Supporting Online Material for:

Chemoenzymatic Elaboration of Monosaccharides Using Engineered Cytochrome $P450_{BM3}$ Demethylases

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Materials and Methods

Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers (Sigma, Aldrich, Fluka, Wako Chemicals, Toronto Research, Carbosynth, Fischer Scientific, MP Biomedicals, TCI America) and used without purification. Solvents used in the synthesis of substrates were obtained from a Seca Solvent System by GlassContour (solvent dried over alumina under a N_2 atmosphere). Chemical reactions were prepared in flame or oven-dried glassware under an inert N_2 atmosphere using either syringe or cannula techniques. Bioconversions were conducted open to the atmosphere in large crystallization dishes with stirring provided by a magnetic stir bar/plate. Silica gel chromatography purifications were carried out using AMD Silica Gel 60, 230-400 mesh. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on either a Varian Mercury 300 spectrometer (300 MHz, 75 MHz, and 282 MHz, respectively), a Bruker DRX-600 spectrometer equipped with a CryoProbe (600 MHz and 150 MHz for ^{1}H and ^{13}C), or a Varian Inova 600 spectrometer (600 MHz and 150 MHz for ^{1}H and ^{13}C), and are internally referenced to residual solvent peak. Data for ^{1}H NMR are reported in the conventional

form: chemical shift (δ ppm), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad), coupling constant (Hz), integration. Data for ^{13}C and ^{19}F NMR are reported in the terms of chemical shift (δ ppm) and multiplicity. High-resolution mass spectra were obtained with a JEOL JMS-600H High Resolution Mass Spectrometer at the California Institute of Technology Mass Spectral Facility or an Agilent 6210 Time of Flight Mass Spectrometer at The Scripps Research Insititue Center for Mass Spectrometry. Reactions were monitored using thin layer chromatography (Merck 60 silica gel plates) using a 5% aqueous H_2SO_4 solution and heat as visualizing agent.

Gas chromatography (GC) analyses were carried out using a Shimadzu GC-17A gas chromatograph, a FID detector, and an Agilent HP5 column (30 m x 0.32 mm x 0.1 μm film). GC-MS analyses were carried out on a Hewlett-Packard 5970B MSD with 5890 GC and a DB-5 capillary column. For permethylated monosaccharides: Start at 35 °C and hold for 10 min, then ramp at 5 °C/min to 150 °C and hold for 5 min, then ramp at 30 °C/min to 300 °C and hold for 5 min. For other compounds: start at 100 °C, hold for 2.5 min, then ramp at 10 °C/min to 168 °C, hold 2 min, then ramp at 0.1 °C/min to 171 °C, hold for 1 min, then 25 °C. min to 300 °C.

Complete analytical data and synthesis procedures have been reported in the literature for: 1,2,3,4,6-pentamethyl-α-glucopyranoside (**1**),[1] 1,2,3,4,6-pentamethyl-α-galactopyranoside (**2**),[2] 1,2,3,4,6-pentamethyl-α-mannopyranoside (**3**),[3] 1-benzyl-2,3,4,6-tetramethyl-β-glucopyranoside (**4**),[4] 1,2,3,4,6-pentamethyl-β-galactopyranoside (**5**),[5] 1-acetyl-2,3,4,6-tetramethyl-β-galactopyranoside (**8**).[6]

Plasmid pCWori[BM3] was used as cloning vector. Oligonucleotides were purchased from Integrated DNA Technologies (IDT, San Diego, CA), and the sequences of the primers used in this study are reported below. Electro-competent *Escherichia coli* cells were prepared following the protocol of Sambrook et al.[7] Restriction enzymes *Bam*HI and *Sac*I, Phusion polymerase, and T4 ligase were purchased from New England Biolabs (NEB, Ipswich, MA). Alkaline phosphatase was obtained from Roche (Nutley, NJ). AmpliTaq was purchased from Applied Biosystems (Foster City, CA). The modified trace metal mix used in expression cultures was reported by Studier.[8]

General Procedures

Enzyme Library Screening. Libraries maintained in our laboratory, including the compilation library and the active site library, are stored at -78 °C as glycerol stocks (Luria-Bertani medium (LB_{amp})), 150 μL, 20% v/v LB/glycerol with 0.1 mg/mL ampicillin) in 96-well plates. These stocks were used to

inoculate 96-well deep-well plates containing 300 μL LB_{amp} medium using a 96-pin stamp. Single colonies from error prone PCR or recombination libraries were picked by a Qpix robot (Genetix, Beaverton, OR) and used to inoculate 300 μl of LB_{amp}. The cells were incubated at 37 °C, 225 rpm, and 80% relative humidity over night. After 16 h, 50 μL aliquots of these over night cultures were transferred into 2 mL, deep-well plates containing terrific broth (TB_{amp}) (800 μL containing 0.1 mg/mL ampicillin and 1 μL/mL trace metal mix) using a Multimek 96-channel pipetting robot (Beckman Coulter, Fullerton, CA). The cultures were incubated at 37 °C for 4 h, and 30 min after reducing the incubation temperature to 25°C (225 rpm and 80% relative humidity), 50 μL isopropyl β-D-1-thiogalactopyranoside (IPTG, 4.5 mM in TB_{amp}) was added, and the cultures were allowed to continue for another 24 h at 25 °C (225 rpm and 80% relative humidity). Cells were then pelleted (3,000xg, 15 min, 4 °C) and stored at -20 °C until further use but at least for 2 h. For cell lysis, plates were allowed to thaw for 30 min at room temperature and then cell pellets were resuspended in 300 μL phosphate buffer (0.1 M, pH = 8, 0.75 mg/mL lysozyme and 40 U/mL DNase I). The lysing cells were incubated at 37 °C for 1 h, and then the cell debris was separated by centrifugation at 5,000xg and 4 °C for 15 min. The resulting crude lysates were then transferred to 96-well microtiter plates for assay.

High Throughput Colorimetric Assay. BM3 variants in cell lysate (40 μL) were diluted with phosphate buffer (200 μL final reaction volume, 0.1 M, pH = 8). The appropriate substrate (1 mM final substrate concentration) in either phosphate buffer (0.1 M, pH = 8) or DMSO (final concentration no greater than 2.5% v/v) was added, and the reactions were initiated by addition of a solution of NADPH in phosphate buffer (0.5 mM final NADPH concentration, 0.1 M phosphate buffer, pH = 8). After 2 h, the reactions were quenched by addition of 50 μL Purpald solution (0.16 M in 2M NaOH), and the absorbance of the mixtures at 550 nm was measured after 30-45 min using a plate reader.[9]

CO Binding Assay. BM3 variants in cell lysate (40 μL) were diluted with 60 μL phosphate buffer (0.1 M, pH = 8). To this solution was added 100 μL sodium dithionite (0.3 M in phosphate buffer, 1 M, pH = 8). The absorbance at 450 and 490 nm was recorded using a plate reader, and the microtiter plates were placed in a vacuum chamber. The chamber was sealed, evacuated to approximately -15 in Hg, purged with CO gas, and incubated for 20 min. The plates were then removed and the aborbance at 450 and 490 nm was again recorded using a plate reader. The difference spectra could then be used to determine the P450 concentration in each well as previously reported.[10]

Site-Directed Mutagenesis. Base pair mutations were introduced into the genes coding for variants 9-10A F87I, 2C6, E12, and B1 by site directed overlap extension (SOE) PCR. For each mutation, two separate PCRs were performed, each using a perfectly complementary flanking primer at the 5' and 3' end of the sequence and a mutagenic primer. The PCR conditions were as follows: Phusion HF buffer 1x, 0.2 mM dNTPs each, 0.5 μM forward primer, 0.5 μM reverse primer, and 0.02 U/μl Phusion polymerase. The resulting two overlapping fragments that contained the base pair substitution were then assembled in a second PCR using the flanking primers resulting in the full length mutated gene. For: F87Idr, 2C6dr, E12r12, B1SYN:

#	Primer Name	Sequence
1	dr_C47R_for	5' - CGA GGC GCC TGG TCG TGT AAC GCG CTA C - 3'
2	dr_C47R_rev	5' - GTA GCG CGT TAC ACG ACC AGG CGC CTC G - 3'
3	dr_I94K_for	5' - GCT GGA CGC ATG AAA AAA ATT GGA AAA AAG CG - 3'
4	dr_I94K_rev	5' - CGC TTT TTT CCA ATT TTT TTC ATG CGT CCA GC - 3'
5	E12r12_V111A_fwd	5' - TAG TCA GCA GGC AAT GAA AGG CTA TCA - 3'
6	E12r12_I141V_fwd	5' - TGA TAG CCT TTC ATT GCC TGC TGA CTA - 3'
7	E12r12_V111A_rev	5' - GAG CAT ATT GAA GTA TCG GAA GAC ATG - 3'
8	E12r12_I141V_rev	5' - CAT GTC TTC CGA TAC TTC AAT ATG CTC - 3'
9	B1SYN_R47S_fwd	5' - AGG CGC CTG GTA GTG TAA CGC GCT ACT - 3'
10	B1SYN_N70Y_fwd	5' - CAC GCT TTG ATA AAT ACT TAA GTC AAG CCT TG - 3'
11	B1SYN_I174N_fwd	5' - CAG CCT CAT CCA TTT AAT ATA AGT ATG GTC - 3'
12	B1SYN_R47S_rev	5' - AGT AGC GCG TTA CAC TAC CAG GCG CCT - 3'
13	B1SYN_N70Y_rev	5' - CAA GGC TTG ACT TAA GTA TTT ATC AAA GCG TG - 3'
14	B1SYN_I174N_rev	5' - GAC CAT ACT TAT ATT AAA TGG ATG AGG CTG - 3'
15	Heme Fwd1	5' - CAG GAA ACA GGA TCA GCT TAC TCC CC - 3'
16	Heme Rev1	5' - CGC GCC GTT CCT TCA GCT GTT CCC - 3'

Radom Mutagenesis. A library of 2C6dr mutants was created by error prone PCR using the GeneMorph II Random Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer's protocol, using 75 ng template DNA (2C6dr) to generate approximately 2.5 nucleotide (nt)

mutations/gene and flanking primers HF1 and HR2. After transforming electro-competent *E. coli* DH5α cells with the desalted ligation mixture, cells were allowed to recover in SOC medium (37 °C, 45 min), plated then onto LB_{amp} agar plates (6.25 g LB powder mix, 4 g agar, 250 mL DDI water, 0.1 mg/mL ampicillin), and incubated at 37 °C for 16 h. Approximately 3,000 clones from this library were picked as previously described.

Variants B1, D3, A8, A11, and E12 were obtained from this library. Site directed mutagenesis using SOE PCR was then carried out as described above in order to introduce N70Y, I174N, and R47S into the gene encoding B1 to create variant B1SYN. Similarly, A111V and V141I were reverted in E12 in order to create E12R12, and F87V was introduced in order to create E12r12 F87V.

#	Variant	Mutation
1	B1	I263M
2	D3	N70Y
3	A8	I174N
4	A11	R47S, W96R
5	E12	A111V, V141I, A180V

A library of E12r12 F87V mutants was created by error prone PCR using AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA) and varying amounts of $MnCl_2$ to introduce the desired error rate. The following PCR conditions gave the best fraction folded while introducing 2.5 nt exchanges per gene: 1x reaction buffer, 0.4 mM of each dNTP, 4 U AmpliTaq, 7 mM $MgCl_2$, 0.15 mM $MnCl_2$, 0.2 mM of each primer HF1 and HR2. The transformed cells were plated on LB_{amp} agar plates as decribed above, and approximately 3,600 variants were picked. Variants 3E11, 7F3, 9C7, 32E11, and 33A8 were obtained from this library.

#	Variant	Mutation
1	3E11	F158L
2	7F3	V87A, K210M, H266Q
3	9C7	V87G, Y345C
4	32E11	V87A, K241R, N283Y
5	33A8	N70Y, M112T

Recombination of Active Variants. Mutations F158L, K210M, H266Q, Y345C, K241R, N283Y, N70Y, and M112T were recombined with 9C7 as template. The recombination library was constructed using SOE PCR introducing the eight mutations found in the five variants while allowing for the 9C7 sequence as well. Nine fragments were generated using either a combination of a flanking primer and a mutagenic primer or two mutagenic primers. Fragments 1-3, 4-6, and 7-9 were assembled in three separate reactions, and the resulting fragments 1', 2', and 3' were assembled in a final PCR step.

After transforming electro-competent *E. coli* DH5α cells with the desalted ligation mixture, cells were allowed to recover in SOC medium (37 °C, 45 min), plated then onto LB_{amp} agar plates (6.25 g LB powder mix, 4 g agar, 250 mL DDI water, 0.1 mg/mL ampicillin), and were incubated at 37 °C for 16 h. Approximately 1,000 clones from this library were picked as previously described from which variant 5E5 was obtained.

#	Primer Name	Sequence
1	N70recfwd	CAC GCT TTG ATA AAW ACT TAA GTC AAG CCT T
2	N70recrev	AAG GCT TGA CTT AAG TWT TTA TCA AAG CGT G
3	M112recfwd	TAG TCA GCA GGC AAY GAA AGG CTA TCA T
4	M112recrev	ATG ATA GCC TTT CRT TGC CTG CTG ACT A
5	F158recfwd	GGT CTT TGC GGC TTT KAC TAT CGC TTT AAC
6	F158recrev	GTT AAA GCG ATA GTM AAA GCC GCA AAG ACC
7	K210recfwd	GTC AAG AAG ATA TCA WGG TGA TGA ACG ACC TA
8	K210recrev	TAG GTC GTT CAT CAC CWT GAT ATC TTC TTG AC
9	K241recfwd	GAT GCT AAA CGG AAR AGA TCC AGA AAC G
10	K241recrev	CGT TTC TGG ATC TYT TCC GTT TAG CAT C
11	H266recfwd	TTA ATT GCG GGA CAK GAA ACA ACA AGT
12	H266recrev	ACT TGT TGT TTC MTG TCC CGC AAT TAA
13	N283recfwd	TAT TTC TTA GTG AAA WAT CCA CAT GTA TTA CAA
14	N283recrev	TTG TAA TAC ATG TGG ATW TTT CAC TAA GAA ATA
15	Y345recfwd	GCT TGG AGG AGA ATR CCC TTT AGA AAA AG
16	Y345recrev	CTT TTT CTA AAG GGY ATT CTC CTC CAA GC

Summary of enzymes utilized for synthesis of monosaccharide derivatives.

Enzyme	Amino acid mutations compared to wildtype $P450_{BM3}$
9-10A[11]	R47C, V78A, K94I, P142S,T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
2C6	9-10A A78L F87A V184T G315S A330V
2C6dr	9-10A C47R A78L F87A I94K V184T G315S A330V
E12	2C6dr A111V V141I A180V
E12r12	2C6dr A180V
B1	2C6dr I263M
B1SYN	B1 R47S N70Y I174N
9-10A F87Idr	9-10A R47S F87I I174N
9-10A F87Vdr	9-10A R47S F87V I174N
9C7	E12r12 A87G Y345C
5E5	9C7 N70Y N159D K241R H266Q C345Y

P450 Expression and Purification. For the chemo-enzymatic transformations, P450BM3 enzyme variants were used in purified form. Enzyme batches were prepared as follows. Two liters TB_{amp} were inoculated with an overnight culture (100 mL, LB_{amp}) of recombinant *E. coli* DH5α cells harboring a pCWori plasmid encoding for the P450 variant under the control of Plac promoter. At an OD_{600} of 1.8 (ca. 3-4 h), the incubation temperature was reduced to 25˚C (30 min), and the cultures were induced by adding IPTG to a final concentration of 0.1 mM. The cultures were allowed to continue for another 24

hours at this temperature. After harvesting the cells by centrifugation (4 °C, 15 min, 3000xg), the cell pellet was resuspended in 25 mM TRIS-HCl buffer (pH 8.0) and cells were disrupted by sonication (4x1min, 50% duty cycle). Cell debris was removed by centrifugation for 20 min at 4 °C and 20000xg and the resulting cell lysate was loaded onto a Q resin and the column was washed with three column volumes (cv) of 25 mM TRIS-HCl (pH 8.0), 150 mM NaCl. Bound protein was eluted with 25 mM TRIS-HCl (pH 8.0), 340 mM NaCl and concentrated using Millipore Centricon tubes. After buffer exchange with 100 mM KPi (pH 8.0), protein samples were frozen and stored at -80 °C. Protein concentration was determined in duplicate from CO-difference spectra as previously described. Yields typically ranged between 100 and 500 mg protein per liter depending on the variant.

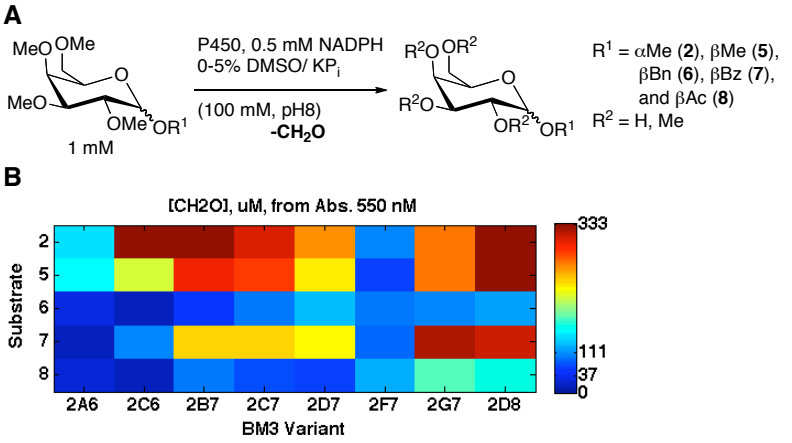
Typical Procedure for Medium-Scale Bioconversions. To a well of a 24-well plate was added potassium phosphate buffer (100 mM, pH 8, 0.5-1 mL final reaction volume) and a solution of the desired substrate in phosphate buffer or DMSO (1 equiv, 1 mM final concentration). A solution of $NADP^+$ (0.5 equiv, 0.5 mM final concentration), glucose-6-phosphate (40 equiv, 40 mM final concentration), glucose-6-phosphate dehydrogenase (2 units/mL final reaction volume), and superoxide dismutase (100 units/mL final reaction volume) was added. A solution of the appropriate BM3 variant (1 μM final concentration) was added, the plate was loosely fitted with its lid, and the plate was shaken at 35 rpm. Following the desired reaction time, a 0.4 mL aliquot was removed and combined with 0.2 mL $CHCl_3$ containing approximately 1 mM tetradecane. The mixture was thoroughly vortexed and the resulting suspension was centrifuged at 10,000 rpm for 1 min. The organic phase was transfered to a GC vial and analyzed by GC.

Typical Procedure for Preparative-Scale Bioconversions. To a 100 x 50 mm crystallizing dish (ie. ChemGlass #CG-8276-100) was added potassium phosphate buffer (100 mM, pH 8) and a solution of the desired substrate in phosphate buffer or DMSO (1 equiv, 5 mM final concentration). A solution of $NADP^+$ (0.1 equiv, 0.5 mM final concentration), glucose-6-phosphate (10 equiv, 50 mM final concentration), and glucose-6-phosphate dehydrogenase (2 units/mL final reaction volume) was added. A solution of the appropriate BM3 variant (0.001 equiv, 5 μM final concentration) was added, the dish was loosely covered with aluminum foil, and stirred using a magnetic stir bar. The moderate stir rate was utilized to allow efficient O_2 transfer to the reaction mixture while avoiding foaming. The reaction progress could be monitored by analyzing aliquots of the reaction mixture as described for the medium scale bioconversions. After 12 h, the reaction mixture was transferred to a separatory funnel. The

mixture was extracted with 4 x 75 mL CH_2Cl_2. Emulsions formed following agitation of the separatory funnel and complete phase separation was not typically observed. The mixture was allowed to sit approximately 5-10 min after each agitation in order to obtain a distinct boundary between the aqueous phase and the emulsion. The combined emulsion layers were then filtered through Celite to break the emulsion and the Celite pad was rinsed with 3x20 mL CH_2Cl_2. The resulting biphasic mixture was transfered to a separatory funnel and the organic phase was removed. The remaining aqueous phase was re-extracted with 3x10 mL CH_2Cl_2. The combined organic extracts were dried with sodium sulfate, filtered, and concentrated. The resulting residue was purified by SiO_2 chromatography eluting with ethylacetate/hexanes.

