Synthesis of a Suite of Click-Compatible Sugar Analogs for Probing Carbohydrate Metabolism

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Abstract

Metabolic labeling based on the click chemistry between alkynyl and azido groups offers a powerful tool to study the function of carbohydrates in living systems, including plants. Herein, we describe the chemical synthesis of six alkynyl-modified sugars designed as analogs to D-glucose, D-mannose, L-rhamnose and sucrose present in plant cell walls. Among these new alkynyl probes, four of them are the 6-deoxy-alkynyl analogs of the corresponding sugars and do

not possess any 6-OH groups. The other two are based on a new structural design, in which an ethynyl group is incorporated at the C-6 position of the sugar and the 6-OH group remains. The synthetic routes for both types of probes share common aldehyde intermediates, which are derived from the corresponding 6-OH precursor with other hydroxy groups protected. The overall synthesis sequence of these probes is efficient, concise, and scalable.

Keywords: click chemistry, labeling probe, carbohydrate metabolism

1. Introduction

Organisms throughout the tree of life coat their cell exteriors with carbohydrates. These coatings are heterogeneous, complex, and dynamic. Carbohydrates can have multiple levels of structural complexity: unlike linear peptides and nucleic acids, polysaccharides are often branched and can be constructed from many building-block monosaccharide monomers¹⁻⁴. Despite the structural complexity of carbohydrate polymers, monosaccharides are quite uniform from a functional-group perspective. This presents a dilemma to researchers interested in detecting and distinguishing individual types of polysaccharides in cells, often forcing them to rely on higher-order carbohydrate organizations that can form epitopes for immunodetection. A new and powerful method to detect specific carbohydrates is through orthogonal metabolic labeling⁵⁻⁷. In this approach, synthetic sugar analogs serve as chemical reporters. They are introduced to cells, which then incorporate them into native polysaccharides, installing a functional group that is not otherwise present in the organism. This functional group can then be detected by taking advantage of a bioorthogonal ‘click’ reaction to couple it to a small-molecule detection probe. This strategy, termed ‘click-labeling’, has enabled carbohydrate imaging in diverse taxa, including vertebrates, bacteria, and plants⁸⁻¹⁰.

A) 6-Alkynyl fucose probe

B) Expanding the toolbox of alkynyl sugars

Scheme 1. Expanding the toolbox of 6-deoxy-alkynyl sugar probes for metabolic click labeling of oligosaccharides.

The plant cell wall is a particularly enigmatic and interesting structure, which must be strong to ensure the mechanical integrity of plant tissues, but must also permit tightly-controlled nanoscale loosening that results in cell expansion and determines cell shape^{11–13}. The walls of growing plant cells contain four major components: rigid, load-bearing cellulose¹⁴; other glycans grouped as hemicelluloses¹⁵; acidic gel-forming pectins¹⁶; and a variety of proteins¹⁷. Despite intense current interest in understanding plant cell wall structure and how it changes *in vivo*, only three examples of click-labeling plant cell wall components have been reported^{8,18}. In the first study, researchers used a fucose analog **1**, 6-deoxy-alkynyl fucose (6AF), as a chemical reporter for pectin (Scheme 1A)⁸. Alkyne groups in the apoplast were then detected with azide-modified probes, taking advantage of the rapid, selective copper-catalyzed alkyne-azide cyclization (CuAAC) reaction. With this method, pectin delivery and distribution can be monitored in diffusely-expanding cells. In the second study, the authors used azido 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) to label another form of pectin¹⁸, and in the third study, we reported the use of 6-deoxy-6-alkynyl glucose to label an unknown wall component in the tips of root hairs¹⁹. In the present study, we describe the chemical synthesis of six additional

alkyne-carried sugars designed as analogs to three other sugars that present in plant cell walls (Scheme 1B): glucose, which forms the backbone of cellulose and xyloglucan; rhamnose, which is a 6-deoxy-sugar component of two pectin polysaccharides, rhamnogalacturonan-I and rhamnogalacturonan-II^{20–22}; and mannose, which is present in glycosylated proteins and also in mannans, a hemicellulose class that is enriched in walls that are deposited after the cessation of cell growth⁴. Among these new alkynyl probes, four are the 6-deoxy-alkynyl analogs of the corresponding sugars and therefore do not possess 6-OH groups. The other two are based on a new structural design, in which an ethynyl group is incorporated at the C6 position of sugar, and the 6-OH group remains. The synthetic routes we developed featuring the use of methoxymethyl (MOM) and triethylsilyl (TES) protecting groups are efficient and concise. Although they were originally designed to report on plant cell wall polysaccharides, these sugar analogs could prove to be useful reporters for polysaccharides in diverse living systems.

