C*-glycosyl compounds used in this study are outlined as follows. Methyl (tetra-*O*-benzyl)-α-D-mannopyranoside (**1**) was treated with allyltrimethylsilane in acetonitrile using trimethylsilyl triflate (Me_3SiOTf) as a catalyst according to conditions reported by Hosomi *et al.*[13,14]

The *C*-glycosyl compounds **2** and **3** were obtained in a >15:1 mixture in an overall yield of 91%. Compound **2** was deprotected and the product hydrogenolyzed (H_2, Pd–C) to give alkane **4**.

Compounds **6**–**8** were synthesized from alcohol **5**, which was obtained by hydroboration (9-BBN) of compound **2**. Oxidation of alcohol **5** using Jones' reagent (CrO_3, H_2SO_4) followed by hydrogenolysis (H_2, Pd–C) gave the free acid **6**. The amine hydrochloride salt **7** was synthesized from compound **5** by mesylation of the primary alcohol (MsCl, Et_3N) followed by reaction with tetrabutylammonium azide[15] in acetonitrile (Bu_4NN_3, CH_3CN) and hydrogenolysis [H_2, $Pd(OH)_2$, HCl]. Debenzylation (H_2, Pd–C)



* Mannosidases are not present under the conditions of the agglutination assays used in this study, but enzymic stability is desirable in the design of ligands that can prevent bacterial adhesion *in vivo*. Although thioglycosides are also relatively stable to enzymic hydrolysis, *C*-glycosyl compounds are more conveniently synthesized from simple carbohydrate starting materials. Furthermore, the carbon linkage is compatible with a broader range of chemical reactions (such as oxidations, reductions or strong acid) allowing for the synthesis of a variety of derivatives for use in protein targeting. *C*-glycosyl compounds have been previously used to inhibit carbohydrate processing enzymes. To our kowledge, however, they have never been used to interfere with cell adhesion[12].



of compound **5** directly gave alcohol **8**. Reaction of compound **7** with *N*-hydroxysuccinimidobiotin and Et_3N in 1:1 DMF–MeOH gave conjugate **9**.

It has been reported[5] that β-D-mannopyranosides do not bind to type 1 pili receptors. Therefore, as a control for the cell-surface binding studies, we synthesized the β-*C*-glycosyl compound **3** using a modification of a procedure developed by Lewis *et al.*[16] The addition of allylmagnesium bromide to lactone **10** [synthesized from methyl tetra-*O*-benzyl-α-D-mannopyranoside by hydrolysis (AcOH, H_2O) of the glycoside followed by Jones oxidation (CrO_3, H_2SO_4) in acetone] gave hemiacetal **11** as a mixture of anomers. Stereoselective reduction using triethylsilane and boron trifluoride etherate (Et_3SiH, BF_3OEt_2) in acetonitrile gave a 1:10 mixture of *C*-glycosyl compounds **2** and **3**. Compound **3** was deprotected and reduced by hydrogenolysis to give glycosylalkane **12**.

Compounds **4**, **6–9**, and **12** were assayed for bacterial receptor binding using agglutination studies with yeast cells, and their inhibitory activity was compared to that of methyl α-D-mannopyranoside (**13**)[6,8,17]. The bacterial strain used was a systemically invasive *Escherichia coli* K1 *pilA+::tetR* strain that is responsible for sepsis and meningitis in human infants[11]. A summary of our results is given in Table I.

Three important conclusions may be drawn: (*1*) Carbon-linked glycoside analogs bind to bacterial mannose-specific lectins and inhibit the attachment of *E. coli* K1 cells to yeast. As the 28 kD lectin is highly conserved in its morphology as determined by its cross reactivity with monoclonal and polyclonal antibodies[9], these compounds should

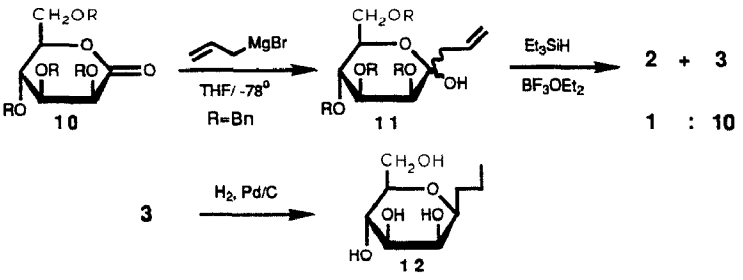


TABLE I

Inhibitory activity of *C*-mannopyranosyl derivatives on the bacterial receptor-mediated agglutination of yeast cells[a]