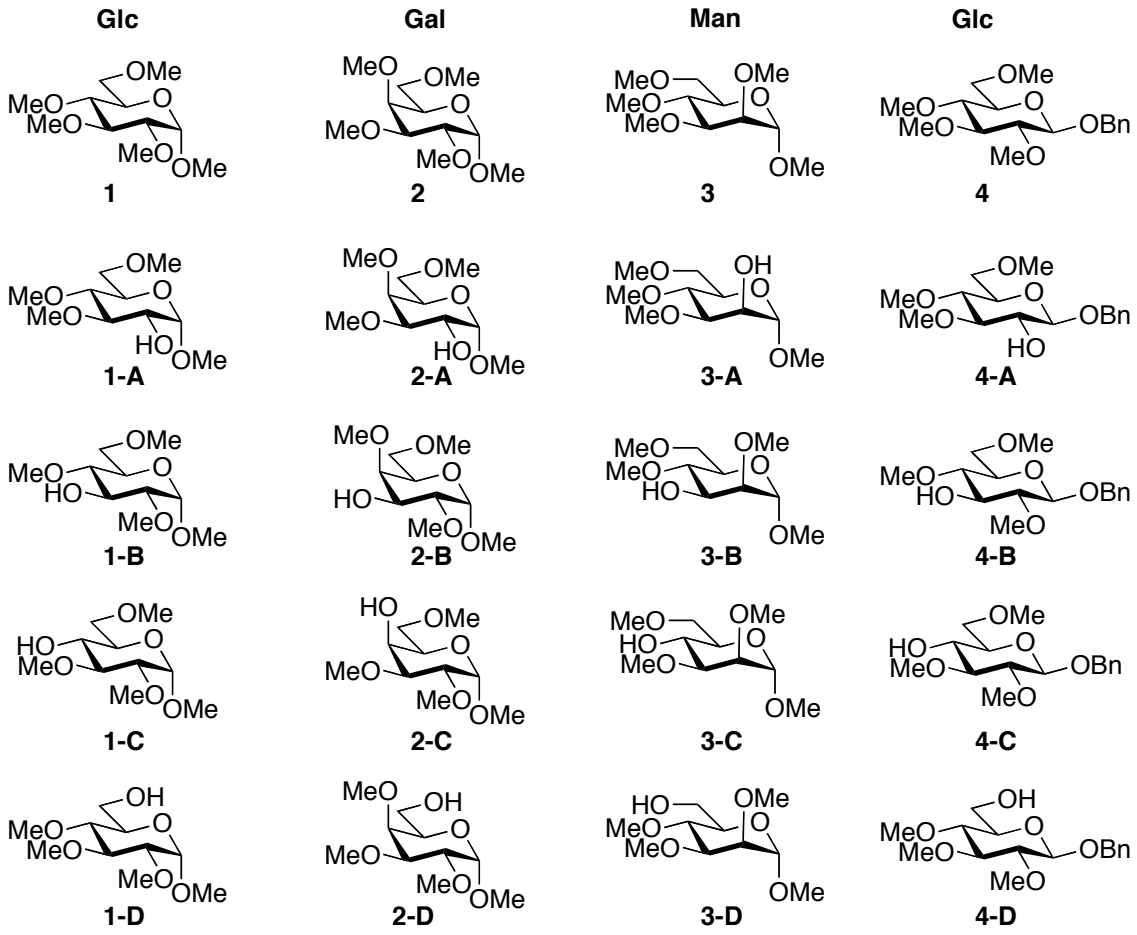
We have subsequently found that the workup procedure can be greatly simplified by adjusting the pH of the reaction mixture to <4 and saturating the solution with brine. This has the effect of precipitating most of the protein from solution and improving the efficiency for the extraction of the hydrophilic monosaccharide product from the mixture. The resulting suspension is then filtered through Celite, which is rinsed with methylene chloride in order to provide a biphasic mixture. The organic phase can then be cleanly separated, and the aqueous phase can be extracted without any complication from emulsions.

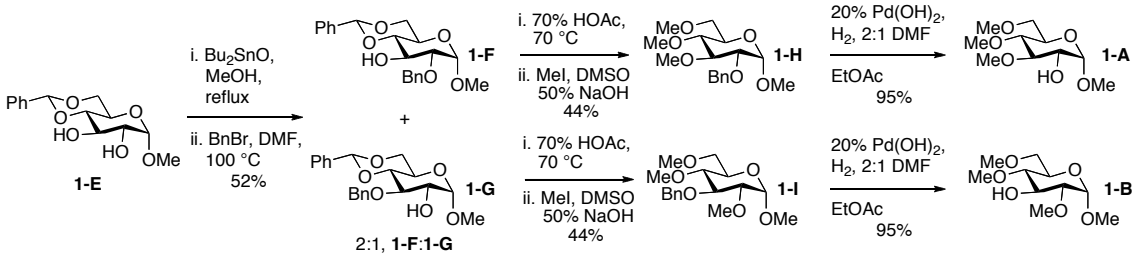
Colorimetric analysis of formaldehyde produced from various galactose substrates



(A) Reaction conditions used for colorimetric analysis. **(B)** [CH_2O] determined by colorimetric analysis of CH_2O-purpald adduct at 550 nm relative known concentrations of this species within the linear range of the assay. 1 μM (0.1 mol%) BM3 variant used.

Synthesis of Substrates and Standards for Initial Screens





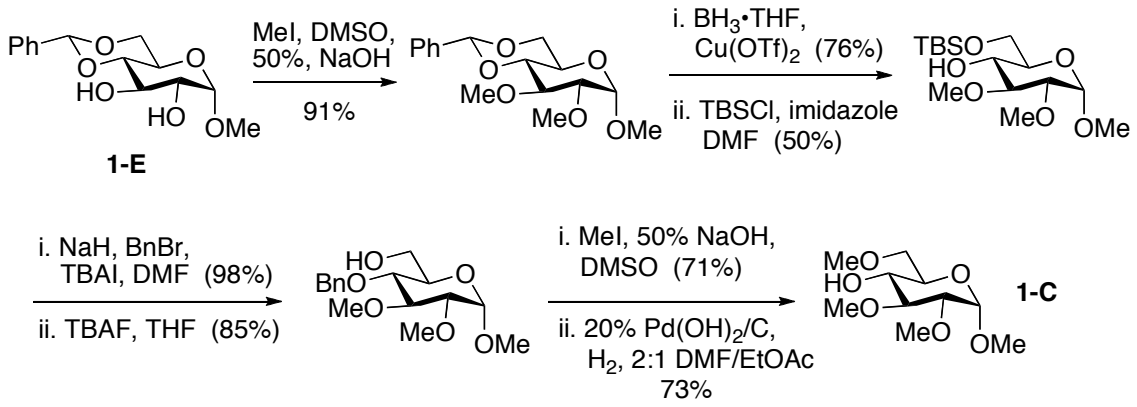
α-methyl-3,4,6-triemthylglucopyranoside (1-A). 1-Methyl-4,6-O-(phenylmethylene)-α-D-glucopyranoside, **1-E**, (200 mg, 0.7 mmol) and Bu_2SnO (192 mg, 760 mmol) were suspended in 6 ml MeOH and heated to reflux. After 1.5 h, the solution was cooled to room temperature and concentrated. The resulting oil was pumped on under high vacuum for 2.5 h, then dissolved in 6 ml DMF, treated with benzyl bromide (166 μl, 1.4 mmol) and heated to 100 °C overnight. In the morning, the reaction was cooled to room temperature and poured into a mixture of ethyl acetate (10 ml) and saturated $NaHCO_3$ (5 ml). The layers were separated, and the organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 20%-30% ethyl acetate/hexanes)

afforded compounds **1-F** (90 mg, 35%) and **1-G** (46 mg, 17%), which were taken forward without any further purification.

Compound **1-F** (81 mg, 0.2 mmol) was suspended in 2 ml 70% aqueous HOAc and heated to 70 °C overnight. In the morning, the reaction was cooled to room temperature, concentrated and coevaporated with toluene (3x). The resulting residue was dissolved in 0.77 ml DMSO and treated with 0.06 ml 50% aqueous NaOH and 0.09 ml MeI. After 3 h the reaction was diluted with water and extracted with ethyl acetate (3X). The pooled organics were washed with H_2O and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The resulting residue was passed through a silica gel column, eluting with 1:1 ethylacetate:hexanes to afford, after removal of the solvent, 32 mg of an oil. This oil was dissolved in 0.6 ml DMF and 0.3 ml ethyl acetate, treated with 19 mg 20% $Pd(OH)_2/C$ and placed under an H_2 atmosphere. After 14 h the reaction was treated with 0.15 mL ethyl acetate, 2 drops HOAc and 8 mg 20% $Pd(OH)_2/C$. After stirring for an additional 10 h the reaction was filtered through Celite and concentrated. Flash column chromatography (SiO_2 7:3 ethyl acetate:hexanes) afforded compound **1-A** (20 mg, 95%) ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.75 (d, *J* = 3.5 Hz, 1H), 3.64 (s, 3H), 3.60-3.54 (m, 4 H), 3.51 (s, 3H), 3.40 (s, 6H), 3.34 (t, *J* = 8 Hz, 1H), 3.19 (t, *J* = 8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 99.2, 84.4, 79.3, 72.3, 70.9, 70.2, 60.9, 60.3, 59.2, 55.3; HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1158.

α-methyl-2,4,6-triemthylglucopyranoside (1-B). Compound **1-G** (66 mg, 0.18 mmol) was suspended in 70% aqueous HOAc (1.5 ml) and heated to 70 °C overnight. In the morning, the reaction was cooled to room temperature and concentrated. The resulting residue was dissolved in DMSO (0.64 ml) and treated with 50% aqueous NaOH (0.07 ml) and MeI (0.07 ml). After 3 h the reaction was diluted with H_2O and extracted with ethyl acetate (3X). The pooled organic layers were washed with H_2O and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The resulting residue was passed through a silica gel plug eluting with ethyl acetate. Following removal of the solvents, the residue was dissolved in DMF (0.5 ml) and ethyl acetate (0.25 ml), treated with 20% $Pd(OH)_2/C$ and placed under a H_2 atmosphere. After 24 h, the reaction was filtered through Celite and concentrated *in vacuo*. Flash column chromatography (SiO_2, 7:3 ethyl acetate:hexanes) afforded **1-B** (16 mg, 38%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.89 (d, *J* = 3.5 Hz, 1H), 3.91 (t, *J* = 9 Hz, 1H), 3.62-3.59 (m, 3H), 3.56 (s, 3H), 3.49 (s, 3H), 3.42 (s, 3H), 3.41 (s, 3H), 3.28-3.25 (m, 1H), 3.20 (dd, *J* = 10 Hz, 3.5 Hz, 1H). ^{13}C NMR

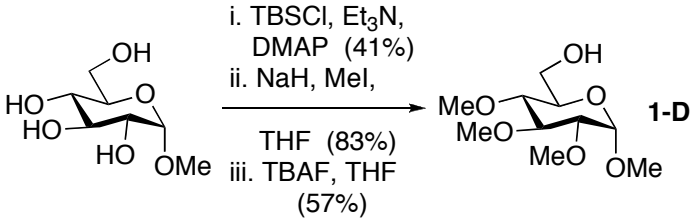
($CDCl_3$, 126 MHz): δ = 97.1, 81.7, 79.5, 73.6, 71.5, 70.1, 60.9, 59.6, 58.8, 55.7. HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1155.



α-methyl-2,3,6-triemthylglucopyranoside (1-C). Compound **1-E** (100 mg, 0.33 mmol) was placed in a sealed tube which had been purged with argon and treated with a 1 M solution of BH_3·THF in THF (1.65 ml). After 10 min the reaction was treated with $Cu(OTf)_2$ (6 mg, 16 μmol) and sonicated. After an additional 10 min the reaction was treated with 6 mg $Cu(OTf)_2$. After an additional 30 min the reaction was quenched with Et_3N, diluted with MeOH and concentrated. The resulting residue was passed through a silica plug eluting with ethyl acetate to afford a solid (59 mg, 76%). This solid was dissolved in DMF (4.6 ml) and treated with TBSCl (59 mg, 0.39 mmol) and imidazole (51 mg, 0.76 mmol). After 3 h, the reaction was diluted with ethyl acetate and washed with H_2O (2X). The washes were back extracted with ethyl acetate (2X) and the pooled organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 3:7 ethyl acetate:hexanes) afforded an oil (45 mg). This oil was dissolved in THF (1 ml) and treated with 60% NaH (30 mg, 0.75 mmol). After stirring for 1 h the reaction was treated with benzyl bromide (70 μl, 0.59 mmol) and TBAI (7 mg, 21 μl). The reaction was stirred for an additional 48 h, then cooled to 0 °C, quenched with MeOH and concentrated. The residue was dissolved in ethyl acetate, washed with H_2O (2X), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2. 1:9 ethyl acetate:hexanes) afforded an oil. The oil was dissolved in THF (1.7 ml), cooled to 0 °C and treated with a 1 M solution of TBAF in THF (0.13 ml, 0.13 mmol). After 1 h the reaction was warmed to room temperature, stirred for 1 h, then treated with 0.6 ml TBAF solution. This operation was repeated one more time, the reaction was diluted with ethyl acetate, washed with saturated aqueous NH_4Cl and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column

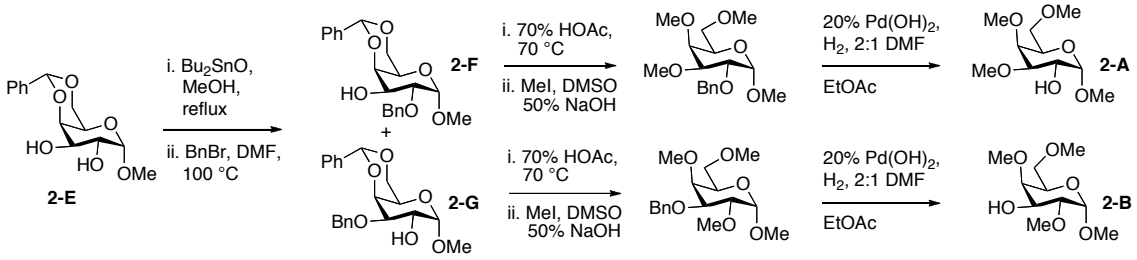
chromatography (SiO_2, 7:3 ethyl acetate:hexanes) afforded an oil (32 mg, 42%) which was carried forward without any further purification.

The oil was dissolved in DMSO (1 ml) and treated with 50% aqueous NaOH (0.1 ml) and MeI (1.2 ml). After 1 h, the reaction was diluted with H_2O and extracted with ethyl acetate (3X). The pooled organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The resulting residue (19.8 mg, 0.54 mmol) was dissolved in DMF (0.4 ml) and ethyl acetate (0.2 ml), treated with 20% $Pd(OH)_2/C$ and placed under a H_2 atmosphere. After 24 h, the reaction was treated with an additional 12 mg 20% $Pd(OH)_2/C$ and 2 drops HOAc. After an additional 4h, the reaction was filtered through Celite and concentrated. Flash column chromatography (SiO_2, 7:3 ethyl acetate:hexanes) afforded **1-C** (9.3 mg, 73%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 4.86 (d, *J* = 3.6 Hz, 1H), 3.71-3.96 (m, 1H), 3.64-3.62 (m, 2H), 3.63 (s, 3H), 3.55-3.50 (m, 1H), 3.51 (s, 3H), 3.49-3.45 (m, 1H), 3.46 (s, 3H), 3.43 (s, 3H), 3.26-3.24 (m, 1H); ^{13}C NMR ($CDCl_3$, 600 MHz): 98.4, 83.6, 82.6, 72.8, 71.5, 70.5, 62.1, 60.4, 59.4, 56.1; HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1154.



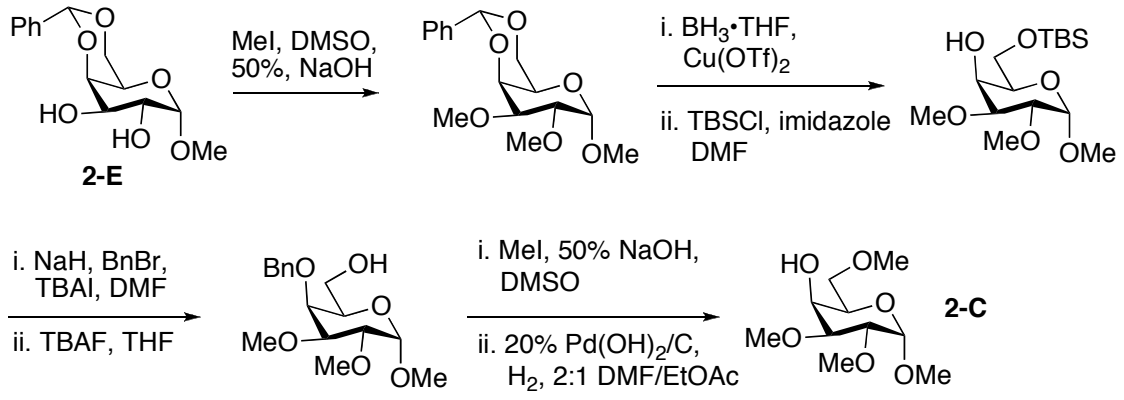
α-methyl-2,3,4-triemthylglucopyranoside (1-D). α-methyl glucose (500 mg, 2.5 mmol) was suspended in CH_2Cl_2 (5 ml) and treated with Et_3N (0.8 ml, 5.7 mmol), DMAP (31 mg, 0.25 mmol) and TBSCl (425 mg, 2.8 mmol). After stirring overnight, the solution was filtered and concentrated. The resulting residue was passed through a plug of silica gel eluting with 7:13 ethyl acetate:hexanes to afford 317 mg (41%) of an oil. This oil was dissolved in THF (14 ml), cooled to 0 °C and treated with 60% NaH (293 mg, 12.2 mmol). After 10 min, MeI (2.5 ml, 41 mmol) was added and the reaction allowed to warm to room temperature overnight. In the morning, the reaction was poured into ice cold saturated aqueous NH_4Cl solution and extracted with ethyl acetate (3X). The pooled organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was passed through a silica gel plug eluting with 1:1 ethyl acetate:hexanes and concentrated. The residue was dissolved in THF (5 ml), cooled to 0 °C and treated with a 1 M solution of TBAF (0.54 ml). The solution was stirred for 1.5 h, then treated with additional TBAF solution (0.18 ml). After an additional 2 h, the reaction was diluted with ethyl

acetate, washed with saturated aqueous NH_4Cl solution and brine, dried over Na_2SO_4 filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, ethyl acetate) afforded **1-D** (39 mg, 27%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.88 (d, *J* = 3.5 Hz, 1H), 3.92-3.88 (m, 1H), 3.84-3.78 (m, 1H), 3.72 (s, 3H), 3.64 (s, 3H), 3.63-3.58 (m, 2H), 3.59 (s, 3H), 3.69 (s, 3H), 3.26-3.21 (m, 2H); ^{13}C NMR ($CDCl_3$. 126 MHz): δ = 97.9, 83.8, 82.3, 80.1, 70.9, 62.4, 61.3, 61.0, 59.5, 55.6; HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1150.