2. Results and discussion

2.1. Synthesis of 6-alkynyl aldohexoses 2, 3 and 4

Although a number of azido-substituted analogs for common monosaccharides have been synthesized and even commercially available, we previously found that 6-deoxy-azidofucose is not efficiently incorporated into plant cell walls as compared to 6-alkynyl fucose **1**(6AF).⁸ Thus, we have sought to expand the toolbox of 6-alkynyl monosaccharides to allow for the study of wall polysaccharides that contain other aldohexoses and deoxyaldohexoses, such as glucose, galactose, xylose, rhamnose, and arabinose. An initial strategy to prepare the 6-alkynyl aldohexoses is to first selectively oxidize the 6-OH group to an aldehyde and then use the Seyferth-Gilbert reaction to install the terminal alkynyl group (Scheme 2A). The key to achieve an efficient and practical synthesis of these compounds is to use proper OH protecting groups, which are compatible with the homologation reaction and also allow facile protection and deprotection. Acetate and benzyl, two of the mostly commonly used protecting groups for sugar

synthesis, cannot be used due to the basic conditions required for the Seyferth-Gilbert reaction and the sensitivity of alkynyl group under hydrogenation conditions. Use of two acetonide protecting groups has been successfully applied for the synthesis of 6-alkynyl fucose **1** (Scheme 2B)⁵. However, most of the other hexoses cannot be properly protected by acetonides, so new protecting strategies are necessary.

A) General synthesis strategy

B) Reported synthesis of fucose **1**

C) 1st generation synthesis of 6-deoxy-alkynyl mannose **3**

D) 2nd generation synthesis of **3**

Scheme 2. Synthesis of 6-deoxy-alkynyl mannose **3**. Reagents and conditions: a)

2-(Trimethylsilyl)ethanol, TMSOTf, dichloromethane (DCM), rt, 0.5 h, 92%; b) K₂CO₃, MeOH, rt, 1h, then PivCl, pyridine (Py), 0 °C, 12h, 52% over two steps; c) TESOTf, triethylamine (TEA), DCM, rt, 3 h, 86%; d) DIBAL-H, DCM, -78 °C, 1h then DMP, DCM, 5h, 91%; e) **8**, K₂CO₃, MeOH, rt, 12h; f) TBAF, THF, rt, 1 h, 72% over two steps; g) 0.1% aq. H₂SO₄, 80 °C, 12h, 79%; h) Ac₂O, Py, 12h, 88%; i) TBSCl, Py, 12h, 50%; j) MOMCl, TBAI, THF, reflux, 12h, then TBAF, THF, rt, 1h, 83% over two steps; k) DMP, DCM, 5h; l) **8**, K₂CO₃, MeOH, rt, 60% over two steps; m) 0.1% aq. H₂SO₄, 80 °C, 12h, 68%.

To synthesize 6-deoxy-alkynyl-D-mannose (6dAM) **3**, we firstly tested a silyl-based protecting group strategy (Scheme 2C). Benzoyl (Bz) protected D-mannosyl tricholoacetimidatedonor **9** prepared following the reported procedure²³ reacted with trimethylsilylethanol to give compound **10**. The OBz groups of **10** were then removed by the treatment of K₂CO₃ in MeOH and the 6-OH group was selectively protected by pivaloyl chloride (PivCl). The remaining OH groups were then protected with triethylsilyltrifluoromethanesulfonate (TESOTf) to give compound **12**. Treatment of **12** with diisobutylaluminium hydride (DIBAL-H) selectively removed OPiv, and the 6-OH intermediate was oxidized by Dess-Martin periodinane (DMP) to give compound **13**. Reaction of **13** with Bestmann's reagent **8** under the typical Seyferth-Gilbert conditions gave compound **14** along with a mixture of partially TES-removed products. Treatment of the mixed products with tetra-*n*-butylammonium fluoride (TBAF) at room temperature (rt) gave compound **15** in good yield. Treatment of **15** with 0.1% aq. H₂SO₄ cleanly gave the desired product 6dAM **3** as an anomeric mixture. Treatment of **3** with Ac₂O gave the corresponding peracetylated product **16**.