Entry	*Compound*	*Concentration (mM)*[b]	*Relative inhibitory activity*[c]
1	**13**	67	1
2	**6**	47	1.4
3	**7**	40	1.7
4	**8**	13	5.2
5	**4**	7	9.6
6	**9**	7	9.6
7	**14**	1.6	42
8	**9** + streptavidin	0.6[d]	
9	**9** + avidin	0.05 (50μM)	1340

[a] *E. Coli* K1 *pilA* + *::tetR* were grown for 24 h at 37° on solid LB media supplemented with tetracycline and were suspended with a cotton swab in 4 mL of Dulbecco's PBS to a final dilution of 2×10^8 cells/mL. Yeast (*Saccharomyces cerevisiae*, wild type) were grown for 36 h on solid YPD media at 30° and were suspended with a cotton swab in 4 mL of Dulbecco's PBS to a final dilution of 1×10^8 cells/mL. Protein concentrations were determined by BCA Protein Assay (Pierce). Agglutination assays were performed on a 20-well ceramic ring plate. Typically, 90 μL of a solution of the test compound was combined with 30 μL of the bacterial suspension. After 30 s, 30 μL of the yeast suspension was added to give a final volume of 150 μL and the wells were allowed to develop for 3 min with agitation. A 5 μL aliquot was removed from each well and spread onto a standard microscope slide. The slides were quickly heat fixed and mounted with 10 μL of glycerol. The slides were examined under phase contrast at 500 × magnification using a Zeiss Axioskop microscope. Agglutination was observed as clusters of cells. Total inhibition of agglutination was determined by the observation of single cells only. [b] Concentration causing total inhibition of yeast agglutination. [c] These numbers represent the concentration of methyl α-D-mannopyranoside divided by the concentrations listed in column 3. [d] Only partial inhibition of agglutination was achieved at this concentration.

also bind to other type 1 pili receptors. The β-C-glycosyl compound **12** shows no inhibitory activity at a concentration of 100mM, demonstrating that the α-specificity of the receptor observed with naturally occurring glycosides is maintained with the *C*-glycosyl analogs. (*2*) The binding of *C*-glycosyl compounds is stronger than that of methyl α-D-mannopyranoside*. The increase in binding affinity seems to be a function

* The agglutination assay measures the ability of the test compound to effectively compete with yeast mannan for the receptor binding site. Although the inhibitory concentrations obtained in this assay do not represent binding constants, they can be used to assess in qualitative terms the relative affinities of the test compounds for the receptor.

of the hydrophobicity of the carbon-linked side chain. For example, the charged compounds **6** and **7** (entries 2 and 3) may be compared with the neutral, hydrophobic compounds **4** and **9** (entries 5 and 6). Compounds **4** and **9** inhibit agglutination at a concentration that is ~6 times lower than that of the charged compounds **6** and **7**. We consider that the poor solvation of the hydrophobic side chain in water increases the affinity of compounds **4** and **9** for the receptor binding site. Transfer of the ligand from solution to the environment of the receptor binding site results in a favorable entropic contribution to the overal binding energy. This "hydrophobic effect" is also observed with *p*-nitrophenyl-α-D-mannopyranoside (compound **14**, entry 7).

Finally, we observed an increase in inhibitory activity for the biotin–streptavidin (avidin) system (entries 8 and 9, Table I). Unfortunately, streptavidin has limited solubility under the conditions of our assay. Despite the apparent increase in affinity of the streptavidin–compound **9** complex for the bacterial receptors, we could not achieve total inhibition at this concentration (entry 8). Therefore, avidin, a more soluble protein, was used in place of streptavidin. We observed that avidin alone has an intrinsic affinity for the bacterial receptors which is probably due to its glycosylation pattern (total inhibition of agglutination by avidin is achieved at a concentration of 0.4mM)[18]. *However, the conjugate of avidin with compound* **9** *(entry 9) inhibits agglutination at a concentration of 0.05mM (50μM), an order of magnitude less than avidin alone, and was the tightest binding C-glycosyl conjugate in our study.* Since avidin has four binding sites, the resulting complex with compound **9** is a multivalent ligand with four *C*-glycosyl attachment sites. Biotinylation of ligands that bind to cell-surface receptors can be used as a general approach to create multivalent ligand arrays that bind to cell surfaces with high affinity with control over their spatial arrangement.

