Supplementary Information

ANTIFOULING GLYCOCALYX-MIMETIC PEPTOIDS

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Contents

List of Tables

List of Figures

Experimental

Materials

2-methoxyethylamine (MEA), N,N'-Diisopropylcarbodiimide (DIC), bromoacetic acid (BAA), diisopropylethylamine (DIPEA), piperidine, triisopropylsilane (TIPS), trifluoroacetic acid (TFA), acetic anhydride, pyridine, 2-propanol, anhydrous dichloromethane (DCM), methanol, and 2,5-dihydroxybenzoic acid (DHB) matrix were purchased from Aldrich (Milwaukee, WI). Rink amide-MBHA resin, Fmoc-Lys(Boc)-OH, Fmoc-DOPA(acetonide)-OH, and *N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) were purchased from EMD4Biosciences (San Diego, CA). Copper (II) sulfate, sodium azide, 2-bromoethanol, propargylamine, 1-azido-1-deoxy-β-D-glucopyranoside, β-D(+) maltose monohydrate, sodium methoxide, and Dorex50WX4-400 resin were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. HPLC grade acetonitrile (ACN), dimethylformamide (DMF), and N-methylpyrrolidone (NMP) were purchased from J.T. Baker (Pittsburgh, PA). Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plate (0.25 mm thickness). Silica gel column chromatography was performed using silica gel 60 Å (60-100 mesh). TLC plate, silica gel powder, silver nitrate, and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO), and ACS grade of hexane, methanol and ethylacetate were purchased from Fisher Scientific Co. (Pittsburgh, PA) and used as received. All organic reactions were run in dry glassware under Ar or N_2 gas. Ultrapure water (resistivity ≥ 18.2 MΩ-cm) used for all experiments was obtained from a NANOpure Infinity System from Barnstead/Thermolyne Corporation (Dubuque, IA).
Four inch prime silicon wafers were obtained from Ultrasil Corporation Hayward, CA). N-morpholinopropanesulfonic acid (MOPS) buffer salt, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer salt, Tris buffer salt, citrate, sodium chloride, and lyophilized fibrinogen were purchased from Sigma-Aldrich (St. Louis, MO). 3T3-Swiss albino fibroblasts, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, trypsin-EDTA, *Escherichia coli* (ATCC 35218), *Staphylococcus epidermidis* RP62A (ATCC 12228), and *Pseudomonas aeruginosa* (ATCC 27853) were obtained from American Type Culture Collection (ATCC) (Manassas, VA). Tryptic soy broth and tryptic soy agar, Luria-Bertani (LB) broth and LB agar, and R2A agar were purchased from Becton, Dickinson and Co. (Franklin Lakes, NJ). Calcein-AM, SYTO 9 and propidium iodide were purchased from Molecular Probes (Eugene, OR). Glass microscope slides were purchased from Fisher Scientific (Pittsburgh, PA).

Synthesis of glycopeptoids

Solid Phase Synthesis of peptoid backbone

The peptidomimetic polymer backbone with peptide anchor was synthesized as previously described[1] using an automated peptide synthesizer (CSBio Co., Menlo Park, CA). C-terminal DOPA-Lys-DOPA-Lys-DOPA peptide anchor was synthesized on Rink amide-MBHA resin following the conventional Fmoc strategy of solid phase peptide synthesis, after which the polypeptoid portion is synthesized using a two-step submonomer reaction (acylation with bromoacetic acid and nucleophilic displacement with methoxyethylamine (MEA)[2]. These two cycles are alternated 20 times. Then, alkyne functionality was

introduced to the end of the peptoid by submonomer addition of 10% propargylamine after bromoacetylation. Following completion of the peptoid backbone synthesis (M20A), the N-terminus was acetylated with acetic anhydride.

Synthesis of 2-azidoethyl-β-D maltosepyranose (Mal(OH)-N_3)

2'-azidoethyl-β-D maltopyranose (Mal(OH)-N_3) was prepared from β-D-maltose-monohydrate via a four-step synthesis (acetylation of hydroxyl groups, bromoethylation, azidolysis, and deacetylation) as shown in Scheme 1. The products were characterized by nuclear magnetic resonance (NMR) and liquid chromatography electrospray ionization mass spectrometry (LC-ESI) (Agilent 1100 MSD) after each step of synthesis (spectra are provided in the supporting Information). NMR data were acquired at room temperature on a 500 MHz Bruker Avance-III spectrometer equipped with a DCH cryoprobe. Experiments included 1D ^{1}H, 1D ^{13}C, TOCSY (20 and 80 msec mixing times), gCOSY, and ^{1}H-^{13}C HSQC. Chemical shifts were referenced to internal TMS.

<u>*a-D-maltopyranose octaacetate (2):*</u> Sodium acetate (5.4 g, 66.6 mmol, 3 eq.) and zinc chloride (cat. 0.6 g) were added to a solution of acetic anhydride (50 ml), then refluxed to 65 °C for 12 minutes. β-D-maltose-monohydrate (1) (7.992 g, 22.2 mmol) was added to the mixture and stirred at 65 °C for 12 hours. TLC analysis was performed to confirm a complete conversion of maltose (R_f = 0.66, Ethyl Acetate: Ethanol: Water = 45:5:0.1). The mixture was concentrated under vacuum, dissolved in methanol, and recrystallized with cold water to yield a white solid. The precipitates were redissolved in methanol, filtered, recrystallized against water, and dried to afford (2).

<u>*2-bromoethyl- b-D-maltopyranose heptaacetate (3):*</u> β-D-maltopyranose octaacetate (2) (6.782 g, 10 mmol, 1 eq.) was added to anhydrous dichloromethane (DCM) (30 ml) in a round bottom flask (RBF). 2-bromoethanol (0.94 ml, 13 mmol, 1.3 eq.) was added followed by dropwise addition of boron trifluoride diethyl etherate (1.60 ml, 13 mmol, 1.3 eq.) at 0 °C over the course of 20 minutes. The mixture was stirred under inert atmosphere (Ar gas) for 18 hr at room temperature. Upon completion of the reaction, the mixture was extracted with anhydrous DCM and washed with ice-water and saturated sodium bicarbonate ($NaHCO_3$) two times. The organic layers were combined, concentrated under reduced pressure. The crude product was recrystallized in n-hexane (200 ml), filtered, and dried under vacuum to produce a white solid (3).

<u>*2-azidoethyl-b-D-maltopyranose heptaacetate (4):*</u> 2-bromoethyl-β-D-maltopyranose octaacetate (3) (5.936 g, 8 mmol) and sodium azide (1.04 g, 16 mmol) were dissolved in a dimethylformamide (DMF) and dichloromethane (DCM) mixture 6:1 (v/v) at 0 °C. The mixture was stirred under inert atmosphere (Ar gas) overnight (18 hours) at room temperature. Upon completion, the mixture was extracted with DCM and water twice. The organic layer was collected and DCM was removed under reduced pressure. The concentrated solution was dried under vacuum to yield a white solid and then purified by silica gel column (ethyl acetate: methanol = 1:1) to afford (4).

<u>*2-azidoethyl-β-D-maltopyranose (5):*</u> 2-azidoethyl-β-D-maltopyranose octaacetate (4) (4.23 g, 6 mmol) and sodium methoxide (43 mg, 0.8 mmol, 7.5 eqv.) were dissolved in anhydrous methanol and stirred at room temperature overnight. The reaction mixture was

neutralized with Dorex50WX4-400 resin, filtered, and dried under reduced pressure to afford a white product (5). The final product was purified by RP-HPLC using Pheomenex Synergi™ 4 μm Polar RP column at 2.5% acetonitrile/97.5% water with 0.1% formic acid.