Although this route provided the desired products, it required lengthy protecting group manipulations. A 2nd generation synthesis of compound **3** featuring the use of methoxymethyl (MOM) group was then developed. The 6-OH group of D-mannose was first selectively protected by *tert*-butyldimethylsilyl (TBDMS) group to give compound **17**. The remaining OH groups of **17** were then protected by MOM, followed by the removal of the TBDMS group by

TBAF to liberate the 6-OH group to give **18**. The 6-OH group of **18** was oxidized by DMP to form the corresponding aldehyde **19**. The aldehyde was then converted to alkyne **20** under the standard Seyferth-Gilbert conditions with Bestmann's reagent **8**. Global deprotection of MOM groups by the treatment of 0.1% aq. H₂SO₄ gave the desired product **3** in excellent yield and purity. Compared to the 1st generation synthesis of **3**, the new route only involves six total steps, uses two common protecting groups TBDMS and MOM, requires three silica gel chromatograph separations, and is suitable for gram scale synthesis.

Scheme 3. Synthesis of 6-deoxy-alkynyl glucose **2** and 6-alkynyl rhamnose **4**. Reagents and conditions: a) TBSCl, Py, 12h, 46% for D-glucose, 54% for L-mannose; b) MOMCl, TBAI, THF, reflux, 12h, then TBAF, THF, rt, 1h, 88% for **21**, 76% for **24**; c) DMP, DCM, 5h; d) **8**, K₂CO₃, MeOH, rt, 12h, 67% for **22**, 55% for **25**; e) 0.1% H₂SO₄, 80 °C, 12h, 80% for **2**, 70% for **4**.

As shown in Scheme 3, the new synthesis strategy for 6-deoxy-alkynyl sugar can be readily applied to the synthesis of 6-deoxy-alkynyl-glucose (6dAG) **2** and 6-alkynyl-rhamnose (6AR) **4**. It should be noted that 6AR **4** was prepared from a L-mannose precursor.

2.2. Synthesis of 6-deoxy-alkynyl sucrose **5**

Scheme 4. Synthesis of 6-deoxy-alkynyl sucrose **5**. Reagents and conditions: a) triethylorthoacetate, *p*-TsOH, DMF, rt, 3h; b) H₂O, rt, 1h (one pot operation for steps a and b); c) *t*-BuNH₂, rt, 1.5h, 44% over three steps; d) TESOTf, TEA, DCM, rt, 12h, then DIBAL-H, DCM, -78 °C, 1h, 88%; e) DMP, DCM, 5h, rt; f) **8**, K₂CO₃, MeOH, rt, 12h, then TBAF, THF, rt, 1h; g) Ac₂O, Py, 12h, 40% over four steps.

In addition to the monosaccharides mentioned above, the disaccharide sucrose is the major transport form for photo-assimilated carbon and is also a source of carbon skeletons for plant organs unable to perform photosynthesis²⁴. We hypothesized that sucrose carrying a click-compatible groups could also be potentially used by the plant cell. The synthesis of the corresponding 6-deoxy alkynyl analog **5** was developed accordingly as shown in Scheme 4. The 4,6-diol moiety of glucose unit in sucrose was selectively protected by orthoester to give compound **27**, which can be then hydrolyzed under acidic conditions to give a mixture of 4-OAc compound **28** and 6-OAc compound **29**. Treatment of the mixture of **28** and **29** with *t*-BuNH₂ affected the acyl migration from O-4 to O-6 in **28**, giving 6-OAc compound **29** as the major product. The remaining OH groups of **29** were then protected with TES groups to give **30**. The OAc group of **30** was then selectively removed and the liberated 6-OH group was oxidized to give the corresponding aldehyde. Reaction of **31** with Bestmann's reagent **8** under the standard

Seyferth-Gilbert conditions gave the desired 6-deoxy-alkynyl sucrose (6dAS) **5**. Treatment of **5** with acetic anhydride gave the per-acetylated product **32**.