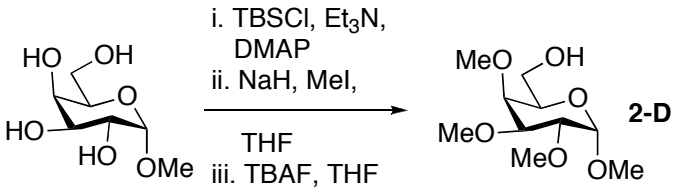


α-methyl-3,4,6-triemthylgalactopyranoside (2-A). Synthesized according to the general procedure for **1-A** from 1-Methyl-4,6-O-(phenylmethylene)-α-D-galactopyranoside, **2-E**. ^{1}H NMR ($CDCl_3$. 500 MHz): δ = 4.83 (d, *J* = 5.0 Hz, 1H), 3.99 (dd, *J* = 12.5, 5.0 Hz, 1H), 3.79 (d, *J* = 2.5 Hz, 1H), 3.59-3.55 (m, 1H), 3.57 (s, 3H), 3.54-3.51 (m, 1H), 3.52 (s, 3H), 3.44-3.41 (m, 2H) 3.43 (s, 3H), 3.41 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 99.91, 81.53, 75.45, 71.56, 69.65, 68.96, 61.52, 59.60, 58.02, 55.83. HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1157.

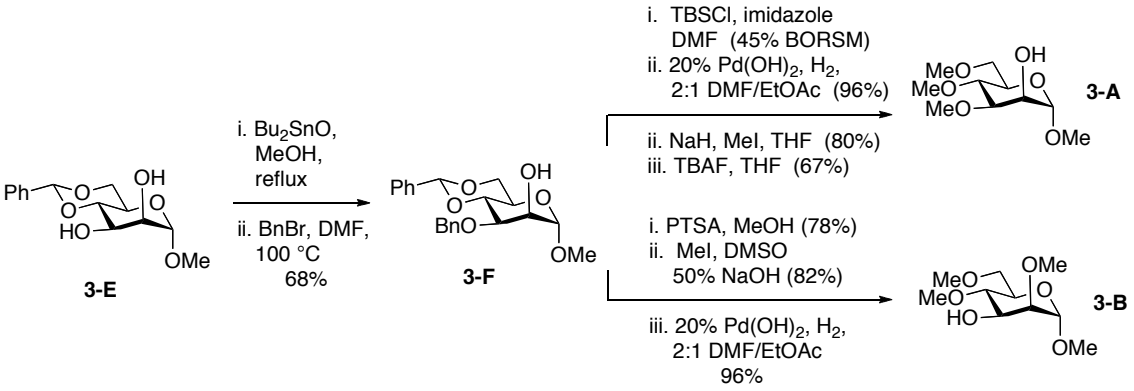
α-methyl-2,4,6-triemthylgalactopyranoside (2-B). Prepared according to the general procedure for **1-B** from **2-E**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.92 (d, *J* = 3.5 Hz, 1H), 3.98-3.94 (m, 1H), 3.91 (ddd, *J* = 6.0 Hz, 6.0 Hz, 1.0 Hz, 1H), 3.61 (m, 1H), 3.59 (s, 3H), 3.56-3.54 (m, 2H), 3.51-3.49 (m, 1H), 3.50 (s, 3H), 3.42 (s, 3H), 3.41 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 97.6, 79.4, 79.3, 71.7, 70.5, 69.3, 62.1, 59.6, 58.7, 55.8. HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1151.



α-methyl-2,3,6-triemthylgalactopyranoside (2-C). Prepared according to the general procedure for **1-C** from **2-E**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.91 (d, *J* = 3.5 Hz, 1H), 4.12 (m, 1H), 3.87 (t, *J* = 5.5 Hz, 1H), 3.69-3.64 (m, 2H), 3.60 (dd, *J* = 10 Hz, 3.5 Hz, 1H), 3.55-3.52 (m, 1H), 3.51 (s, 3H), 3.50 (s, 3H), 3.44 (s, 3H), 3.42 (s, 3H). ^{13}C NMR ($CDCl_3$, 126MHz): δ = 98.3, 79.6, 77.7, 72.8, 68.6, 67.5, 59.9, 59.4, 58.1, 55.6; HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1148.



α-methyl-2,3,4-triemthylgalactopyranoside (2-D). Prepared according to the general procedure for **1-D**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 4.92 (d, *J* = 3.6 Hz, 1H), 3.89-3.86 (m, 1H), 3.78-3.76 (m, 1H), 3.69 (m, 1H), 3.66-3.64 (m, 1H), 3.57-3.54 (m, 2H), 3.57 (s, 3H), 3.51 (s, 6H), 3.41 (s, 3H); ^{13}C NMR ($CDCl_3$, 600 MHz): δ = 98.8, 81.3, 78.7, 78.2, 71.2, 63.5, 62.2, 59.9, 59.3, 56.3; HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1157.



α-methyl-3,4,6-triemthylmannopyranoside (3-A). 1-Methyl-4,6-O-[(R)-phenylmethylene]-α-D-mannopyranoside (1.0 g, 3.0 mmol) and Bu_2SnO (810 mg, 3.3 mmol) were dissolved in MeOH (25 ml) and heated to reflux. After 1.5 h the reaction was cooled to room temperature, concentrated and pumped on under high vacuum for 2.5 h. The residue was then dissolved in DMF (25 ml), treated with benzyl bromide (720 μl, 6 mmol) and heated to reflux overnight. In the morning, the reaction was poured into ethyl acetate (50 ml) and saturated aqueous $NaHCO_3$ (25 ml) and the layers separated. The organic layer was washed with brine (2X), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column

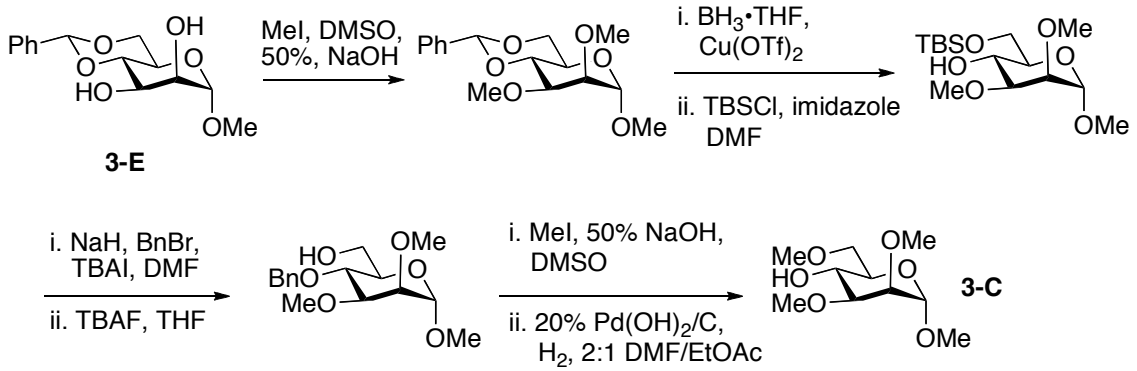
chromatography (SiO_2, 15:6 ethyl acetate:hexanes) afforded a white foam (768 mg, 68%) which was carried on without further purification.

The foam (384 mg, 1 mmol) was dissolved in DMF (19 ml) and treated with TBSCl (330 mg, 2 mmol) and imidazole (277 mg, 4 mmol). After 48 h, the reaction was diluted with ethyl acetate and washed with water (2X). The washes were back extracted with ethyl acetate (3X), and the pooled organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 1:9 ethyl acetate:hexanes) afforded an oil (79 mg, 14%) which was carried on without further purification.

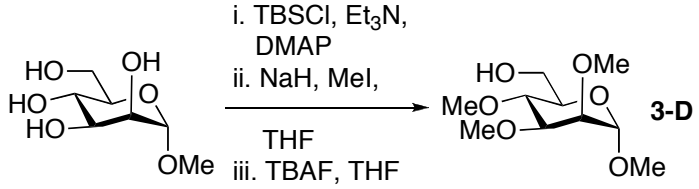
This oil (79 mg, 0.16 mmol) was dissolved in 0.81 ml DMF and 0.4 ml ethyl acetate, treated with 20% $Pd(OH)_2/C$ and placed under a H_2 atmosphere. After 48 h the reaction was filtered through Celite and concentrated. The resulting residue was passed through a plug of silica gel eluting with ethyl acetate. Following concentration, the residue was dissolved in THF (1.2 ml), cooled to 0 $^{\circ}C$, treated with 60% NaH (62 mg, 1.5 mmol) and MeI (31 μl, 5.0 mmol) and allowed to warm to room temperature overnight. In the morning, the reaction was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate (3X). The pooled organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Flash column chromatography (SiO_2, 1:1 ethyl acetate:hexanes) afforded an oil (45 mg, 80%) which was carried on without further purification.

The oil (36 mg, 0.1 mmol) was dissolved in 0.7 ml THF, treated with a 1 M solution of TBAF in THF (112 μl, 0.1 mmol) and stirred overnight. In the morning, the reaction was diluted with ethyl acetate, washed with saturated aqueous NH_4Cl and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, ethyl acetate) afforded **3-A** (16 mg, 67%) as a colorless oil. ^1H NMR ($CDCl_3$, 500 MHz): δ = 4.83 (d, *J* = 2.0 Hz, 1H), 3.81 (t, *J* = 12.0 Hz, 1H), 3.71-3.60 (m, 4H), 3.48 (s, 3H), 3.47 (s, 3H), 3.46-3.44 (m, 1H), 3.42 (s, 3H), 3.40 (s, 3H). HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1154.

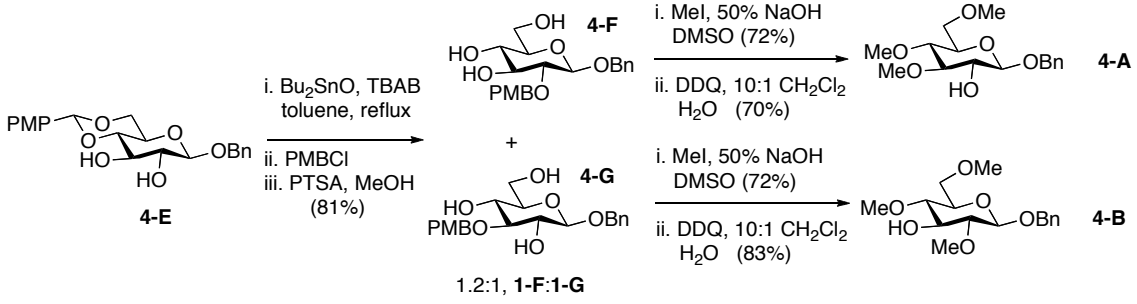
α-methyl-2,4,6-triemthylmannopyranoside (3-B). Prepared according to the general procedure for **1-B**. ^1H NMR ($CDCl_3$, 500 MHz): δ = 4.8 (d, *J* = 1.5 Hz, 1H), 3.83 (d, *J* = 8.0 Hz, 1H), 3.61 (d, *J* = 3.5 Hz, 1H), 3.58-3.54 (m, 1H), 3.55 (s, 3 H), 3.52-3.49 (m, 1H), 3.47 (s, 3H), 3.45 (dd, *J* = 4.0 Hz, 1.5 Hz, 1 H), 3.42 (s, 3H), 3.37 (s, 3H), 3.30 (t, *J* = 9.5 Hz, 1H); ^{13}C NMR ($CDCl_3$. 126 MHz): δ = 97.7, 80.7, 78.3, 71.9, 71.9, 70.8, 61.1, 59.6, 59.2, 55.3. HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1148.



α-methyl-2,3,6-triemthylmannopyranoside (3-C). Prepared according to the general procedure for **1-C**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.83 (d, *J* = 1.5 Hz, 1H), 3.81 (ddd, *J* = 9.5 Hz, 9.5 Hz, 1.5 Hz, 1H), 3.73-3.60 (m, 5H), 3.48 (s, 3H), 3.47 (s, 3H), 3.41 (s, 3H), 3.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 98.8, 81.4, 76.1, 73.1, 71.3, 67.9, 59.9, 59.4, 57.5, 55.4; HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1158.



α-methyl-2,3,4-triemthylmannopyranoside (3-D). Prepared according to the general procedure for **1-D**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.77 (d, *J* = 1.8 Hz, 1H), 3.84-3.82 (m, 1H), 3.79-3.75 (m, 1H), 3.58-3.57 (m, 1H), 3.55 (s, 3H), 3.53-3.44 (m, 3H), 3.49 (s, 6H), 3.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 600 MHz): δ = 98.1, 81.2, 77.0, 76.5, 71.8, 62.3, 60.7, 59.1, 57.7, 54.9 pp; HRMS (ESI-TOF) calcd for $C_{10}H_{20}O_6Na$: 259.1152 (M+Na), found: 259.1164.



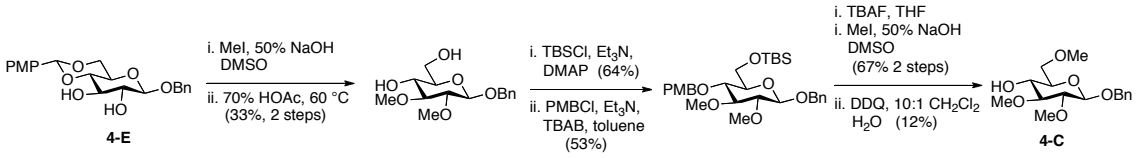
β-benzyl-3,4,6-triemthylglucopyranoside (4-A). Compound **4-E** (1.0 g, 2.6 mmol), Bu_2SnO (650 mg, 2.6 mmol) and Bu_4NBr (84 mg, 2.6 mmol) were suspended in toluene (8.8 ml) and heated to reflux. After 3 h, the solution was treated with PMBCl (0.42 ml, 3.1 mmol). After 48 h, the reaction was cooled

to room temperature, diluted with ethyl acetate, washed with H_2O and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 1:3 ethyl acetate:hexanes) afforded a mixture of 2- and 3-position isomers. This mixture was suspended in MeOH (15 ml) and treated with *p*-toluenesulfonic acid (65 mg). After 2 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and concentrated *in vacuo*. The residue was dissolved in ethyl acetate and washed with brine. The aqueous layer was back extracted with ethyl acetate (2X), and the pooled organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2 8:2 ethyl acetate:hexanes then ethyl acetate) afforded C-2 isomer **4-F** (147 mg, 26%) and C-3 isomer **4-G** (309 mg, 55%).

Compound **4-F** was dissolved in DMSO (1.35 ml) and treated with 50% aqueous NaOH (0.14 ml) and MeI (0.15 ml) and stirred overnight. In the morning, the reaction was diluted with H_2O and extracted with ethyl acetate (3X). The pooled organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in CH_2Cl_2 (3 ml) and H_2O (0.3 ml) and treated with DDQ (75 mg, 0.31 mmol). After 30 min, the reaction was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ (2X) and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2 6:4 ethyl acetate:hexanes) afforded **4-A** (60 mg, 70%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.37-7.28 (m, 5H), 4.93 (d, *J* = 12.0 Hz 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.31 (d, *J* = 8.0 Hz, 1H), 3.66 (dd, *J* = 10.5 Hz, 2.0 Hz, 1H), 3.64 (s, 3H), 3.61-3.58 (m, 1H), 3.53 (s, 3H), 3.46 (t, *J* = 8.0 Hz, 1H), 3.42 (s, 3H), 3.34-3.31 (m, 1H), 3.23 (t, *J* = 9.0 Hz, 1H), 3.19 (t, *J* = 9.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 137.0, 128.4, 128.1,.127.9, 101.5, 85.9, 79.3, 74.9, 74.0, 71.2, 70.9, 60.6, 60.3, 59.3; HRMS (ESI-TOF) calcd for $C_{16}H_{24}O_6Na$: 335.1465 (M+Na), found: 335.1466.

β-benzyl-2,4,6-triemthylglucopyranoside (4-B). Compound **4-G** (309 mg, 0.79 mmol) was dissolved in DMSO (2.74 ml) and treated with 50% aqueous NaOH (0.28 ml) and MeI (0.29 ml) and stirred overnight. In the morning, the reaction was diluted with H_2O and extracted with ethyl acetate (3X). The pooled organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in CH_2Cl_2 (6.7 ml) and H_2O (0.67 ml) and treated with DDQ (170 mg, 0.71 mmol). After 30 min, the reaction was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ (2X) and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2 6:4 ethyl acetate:hexanes) afforded **4-B** (159 mg, 83%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.37-7.32 (m, 4H), 7.30-7.27 (m, 1H), 4.95 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.38 (d, *J* = 8.0 Hz,

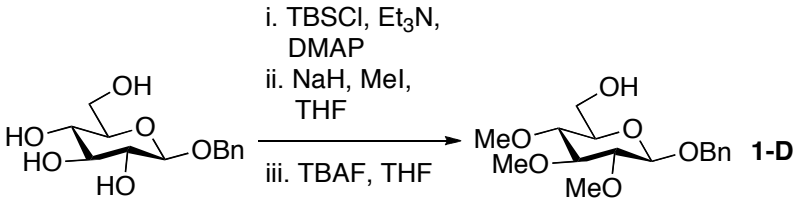
1H), 3.67 (dd, *J* = 10.5 Hz, 2.0 Hz, 1H), 3.62-3.59 (m, 1H), 3.61 (s, 3H), 3.57 (s, 3H), 3.54 (dd, *J* = 9.0 Hz, 1.5 Hz, 1H), 3.43 (s, 3H), 3.32 (ddd, *J* = 9.5 Hz, 4.5 Hz, 2.0 Hz, 1H), 3.23 (t, *J* = 9.0 Hz, 1H), 3.06 (dd, *J* = 9.0 Hz, 7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 137.4, 128.3, 127.7, 127.6, 102.1, 83.2, 79.0, 76.4, 74.7, 71.4, 70.9, 60.7, 60.5, 59.4; HRMS (ESI-TOF) calcd for $C_{16}H_{24}O_6Na$: 335.1465 (M+Na), found: 335.1465.



β-benzyl-2,3,6-triemthylglucopyranoside (4-C). Compound **4-E** (500 mg, 1.3 mmol) was dissolved in DMSO (2.1 ml) and treated with 50% aqueous NaOH (0.21 ml) and MeI (0.24 ml). After stirring overnight, the reaction was diluted with H_2O and extracted with ethyl acetate (3X). The pooled organic layers were washed with H_2O, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was dissolved in 70% aqueous HOAc (12 ml) and heated to 70 °C overnight. In the morning, the reaction was cooled to room temperature, concentrated and coevaporated with toluene (3X). The residue (172 mg) was dissolved in CH_2Cl_2 (1.5 ml), treated with Et_3N (0.18 ml, 1.3 mmol), DMAP (0.6 mg, 5 μmol) and TBSCl (97 mg, 0.64 mmol) and stirred overnight. In the morning, the reaction was filtered through Celite and concentrated. Flash column chromatography (SiO_2, 1:3 ethyl acetate:hexanes) afforded 190 mg of a foam which was used without any further purification.