Oligosaccharide coupling by copper catalyzed alkyne-azide cycloaddition

For synthesis of M20Glu(OH), M20A-acetylated resin (0.4 mmol) was reacted with sodium ascorbate (1.981g), glucose azide (Glu(OH)-N_3, 328.24 mg), and copper (II) sulfate (249.68 mg). in 28 ml of DMF: pyridine 6:1 (v/v) for 2 days with stirring at room temperature. The reaction ratio used for this coupling reaction was alkyne:azide: Cu(II): ascorbic acid = 1:4:2.5:25. Upon completion of the reaction, the resins were collected, washed with DMF, DCM, IPA, and methanol, EDTA containing water and dried under vacuum. Glycopeptoids were then cleaved from the resin by treating with 95% TFA cleavage solution (TFA: TIPS: Water = 95:2.5:2.5) for 2 hours and filtered. Solvents were removed using a rotary evaporator to a viscous solution, ether precipitated, and then dried under pressure. M20Mal(OH) synthesis was performed following the same procedures using maltose-azide (Mal(OH)-N_3) instead of Glu(OH)-N_3. The oligosaccharide coupling reaction scheme is shown in Figure 1, and the chemical structure of the final products are shown in Figure 2. The crude product was purified by preparative RP–HPLC (Waters, Milford, MA) using a Vydac C18 column and acetonitrile/water/0.1%TFA as a mobile phase. The purity of the each final product was confirmed by analytical RP–HPLC and matrix-assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics Apex III, Billerica, MA) using 2,5-dihydroxybenzoic acid (DHB) as a matrix (Figure 2).

Surface modification and characterization

Antifouling polymer coating

TiO_2 native oxide films were prepared by depositing 4 nm of Ti film on silicon wafers by electron beam evaporation (Edward Auto500, $6x10^{-6}$ Torr, 0.1 nm/s) and cutting into 11 x 7 mm^2 pieces. The substrates were cleaned ultrasonically in acetone, 2-propanol, and deionized water (10 minutes each), dried under N_2, and then exposed to O_2 plasma (Harrick Scientific, Ossining, NY) at 120 mTorr and 100 W for 3 minutes to produce a clean titanium oxide surface. Clean substrates were immersed in 0.3 mM polymer solution (either M20Glu(OH) or M20Mal(OH)) in 3M NaCl buffered with 0.1M *N*-morpholinopropanesulfonic acid (MOPS) (pH 6.0) at 50°C for 18 hours with mechanical shaking at 100 rpm. For each condition, samples were prepared in triplicate. After modification, substrates were extensively rinsed with U.P. H_2O to remove any unbound polymer and then dried in a stream of filtered N_2.

Spectroscopic Ellipsometry Measurements

Prior to incubation with polymer solution, unmodified TiO_2 substrate thickness was measured in air as a reference using an M-2000 spectroscopic ellipsometer (J.A.Woollam, Lincoln, NE). After modification, substrates were extensively rinsed with ultrapure H_2O and dried under N_2. All measurements were made at 65°, 70° and 75° at 377-1000 nm, and the spectra were fitted with multilayer slab models in the CompleteEASE software (J.A.

Woollam). Optical properties of TiO_2 substrates were fit using a standard Si and TiO_2 model (on average, measured n was ~ 2.3 + 0.04/λ2 for TiO_2), while properties of the polymer layer were fitted using a Cauchy model ($A_n = 1.45$, $B_n = 0.01$, $C_n = 0$)[3] as an ad-layer to the TiO_2 layer to determine the 'dry' polymer film thickness. The average thickness and standard deviation of three or more substrates is reported for each polymer.

Water Contact Angle

Advancing and receding water contact angles were measured to determine the change in substrate wettability due to surface modification using a contact-angle goniometer with an auto-pipetting system (Model 190 CA, Ramé-Hart, Succasunna, NJ) and DropImage software (Ramé-Hart, Succasunna, NJ). After calibration and alignment of the system, live video images of the water drop on the surface were monitored to determine the contact angle. For advancing angle measurements, 1 μl of U.P. H_2O was applied to the sample surface and the drop volume was increased stepwise at 0.08 μl/step. The contact angle value at the maximum volume permitted without increasing the solid/liquid interfacial area was recorded as the advancing angle. Receding angles were measured by reducing the drop volume stepwise (0.08 μl/step) from 4.2 μl. The smallest angle measured without reducing the solid/liquid interfacial area was recorded as the receding angle.

X-ray Photoelectron Spectroscopy (XPS)

Survey and high-resolution XPS spectra were collected at ultrahigh vacuum ($<10^{-8}$ Torr) on an Omicron ESCALAB (Omicron, Taunusstein, Germany) with a monochromatic Al Kα (1486.8 eV) 300W X-ray source with a spot size of 1.5 mm and a fixed take off angle of 45°. All binding energies were calibrated using the C1s peak (284.6 eV). Broad survey scan spectra was acquired from 0-1100 eV and detailed high-resolution spectra were acquired at 280-292 eV for C(1s), 394-406 eV for N(1s), 524-536eV for O(1s), and 455-467 eV for Ti(2p). The spectra were fitted using XPSpeak41 software and a Shirley background subtraction was performed. Atomic compositions were calculated from high-resolution spectral peak areas after normalizing with individual atomic sensitivity factors.[4]

Evaluation of antifouling performance

Protein adsorption experiments on polymer-coated substrates

Lyophilized fibrinogen from human plasma was dissolved in HEPES buffer (10 mM HEPES with 150 mM NaCl, pH 7.4) at a concentration of 3 mg/ml and warmed to 37°C. Modified and unmodified TiO_2 substrates were placed in sterile polystyrene 24 well plates (one substrate per well) and pre-equilibrated with HEPES buffer for 30 minutes at 37°C. After removal of the equilibration buffer, fibrinogen solution or control buffer was applied to the corresponding wells and incubated at 37°C for different durations (20 minutes, 1 day and 3 days). The control buffer was prepared by adding 1.3 mM citrate salt to HEPES buffer to balance the contents of citrate salt contained in the 3 mg/ml of fibrinogen solution. After treatment, substrates were rinsed with HEPES buffer and then U.P. H_2O, and the final dry thickness of each substrate was measured by ellipsometry and fit using a Cauchy model assuming n = 1.45 + 0.01/λ2. The amounts of adsorbed fibrinogen for each individual sample were calculated from the film thickness change measured by ellipsome-

try due to fibrinogen adsorption. The measured thickness was converted to adsorbed mass density using a previously measured value (a thickness of 3.7 nm corresponded to mass density of 521 ng/cm^2 on bare TiO_2).[5]

Mammalian cell attachment on polymer-coated substrates

3T3-Swiss albino fibroblasts (CCL-92, ATCC, Manassas, VA) were cultured for 4 days in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 100 U/ml of penicillin/streptomycin at 37ºC and 5% CO_2. In a 12-well tissue culture plate, modified and unmodified substrates were placed and sterilized by exposure to UV light for 30 minutes, after which the substrates were pre-equilibrated with DMEM with 10% FBS (1ml/well) for 30 minutes at 37ºC and 5% CO_2. In the meantime, fibroblasts (passage 12-16) were harvested by treating with 0.25% trypsin-EDTA, and resuspended in DMEM with 10% FBS and counted using a hemocytometer. Then, the fibroblast cell stock was diluted to 2.6 x 10^3 cells/cm^2 with DMEM with 10 % FBS, and 1 ml was applied to each well after equilibration. After 4 hours, the media was aspirated from each well to remove any non-adherent cells and PBS was used to rinse the substrates and wells. Adherent cells were stained with 2.5 μM calcein-AM in complete PBS for 1 hour at 37 ºC, after which the substrates were transferred to a new culture plate with fresh media and imaged using a Leica epifluorescent microscope (W. Nuhsbaum Inc., McHenry, IL) equipped with a SPOT RT digital camera (Diagnostics Instruments, Sterling Heights, MI). After imaging, substrates were transferred to a new culture plate with fresh DMEM media with 10% FBS that was pre-equilibrated at 37ºC and 5% CO_2. Fibroblast cell suspension was seeded at a density of 2.6 x 10^3 cells/cm^2 and placed back into the incubator until the next time point. Four images (40X magnification) were taken from random locations on each substrate and three identical substrates for each experiment were analyzed for statistical purposes, resulting in a total of 12 images per time point for each modification. The microscopy images were quantified using threshold and area analysis in ImageJ64.