The biotin–avidin system also allows us to target molecules to the surface of pathogenic organisms. As the binding of this conjugate alters the antigenic properties of the bacterial surface, this strategy may be used to target anti-avidin antibodies to the pathogen that would not otherwise recognize the organism. The antibody-binding studies will be reported in a future publication.

In summary, we report the first example of the use of *C*-glycosyl compounds to inhibit the attachment of pathogenic organisms to cells and have demonstrated their capability to target molecules to an infectious agent *via* its cell-surface carbohydrate receptor.

EXPERIMENTAL

General Procedures. — Unless otherwise noted, materials were commercial products and were used without further purification. Dichloromethane, acetonitrile and triethylamine were distilled from calcium hydride immediately prior to use. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl and dimethylformamide (DMF) was distilled from calcium sulfate into 3 Å molecular sieves immediately prior to use. Methanol was distilled from Mg/I_2 immediately prior to use. Methanesulfonyl chloride was distilled from P_2O_5 under diminished pressure in an inert atmo-

sphere. Distilled water was used in all manipulations. Melting points (pyrex capillary) are uncorrected. The silica gel used in column chromatography was Universal Adsorbents DCC. All high-pressure liquid chromatography (h.p.l.c.) separations were conducted with a Rainin semi-preparative instrument with Rainin Dynamax® columns and either a Knauer differential refractometer or a Knauer variable wavelength u.v. detector. I.r. spectra were determined with a Nicolet 5DX F.t. infrared spectrophotometer. ^{1}H-n.m.r. spectra were determined at 400 or 500 MHz on Bruker superconducting F.t. spectrometers. ^{13}C-N.m.r. spectra were proton decoupled and measured at 100.6 or 125.7 MHz on the Bruker spectrometers. Chemical shifts are reported in δ values, from Me_4Si. Coupling constants are given in Hz. The internal reference for ^{1}H- and ^{13}C-n.m.r. spectra determined in $CDCl_3$ and CD_3OD was Me_4Si. The internal reference for ^{1}H- and ^{13}C-n.m.r. spectra determined in D_2O was sodium 4,4-dimethyl-4-silapentanoate-2,2,3,3-d_4. Fast atom bombardment (f.a.b.$^+$) mass spectra were recorded at the U.C. Berkely Mass Spectral Laboratory on an AE1 M512 mass spectrometer. Mass spectral data are reported as *m/z* for the molecular ion. Elemental analyses were performed by the Microanalytical Laboratory, operated by the College of Chemistry, University of California, Berkeley, California.

*3-(Tetra-*O*-benzyl-α-*D*-mannopyranosyl)propene* (**2**). — A solution of 20.7 g (38.0 mmol) of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside in 75 mL of dry MeCN was cooled to 0° under N_2. Allyltrimethylsilane (8.69 g, 76.1 mmol) and trimethylsilyl triflate (4.22 g, 19.0 mmol) were added to the solution with a syringe. The solution was stirred for 16 h at 0° and then for 4 h at room temperature. The resulting deep-orange solution was diluted with 250 mL of CH_2Cl_2 and quenched with 150 mL of saturated $NaHCO_3$ solution. The layers were separated and the aqueous layer was washed with two 50-mL portions of CH_2Cl_2. The combined organic washings were dried over $MgSO_4$, and the solvents removed *in vacuo* to afford a crude orange syrup. Purification by silica gel chromatograpy eluting with 35:1 cyclohexane–EtOAc afforded 19.2 g (91%) of a colorless syrup; ν_{max}^{film} 3087, 3062, 3030, 3006, 2906, 2867, 1951, 1875, 1812, 1641, 1604, 1584, 1496, 1452, 1362, 1206, 1096, 1027, 913, 737, and 698 cm^{-1}; ^{1}H-n.m.r. (400 MHz, $CDCl_3$): δ 2.33 (m, 2 H), 3.63 (dd, 1 H, *J* 3.1, 4.7), 3.71 (dd, 1 H, *J* 3.6, 10.0), 3.77 (m, 2 H), 3.85 (m, 2 H), 4.05 (m, 1 H), 4.50–4.60 (m, 7 H), 4.70 (d, 1 H, *J* 11.0), 5.00 (m, 1 H), 5.03 (app s, 1 H), 5.75 (m, 1 H), and 7.19–7.38 (m, 20 H); ^{13}C-n.m.r. ($CDCl_3$): δ 34.59, 69.07, 71.45, 71.99, 72.36, 73.21, 73.63, 73.76, 74.81, 75.09, 76.81, 117.13, 127.40, 127.56, 127.63, 127.66, 127.81, 127.91, 127.94, 128.22, 128.25, 128.31, 134.26, 138.10, 138.19, 138.21, and 138.36; *m/z* (f.a.b.$^+$): 587 $(M + Na)^+$.