The foam was dissolved in toluene (2.6 ml) and treated with KOH (30 mg, 0.5 mmol), Bu_4NBr (10 mg, 0.03 mmol) and PMBCl (0.7 ml, 0.5 mmol). After 48 h, the reaction was filtered through Celite and concentrated. Flash column chromatography (SiO_2, 1:3 ethyl acetate:hexanes) afforded 128 mg product which was used without any further purification. This product was dissolved in THF (1.6 ml) and treated with 1 M TBAF in THF (0.26 ml). After 12 h, the rxn was diluted with ethyl acetate, washed with saturated aqueous NH_4Cl and brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was dissolved in DMSO (0.82 ml) and treated with 50% aqueous NaOH (0.08 ml) and MeI (0.9 ml). After 2 h, the reaction was diluted with H_2O and extracted with ethyl acetate (3X). The pooled organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was passed through a plug of silica eluting with 1:1 ethyl acetate: hexanes and concentrated to an oil. The oil was dissolved in CH_2Cl_2 (10 ml) and H_2O (1 ml) and treated with DDQ (254 mg, 1.0 mmol). After 1 h, the reaction was diluted with

CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ (2X) and brine, dried (Na_2SO_4), filtered and concentrated. Flash column chromatography (SiO_2, 8:2 ethyl acetate:hexanes) afforded **4-C** (33 mg, 12%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.38-7.27 (m, 5H), 4.94 (d, *J* = 12.0 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.38 (d, *J* = 7.8, 1H), 3.67 (dd, *J* = 10.7, 2.0, 1H), 3.63 (s, 3H), 3.63-3.58 (m, 1H), 3.59 (s, 3H), 3.54 (dd, *J* = 9.1, 1.4, 1H), 3.42 (s, 3H), 3.41-3.38 (m, 1H), 3.15-3.09 (m, 1H), 3.06 (dd, *J* = 9.2, 7.8, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 128.76, 128.15, 128.08, 102.58, 83.57, 79.46, 76.79, 75.11, 71.78, 71.32, 61.14, 60.87, 59.80. HRMS (ESI-TOF) calcd for $C_{16}H_{24}O_6Na$: 335.1465 (M+Na), found: 335.1461.



β-benzyl-2,3,4-triemthylglucopyranoside (4-D). Starting from benzyl-β-D-glucopyranoside, **4-E**, prepared using the general procedure described for compound **1-D**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.37-7.28 (m, 5H), 4.87 (d, *J* = 12.0 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.40 (d, *J* = 7.5 Hz, 1H), 3.86 (dd, *J* = 11.5 Hz, 2.5 Hz, 1H), 3.70 (dd, *J* =12.0 Hz, 4.5 Hz, 1H), 3.62 (s, 3H), 3.60 (s, 3H), 3.55 (s, 3H), 3.24-3.19 (m, 1H), 3.18-3.13 (m, 2H), 3.06-3.03 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 137.8, 128.9, 128.3, 128.2, 103.0, 86.7, 84.3, 79.9, 75.3, 71.9, 62.5, 61.3, 61.0, 60.9; HRMS (ESI-TOF) calcd for $C_{16}H_{24}O_6Na$: 335.1465 (M+Na), found: 335.1464.

Synthesis of Monosaccharide Substrates

See materials section for references to substrates previously reported in the literature.

1-benzyl-2,3,4,6-tetramethyl-β-galactopyranoside (6). To a suspension of 0.152 g 2,3,4,6-tetramethylgalactose (0.630 mmol, 1 equiv), 0.0641 g KOH (1.14 mmol, 1.8 equiv), and 0.0067 g 18-crown-6 (0.025 mmol, 0.04 equiv) in THF (5 mL,) under N_2 was added 0.084 mL of benzyl bromide (0.70 mmol, 1.1 equiv). The mixture was allowed to stir at room temperature for 19 h, and diluted with water (50 mL). The resulting solution was extracted with 3x25 mL CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to provide a yellow oil. The crude product was purified by SiO_2 chromatography eluting with ethyl acetate hexanes to provide 0.148g (71%) **6** as a

5:1 (β/α) mixture of anomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23-7.44 (m, 7H), 5.05 (d, *J* = 3.03 Hz, 0.2H, α anomer), 4.94 (d, *J* = 12.1 Hz, 1H, β anomer), 4.72 (d, J = 12.2 Hz, 0.28H, α anomer), 4.53-4.67 (m, 1.2 H, doublet for β anomer at δ 4.62 wth *J* = 12.1 Hz), 4.35 (d, *J* = 7.6 Hz, 1H, β anomer), 3.38-3.69 (m, 24H, methyl singlets for β anomer at δ 3.42, 3.53, 3.58, and 3.61), 3.14 (dd, J_1 = 9.7 Hz, J_2 = 3.1 Hz, 1H, β anomer). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.89, 128.49, 127.86, 127.72, 102.83, 83.98, 80.90, 73.26, 75.04, 70.97, 70.82, 61.53, 61.15, 59.49, 58.64. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{17}H_{25}O_6$, 325.1651; found, 325.1636.

1-benzoyl-2,3,4,6-tetramethyl-β-galactopyranoside (7). To a solution of 0.503 g 2,3,4,6-tetramethylgalactose (2.10 mmol, 1 equiv) and 0.90 mL triethylamine (6.3 mmol, 3 equiv) in 10 mL CH_2Cl_2 was added in a drop-wise fashion 0.30 mL benzoyl chloride (2.5 mmol, 1.2 equiv). The solution was allowed to stir at room temperature for 15 h, and the reaction mixture was quenched with 50 mL water. The organic layer was removed and the aqueous layer was extracted with 3x25 mL CH_2Cl_2. The combined organic layers were dried using $MgSO_4$, filtered, and concentrated to provide a yellow oil. The crude product was purified by SiO_2 chromatography eluting with 30% ethyl acetate/hexanes to provide 0.546 g **7** (75%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 – 8.05 (m, 2H), 7.63 – 7.52 (m, 1H), 7.50 – 7.38 (m, 2H), 5.71 (d, J = 8.1, 1H), 3.78 – 3.63 (m, 4H), 3.62 (s, 3H), 3.57 (s, 6H), 3.52 (dd, J = 10.7, 5.2, 1H), 3.38 (s, 3H), 3.31 (dd, J = 9.7, 3.1, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.13, 133.73, 130.30, 129.54, 128.63, 95.14, 84.09, 79.84, 74.73, 73.97, 70.19, 61.68, 61.29, 59.42, 58.70. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{17}H_{23}O_7$, 339.1428; found, 339.1444.

1,2,3,4-tetramethoxymethyl-α-xylopyranoside (21). Initial studies using a mixture of α- and β-tetramethoxymethylxylopyranosides suggested that only the α-anomer was consumed in the bioconversion. This α/β-mixture was prepared directly from xylose and MOMCl as described below. In order to unambiguously determine the substrate specificity of this reaction, the pure α-anomer was also prepared.

Trimethylsilyl-2,3,4-tri-O-acetyl-α-D-xylopyranoside (**21-B**). To a solution of 2,3,4-tri-O-acetyl-D-xylopyranoside (2.0 g, 7.2 mmol) in dry DCM (20 mL) was added Et_3N (1.5 mL, 10.8 mmol) and then TMSCl (1.1 mL, 8.6 mmol). After 1 h, **21-A** (2.2 g, 88% of α-/β-mixture) was obtained by filtration in Al_2O_3 with DCM as solvent and used directly in the next step without any further purification.

TMSOTf (37 μL, 0.2 mmol) was added to a solution of **21-A** (1.4g, 4 mmol) in dry DCM (20 mL). The mixture was stirred at room temperature for 8 h. After addition of Et_3N (140 μL, 1.0 mmol), the mixture was dried *in vacuo* and **21-B** (1.22g, 88%) was obtained as colorless oil by flash chromatography (SiO_2 1:7 ethyl acetate:hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 (t, *J* = 9.7, 1H), 5.26 (d, *J* = 3.2, 1H), 4.91 (dd, *J* = 17.4, 8.8, 1H), 4.71 (dd, *J* = 10.0, 3.3, 1H), 3.71 (d, *J* = 8.2, 2H), 2.01 (s, 3H), 1.99 (s, 6H), 0.12 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.43, 170.40, 170.22, 90.58, 72.27, 69.90, 69.76, 58.38, 21.05 (2C), 20.97, 0.00 (3C). HRMS (ESI-TOF) calcd for $C_{14}H_{24}O_8Si$: 371.1133 (M+Na), found: 371.1145.

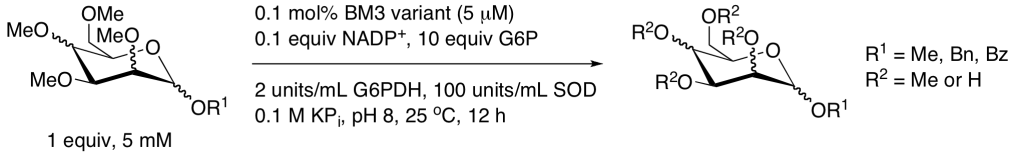
Methoxymethyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside (**21-C**) To a solution of **21-B** (800 mg, 2.3 mmol), dimethoxylmethane (1.0 mL, 11.5 mmol) and acetone (0.85 mL, 11.5 mmol) in dry DCM (10 mL) at 0 °C was added TMSOTf (40 μL, 0.23 mmol). The mixture was finished in few minute and quenched by addition of Et_3N (160 μL, 1.15 mmol). The solution was diluted with DCM (50 mL), washed with saturated $NaHCO_3$ and brine, and dried with $MgSO_4$. Concentration *in vacuo* and flash chromatography (SiO_2 2:3 ethyl acetate:hexanes) afforded **21-C** (660 mg, 90%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.51 (t, *J* = 9.8, 1H), 5.24 (d, *J* = 3.7, 1H), 4.99 (ddd, *J* = 10.6, 9.5, 6.0, 1H), 4.89 (dd, *J* = 10.3, 3.8, 1H), 4.87 (d, *J* = 6.7, 1H), 4.59 (d, *J* = 6.6, 1H), 3.82 (dd, *J* = 10.9, 6.0, 1H), 3.68 (t, *J* = 10.8, 1H), 3.40 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.27, 170.13, 170.10, 93.43, 92.73, 70.79, 69.72, 69.44, 59.07, 56.12, 20.95, 20.89, 20.80. HRMS (ESI-TOF) calcd for $C_{13}H_{20}O_9$: 343.0999 (M+Na), found: 343.1016.

Methoxymethyl α-D-xylopyranoside (**21-D**) **21-C** (200 mg, 0.625 mmol) was dissolved in 10 mM NaOMe/MeOH (3 mL). After 1h, **21-D** (120 mg, 99%) was obtained as colorless oil by chromatography (iatro beads, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29 (d, *J* = 2.4, 1H), 5.06 (d, *J* = 3.7, 1H), 4.88 (d, *J* = 6.4, 1H), 4.74 (d, *J* = 2.6, 1H), 4.64 (d, *J* = 6.4, 1H), 4.32 (d, *J* = 7.9, 1H), 3.77 – 3.48 (m, 2H), 3.43 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 95.71, 93.66, 74.58, 71.90, 70.12, 62.33, 56.42. HRMS (ESI-TOF) calcd for $C_7H_{14}O_6$: 217.0683 (M+Na), found: 217.0683.

1,2,3,4-tetra-O-methoxymethyl-α-D-xylopyranoside (**21**) DIEA (1.0 mL, 6 mmol) and MOMCl (340 μL, 4.5 mmol) were added to a solution of **21-D** (100 mg, 0.5 mmol) in DCM (5 mL) at 0 °C. The reaction was kept at room temperature overnight untill disappearance of starting material as judged by TLC. The residue was dried *in vacuo* and submit to flash chromatography (SiO_2 2:3 ethyl

acetate:hexanes) to give **21** (140 mg, 86%) as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 (d, *J* = 3.6, 1H), 4.91 (d, *J* = 6.5, 1H), 4.83 (dd, *J* = 25.0, 6.3, 2H), 4.78 (t, *J* = 6.8, 2H), 4.71 (d, *J* = 6.6, 1H), 4.67 (d, *J* = 6.8, 1H), 4.63 (d, *J* = 6.5, 1H), 3.94 – 3.86 (m, 1H), 3.83 – 3.74 (m, 1H), 3.64 – 3.52 (m, 3H), 3.46 (s, 3H), 3.42 (s, 3H), 3.41 (s, 3H), 3.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 98.32, 97.78, 97.55, 95.10, 93.82, 77.64, 77.50, 77.36, 61.50, 56.62, 56.28, 56.00, 55.93. HRMS (ESI-TOF) calcd for $C_{13}H_{26}O_9$: 349.1469 (M+Na), found: 349.1453.

Preparative Scale Bioconversions



1,3,4,6-tetramethyl-α-galactopyranoside (9). Prepared using 162.2 mg **2** (0.6479 mmol, 1 equiv), 8.0 mL 5E5 (81 μM, 0.001 equiv), 49.59 mg $NADP^+$ (0.06479 mmol, 0.1 equiv), 1.9927 g glucose-6-phosphate (6.479 mmol, 10 equiv), 150 μL glucose-6-phosphate dehydrogenase (2 u/mL total volume) and 57 mL KP_i. The product was purified by SiO_2 chromatography eluting with ethyl acetate to provide 0.0741 g **11** (48%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.81 (d, J = 3.9, 1H), 4.00 – 3.94 (m, 1H), 3.84 (t, J = 6.5, 1H), 3.72 (d, J = 2.1, 1H), 3.58 – 3.55 (m, 1H), 3.55 (s, 3H), 3.51 (s, 3H), 3.52 – 3.48 (m, 1H), 3.41 (s, 3H), 3.43 – 3.37 (m, J = 11.8, 1H), 3.39 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 99.47, 81.12, 74.99, 71.13, 69.24, 68.57, 61.11, 59.19, 57.61, 55.43. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{10}H_{21}O_6$, 237.1338; found, 237.1332.

1,2,4,6-tetramethyl-β-galactopyranoside (11). Prepared using 56.70 mg **5** (0.225 mmol, 1 equiv), 4.0 mL E12r12 (56 μM, 0.001 equiv), 16.73 mg $NADP^+$ (0.0225 mmol, 0.1 equiv), 0.6842 g glucose-6-phosphate (2.25 mmol, 10 equiv), 44 μL glucose-6-phosphate dehydrogenase (2 u/mL total volume) and 45 mL KP_i. The product was purified by SiO_2 chromatography eluting with ethyl acetate to provide 0.0347 g **11** (65%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.13 (d, J = 7.6, 1H), 3.62 – 3.59 (m, 1H), 3.59 (s, 3H), 3.56 (s, 3H), 3.56 - 3.53 (m, 4H), 3.50 (s, 3H), 3.39 (s, 3H), 3.17 (dd, J = 9.2, 7.6, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 104.65, 81.82, 78.12, 74.08, 73.54, 70.99, 61.88, 61.09, 59.44, 56.91. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{10}H_{21}O_6$, 237.1338; found, 237.1340.

1-benzoyl-2,4,6-trimethyl-β-galactopyranoside (12). Prepared using 0.1020 g **7** (0.294 mmol, 1 equiv) dissolved in 3.6 mL DMSO, 3.68 mL B1SYN (40 μM, 0.0005 equiv), 54.64 mg $NADP^+$ (0.0735 mmol, 0.25 equiv), 0.8940 g glucose-6-phosphate (2.940 mmol, 10 equiv), 143 μL glucose-6-phosphate dehydrogenase (2 u/mL total volume), 2.92 mg superoxide dismutase (100 units/mL total volume), and 145 mL KP_i. The product was purified by SiO_2 chromatography eluting with 50% ethyl acetate/hexanes to provide 0.0988 g **12** (99%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 – 8.04 (m, 2H), 7.58 (dd, J = 10.4, 4.4, 1H), 7.45 (t, J = 7.6, 2H), 5.71 (d, J = 8.0, 1H), 3.85 – 3.78 (m, 1H), 3.77 – 3.66 (m, 2H), 3.63 (s, 3H), 3.59 (s, 3H), 3.58 – 3.51 (m, J = 9.4, 5.8, 2H), 3.38 (s, 3H), 2.50 (d, J = 6.0, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.98, 133.83, 130.27, 129.45, 128.70, 95.06, 80.91, 77.85, 74.25, 74.10, 70.19, 62.08, 61.30, 59.37. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{16}H_{23}O_7$, 327.1444; found, 327.1441.

1,2,3,4-tetramethyl-α-mannopyranoside (13). Prepared using 0.1020 g **3** (0.4 mmol, 1 equiv), 3.25 mL 9-10A F87Idr (118 μM, 0.001 equiv), 29.81 mg $NADP^+$ (0.04 mmol, 0.1 equiv), 1.253 g glucose-6-phosphate (4.01 mmol, 10 equiv), 78 μL glucose-6-phosphate dehydrogenase (2 u/mL total volume), 1.90 mg superoxide dismutase (100 units/mL total volume), and 80 mL KP_i. The product was purified by SiO_2 chromatography eluting with ethyl acetate to provide 0.0687 g **13** (71%) as a white solid. ^1H NMR (600 MHz, $CDCl_3$) δ 4.76 (d, J = 1.6, 1H), 3.82 (d, J = 9.6, 1H), 3.74 (d, J = 8.2, 1H), 3.56 (dd, J = 3.1, 1.8, 1H), 3.53 (s, 3H), 3.51 – 3.49 (m, 1H), 3.48 (s, 3H), 3.48 (s, 3H), 3.47 – 3.38 (m, 2H), 3.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 98.38, 81.41, 77.46, 76.75, 72.00, 62.57, 61.01, 59.37, 57.94, 55.14. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{10}H_{19}O_6$, 235.1182; found, 235.1188.

1-benzyl-2,3,6-trimethyl-β-glucopyranoside (14). Prepared using 0.1041 g **4** (0.3192 mmol, 1 equiv) dissolved in 1.5 mL DMSO, 0.5 mL 9-10A F87Idr (118 μM, 0.001 equiv), 22.50 mg $NADP^+$ (0.0303 mmol, 0.1 equiv), 0.8754 g glucose-6-phosphate (2.879 mmol, 10 equiv), 60 μL glucose-6-phosphate dehydrogenase (2 u/mL total volume) and 60 mL KP_i. The reaction was stopped after only 4 hours in order to avoid subsequent demethylation of the desired product. The product was purified by SiO_2 chromatography eluting with 60% ethyl acetate/hexanes to provide 0.0441 g **14** (44%) as a white solid. ^1H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 4H), 7.30 – 7.26 (m, 1H), 4.92 (d, J = 12.0, 1H), 4.63 (d, J = 12.0, 1H), 4.42 – 4.38 (m, 1H), 3.71 – 3.63 (m, 2H), 3.62 (s, 3H), 3.58 (s, 3H), 3.57 – 3.48 (m, 1H), 3.42 (s, 3H), 3.41 – 3.37 (m, 1H), 3.12 – 3.09 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.41,

128.34, 127.71, 127.67, 104.98, 85.66, 83.47, 73.77, 72.63, 71.11, 71.01, 60.82, 60.34, 59.59. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{16}H_{25}O_6$, 313.1655; found, 313.1651.

1-benzyl-2,3,6-trimethyl-β-galactopyranoside (15). Prepared using 0.1022 g **6** (0.3066 mmol, 1 equiv) dissolved in 1.5 mL DMSO, 2.75 mL 9-10A F87Idr (118 μM, 0.001 equiv), 24.50 mg $NADP^+$ (0.0330 mmol, 0.1 equiv), 0.9368 g glucose-6-phosphate (2.879 mmol, 10 equiv), 60 μL glucose-6-phosphate dehydrogenase (2 u/mL total volume) and 60 mL KP_i. The product was purified by SiO_2 chromatography eluting with 75% ethyl acetate/hexanes to provide 0.0609 g **15** (64%) as a colorless oil. ^1H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.22 (m, 5H), 4.92 (d, J = 12.0, 1H), 4.63 (d, J = 12.0, 1H), 4.35 (d, J = 7.7, 1H), 4.03 (d, J = 2.6, 1H), 3.74 – 3.67 (m, 1H), 3.67 – 3.61 (m, 1H), 3.57 (s, 3H), 3.51-3.48 (m, 1H), 3.47 (s, 3H), 3.40 (s, 3H), 3.32 (dd, J = 9.3, 7.8, 1H), 3.13 (dd, J = 9.4, 3.4, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.73, 128.54, 127.92, 127.84, 102.76, 82.84, 80.43, 73.15, 71.95, 70.99, 66.17, 61.14, 59.70, 57.96. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{16}H_{25}O_6$, 313.1655; found, 313.1651.