Bacterial cell attachment on polymer-coated substrates

For all bacterial experiments, aseptic techniques were used. *E. coli* were streaked from frozen stocks onto LS agar and incubated overnight at 37°C with moderate shaking. A few colonies were then used to inoculate 50 ml of sterile LB broth, and grown overnight at 37°C. *S. epidermidis* and *P. aeruginosa* were cultured in the same manner but using different agar or broth. For *S. epidermidis,* tryptic soy agar and tryptic soy broth (TSB) was used whereas R2A agar and TB agar were used for *P. aeruginosa* culture. TiO_2 substrates (11 x 7 mm^2) were modified with the polymers as previously described, sterilized by exposure to UV light for 30 minutes in a 24-well cell culture plate, and equilibrated with culture medium. The bacterial suspension was diluted to 1x10^8 CFU/ml with 0.85% NaCl in U.P. H_2O, seeded on modified and unmodified TiO_2 substrates (1 ml each for all three bacterial strains), and placed in a humidified incubator at 37 ºC; After 24 or 96 hours, the bacterial suspensions were carefully removed by aspiration without disturbing adherent bacteria. The attached bacteria were then stained with 4 μL/mL Syto 9 and propidium iodide each for 15 minutes at 37ºC, briefly rinsed with 0.85% NaCl to remove excess propidium iodide, and visualized using a Leica epifluorescent microscope (W. Nuhsbaum Inc., McHenry, IL) equipped with a SPOT RT digital camera (Diagnostics Instruments, Sterling Heights, MI) using appropriate filters. Four images (40X magnification) were

taken from random locations on each substrate and three identical substrates for each experiment were analyzed for statistical purposes, resulting in a total of 12 images per time point for each modification. The microscopy images were quantified using threshold analysis and area analysis in ImageJ64.

Numerical Methods

All-atom molecular dynamics (MD) simulations were carried out for glycopeptoid 20mer chains grafted on a TiO_2 surface. The simulated systems were prepared by generating 16 glycopeptoid (M20Glu(OH) or M20-Mal(OH)) chains in a hexagonal array on (110) surface of a non-hydroxylated rutile TiO_2 crystal. The surface chain density of the system was 0.65 nm^{-2}. From experimental measurement, a slight fibrinogen adsorption occurred on M20Glu(OH) at ~0.65 nm^{-2}, whereas no protein adsorption has occurred on M20Mal(OH). Thus, this chain density was chosen to investigate the effects and mechanism of oligosaccharides on antifouling performance by comparison with theoretical analysis. The surface grafting of each glycopeptoid chain was implemented by fixing the z-coordinate of a hydrogen atom in the catechol hydroxyl functional group of the terminal DOPA residue during simulation. Explicit water molecules modeled by the SPC water potential filled the simulation box, and 32 Cl^- ions were added to neutralize the net positive charge from the protonated Lys residues.

The system was first energetically stabilized by the steepest descent algorithm, which was followed by a 100 ns simulation for production at temperature 300 K at constant volume on GROMACS package, version 4.5.4.[6] The positions of the Ti and O atoms in the TiO_2 surface were constrained to remain fixed in the simulations. The OPLS force field parameters for peptides,[7] peptoids[8] and carbohydrates[9] were used to model the glycopeptoid chains, and the formal charges of the triazole linker were assigned in close reference to those of 1,2,4-triazole.[10] The atomic formal charges and the Lennard-Jones parameters of Ti and O atoms in the surface were taken from Kang et al.[11]

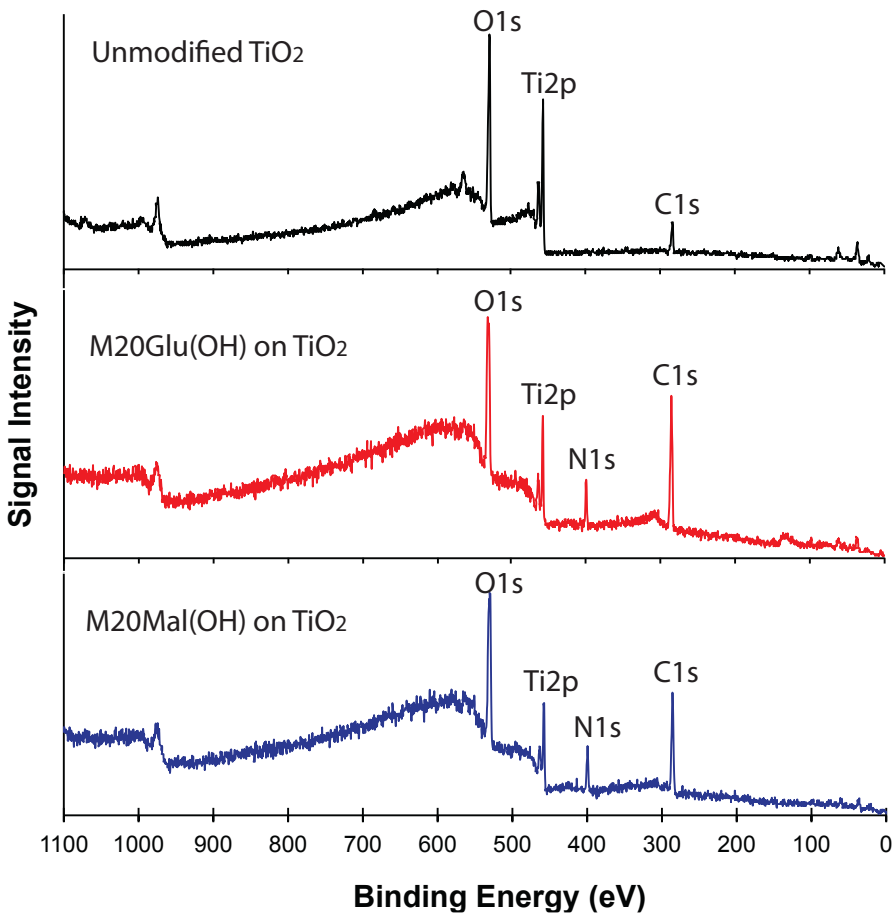
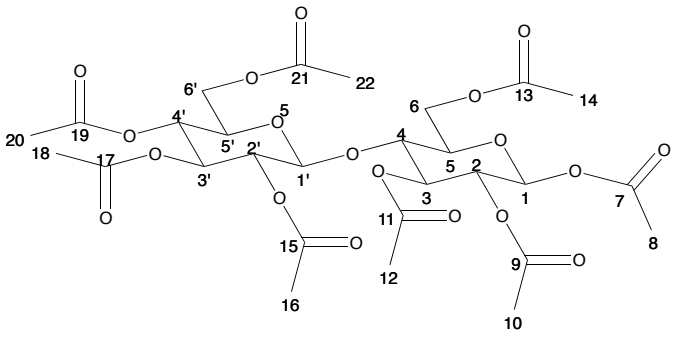


Figure S1. XPS survey scan spectra of unmodified (top), M20Glu(OH)- (middle), and M20Mal(OH)- (bottom) modified TiO_2 substrates.

Mal(OAc) : α-D-maltopyranoside octaacetate (2)



^{1}H NMR (500 MHz, Chloroform-d): δ ppm 5.79 – 5.73 (d, *J* = 8.2 Hz, 1H), 5.45 – 5.27 (m, 3H), 5.12 – 5.04 (t, *J* = 9.9 Hz, 1H), 5.03 – 4.96 (dd, *J* = 9.2, 8.2 Hz, 1H), 4.91 – 4.84 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.51 – 4.44 (dd, *J* = 12.3, 2.5 Hz, 1H), 4.29 – 4.21 (m, 2H), 4.10 – 4.02 (m, 2H), 3.99 – 3.92 (m, 1H), 3.91 – 3.82 (ddd, *J* = 9.6, 4.3, 2.5 Hz, 1H), 2.20 – 1.97 (m, 24H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ ppm 170.60, 170.56, 170.48, 170.10, 169.92, 169.64, 169.48, 168.85, 95.69, 91.24, 77.30, 77.24, 77.04, 76.79, 75.25, 72.96, 72.33, 70.91, 69.98, 69.27, 68.56, 67.89, 62.49, 61.41, 20.90, 20.85, 20.72, 20.64, 20.62, 20.58.