Anal. Calc. for $C_{37}H_{40}O_5$: C, 78.70; H, 7.14. Found: C, 78.93; H, 7.18.

Lit.[14] ^{1}H-n.m.r. (90 MHz, CCl_4): δ 7.33–7.03 (br s, 20 H), 6.03–5.53 (m, 1 H), 5.13–4.86 (m, 2 H), 4.73–4.33 (m, 7 H), 4.10–3.53 (m, 8 H), 2.30 (br t, 2 H, *J* 7).

*1-(α-*D*-Mannopyranosyl)propane* (**4**). — To a stirring solution of 1.40 g (2.50 mmol) of compound **2** in 15 mL of abs. EtOH were added 0.250 g of 10% Pd–C and a catalytic amount of concentrated HCl. Hydrogen gas was bubbled through the suspension for 30 min after which the mixture was stirred under H_2 for 16 h. The mixture was filtered through Celite and the catalyst rinsed with MeOH. Concentration of the filtrate

afforded a colorless syrup which was dissolved in water and washed with petroleum ether. Concentration of the water layer afforded 0.479 g (93%) of a colorless syrup; ν_{max}^{film} 3370, 2935, 2507, 1718, 1382, 1256, 1071, 965, 911, 872, 838, and 787 cm^{-1}; ^{1}H-n.m.r. (400 MHz, CD_3OD): δ 0.94 (t, 3 H, *J* 7.1), 1.30–1.53 (m, 3 H), 1.68–1.78 (m, 1 H), 3.38 (ddd, 1 H, *J* 2.8, 5.7, 8.6), 3.59 (app t, 1 H, *J* 8.7), 3.63–3.71 (m, 3 H), 3.76 (dd, 1 H, *J* 2.7, 11.7), and 3.83 (ddd, 1 H, *J* 2.1, 4.3, 9.9); ^{13}C-n.m.r. (CD_3OD): δ 14.16, 20.14, 31.79, 63.10, 69.29, 72.87, 73.14, 75.53, and 78.74; high-resolution mass spectrum (f.a.b.$^+$) calc. for $C_9H_{19}O_5$ $(MH)^+$: 207.1233. Found: 207.1228.

3-(Tetra-O-benzyl-α-D-mannopyranosyl)-1-propanol (**5**). — A solution of 8.90 g (15.8 mmol) of compound **2** in 50 mL of dry THF was cooled to 0° under N_2. A 0.5M solution of 9-BBN in THF (41.0 mL, 20.5 mmol) was added with a syringe. The solution was warmed to room temperature and heated at reflux for 3 h. The solution was then cooled to room temperature and 3 mL of EtOH was added dropwise followed by 8 mL of 4M NaOH. The solution was cooled to 0° and 8 mL of 30% H_2O_2 was added. The resulting suspension was stirred overnight at room temperature and then diluted with 50 mL of brine and 150 mL of ether. The organic layer was washed twice with brine, dried, and concentrated *in vacuo* to afford a colorless syrup. Purification by silica gel chromatography eluting with 3:1 cyclohexane–EtOAc afforded 8.78 g (95%) of a waxy solid; m.p. 47–50°; ν_{max}^{film} 3456, 3064, 3030, 2866, 1952, 1877, 1810, 1496, 1452, 1362, 1209, and 1093 cm^{-1}; ^{1}H-n.m.r. (400 MHz, $CDCl_3$): δ 1.60–1.75 (m, 4 H), 2.37 (br s, 1 H), 3.61 (dd, 1 H, *J* 2.6, 5.0), 3.64 (m, 2 H), 3.71 (dd, 1 H, *J* 3.6, 10.2), 3.77–3.84 (m, 3 H), 3.87–3.91 (m, 1 H), 4.00–4.04 (m, 1 H), 4.53 (d, 1 H, *J* 11.4), 4.56–4.61 (m, 6 H), 4.70 (d, 1 H, *J* 11.5), 7.21–7.23 (m, 2 H), and 7.28–7.40 (m, 18 H); ^{13}C-n.m.r. ($CDCl_3$): δ 26.07, 29.34, 62.09, 68.93, 71.48, 72.07, 72.73, 73.19, 73.29, 73.55, 74.78, 76.12, 127.46, 127.56, 127.62, 127.68, 127.75, 127.83, 127.87, 128.00 128.23, 128.25, 128.28, 138.03, 138.06 and 138.09; *m/z* (f.a.b.$^+$): 583 $(MH)^+$.