1,2,4-trimethoxymethyl-α-xylopyranoside (22). Prepared using 0.1139 g **21** (0.349 mmol, 1 equiv) dissolved in 1.4 mL DMSO, 4.9 mL 9-10A F87Vdr (107 μM, 0.0015 equiv), 51.40 mg $NADP^+$ (0.0698 mmol, 0.2 equiv), 2.1196 g glucose-6-phosphate (6.98 mmol, 20 equiv), 140 μL glucose-6-phosphate dehydrogenase (2 u/mL total volume) and 70 mL KP_i. The product was purified by SiO_2 chromatography eluting with 60% ethyl acetate/hexanes to provide 0.0380 g **22** (40%) as a colorless oil. ^1H NMR (600 MHz, $CDCl_3$) δ 5.06 (d, J = 3.7, 1H), 4.85 (d, J = 6.5, 1H), 4.78 (d, J = 6.8, 1H), 4.72 (d, J = 6.9, 1H), 4.68 (dd, J = 14.7, 6.8, 1H), 4.57 (d, J = 6.5, 1H), 3.84 (t, J = 9.1, 1H), 3.74 – 3.66 (m, 2H), 3.55 (t, J = 10.9, 1H), 3.51 – 3.44 (m, 2H), 3.40 (s, 3H), 3.39 (s, 3H), 3.36 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 97.28, 97.25, 94.17, 93.26, 78.49, 78.22, 71.60, 60.32, 56.01, 55.66, 55.58. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{13}H_{25}O_9$, 325.1498; found, 325.1485.

Functionalization of Mono-demethylated Monosaccharide Derivatives

6-fluoro-1,2,3,4-tetramethyl-α-mannopyranoside (16). To a solution of 0.0605 g **13** (0.26 mmol, 1 equiv) in 5 mL CH_2Cl_2 at 0 °C under N_2 was added drop-wise 0.08 mL (diethylamino)sulfur trifluoride (DAST, 0.61 mmol, 2.3 equiv). The reaction was allowed to warm to room temperature over four hours and quenched with 5 mL methanol and 25 mL water. The mixture was extracted with 3x25 mL CH_2Cl_2, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give a yellow oil.

The crude product was purified by SiO_2 chromatography eluting with 40% ethyl acetate/hexanes to provide 0.0348 g **16** (56%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.79 (d, J = 1.6, 1H), 4.74 – 4.61 (m, 1H), 4.57 – 4.46 (m, 1H), 3.58 – 3.54 (m, 1H), 3.53 (s, 3H), 3.52 – 3.49 (m, J = 2.9, 2H), 3.48 (s, 3H), 3.48 (s, 3H), 3.47 – 3.41 (m, J = 9.3, 1H), 3.36 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 98.27 (s), 83.61 (s), 81.40 (d, J = 1.3), 81.32 (s), 75.46 (d, J = 6.7), 71.12 (d, J = 18.2), 61.01 (s), 59.25 (s), 57.93 (s), 55.24 (s). ^{19}F NMR (282 MHz, $CDCl_3$) δ -32.66 (td, J = 47.7, 26.4, 1H). HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{10}H_{19}O_5F$, 238.1216; found, 238.1217.

6-fluoro-1,2,3,4-tetraacetyl-α-mannopyranoside (17). To a solution of 0.0281 g **16** (0.118 mmol, 1 equiv) in 1 mL CH_2Cl_2 at 0 °C under N_2 was added drop-wise 0.18 mL BBr_3 (1.77 mmol, 15 equiv). The reaction was allowed to warm to room temperature over six hours and quenched with 5 mL water. The CH_2Cl_2 was removed by rotary evaporation and the aqueous mixture was purified by anion exchange chromatography (Dowex). Product-containing fractions were combined and concentrated by rotary evaporation to give crude 6-fluoromannopyranoside as a mixture of anomers. To this material was added 0.0010 g dimethylaminopyridine (0.0060 mmol, 0.05 equiv) and a stir bar. The flask was evacuated and purged with N_2 three times, and charged with 2 mL CH_2Cl_2 and 0.15 mL pyridine (1.42 mmol, 12 equiv). Finally, 0.1 mL acetyl chloride (1.18 mmol, 10 equiv) was added to the flask, and the mixture was stirred at room temperature for 12 h. 10 mL 1 M HCl was added, and the resulting mixture was extracted with 3 x 20 mL CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give a yellow oil. The crude product was purified by SiO_2 chromatography eluting with 40% ethyl acetate/hexanes to provide 0.0227 g **17** (55%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.10 (d, J = 2.0, 0.4 H), 5.88 (s, 0.4 H), 5.49 (d, J = 3.1, 0.4 H), 5.38 (m, 1 H), 5.34 - 5.28 (d, J = 9.28, 0.6 H), 5.27 (m, 0.6 H), 5.15 (dd, J = 10.0, 3.2, 0.6 H), 4.64 – 4.50 (m, 1H), 4.47 – 4.35 (m, 1H), 4.17 – 3.89 (m, 0.6 H), 3.89 – 3.66 (m, 0.4 H), 2.21 (s, 1H), 2.17 (m, 3H), 2.11 (m, 2H), 2.08 (m, J = 2.3, 3H), 2.01 (d, J = 0.7, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.18, 169.99, 169.79, 169.74, 169.46, 169.44, 168.34, 168.00, 90.50, 90.24 (2C), 81.75, 80.59, 73.80, 73.67, 71.36, 71.22, 70.61, 68.64, 68.22, 68.08, 20.83, 20.72, 20.66, 20.64, 20.62 (2C), 20.51. ^{19}F NMR (282 MHz, $CDCl_3$) δ -31.45 (td, J = 46.7, 21.3, 0.4 F), -32.58 (td, J = 47.3, 23.1, 0.6 F). HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{14}H_{19}O_9F$, 350.1013; found, 350.0984.

1-benzoyl-3-deoxy-2,4,6-trimethyl-β-galactoopyranoside (18). To a solution of 0.106 g **12** (0.322 mmol, 1 equiv) and 0.0022 g dimethylaminopyridine (0.016 mmol, 0.05 equiv) in 8 mL CH_2Cl_2 under N_2 was added 0.08 mL pyridine (1.0 mmol, 3 equiv) and 0.08 mL *O*-phenyl chlorothionoformate (0.55 mmol, 1.7 equiv). The solution was allowed to stir at room temperature over 12 hours and quenched with 25 mL water. The mixture was extracted with 3 x 25 mL CH_2Cl_2, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give a yellow oil. The crude product was purified by SiO_2 chromatography eluting with 10% ethyl acetate/hexanes to provide 0.135 g of the intermediate thionoester **19** (90%) as a colorless oil.

To a solution of 0.205 g **19** (0.430 mmol, 1 equiv) and 0.0259 g 2,2'-azobis(2-methylpropionitrile) (0.160 mmol, 0.36 equiv) in 20 mL toluene under N_2 was added 0.35 mL tributyltin hydride (1.30 mmol, 3 equiv). The resulting solution was stirred at reflux for four hours. The volatiles were removed by rotary evaporation, and the mixture was purified by SiO_2 chromatography eluting with 10% ethyl acetate/hexanes to provide 0.117 g of **20** (85%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 – 8.04 (m, 2H), 7.62 – 7.51 (m, 1H), 7.50 – 7.38 (m, 2H), 5.81 (d, J = 7.8, 1H), 3.88 (ddd, J = 6.7, 6.1, 1.6, 1H), 3.70 – 3.49 (m, 5H), 3.46 (s, 3H), 3.44 (s, 3H), 3.34 (s, 3H), 2.57 (ddd, J = 13.9, 4.9, 3.6, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.55, 128.67, 128.60, 127.99, 127.96, 102.55, 83.04, 76.35, 74.20, 72.80, 71.52, 71.25, 60.99, 59.86. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{16}H_{21}O_6$, 309.1338; found, 309.1350.

3-deoxy-1,2,4,6-tetraacetylgalactoopyranoside (19). To a solution of 0.0487 g **12** (0.157 mmol, 1 equiv) in 1 mL CH_2Cl_2 at 0 °C under N_2 was added drop-wise 0.23 mL BBr_3 (2.36 mmol, 15 equiv). The reaction was allowed to warm to room temperature over four hours and quenched with 5 mL water. The CH_2Cl_2 was removed by rotary evaporation and the aqueous mixture was purified by anion exchange chromatography (Dowex). Product-containing fractions were combined and concentrated by rotary evaporation to give crude 3-deoxygalactopyranoside (**19**) as a mixture of anomers.

To 0.0233 g of **19** (0.142 mmol, 1 equiv) was added 0.0010 g dimethylaminopyridine (0.0071 mmol, 0.05 equiv) and a stir bar. The flask was evacuated and purged with N_2 three times, and charged with 2 mL CH_2Cl_2 and 0.14 mL pyridine (1.70 mmol, 12 equiv). Finally, 0.101 mL acetyl chloride (1.42 mmol, 10 equiv) was added to the flask, and the mixture was stirred at room temperature for 6 h. 10 mL 1 M HCl was added, and the resulting mixture was extracted with 3 x 20 mL CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give a yellow oil. The crude product was

purified by SiO_2 chromatography eluting with 40% ethyl acetate/hexanes to provide 0.0400 g **20** (85%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.41 (d, J = 3.5, 1H), 5.18 (m, 1H), 5.08 (m, 1H), 4.57 (dd, J = 12.3, 2.4, 1H), 4.23 (m, 2H), 4.12 (dd, J = 12.3, 5.2, 1H), 3.81 (dd, J = 10.7, 1.8, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.88, 169.97, 169.88, 169.57, 77.73, 77.42, 74.80, 72.70, 68.07, 63.53, 21.07, 21.03 (2C), 20.93. HRMS-FAB+ (m/z): $[M]^+$ calcd for $C_{14}H_{21}O_9$, 333.1185; found, 333.1196.

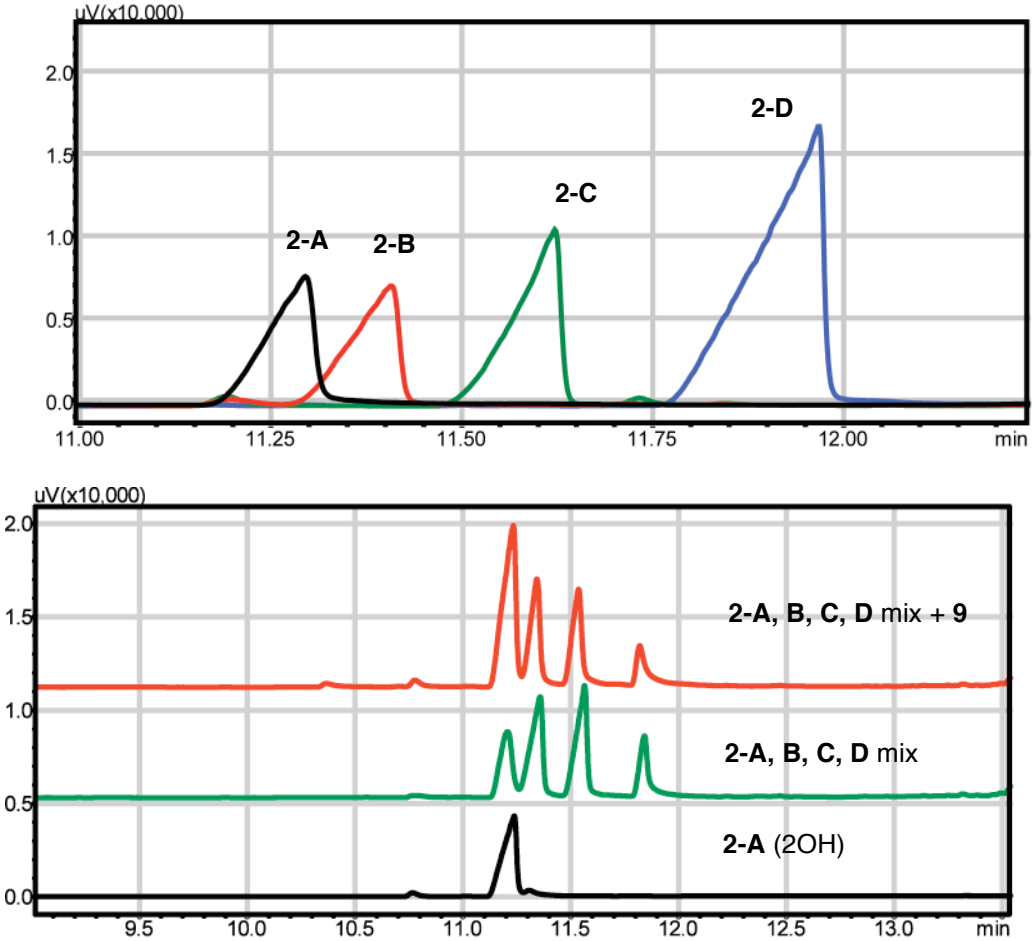
Disaccharide Formation.

3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-1,2,4-tri-O-methoxymethyl-α-D-xylopyranoside (**24**) A mixture of **22** (78 mg, 0.27 mmol), 2,3,4-tetra-O-acetyl-α-D-xylopyranosyl trichloroacetimidate[12] (175 mg, 0.42 mmol) and molecular sieves (250 mg) in dry DCM (5 mL) was stirred at room temperature for 30 min. TMSOTF (5 μL, 27 μmol) was added at -50 °C. After 2 h at this temperature, reaction was quenched by Et_3N (18 μL, 0.13 mmol), then allowed to reach room temperature and filtered through Celite. Flash column chromatography (SiO_2 1:1 ethyl acetate:hexanes) afforded **24** (132 mg, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (ddd, *J* = 8.6, 6.2, 2.7, 1H), 5.09 (d, *J* = 3.6, 1H), 4.95 (td, *J* = 8.8, 5.2, 1H), 4.92 – 4.86 (m, 3H), 4.78 (d, *J* = 6.8, 1H), 4.70 (q, *J* = 6.8, 2H), 4.63 (d, *J* = 6.6, 2H), 4.15 (dd, *J* = 11.8, 5.2, 1H), 4.08 – 4.02 (m, 1H), 3.80 – 3.70 (m, 1H), 3.61 – 3.57 (m, 2H), 3.53 (dd, *J* = 9.8, 3.6, 1H), 3.45 (s, 3H), 3.40 (s, 3H), 3.36 (s, 3H), 3.35 – 3.30 (m, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.28, 170.06, 169.49, 100.63, 98.17, 97.59, 95.28, 93.75, 78.78, 77.68, 75.42, 72.08, 71.69, 69.12, 62.20, 61.12, 56.56, 55.90, 55.69, 20.96, 20.93, 20.90. HRMS (ESI-TOF) calcd for $C_{22}H_{36}O_{15}$: 563.1946 (M+Na), found: 563.1946.

3-O-β-D-xylopyranosyl-D-xylopyranoside (**25**) **24** (11 mg, 20μmol) was dissolved in 10 mM NaOMe/MeOH solution (1 mL). After 1 h, reaction mixture was passed through iatro beads and dried. Then the mixture was dissolved in 2 M HCl in MeOH/H_2O (1:1, 1 mL) and stirred at room temperature overnight. Flash chromatography (RP-C18, H_2O) gives **25** (3.2 mg, 55%) as a colorless oil. ^{1}H NMR (500 MHz, D_2O) δ 5.18 (d, *J* = 3.7, 1H), 4.56 (d, *J* = 7.9, 1H), 3.91 (dd, *J* = 11.5, 5.5, 1H), 3.70 – 3.64 (m, 2H), 3.64-3.57 (m, 2H), 3.50 (dd, *J* = 9.4, 3.7, 1H), 3.42 (dd, *J* = 16.9, 7.6, 2H), 3.34 – 3.28 (m, 1H), 3.21 (dd, *J* = 9.4, 7.9, 1H). ^{13}C NMR (126 MHz, D_2O) δ 97.01, 92.62, 76.21, 74.42, 73.21, 71.85, 69.79, 69.61, 65.57, 61.32. HRMS (ESI-TOF) calcd for $C_{10}H_{18}O_9$: 305.0843 (M+Na), found: 305.0845.

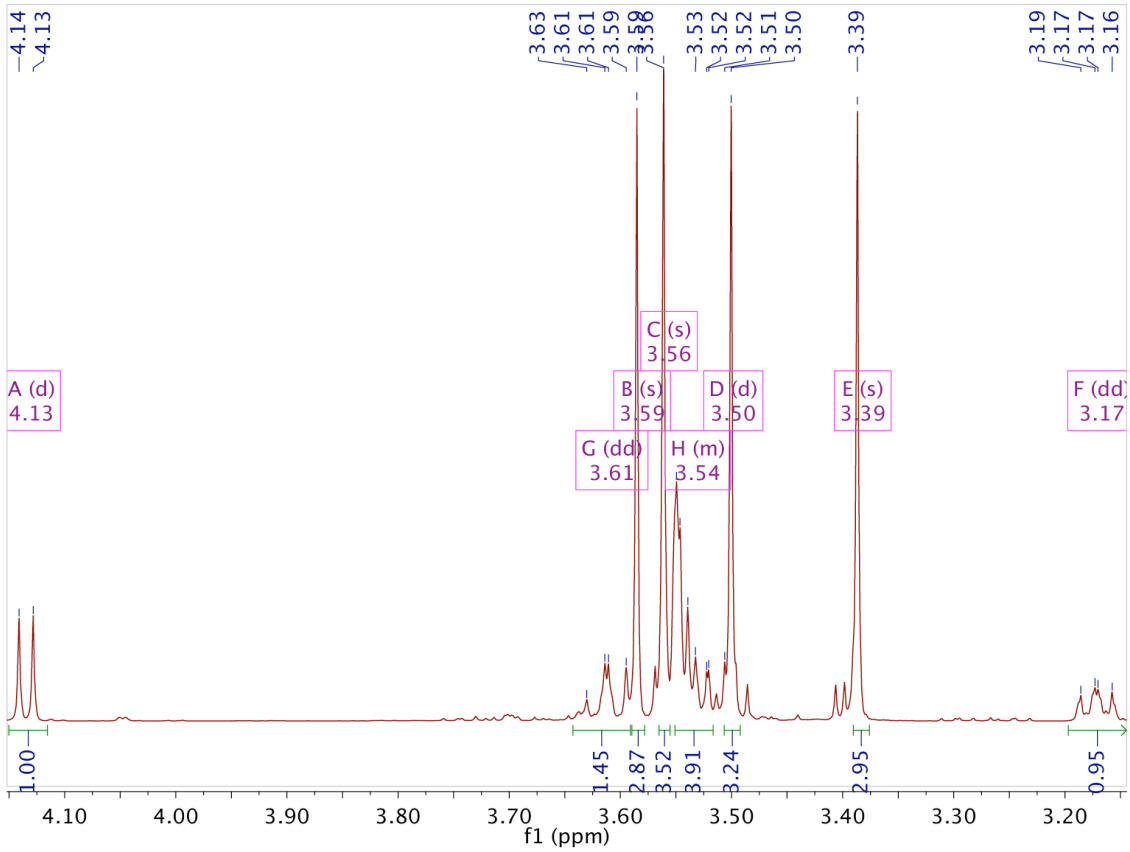
Assignment of Sugar Structures:

1,3,4,6-tetramethyl-α-galactopyranoside (9). The structure of **9** was assigned by comparing its GC chromatogram with those of authentic standards. Chromatograms for the 2, 3, 4, and 6-OH tetramethyl-α-galactopyranosides (**2A-D**) are shown, and a mixture of these compounds was spiked with **9**. The 1-OH tetramethylgalactopyranoside elutes significantly later than the four isomers shown. This assignment was verified by COSY, HSQC, and HMBC NMR spectroscopy as described for **11**, **14**, and **22**. NMR spectra are available upon request.