ESI – MS: calcd for $C_{28}H_{42}O_{19}N$ $[M+NH_4^+]^+$, 696.20 ; found, 696.10

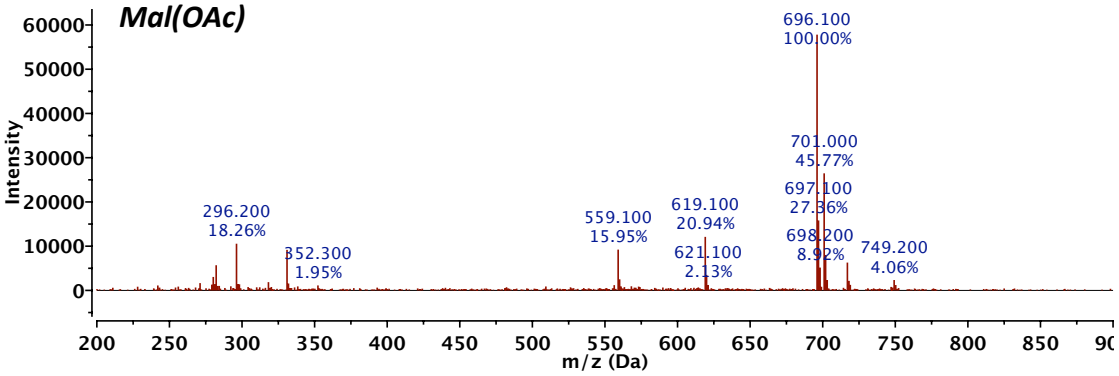


Figure S2. Mass spectrum of α-D-maltopyranoside octaacetate (2).

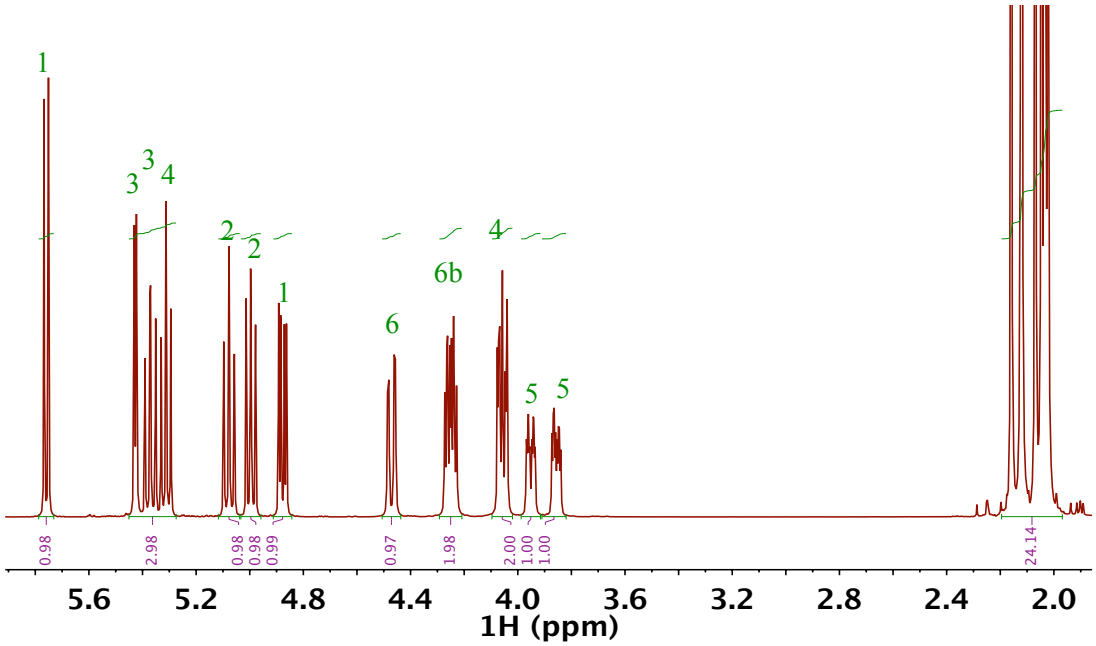


Figure S3. [1]H NMR spectrum of α-D-maltopyranoside octaacetate (2).

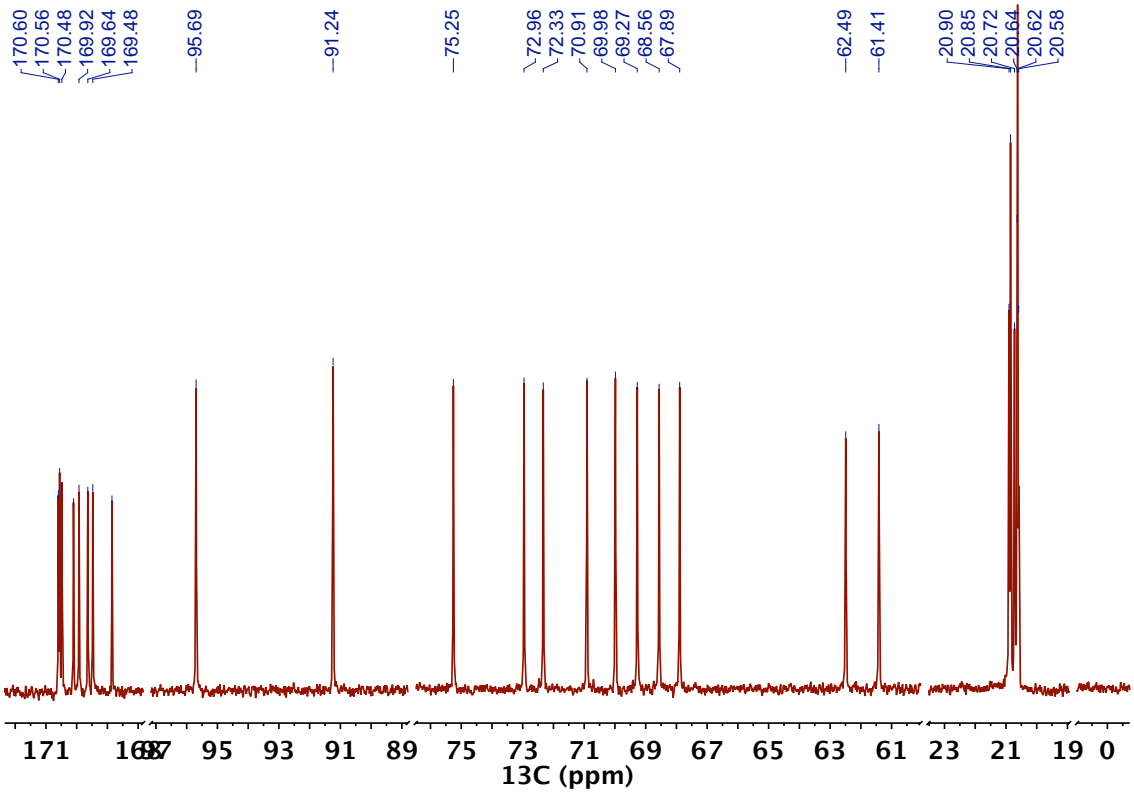
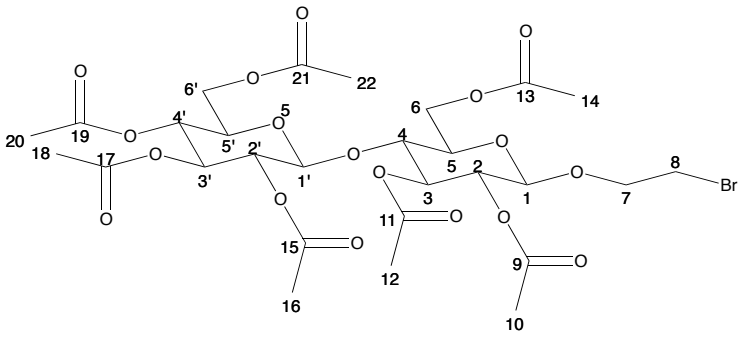


Figure S4. 13C NMR spectrum of a-D-maltopyranoside octaacetate (2).