Anal. Calc. for $C_{37}H_{42}O_6$: C, 76.26; H, 7.26. Found: C, 76.00; H, 7.29.

3-(Tetra-O-benzyl-α-D-mannopyranosyl)propanoic acid. — To a stirring solution of compound **5** (1.00 g, 1.72 mmol) in 10 mL of acetone at 0° was added 2.0 mL (4.0 mmol) of Jones' reagent. The orange solution was warmed to room temperature and stirred for 2.5 h. The excess of Jones' reagent was quenched with MeOH and the solution was diluted with 100 mL of ether. The solution was washed with water and brine and dried over $MgSO_4$. Removal of the solvent afforded a colorless syrup. Purification by silica gel chromatrography eluting with 1:1 EtOAc/cyclohexane gave 0.978 g (95%) of a colorless oil; ν_{max}^{film}: 3100, 3089, 3032, 2870, 1953, 1876, 1812, 1707, 1495, 1454, 1101, 908, 733, and 696 cm^{-1}; ^{1}H-n.m.r. (400 MHz, $CDCl_3$): δ 1.82–1.89 (m, 2 H), 2.34–2.42 (m, 1 H), 2.46–2.54 (m, 1 H), 3.57 (dd, 1 H, *J* 3.0, 5.2), 3.68 (dd, 1 H, *J* 3.4, 10.2), 3.75–3.78 (m, 2 H), 3.80–3.85 (m, 2 H), 3.96–4.00 (m, 1 H), 4.49–4.57 (m, 7 H), 4.65 (d, 1 H, *J* 11.5), 7.17–7.20 (m, 2 H), and 7.24–7.34 (m, 18 H); ^{13}C-n.m.r. ($CDCl_3$): δ 24.82, 30.11, 68.75, 71.32, 71.44, 72.12, 73.19, 73.44, 74.59, 75.82, 76.51, 127.46, 127.62, 127.64, 127.66, 127.77, 127.83, 127.91, 128.18, 128.24, 128.29, 128.37, 137.95, 137.99, 138.06, and 138.14; high-resolution mass spectrum (f.a.b.$^+$) calc. for $C_{37}H_{41}O_7$ $(MH)^+$: 597.2852. Found: 597.2875.

3-(α-D-Mannopyranosyl)propanoic acid (**6**). — To a stirring solution of the compound from the preceding experiment (0.849 g, 1.42 mmol) in 10 mL of abs. EtOH was added 0.300 g of 10% Pd–C and a catalytic amount of concentrated HCl. Hydrogen gas was bubbled through the suspension for 30 min and the suspension was stirred under H_2 for 24 h. The catalyst was filtered through Celite and rinsed with MeOH. Concentration of the filtrate gave a slightly yellow oil (R_F 0.6 in 5:2:1 BuOH–AcOH–H_2O). The crude acid was purified by reversed-phase h.p.l.c. on a 5 × 20 cm column of C_{18} silica gel eluting with 20% aq. MeOH to afford 0.165 g (49%) of a hygroscopic white solid; m.p. 111–115°; ν_{max}^{KBr} 3380, 2933, 1714, 1573, 1403, and 1066 cm^{-1}; ^{1}H-n.m.r. (400 MHz, D_2O): δ 1.76–1.84 (m, 1 H), 2.04–2.14 (m, 1 H), 2.39–2.53 (m, 2 H), 3.49–3.53 (m, 1 H), 3.64 (app t, 1 H, *J* 9.5), 3.72 (dd, 1 H, *J* 5.9, 12.2), 3.81–3.84 (m, 2 H), and 3.89–3.94 (m, 2 H); ^{13}C-n.m.r. (D_2O): δ 26.02, 33.69, 64.08, 70.16, 73.66, 74.26, 76.54, 80.54, 82.87, and 181.20; high-resolution mass spectrum (f.a.b.$^+$) calc. for $C_9H_{16}NaO_7$ $(M + Na)^+$: 259.0794. Found: 259.0797.