2.3. Synthesis of 6-ethynyl sucrose **6** and 6-ethynyl-sucrose **7**

Scheme 5. Synthesis of 6-ethynyl sucrose **6** and 6-ethynyl-sucrose **7**. Reagents and conditions: a) trimethylsilylacetylene, *n*-BuLi, THF, -78 °C, 85% for **33**, 82% for **36**; b) TBAF, THF, rt, 12h, 68%; c) Ac₂O, Py, 12h, 88% for **34**, 53% for **37** from **35**; d) TBAF, THF, rt, 1h, then 0.1% aq. H₂SO₄, 80 °C, 12h, 73%.

Complementary to the above-mentioned 6-deoxy-alkynyl analogs of sugars, we also investigated a new way to incorporate an alkynyl tag to the sugar substrates by installing an ethynyl group at the C-6 position and keeping the 6-OH group. As shown in Scheme 5, nucleophilic addition of TMS-protected ethynylene lithium salt to sucrose aldehyde **31** formed the alcohol compound **33** as a diastereomeric mixture in good yield. Global deprotection of the TES group by TBAF gave the desired 6-ethynyl sucrose **6** in good yield. Treatment of **6** with Ac₂O gave peracetylated **34**. Similarly, reaction of TMS ethynylene with MOM-protected glucose aldehyde **35** gave compound **36** in good yield. Deprotection of TMS and MOM of **36**

followed by per-acetylation gave the corresponding compound **35** in good yield over four steps.

3. Conclusion

We reported the synthesis of six alkynyl-modified sugar probes designed as analogs to sugars present in plant cell walls. Among these new alkynyl probes, four are the 6-deoxy-alkynyl analogs of the corresponding sugars and do not possess any 6-OH groups. The other two are based on a new structural design, in which an ethynyl group is incorporated at the C-6 position of sugar and the 6-OH group remains. The synthetic routes for both types of probes share common aldehyde intermediates. The aldehydes can either undergo a one-carbon homologation to give the deoxy-alkynyl products or undergo a 1,2-addition with an ethylene reagent to introduce a two-carbon unit at the C-6 position. The overall synthesis sequence of these probes is efficient, concise and scalable. We expect that these probes will find broad application in the metabolic labeling studies of carbohydrates in diverse living systems.

4. Experimental sections:

4.1. General methods

All commercial materials were used as received unless otherwise noted. The tetrahydrofuran and dichloromethane for reaction were obtained from a JC Meyer solvent dispensing system and used without further purification. Flash chromatography was performed using 230-400 mesh SiliaFlash 60® silica gel (Silicycle Inc.). Other commonly used reagents were all purchased from Sigma-Aldrich, and used as received without further treatment. NMR spectra were recorded on BrukerAV-3-HD-500 or Bruker CDPX-300 instrument and calibrated using residual solvent peaks as internal reference. Multiplicities are recorded as: s = singlet, d = doublet, t = triplet, dd

= doublet of doublets, m = multiplet, q = quartet. High resolution ESI mass experiments were operated on a Waters LCT Premier instrument.

4.2. Synthesis of compound **3** (6dAM) in Scheme 2C

4.2.1.2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranoside (**10**)