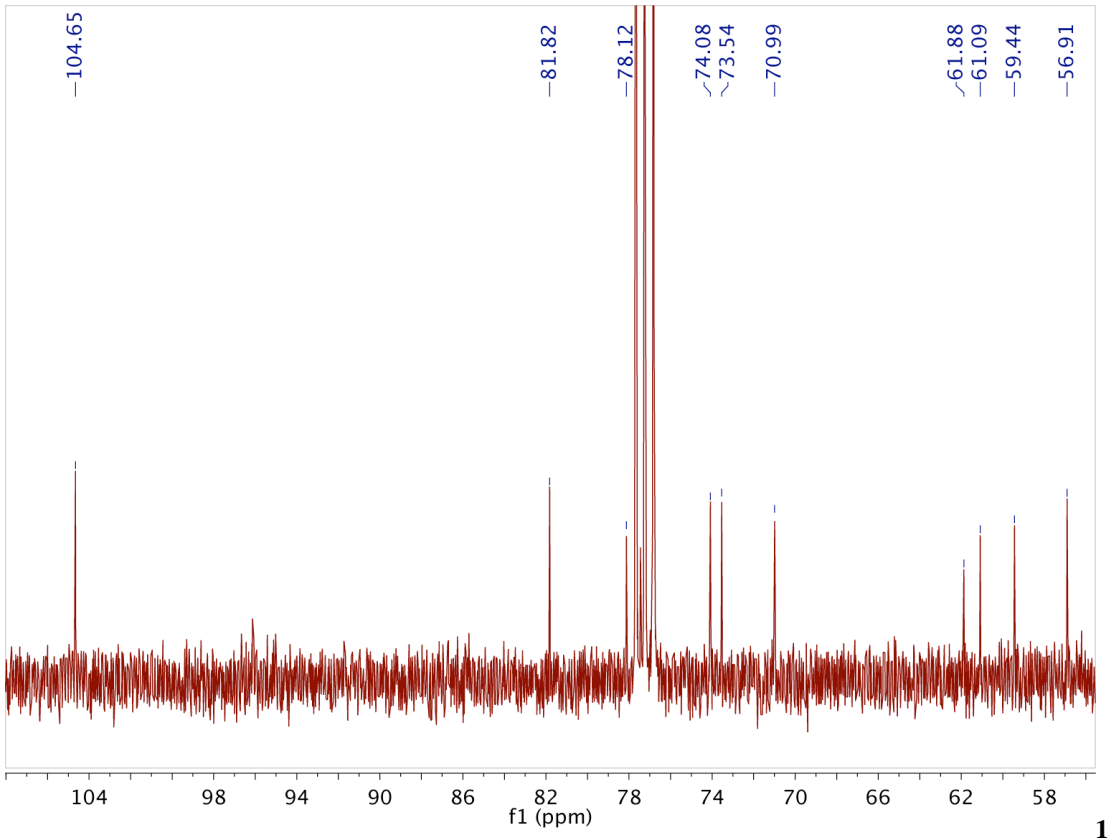


1,2,4,6-tetramethyl-β-galactopyranoside (11). The structure of **11** was assigned by ^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR spectroscopy. Single perspectives of these spectra are shown, and the data files are available upon request.

11-^{1}H:

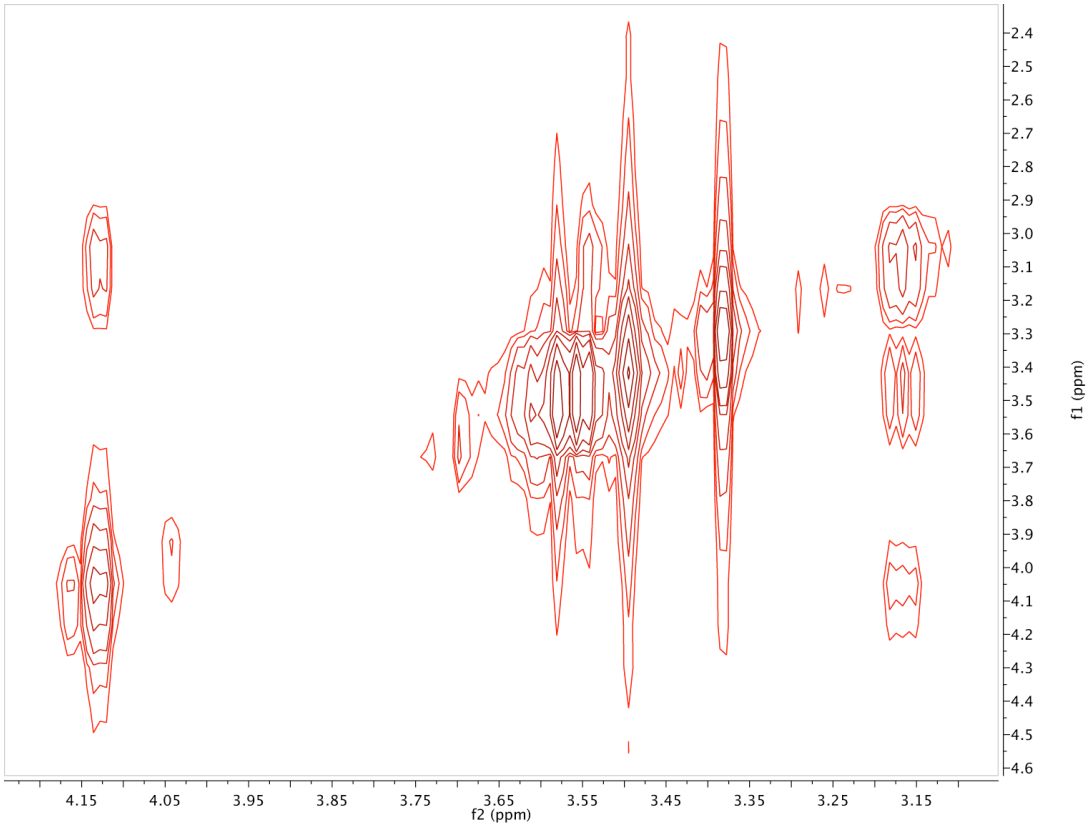


11-^{13}C:

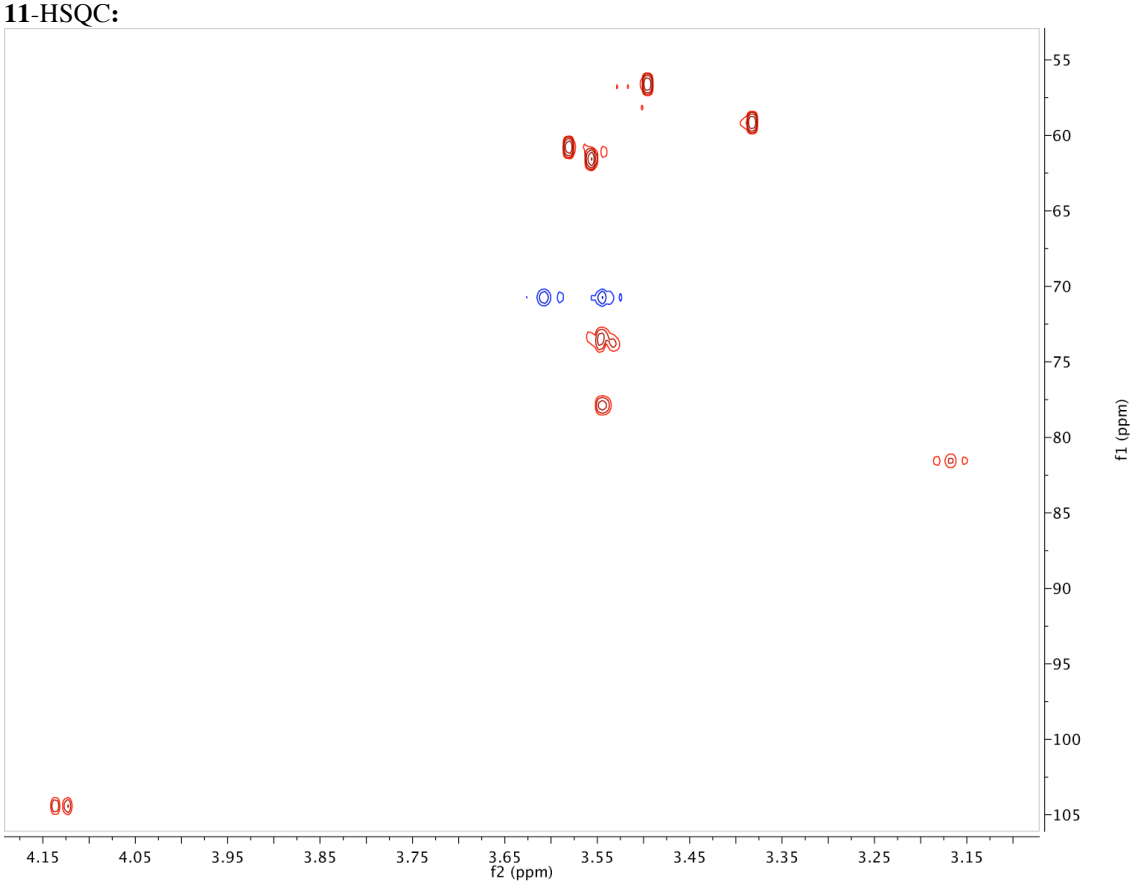


11-

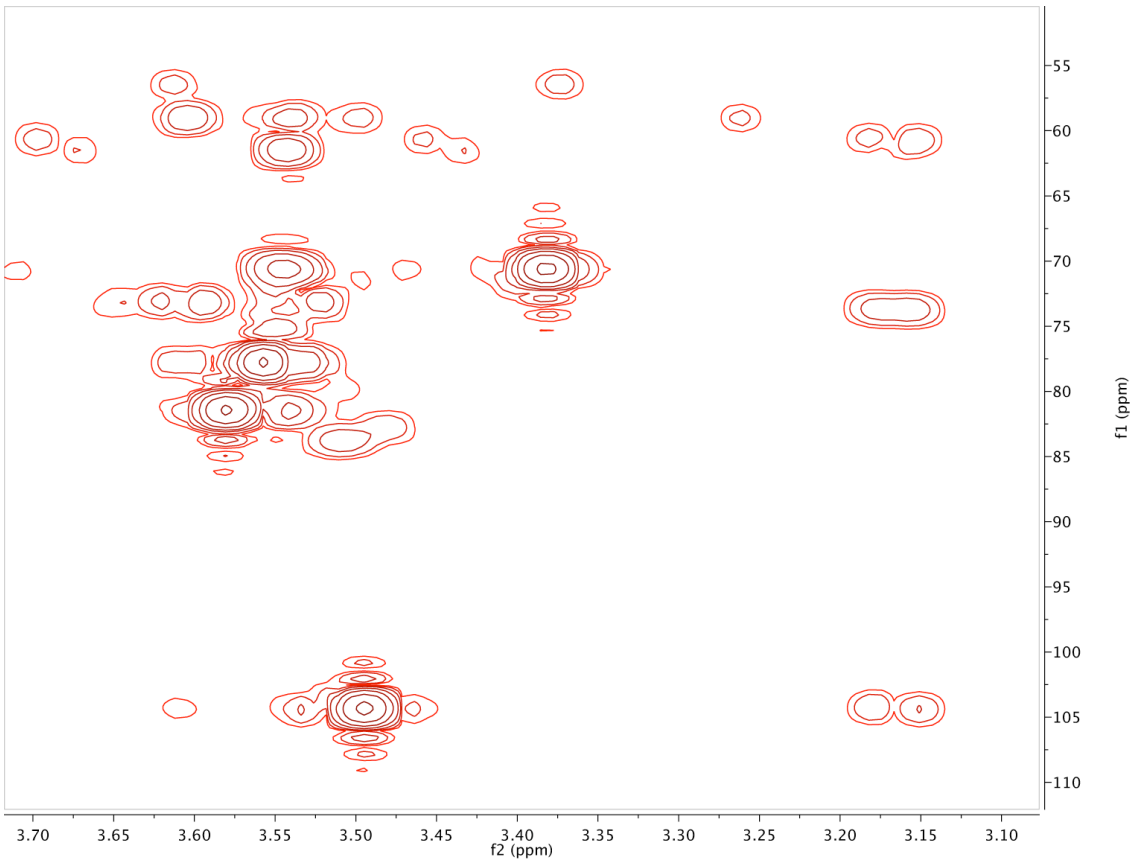
COSY：



11-HSQC：



11-HMBC**:**



1-benzoyl-2,4,6-trimethyl-β-galactopyranoside (12). Single crystals of **12** suitable for X-ray analysis were obtained by slow evaporation of CH_2Cl_2 from a solution of the compound. Crystallographic data have been deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK and copies can be obtained on request, free of charge, by quoting the publication citation and the deposition number 673244.

Crystals were mounted on a glass fiber using Paratone oil then placed on the diffractometer under a nitrogen stream at 100K. Refinement of F_2 against ALL reflections. The weighted R-factor (*w*R) and goodness of fit (S) are based on F_2, conventional R-factors (R) are based on F, with F set to zero for negative F_2. The threshold expression of $F_2 > 2\sigma(F_2)$ is used only for calculating R-factors(gt) etc. and is not relevant to the choice of reflections for refinement. R-factors based on F_2 are statistically about twice as large as those based on F, and R-factors based on ALL data will be even larger. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

Crystal data and structure refinement

Empirical formula	$C_{16}H_{22}O_7$
Formula weight	326.34
Crystallization Solvent	Cyclohexane/dichloromethane
Crystal Habit	Needle
Crystal size	0.41 x 0.12 x 0.11 mm^3
Crystal color Colorless	

Data Collection

Type of diffractometer	Bruker KAPPA APEX II
Wavelength	0.71073 Å MoKα

Data Collection Temperature	100(2) K
θ range for 9998 reflections used in lattice determination	2.76 to 36.01°
Unit cell dimensions	a = 12.5013(6) Å b = 4.6992(2) Å β= 93.488(3)° c = 13.5074(6) Å Volume 792.04(6) Å^3
Z	2
Crystal system	Monoclinic
Space group	$P2_1$
Density (calculated)	1.368 Mg/m^3
F(000)	348
Data collection program	Bruker APEX2 v2.1-0
θ range for data collection	1.63 to 36.47°
Completeness to θ = 36.47°	99.6 %
Index ranges	$-20 \le h \le 20$, $-7 \le k \le 7$, $-22 \le l \le 22$
Data collection scan type	ω scans; 40 settings
Data reduction program	Bruker SAINT-Plus v7.34A
Reflections collected	68204
Independent reflections	7680 [R_{int}= 0.0635]
Absorption coefficient	0.107 mm^{-1}
Absorption correction	None
Max. and min. transmission (calc)	0.9883 and 0.9573

Structure solution and Refinement

Structure solution program	SHELXS-97 (Sheldrick, 1990)
Primary solution method	Direct methods
Secondary solution method	Difference Fourier map
Hydrogen placement	Difference Fourier map
Structure refinement program	SHELXL-97 (Sheldrick, 1997)
Refinement method	Full matrix least-squares on F^2
Data / restraints / parameters	7680 / 1 / 296
Treatment of hydrogen atoms	Unrestrained
Goodness-of-fit on F^2	1.647
Final R indices [I>2σ(I), 6003 reflections]	R1 = 0.0413, *w*R2 = 0.0512
R indices (all data)	R1 = 0.0599, *w*R2 = 0.0523
Type of weighting scheme used	Sigma
Weighting scheme used	$w=1/\sigma^2(Fo^2)$
Max shift/error	0.001
Average shift/error	0.000
Absolute structure determination	Not able to determine reliably
Absolute structure parameter	-0.5(4)
Largest diff. peak and hole	0.340 and -0.397 e.Å^{-3}

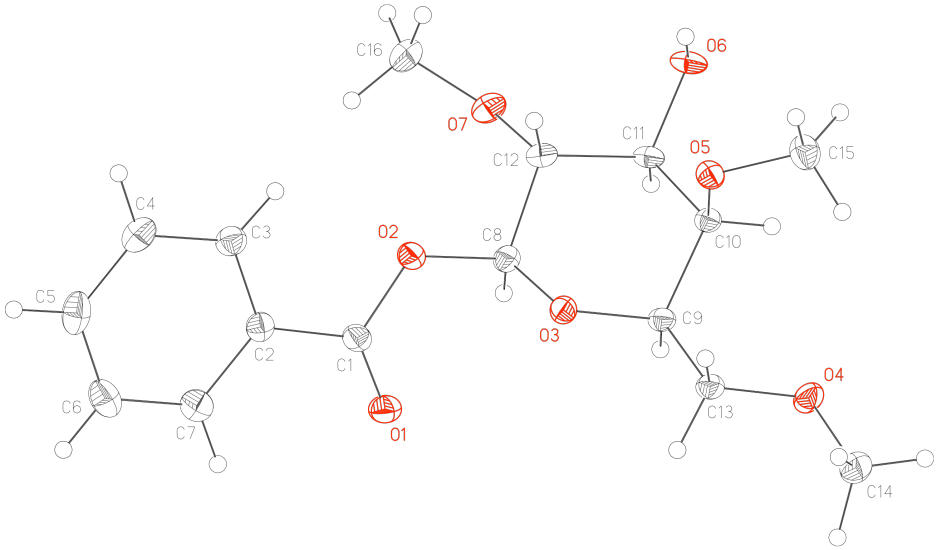
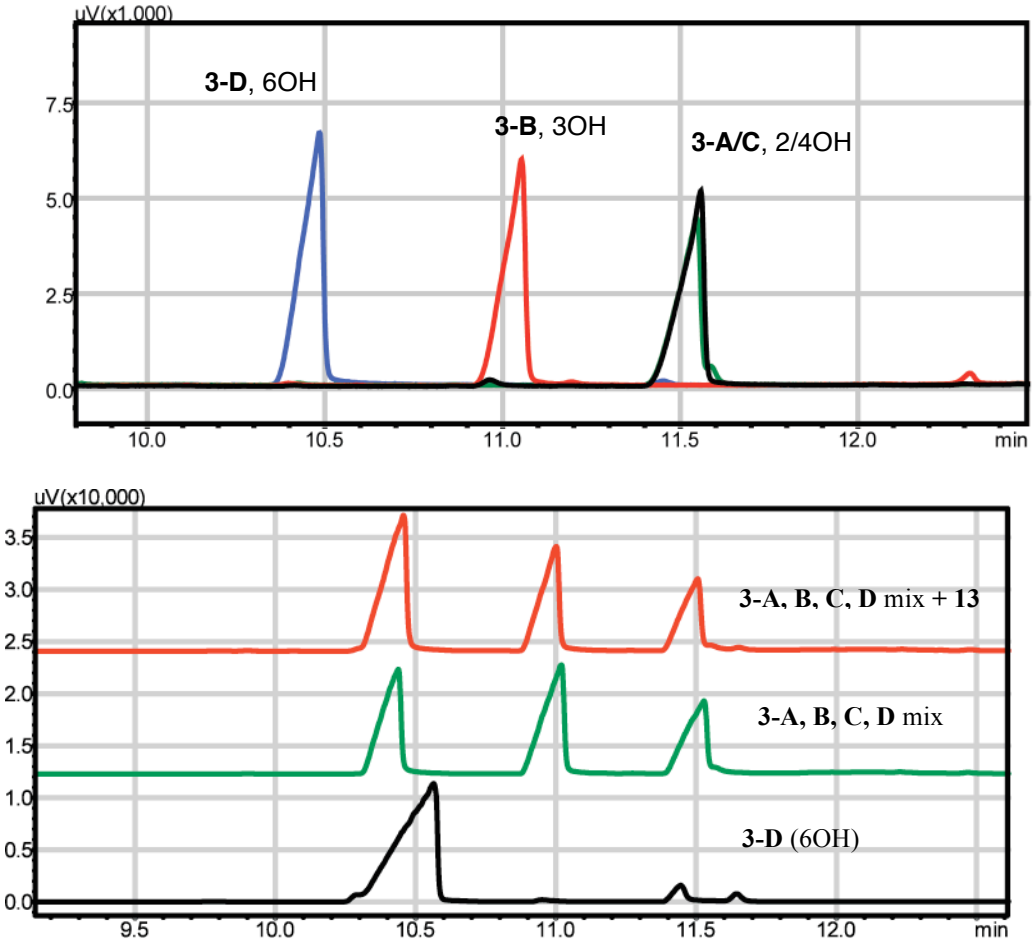