3-(Tetra-O-benzyl-α-D-mannopyranosyl)-1-O-(methylsulfonyl)propanol. — A solution of 2.30 g (3.95 mmol) of compound **5** dissolved in 20 mL of dry CH_2Cl_2 was cooled to 0° under N_2. Dry Et_3N (0.478 g, 4.73 mmol) and methanesulfonyl chloride (0.503 g, 434 mmol) were added with a syringe and the solution was stirred for 1 h at 0°. The mixture was diluted with 10 mL of sat. $NaHCO_3$ and warmed to room temperature. The solution was then diluted with 10 mL of water and the layers were separated. The organic layer was washed again with water and dried over $MgSO_4$. Removal of solvents afforded a slightly yellow wax which was used in the next step without further purification; ^{1}H-n.m.r. (400 MHz, $CDCl_3$): δ 1.66–1.73 (m, 2 H), 1.75–1.86 (m, 1 H), 1.88–1.96 (m, 1 H), 2.91, (s, 3 H), 3.60 (m, 1 H), 3.71 (dd, 1 H, *J* 3.7, 10.0), 3.83–3.85 (m, 3 H), 3.89 (m, 1 H), 3.94–4.01 (m, 1 H), 4.20–4.31 (m, 2 H), 4.53–4.62 (m, 7 H), 4.67 (d, 1 H, *J* 11.6), 7.24–7.26 (m, 2 H), and 7.29–7.38 (m, 18 H); ^{13}C-n.m.r. ($CDCl_3$): δ 25.41, 25.74, 36.96, 68.60, 69.79, 71.09, 71.32, 72.07, 73.05, 73.13, 73.45, 74.48, 75.81, 127.32, 127.40, 127.53, 127.57, 127.68, 127.72, 127.81, 127.86, 128.09, 128.12, 128.16, 128.21, 128.30, 137.85, 137.86, 137.90, and 138.05; high-resolution mass spectrum (f.a.b.$^+$) calc. for $C_{38}H_{45}O_8S$ $(MH)^+$: 661.2852. Found: 661.2830.

1-Azido-3-(tetra-O-benzyl-α-D-mannopyranosyl)propane. — To a stirring solution of 2.61 g (3.95 mmol) of the mesylate from the previous experiment in 30 mL of dry MeCN was added a solution of 1.03 g (4.00 mmol) of Bu_4NN_3 (ref. 15) in dry MeCN. The solution was heated at reflux for 5 h. After cooling to room temperature, the solvent was removed *in vacuo* and the resulting residue was dissolved in ether. The ether solution was washed with two 20-mL portions of water and one 10-mL portion of brine. The organic layer was dried over $MgSO_4$ and the solvent was removed to give a slightly yellow oil. Chromatography on silica gel eluting with 15:1 cyclohexane–EtOAc afforded 2.24 g (93% from alcohol **5**) of a colorless oil; ν_{max}^{film} 3087, 3063, 3030, 2865, 2096, 1951, 1874, 1811, 1496, 1454, 1363, 1256, 1207, 1094, 735, and 697 cm^{-1}; ^{1}H.n.m.r. (400 MHz, $CDCl_3$): δ 1.58–1.66 (m, 3 H), 1.69–1.78 (m, 1 H), 3.25–3.30 (m, 2 H), 3.57 (dd, 1 H, *J* 3.0, 5.3), 3.71 (dd, 1 H, *J* 2.8, 9.7), 3.78–3.85 (m, 4 H), 3.92–3.97 (m, 1 H), 4.52–4.61 (m, 7 H), 4.68 (d, 1 H, *J* 11.4), 7.21–7.23 (m, 2 H), and 7.28–7.35 (m, 18 H); ^{13}C-n.m.r.

($CDCl_3$): δ 25.20, 26.94, 51.17, 68.83, 71.51, 71.76, 72.17, 73.21, 73.44, 73.49, 74.65, 75.95, 76.49, 127.48, 127.66, 127.82, 127.89, 127.93, 128.00, 128.26, 128.33, 128.43, 138.02, 138.05, 138.08, and 138.21; *m/z* (f.a.b.$^+$): 580 $(M - N_2)^+$.

Anal. Calc. for $C_{37}H_{41}N_3O_5$: C, 73.12; H, 6.80; N, 6.91. Found: C, 73.00; H, 6.75; N, 6.80.