Compound **9** was prepared according to the reported procedure²³. To a solution of compound **9** (1.48 g, 2.0 mmol, 1 equiv) and 2-(trimethylsilyl)ethanol (354 mg, 3.0 mmol, 1.5 equiv) in DCM (50 mL) was added TMSOTf (16.3 μ L, 0.1 mmol, 0.05 equiv) at rt. The reaction mixture was stirred at rt for 0.5 h, then quenched by the addition of TEA (100 μ L). The reaction mixture was diluted with DCM (50 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 2:1) to give 1.28 g of compound **10** as a white foam in 92% yield. ¹H NMR (500 MHz, CDCl₃) δ 8.16 – 8.06 (m, 4H), 8.02 – 7.95 (m, 2H), 7.90 – 7.83 (m, 2H), 7.65 – 7.57 (m, 2H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.49 – 7.36 (m, 7H), 7.31 – 7.27 (m, 2H), 6.11 (t, *J* = 9.9 Hz, 1H), 5.96 (dd, *J* = 10.1, 3.3 Hz, 1H), 5.70 (dd, *J* = 3.1, 1.8 Hz, 1H), 5.15 (d, *J* = 1.4 Hz, 1H), 4.72 (dd, *J* = 11.6, 1.8 Hz, 1H), 4.59 – 4.45 (m, 2H), 4.01 – 3.89 (m, 1H), 3.76 – 3.66 (m, 1H), 1.20 – 1.01 (m, 2H), 0.09 (s, 9H); ¹³C NMR (126 MHz, CDCl₃) δ = 166.22, 165.54, 165.52, 165.49, 133.45, 133.15, 133.08, 129.87, 129.84, 129.75, 129.42, 129.16, 129.03, 128.60, 128.46, 128.30, 70.80, 70.14, 68.85, 67.11, 66.20, 63.12, 18.02, -1.36; **HRMS**: calculated for C₃₉H₄₁O₁₀Si⁺ [*M*+H⁺]: 697.2464; found: 697.2450.

4.2.2. 2-(Trimethylsilyl)ethyl 6-*O*-pivaloyl- α -D-mannopyranoside (**11**)

To a solution of compound **10** (1.20 g, 1.7 mmol, 1 equiv) in MeOH (100 mL) was added K₂CO₃ (100 mg), and the resulting solution was stirred at rt for 1. After concentration *in vacuo* to remove the methanol, the residue was re-dissolved in pyridine (50 mL) and cooled in ice-water bath. To the reaction mixture was added pivaloyl chloride (290 μ L, 3.4 mmol, 2 equiv) dropwise.

The resulting mixture was stirred at 0 °C overnight, the reaction was then quenched by the addition of MeOH (1 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 1:1) to give 320 mg of compound **11** as a white foam in 52% yield over two steps. ¹H NMR (300 MHz, CDCl₃) δ 4.78 (s, 1H), 4.60 (s, 1H), 4.48 (s, 1H), 4.40 – 4.20 (m, 2H), 4.09 (s, 1H), 3.88 (s, 1H), 3.84 – 3.67 (m, 3H), 3.61 (t, *J* = 9.2 Hz, 1H), 3.53 – 3.39 (m, 1H), 1.18 (s, 9H), 0.97 – 0.84 (m, 2H), -0.01 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ = 179.56, 99.63, 72.07, 71.26, 70.96, 68.42, 65.45, 64.78, 39.26, 27.64, 18.31, -0.92; **HRMS**: calculated for C₁₆H₃₃O₇Si⁺ [*M*+*H*⁺]: 365.1990; found: 365.1993.

4.2.3. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-triethylsilyl 6-*O*-pivaloyl- α -*D*-mannopyranoside (**12**)

To an ice-water cooled solution of compound **11** (300 mg, 0.82 mmol, 1 equiv) and triethylamine (1.1 mL, 8.2 mmol, 10 equiv) in DCM (30 mL) was added TESOTf (1.1 mL, 4.9 mmol, 6 equiv) dropwise. The reaction mixture was stirred at rt for 3h, and then quenched by the addition of MeOH (5 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 30:1) to give 500 mg of compound **12** as a colorless oil in 86% yield. ¹H NMR (300 MHz, CDCl₃) δ 4.98 (dd, *J* = 7.3, 2.8 Hz, 1H), 4.60 (d, *J* = 3.0 Hz, 1H), 4.08 – 3.91 (m, 2H), 3.85 – 3.68 (m, 3H), 3.61 – 3.50 (m, 1H), 3.50 – 3.36 (m, 1H), 1.23 (s, 10H), 0.99 – 0.89 (m, 29H), 0.67 – 0.53 (m, 18H), 0.01 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ = 178.43, 100.30, 76.07, 75.01, 70.79, 67.52, 65.22, 62.99, 39.37, 27.85, 18.35, 7.33, 7.21, 5.46, 4.95, -0.96; **HRMS**: calculated for C₃₄H₇₅O₇Si₄⁺ [*M*+*H*⁺]: 707.4584; found: 707.4575.