Mal(OAc)-Br: 2'-bromoethyl-β-D-maltopyranoside heptaacetate (3)



^{1}H NMR (500 MHz, Chloroform-d): δ ppm 5.42 (d, *J* = 4.0 Hz, 1H), 5.36 (dd, *J* = 10.5, 9.4 Hz, 1H), 5.27 (dd, *J* = 9.6, 9.2 Hz, 1H), 5.06 (dd, *J* = 10.5, 9.4 Hz, 1H), 4.86 (dd, *J* = 10.5, 4.1 Hz, 1H), 4.85 (dd, *J* = 9.3, 7.9 Hz, 1H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.50 (dd, *J* = 12.1, 2.8 Hz, 1H), 4.26 (dd, *J* = 12.5, 4.0 Hz, 1H), 4.23 (dd, *J* = 12.1, 4.5 Hz, 1H), 4.13 (ddd, *J* = 11.2, 6.0, 5.1 Hz, 1H), 4.05 (dd, *J* = 12.7, 2.2 Hz, 1H), 4.01 (dd, *J* = 9.3, 9.2 Hz, 1H), 3.95 (ddd, *J* = 10.5, 4.0, 2.2 Hz, 1H), 3.82 (ddd, *J* = 11.3, 7.5, 6.1 Hz, 1H), 3.70 (ddd, *J* = 9.6, 4.5, 2.8 Hz, 1H), 3.47 (ddd, *J* = 10.3, 6.0, 5.1 Hz, 1H), 3.44 (ddd, *J* = 10.3, 7.5, 6.1 Hz, 1H), 2.23 - 1.94 (m, 21H).

^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.56, 170.55, 170.46, 170.20, 169.98, 169.72, 169.43, 100.47 (C-1), 95.50 (C-1'), 77.02, 75.14 (C-3), 72.46 (C-4), 72.21 (C-5), 71.86 (C-2), 69.95 (C-2'), 69.79 (C-7), 69.27 (C-3'), 68.48 (C-5'), 67.93 (C-4'), 62.61 (C-6), 61.44 (C-6'), 29.87 (C-8), 20.91, 20.86, 20.73, 20.72, 20.71, 20.63, 20.60, 20.59.

ESI – MS: calcd for $C_{28}H_{39}BrNaO_{18}$ $[M+Na^+]+$, 767.13; found, 767.10

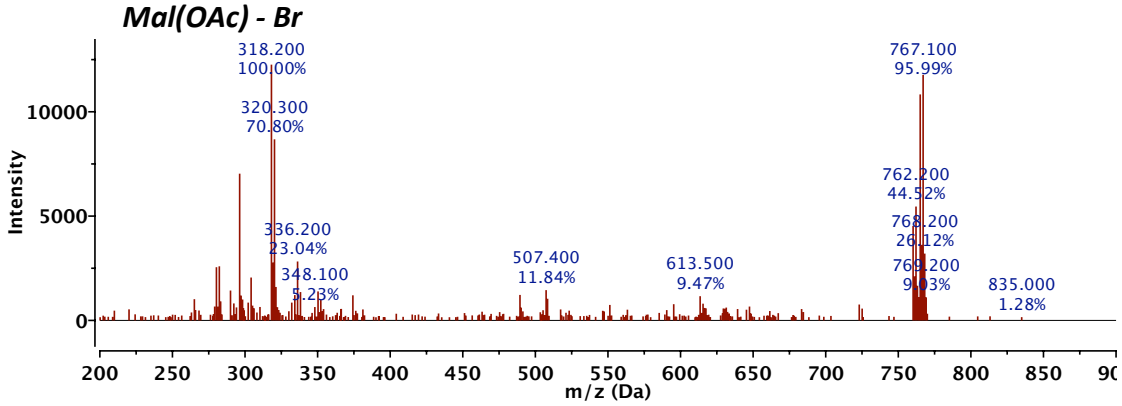


Figure S5. Mass spectrum of 2'-bromoethyl-β-D-maltopyranoside heptaacetate (3).

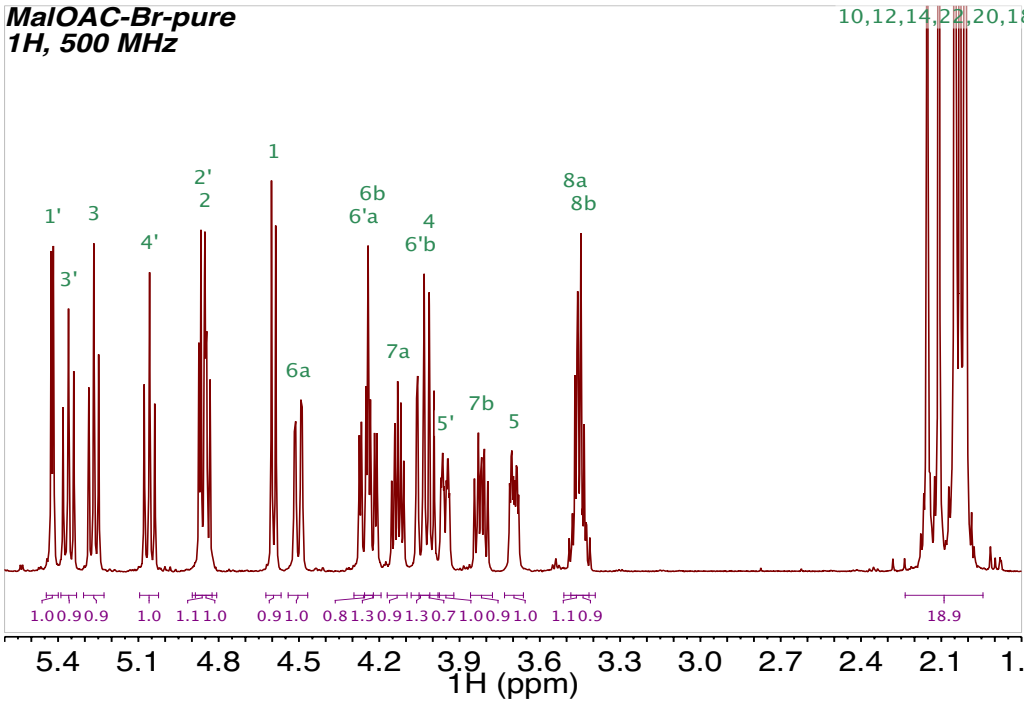


Figure S6. [1]H NMR spectrum of 2'-bromoethyl-β-D-maltopyranoside heptaacetate (3).

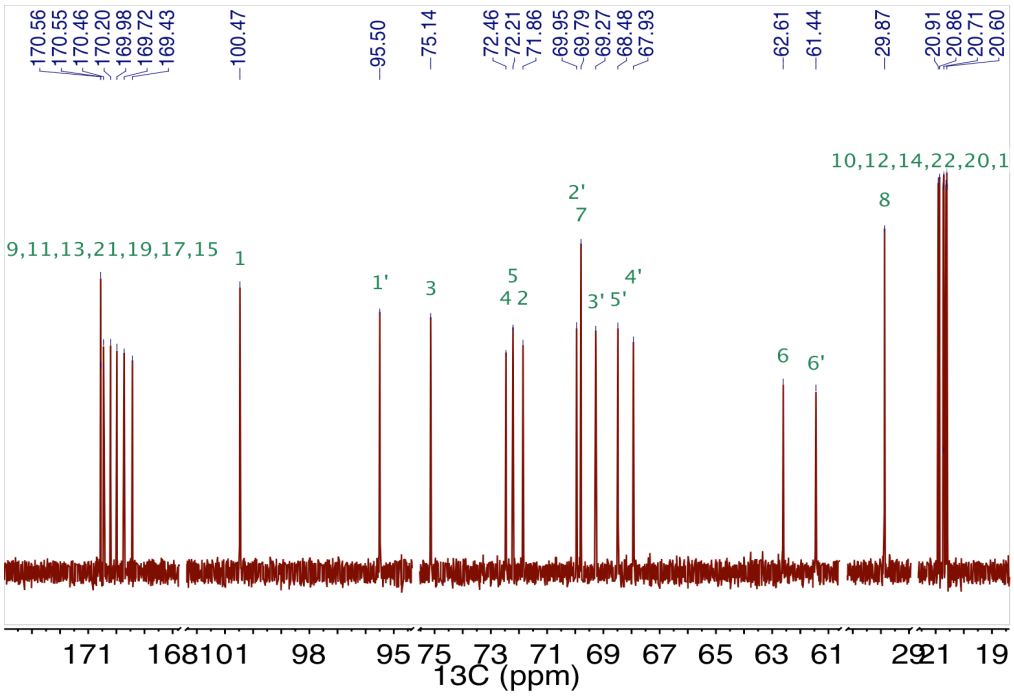
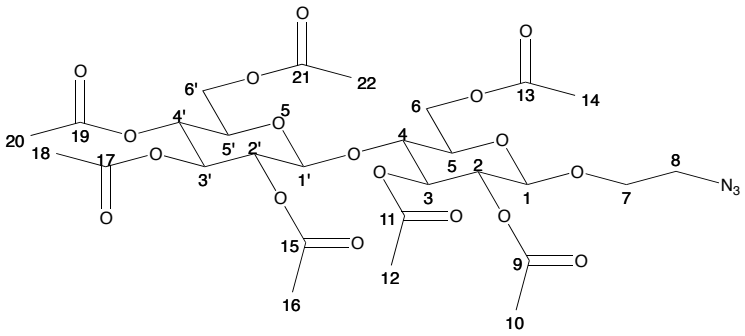