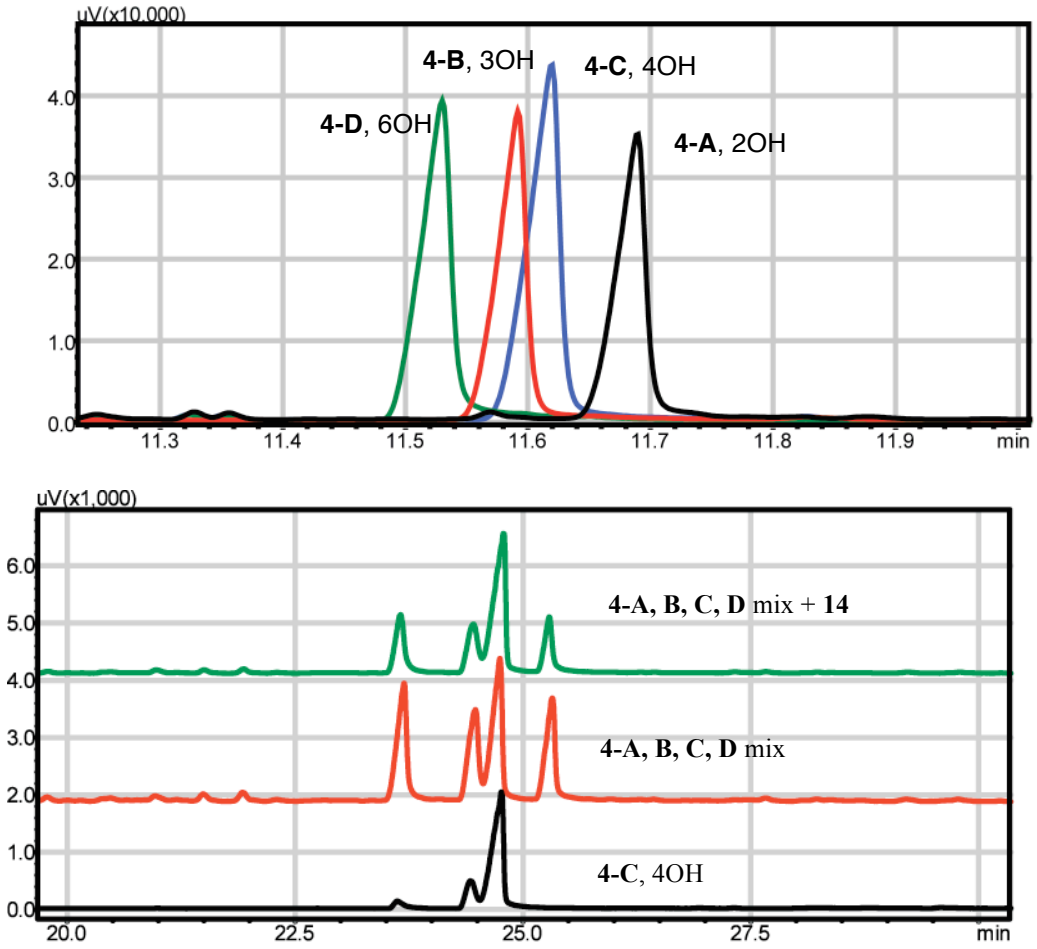
Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for JCL101 (CCDC 673244). U(eq) is defined a the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U_{eq}
O(1)	6561(1)	6646(1)	8841(1)	21(1)
O(2)	7026(1)	8320(1)	7358(1)	17(1)
O(3)	8711(1)	7883(1)	8033(1)	16(1)
O(4)	11334(1)	6167(1)	9089(1)	21(1)
O(5)	10456(1)	8636(1)	6808(1)	15(1)
O(6)	9800(1)	4326(1)	5468(1)	17(1)
O(7)	7547(1)	4571(1)	5765(1)	21(1)
C(1)	6394(1)	8213(2)	8147(1)	16(1)
C(2)	5489(1)	10243(2)	8025(1)	16(1)
C(3)	5325(1)	11960(2)	7187(1)	19(1)
C(4)	4453(1)	13784(2)	7110(1)	23(1)
C(5)	3750(1)	13904(2)	7859(1)	25(1)
C(6)	3914(1)	12219(2)	8695(1)	25(1)
C(7)	4779(1)	10379(2)	8779(1)	21(1)
C(8)	7935(1)	6498(2)	7405(1)	15(1)
C(9)	9668(1)	6199(2)	8156(1)	14(1)
C(10)	10168(1)	5905(2)	7165(1)	13(1)
C(11)	9367(1)	4566(2)	6412(1)	14(1)
C(12)	8305(1)	6179(2)	6360(1)	15(1)
C(13)	10363(1)	7713(2)	8946(1)	16(1)
C(14)	11984(1)	7352(3)	9893(1)	23(1)
C(15)	11568(1)	8985(2)	6675(1)	22(1)
C(16)	6766(1)	6226(3)	5217(1)	27(1)

1,2,3,4-tetramethyl-α-mannopyranoside (13). The structure of **13** was assigned by comparing its GC chromatogram with those of authentic standards. Chromatograms for the 2, 3, 4, and 6-OH tetramethyl-α-mannopyranosides (**3-A-D**) are shown (the 2-OH and 4-OH isomer co-elute), and a mixture of these compounds was spiked with **13**. The 1-OH tetramethylmannopyranoside elutes significantly later than the four isomers shown. This assignment was verified by COSY, HSQC, and HMBC NMR spectroscopy as described for **11**, **14**, and **22**. NMR spectra are available upon request.

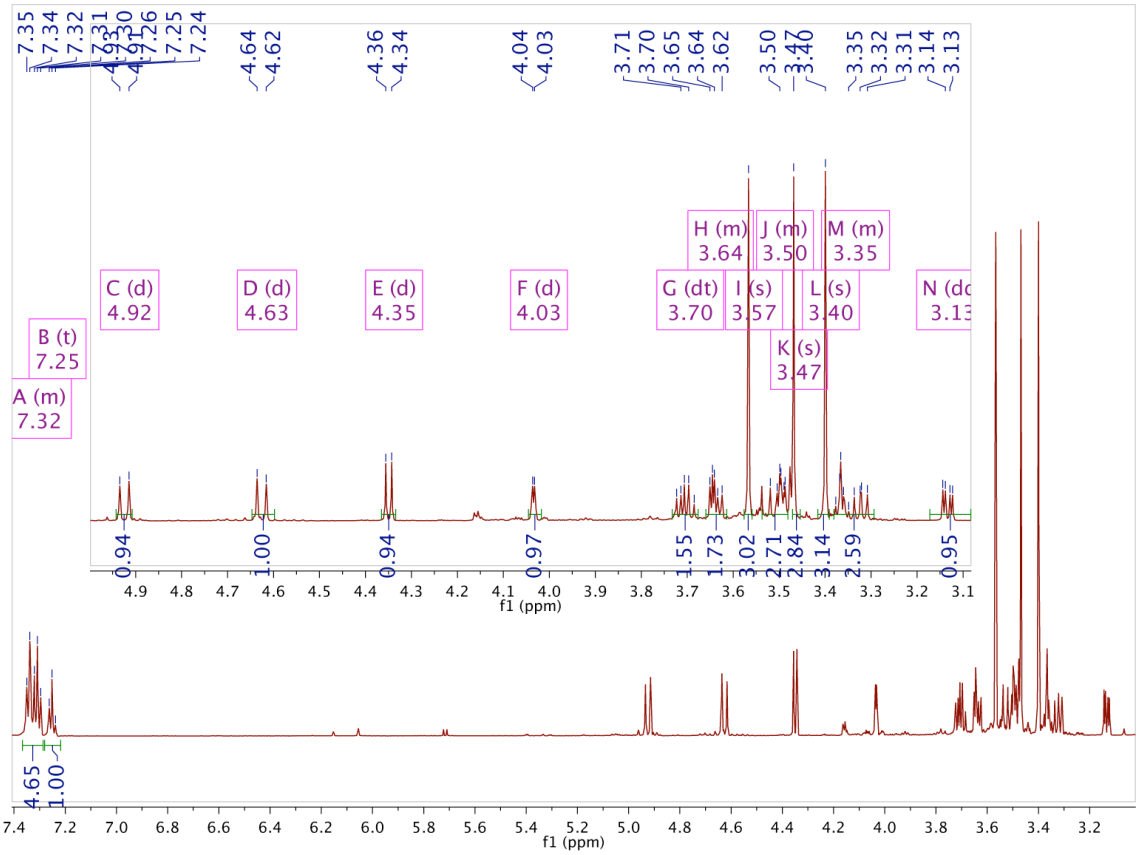


1-benzyl-2,3,6-trimethyl-β-glucopyranoside (14). The structure of **14** was assigned by comparing its GC chromatogram with those of authentic standards. Chromatograms for the 2, 3, 4, and 6-OH 1-benzyltrimethyl-β-glucopyranosides are shown. This assignment was verified by COSY, HSQC, and HMBC NMR spectroscopy as described for **11**, **15**, and **22**. NMR spectra are available upon request.



1-benzyl-2,3,6-trimethyl-β-galactopyranoside (15). The structure of **15** was assigned by ^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR spectroscopy. Single perspectives of these spectra are shown, and the data files are available upon request.

15-^{1}H: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7.34
7.32
7.31
7.30
7.26
7.25
7.24
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4.62
4.36
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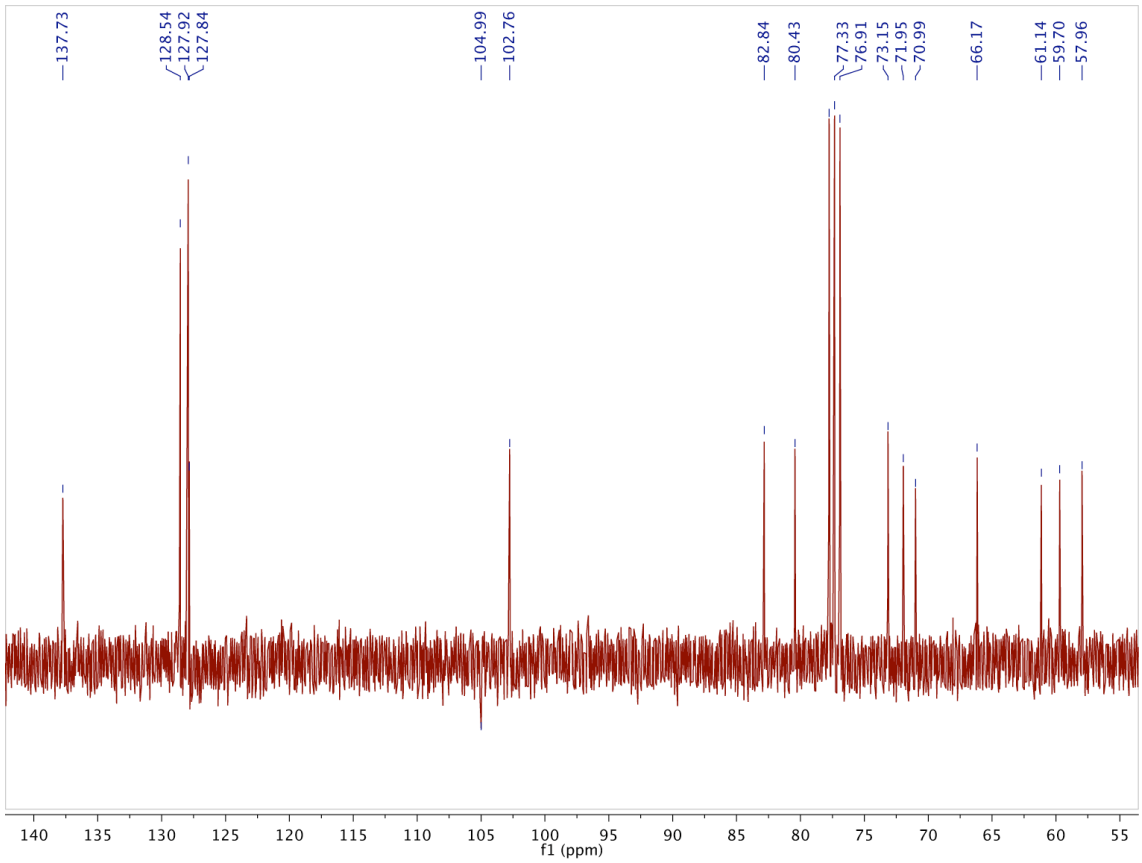
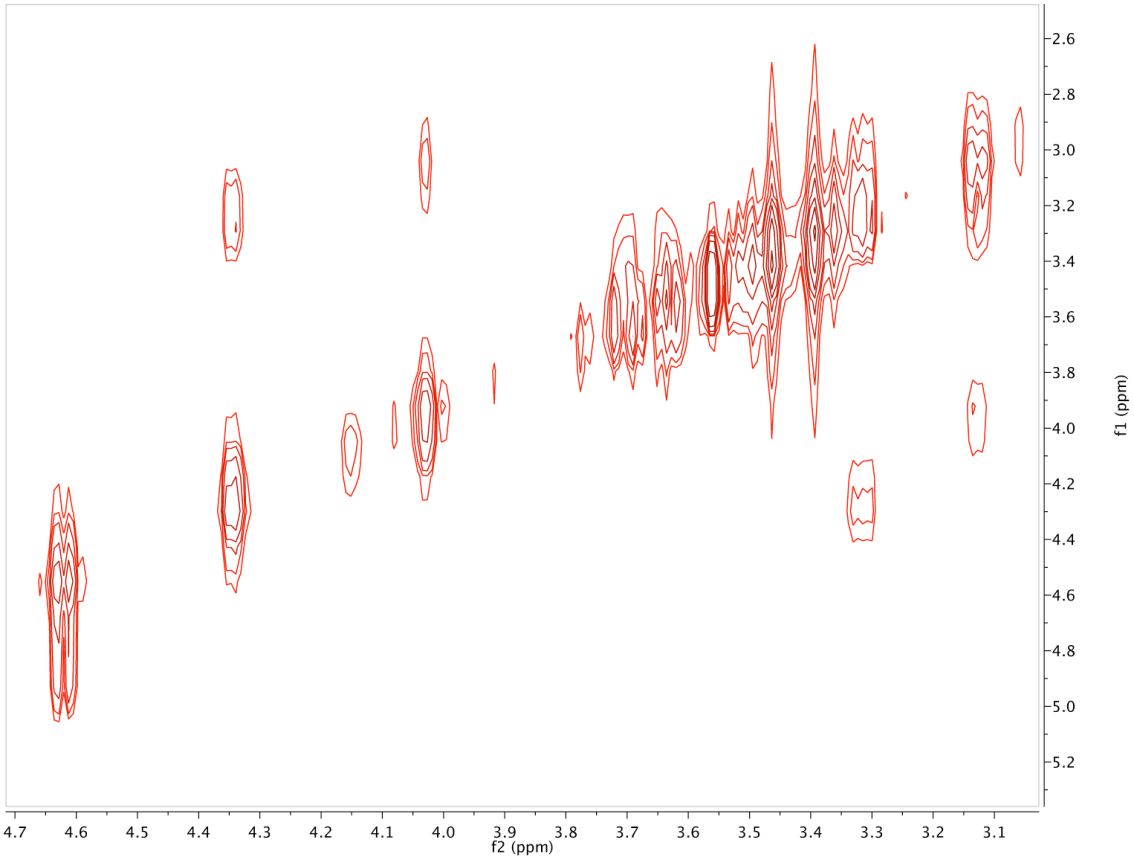
4.04
4.03
3.71
3.70
3.65
3.64
3.62
3.50
3.47
3.40
3.35
3.32
3.31
3.14
3.13
H (m) 3.64
J (m) 3.50
M (m) 3.35
C (d) 4.92
D (d) 4.63
E (d) 4.35
F (d) 4.03
G (dt) 3.70
I (s) 3.57
L (s) 3.40
K (s) 3.47
B (t) 7.25
A (m) 7.32
0.94
1.00
0.94
0.97
1.55
1.73
3.02
2.71
2.84
3.14
2.59
0.95
4.65
1.00
f1 (ppm)

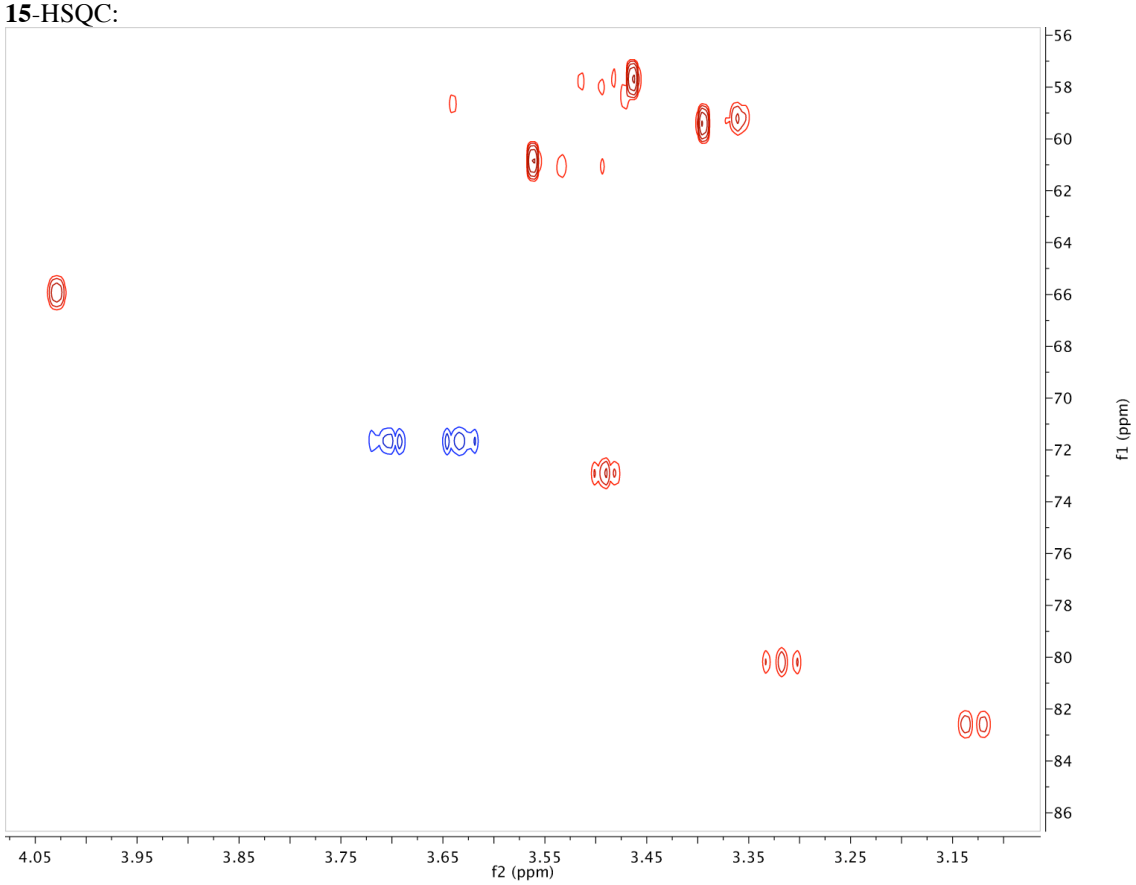
15-^{13}C:


137.73
128.54
127.92
127.84
104.99
102.76
82.84
80.43
77.33
76.91
73.15
71.95
70.99
66.17
61.14
59.70
57.96
f1 (ppm)