1-Amino-3-(α-D-mannopyranosyl)propane hydrochloride (**7**). — To a stirring solution of the azide from the previous experiment (2.24 g, 3.29 mmol) in 27 mL of 13:1 MeOH–EtOH were added 0.638 g of pre-dried $Pd(OH)_2$ (Pearlman's catalyst) and 1.0 mL of concentrated HCl. Hydrogen gas was bubbled through the suspension for 30 min and the mixture was stirred under H_2 for 24 h. The mixture was filtered through Celite and the catalyst was rinsed with MeOH. The filtrate was concentrated to afford an orange syrup. The crude residue was dissolved in water, washed with 10 mL of ether, and loaded onto a 15-mL column of Bio-Rad AG 50W-X4 H^+ resin. The column was rinsed with water and the free amine product was eluted with 1.5M aq. NH_4OH. Concentration of the fractions containing the product (detected with ninhydrin) afforded an amorphous solid which was dissolved in 10 mL of 0.46M aqueous HCl. Concentration of the resulting solution gave the crude hydrochloride salt which was recrystallized from 1:1 MeOH–EtOH affording 0.601 g (71%) of white needles; m.p. 150–152°; ν_{max}^{KBr} 3392, 3236, 2918, 1613, 1505, 1457, 1397, 1250, 1149, 1069, 1040, 973, 932, 837, and 795 cm^{-1}; ^{1}H-n.m.r. (400 MHz, D_2O): δ 1.56–164 (m, 1 H), 1.68–1.96 (m, 3 H), 3.06 (t, 2 H, *J* 7.6), 3.54 (ddd, 1 H, *J* 2.2, 6.5, 9.0), 3.64 (app t, 1 H, *J* 9.4), 3.73 (dd, 1 H, *J* 6.5, 12.1), 3.82 (dd, 1 H, *J* 3.4, 9.2), 3.86–3.91 (m, 2 H), and 3.95 (ddd, 1 H, *J* 1.8, 3.7, 10.5); ^{13}C-n.m.r. (D_2O): δ 26.24, 27.30, 41.89, 64.07, 70.19, 73.50, 74.09, 76.56, and 80.32; high-resolution mass spectrum (f.a.b.$^+$) calc. for $C_9H_{20}NO_5$ $(MH - HCl)^+$: 222.1341. Found: 222.1333.

3-(α-D-Mannopyranosyl)-1-propanol (**8**). — To a stirring solution of compound **5** (2.50 g, 4.29 mmol) in 50 mL of 1:1 EtOH-MeOH was added 0.250 g of 10% Pd–C. A catalytic amount of concentrated HCl was added and hydrogen gas was bubbled through the mixture for 30 min. The mixture was stirred under H_2 for 24 h. The mixture was filtered through Celite and the catalyst was rinsed with MeOH. Concentration of the filtrate afforded a slightly yellow syrup which crystallized upon standing. Recrystallization from EtOH afforded 0.736 g (77%) of pure white needles; mp 134–136°; υ_{max}^{KBr} 3377, 2948, 2916, 2875, 1483, 1435, 1387, 1339, 1259, 1060, 979, 954, 816, 784, 751, and 615 cm^{-1}; ^{1}H-n.m.r. (400 MHz, D_2O): δ 1.52–1.63 (m, 2 H), 1.65–1.75 (m, 1 H), 1.80–1.88 (m, 1 H), 3.54 (ddd, 1 H, *J* 2.2, 6.1, 8.5), 3.61–3.66 (m, 3 H), 3.73 (dd, 1 H, *J* 6.2, 12.1), 3.82–3.88 (m, 2 H), and 3.90–3.95 (m, 2 H); ^{13}C-n.m.r. (D_2O): δ 26.78, 30.64, 64.06, 64.12, 70.20, 73.60, 74.30, 76.29, and 81.02; *m/z* (f.a.b.$^+$): 223 $(MH)^+$.

Anal. Calc. for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 48.62; H, 7.82.