Figure S7. [13]C NMR spectrum of 2'-bromoethyl-β-D-maltopyranoside heptaacetate (3).

Table S1. ^{1}H and ^{13}C NMR atom assignments for Mal(OAc)-Br

No	^{1}H-Chemical Shift (ppm)	Multiplicity (*J* value)	^{3}C- Chemical Shift (ppm)
1'	5.422	d,*J*=4.1 Hz	95.499
3'	5.361	dd,*J*=10.5,9.4 Hz	69.278
3	5.267	dd,*J*=9.6,9.2 Hz	75.124
4'	5.058	dd,*J*=10.5,9.4 Hz	67.939
2'	4.859	dd,*J*=10.5,4.1 Hz	69.952
2	4.850	dd,*J*=9.3,7.9 Hz	71.857
1	4.595	d,*J*=7.9 Hz	100.469
6	4.503,4.225		62.605
6'	4.258,4.046		61.445
7	4.131,3.819		69.792
4	4.014	dd,*J*=9.2 Hz	72.462
5'	3.954	ddd,*J*=10.5,4.0,2.2 Hz	68.483
5	3.696	ddd,*J*=9.6,4.5,2.8 Hz	72.213
8	3.469,3.437		29.870
10	2.060	m	20.719
12	2.060	m	20.719
14	2.060	m	20.719
22	2.060	m	20.719
20	2.060	m	20.719
18	2.060	m	20.719
16	2.060	m	20.719
9		m	170.129
11		m	170.129
13		m	170.129
21		m	170.129
19		m	170.129
17		m	170.129
15		m	170.129

Mal(OAc)-N_3: 2'-azidoethyl-β-D-maltopyranoside heptaacetate (4)



^{1}H NMR (500 MHz, Chloroform-d): δ ppm 5.43 (d, *J* = 4.0 Hz, 1H), 5.36 (dd, *J* = 10.5, 9.5 Hz, 1H), 5.26 (t, *J* = 9.1, 9.1 Hz, 1H), 5.06 (t, *J* = 9.9, 9.9 Hz, 1H), 4.86 (dd, *J* = 10.5, 4.1 Hz, 1H), 4.86 (dd, *J* = 9.2, 7.6 Hz, 1H), 4.62 (d, *J* = 7.9 Hz, 1H), 4.53 (dd, *J* = 12.2, 2.7 Hz, 1H), 4.26 (dd, *J* = 12.5, 3.8 Hz, 1H), 4.22 (dd, *J* = 12.2, 4.3 Hz, 1H), 4.05 (dd, *J* = 12.2, 2.2 Hz, 1H), 4.03 (t, *J* = 9.5, 9.2 Hz, 2H), 4.02 (ddd, *J* = 11.0, 5.0, 3.4 Hz, 0H), 3.96 (ddd, *J* = 10.2, 3.8, 2.3 Hz, 1H), 3.70 (ddd, *J* = 9.5, 4.3, 2.8 Hz, 1H), 3.70 (ddd, *J* = 11.0, 8.3, 3.1 Hz, 1H), 3.49 (ddd, *J* = 13.4, 8.3, 3.3 Hz, 1H), 3.27 (ddd, *J* = 13.4, 4.9, 3.2 Hz, 1H), 2.19 - 1.97 (m, 19H).

^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.56, 170.54, 170.46, 170.25, 169.99, 169.73, 169.42, 100.18 (C-1), 95.50 (C-1'), 75.32 (C-3), 72.43 (C-4), 72.20 (C-5), 71.92 (C-2), 69.96 (C-2'), 69.28 (C-3'), 68.76 (C-7), 68.46 (C-5'), 67.94 (C-4'), 62.53 (C-6), 61.43 (C-6'), 50.46 (C-8), 20.91, 20.86, 20.71, 20.69, 20.63, 20.60, 20.58.

ESI – MS: calcd for $C_{28}H_{39}N_3O_{18}Na$ [M+Na^+]-, 728.22; found, 728.20

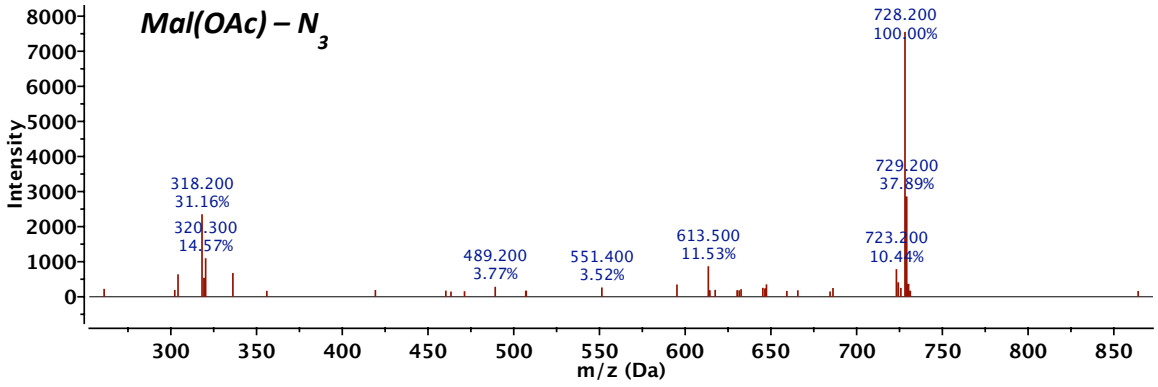


Figure S8. Mass spectrum of 2'-azidoethyl-β-D-maltopyranoside heptaacetate (4).

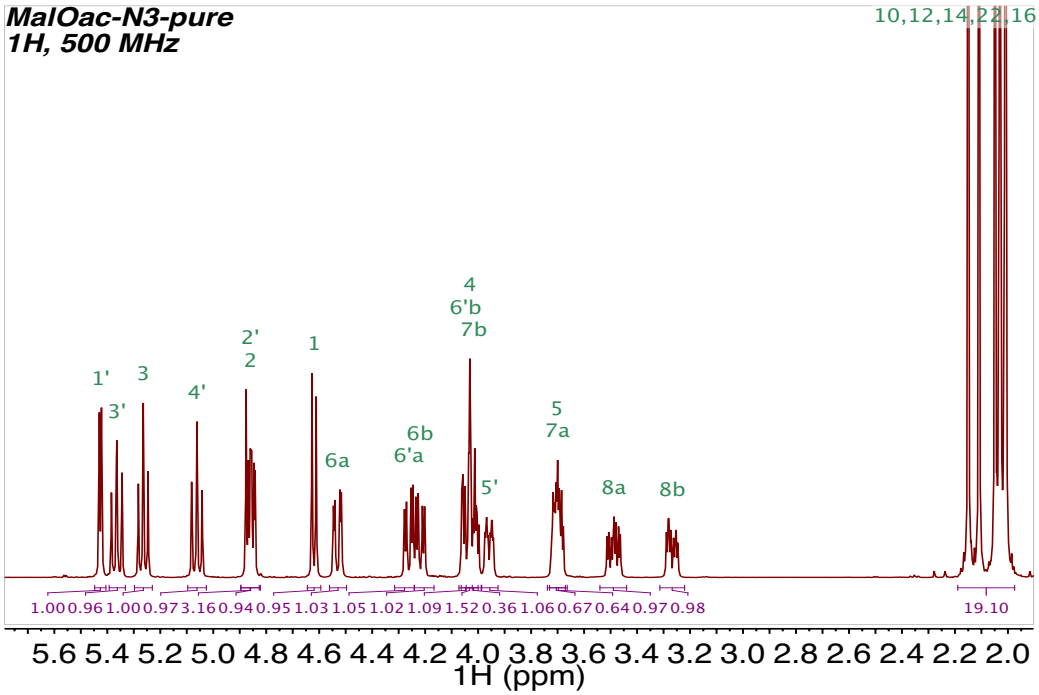


Figure S9. [1]H NMR spectrum of 2'-azidoethyl-β-D-maltopyranoside heptaacetate (4).