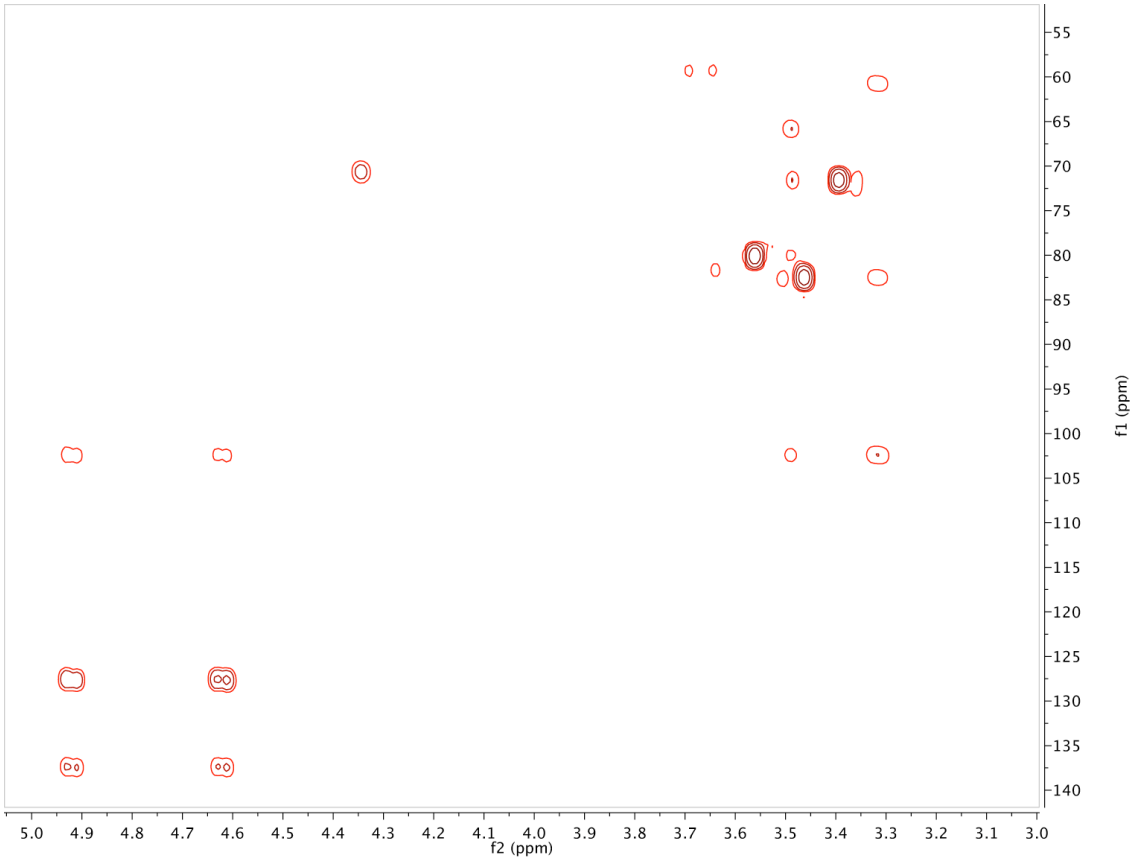
15-COSY:



15-HSQC:

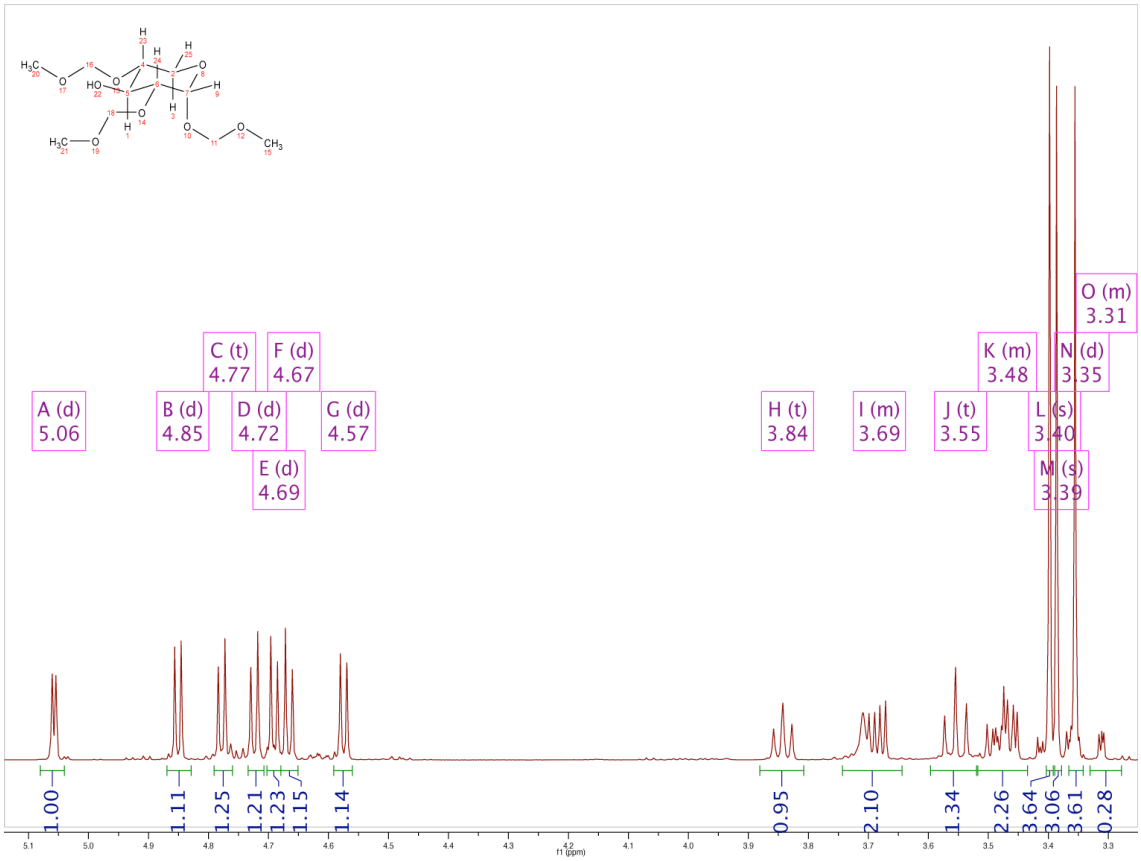


15-HMBC:

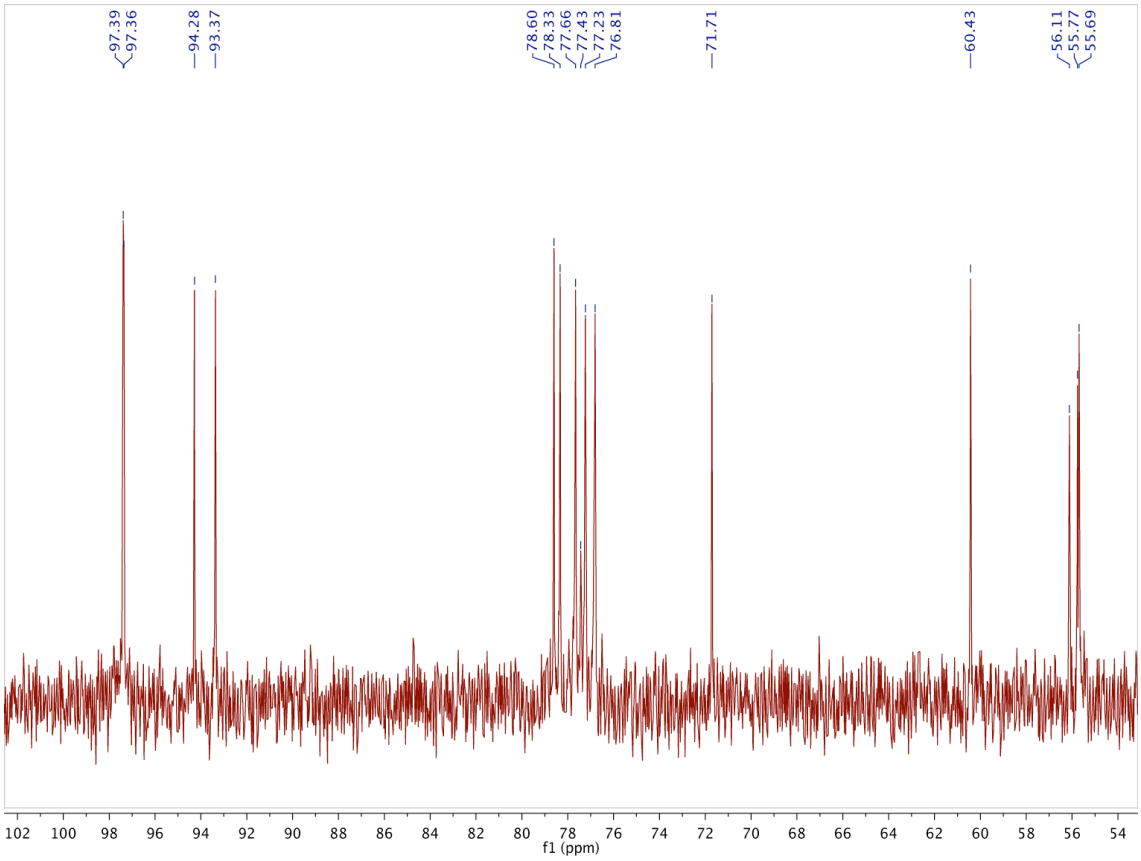


1,2,4-trimethoxymethyl-α-xylopyranoside (22). The structure of **22** was assigned by ^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR spectroscopy. Single perspectives of these spectra are shown, and the data files are available upon request.

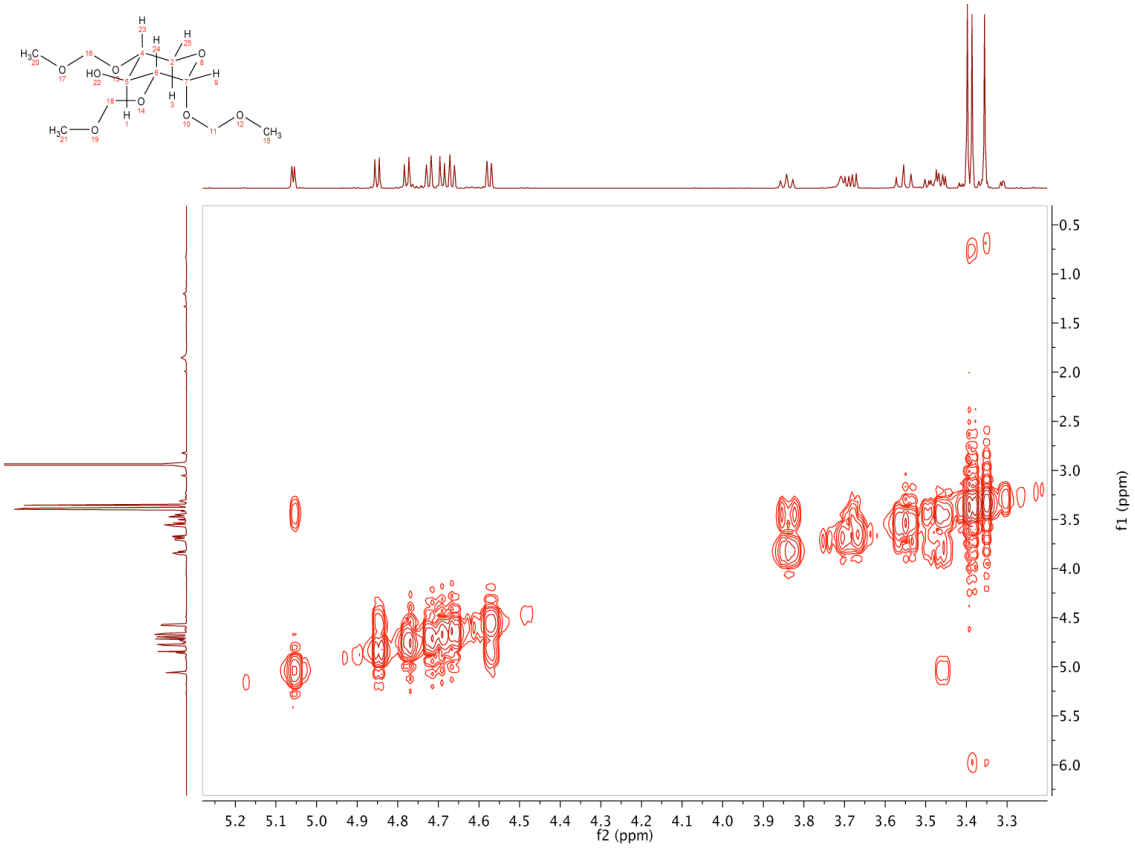
22-^{1}H:


A (d) 5.06
B (d) 4.85
C (t) 4.77
D (d) 4.72
E (d) 4.69
F (d) 4.67
G (d) 4.57
H (t) 3.84
I (m) 3.69
J (t) 3.55
K (m) 3.48
L (s) 3.40
M (s) 3.39
N (d) 3.35
O (m) 3.31
1.00
1.11
1.25
1.21
1.23
1.15
1.14
0.95
2.10
1.34
2.26
3.64
3.06
3.61
0.28
f1 (ppm)

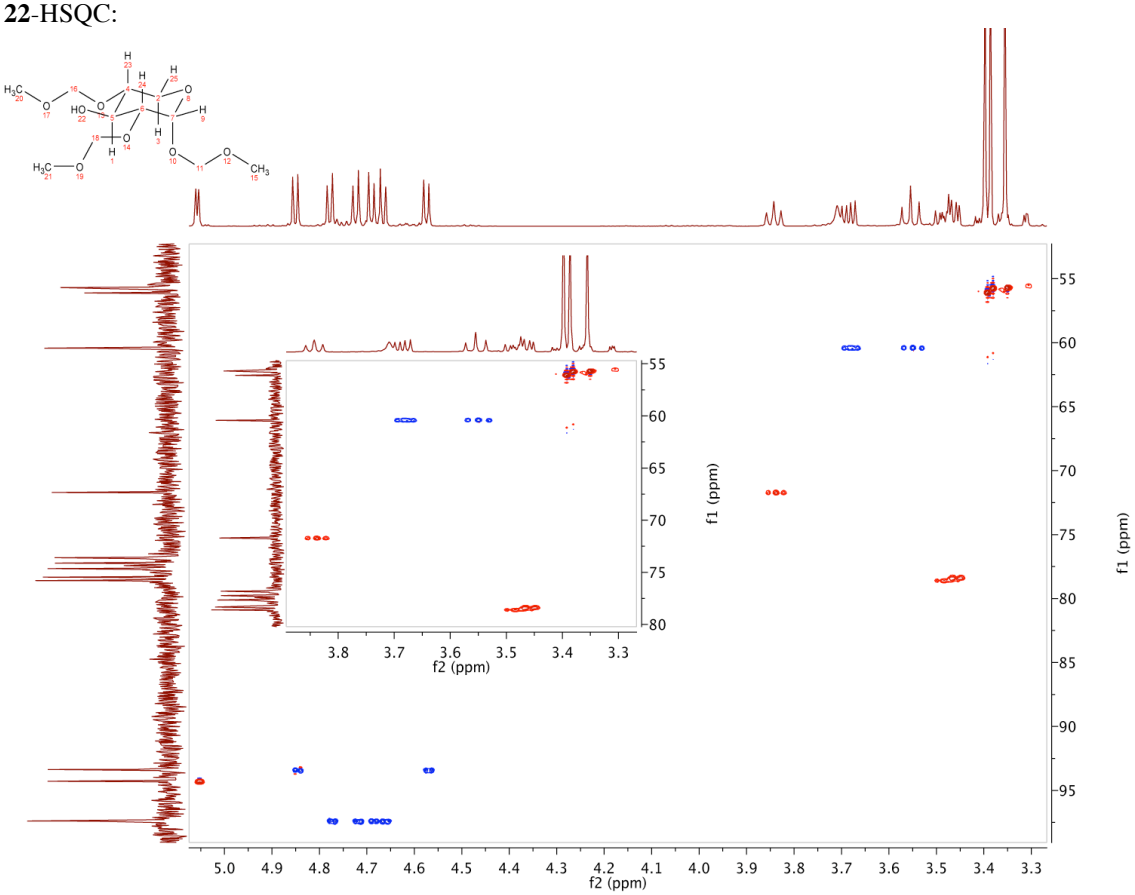
22-^{13}C:


97.39
97.36
94.28
93.37
78.60
78.33
77.66
77.43
77.23
76.81
71.71
60.43
56.11
55.77
55.69
f1 (ppm)

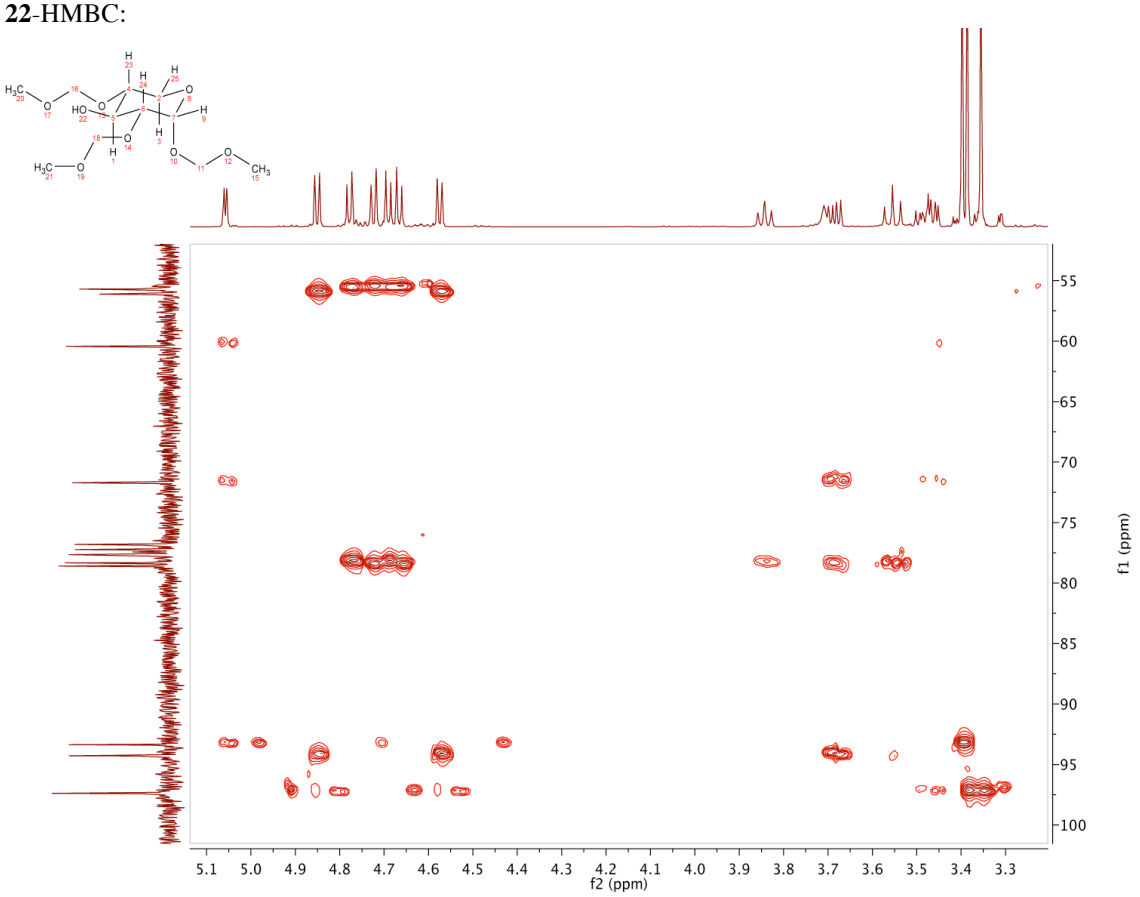
22-COSY:



22-HSQC:



22-HMBC:



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