1-Biotinamido-3-(α-D-mannopyranosyl)propane (**9**). — A suspension of amine hydrochloride **7** (0.100 g, 0.390 mmol) in 2 mL of anhydrous DMF, 1 mL of absolute MeOH and 0.5 mL of dry Et_3N was stirred for 20 min at room temperature. *N*-hydroxysuccinimidobiotin (0.130 g, 0.390 mmol) was added and the suspension clarified over a 15-min period. The solution was stirred overnight at room temperature after which the solvents were removed *in vacuo*. The resulting residue was dissolved in water

and passed through a 3-mL column of Bio-Rad AG50W-X4 H^+ resin. The column was rinsed with water and the eluant was concentrated to give a white solid. The crude product was purified by reversed-phase h.p.l.c. on a 1 × 20 cm column of C_{18} silica gel eluting with 25% aqueous MeCN. The fractions containing the product were combined and concentrated to afford 0.097 g (56%) of a hygroscopic white solid; mp 144°; υ_{max}^{KBr} 3296, 2935, 1693, 1652, 1560, 1401, 1248, 1071, and 843 cm^{-1}; ^{1}H-n.m.r. (500 MHz, D_2O): δ 1.37–1.46 (m, 2 H), 1.50–1.83 (m, 8 H), 2.26 (t, 2 H, *J* 7.1), 2.79 (d, 1 H, *J* 13.0), 3.00 (dd, 1 H, J 4.9, 13.0), 3.24 (t, 2 H, *J* 6.5), 3.34 (m, 1 H), 3.51 (m, 1 H), 3.63 (app t, 1 H, *J* 9.6), 3.72 (dd, 1 H, *J* 6.1, 12.2), 3.81 (dd, 1 H, *J* 3.3, 9.4), 3.85 (dd, 1 H, *J* 2.2, 12.2), 3.89–3.94 (m, 2 H), 4.43 (dd, 1 H, *J* 4.5, 7.9), and 4.61 (dd, 1 H, *J* 4.8, 7.9); ^{13}C-n.m.r. (D_2O): δ 27.72, 27.84, 28.10, 30.60, 30.81, 38.42, 41.71, 42.59, 58.31, 63.17, 64.18, 65.02, 70.22, 73.72, 74.39, 76.39, 80.96, 168.27, and 179.66; high-resolution mass spectrum (f.a.b.$^+$) calc. for $C_{19}H_{34}N_3O_7S$ $(MH)^+$: 448.2117. Found: 448.2116.

1-(β-D-Mannopyranosyl)propane (**12**). — To a stirring solution of 3-(tetra-*O*-benzyl-β-D-mannopyranosyl)propene[16] (0.215 g, 0.380 mmol) in 7 mL of absolute EtOH were added 0.020 g of 10% Pd–C followed by a catalytic amount of concentrated HCl. Hydrogen gas was bubbled through the suspension for 20 min after which the suspension was kept under H_2 for 8 h. The catalyst was filtered through Celite and rinsed with MeOH. Concentration of the filtrate gave a colorless syrup which was dissolved in water and washed with petroleum ether. The aqueous layer was lyophilized to afford 0.082 g (100%) of a foamy residue. The crude product was purified by h.p.l.c. on a 1 × 20 cm column of aminopropyl silica gel eluting with 50% aq. MeOH. Concentration of the fractions containing the product (refractive-index detection) afforded 0.056 g (72%) of a colorless residue; ^{1}H-n.m.r. (400 MHz, CD_3OD): δ 0.94 (t, 3 H, *J* 7.2), 1.33–1.57 (m, 3 H), 1.63–1.72 (m, 1 H), 3.15 (ddd, 1 H, *J* 2.4, 5.6, 9.5), 3.38 (ddd, 1 H, *J* 0.75, 5.6, 7.5), 3.43 (dd, 1 H, *J* 3.3, 9.4), 3.54 (app t, 1 H, *J* 9.5), 3.67–3.70 (m, 2 H), and 3.82 (dd, 1 H, *J* 2.4, 11.7); ^{13}C-n.m.r. (CD_3OD): δ 14.43, 19.91, 34.10, 62.98, 68.83, 72.40, 76.68, 79.34, and 81.87; high-resolution mass spectrum (f.a.b.$^+$) calc. for $C_9H_{19}O_5$ (MH^+): 207.1233. Found: 207.1226.

Preparation of compound **9***–avidin and –streptavidin conjugates.* — Avidin and streptavidin were incubated overnight at 0° with a ten-fold excess of compound **9** in PBS. The solutions were dialyzed 4 times against 400 mL of PBS (6 000–8 000 mol. wt. cutoff) and protein concentrations were determined by BCA protein assay (Pierce) and by $E_{280}^{1\%}$.

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