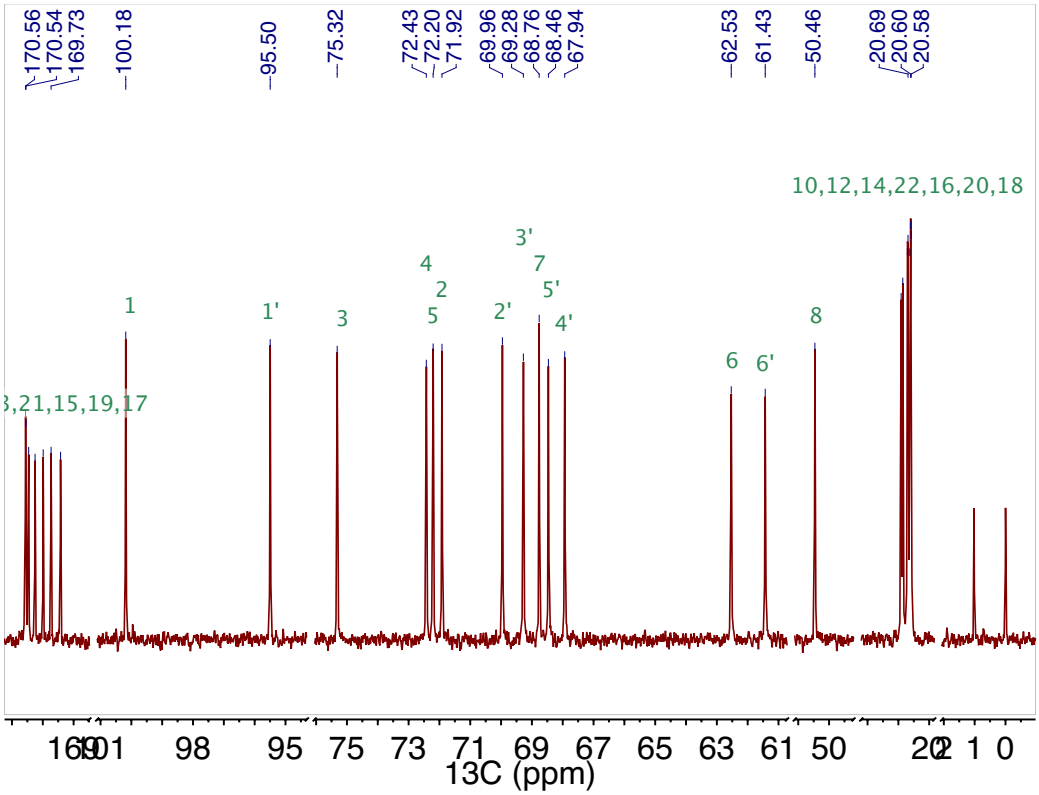
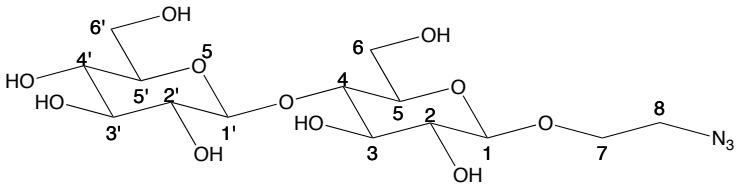


Figure S10. [13]C NMR spectrum of 2'-azidoethyl-β-D-maltopyranoside heptaacetate (4).

Table S2. ^{1}H and ^{13}C NMR atom assignments for Mal(OAc)-N_3

No	^{1}H-Chemical Shift (ppm)	Multiplicity (*J* value)	^{13}C- Chemical Shift (ppm)
1'	5.43	d,*J*=3.97 Hz	95.50
3'	5.36	dd,*J*=10.53,9.52 Hz	69.27
3	5.26	t,*J*=9.12 Hz	75.14
4'	5.06	t,*J*=9.86 Hz	67.96
2'	4.86	dd,*J*=9.19,7.64 Hz	69.96
2	4.86	dd,*J*=9.19,7.64 Hz	71.92
1	4.62	d,*J*=7.87 Hz	100.05
6	4.53,4.22		62.50
6'	4.26,4.05		61.43
4	4.03	t,*J*=9.50 Hz	72.42
5'	3.96	ddd,*J*=10.25,3.83,2.32 Hz	68.39
5	3.70	ddd,*J*=9.50,4.30,2.80 Hz	72.20
7	3.70,4.02		68.76
8	3.49,3.27		50.43
10	2.07	m	20.71
12	2.07	m	20.71
14	2.07	m	20.71
22	2.07	m	20.71
16	2.07	m	20.71
20	2.07	m	20.71
18	2.07	m	20.71
9			170.14
11			170.14
13			170.14
21			170.14
15			170.14
19			170.14
17			170.14

Mal(OH)-N_3: 2'-azidoethyl-β-D-maltopyranoside (5)



^{1}H NMR (500 MHz, DMSO-d6): δ ppm 5.48 (d, *J* = 5.5 Hz, 1H), 5.15 (d, *J* = 6.2 Hz, 1H), 5.01 (d, *J* = 3.8 Hz, 1H), 4.93 (s, 2H), 4.62 - 4.45 (m, 2H), 4.24 (d, *J* = 7.8 Hz, 1H), 3.89 (ddd, *J* = 11.0, 5.9, 4.2 Hz, 1H), 3.71 (dd, *J* = 11.8, 1.5 Hz, 1H), 3.65 (ddd, *J* = 10.9, 6.0, 4.5 Hz, 1H), 3.61 (d, *J* = 10.1 Hz, 1H), 3.55 (dd, *J* = 11.8, 5.4 Hz, 1H), 3.51 – 3.46 (m, 1H), 3.47 – 3.43 (m, 2H), 3.45 – 3.39 (m, 1H), 3.41 (dd, *J* = 9.4, 8.0 Hz, 1H), 3.37 – 3.32 (m, 3H), 3.30 (dd, *J* = 9.7, 8.6 Hz, 1H), 3.24 (ddd, *J* = 9.7, 5.4, 1.9 Hz, 1H), 3.21 – 3.19 (m, 0H), 3.06 (t, *J* = 9.3, 9.3 Hz, 1H), 3.02 (t, *J* = 8.3, 8.3 Hz, 1H), 1.23 (d, *J* = 4.4 Hz, 0H).

^{13}C NMR (126 MHz, DMSO-d6) : δ ppm 102.82 (C-1), 100.84 (C-1'), 79.64 (C-4), 76.46 (C-3), 75.28 (C-5), 73.52 (C-5'), 73.29 (C-3'), 72.96 (C-2), 72.45 (C-2'), 69.85 (C-4'), 67.48 (C-7), 60.77 (C-6'), 60.66 (C-6), 50.38 (C-8).

ESI – MS: calcd for $C_{14}H_{25}N_3O_{11}Na$ [M + Na]+, 434.15; found, 434.20

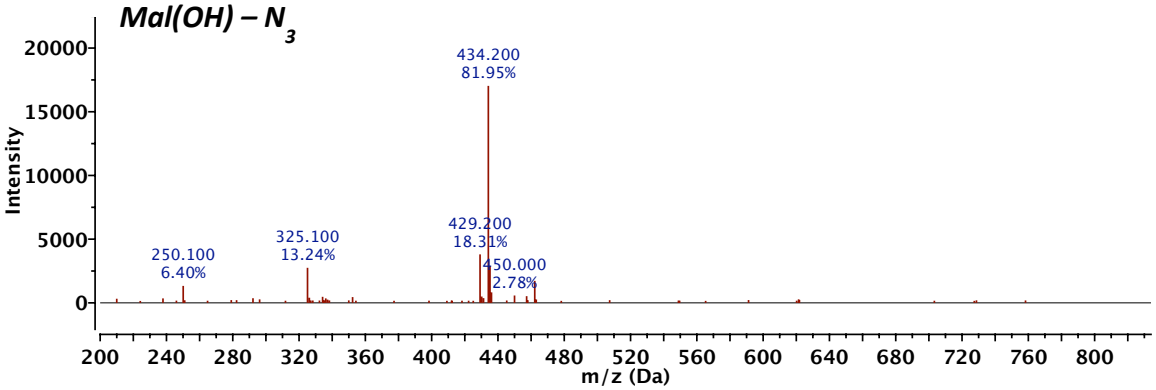


Figure S11. Mass spectrum of 2'-azidoethyl-β-D-maltopyranoside (5).

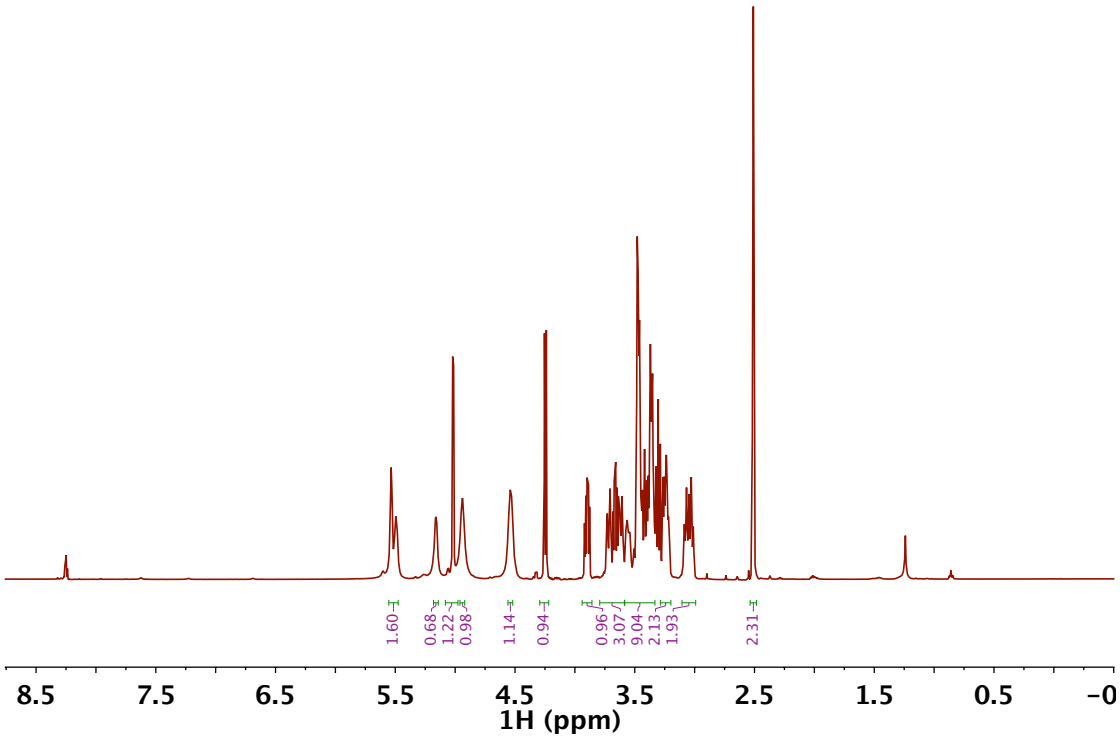


Figure S12. ^{1}H NMR spectrum of 2'-azidoethyl-β-D-maltopyranoside (5)

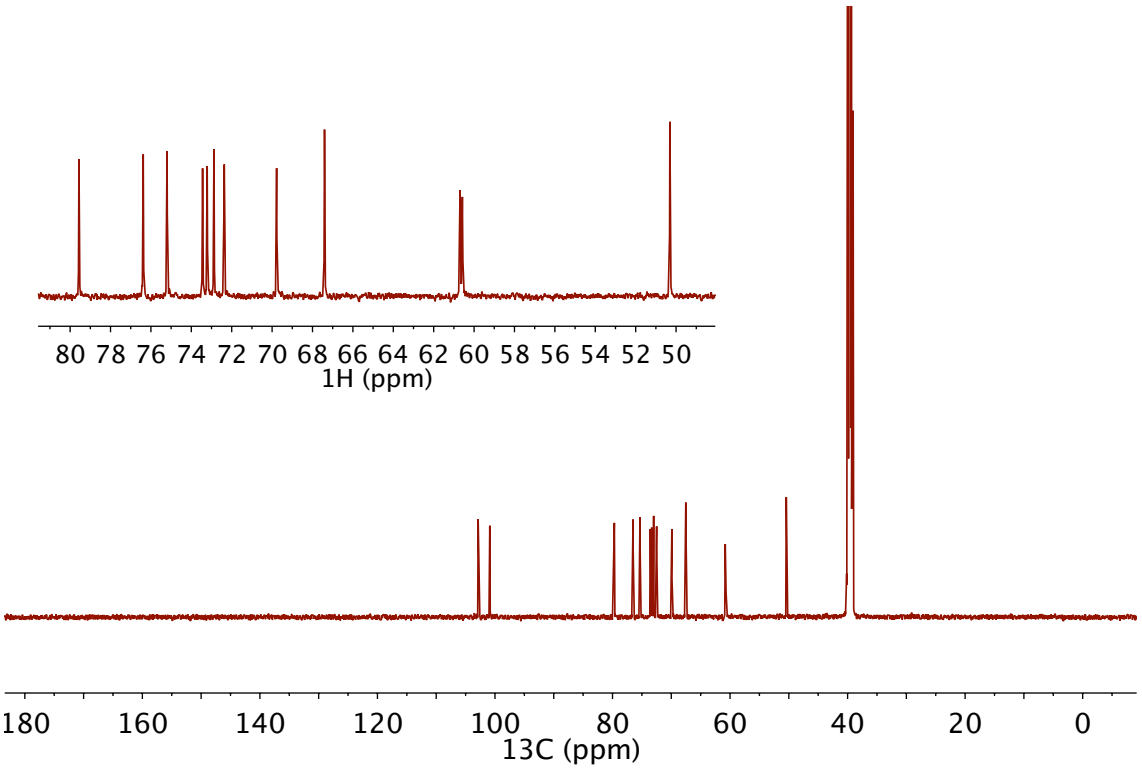


Figure S13. ^{13}C NMR spectrum of 2'-azidoethyl-β-D-maltopyranoside (5).

Table S3. ^{1}H and ^{13}C NMR atom assignments for Mal(OH)-N_3

No	^{1}H-Chemical Shift (ppm)	Multiplicity (*J* value)	^{13}C- Chemical Shift (ppm)
62	5.48	d,*J*=5.53 Hz	
52	5.15	d,*J*=6.15 Hz	
1'	5.01	d,*J*=3.79 Hz	100.84
1	4.24	d,*J*=7.78 Hz	102.82
7	3.89,3.65		67.51
6	3.71,3.55		60.66
6'	3.61,3.46		60.77
5'	3.47	m	73.52
8	3.47	m	50.38
3	3.41	dd,*J*=9.37,8.03 Hz	76.46
3'	3.36		73.29
4	3.30	dd,*J*=9.67,8.60 Hz	79.63
5	3.24	ddd,*J*=9.70,5.43,1.90 Hz	75.28
2'	3.22		72.45
4'	3.06	t,*J*=9.28 Hz	69.85
2	3.02	t,*J*=8.33 Hz	72.96

APPENDIX

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