4.2.4. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-triethylsilyl α -*D*-manno-hexodialdo-1,5-pyranoside (**13**)

To a solution of compound **12** (400 mg, 565.5 μ mol, 1 equiv) in DCM (30 mL) at -78 °C was added DIBAL-H (0.69 mL, 1.1 mmol, 2 equiv, 1.6 M solution in hexanes) dropwise. The reaction was stirred at -78 °C for 1h, and then quenched by the addition of saturated aq. NH₄Cl

solution (10 mL). The reaction was diluted with DCM (50 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was used for next step without further purification. To a solution of the residue in DCM (20 mL) at rt was added Dess–Martin periodinane (480 mg, 1.1 mmol, 2 equiv), and the resulting reaction mixture was stirred at rt for 5h. The reaction mixture was diluted with DCM (50 mL), and washed thoroughly with saturated aq. sodium thiosulfate solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 50:1) to give 320 mg of compound **13** as a colorless oil in 91% yield. ^1H NMR (300 MHz, CDCl_3) δ 9.69 (s, 1H), 4.91 (s, 1H), 4.16 – 3.91 (m, 3H), 3.86 (dd, J = 4.4, 2.2 Hz, 1H), 3.79 (dd, J = 7.3, 2.1 Hz, 1H), 3.57 (dd, J = 16.5, 10.0 Hz, 1H), 1.00 – 0.85 (m, 29H), 0.70 – 0.55 (m, 18H), 0.01 (s, 9H); ^{13}C NMR (75 MHz, CDCl_3) δ = 198.42, 98.61, 83.85, 75.25, 72.95, 69.01, 67.53, 18.64, 7.21, 7.14, 5.27, 5.07, 4.98, -1.12; **HRMS**: calculated for $\text{C}_{29}\text{H}_{65}\text{O}_6\text{Si}_4^+[\text{M}+\text{H}^+]$: 621.3853; found: 621.3854.

4.2.5.2-(Trimethylsilyl)ethyl6,7-dideoxy- α -D-manno-hept-6-ynopyranoside (**15**)

To an ice-water cooled solution of compound **13** (300 mg, 483 μmol , 1 equiv) and Bestmann's reagent **8** (139 mg, 724 μmol , 1.5 equiv) in MeOH (15 mL) was added K_2CO_3 (133 mg, 966 μmol , 2 equiv), and the resulting mixture was gradually warmed up to rt and stirred overnight. The reaction was diluted with EtOAc (50 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was used for next step without further purification. To a solution of the residue in THF (10 mL) was added TBAF (1.4 mL, 1.4 mmol, 3 equiv, 1M solution in THF), and the resulting solution was stirred at rt for 1h. The reaction was diluted with EtOAc (50 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 1:1) to give 95 mg of compound **15** as a white solid in 72% yield over

two steps. ^1H NMR (500 MHz, CDCl_3) δ 4.88 (s, 1H), 4.32 (dd, J = 9.4, 1.7 Hz, 1H), 3.97 (s, 1H), 3.93 – 3.81 (m, 3H), 3.59 – 3.52 (m, 1H), 2.62 – 2.55 (m, 1H), 1.05 – 0.87 (m, 2H), 0.05 (s, 9H); ^{13}C NMR (126 MHz, CDCl_3) δ = 99.64, 80.58, 74.55, 71.08, 70.99, 70.61, 65.92, 63.35, 17.98, -1.33; **HRMS**: calculated for $\text{C}_{12}\text{H}_{23}\text{O}_5\text{Si}^+$ [$\text{M}+\text{H}^+$]: 275.1309; found: 275.1310.

4.2.6.6-alkynyl-*D*-mannose (6dAM, **3**)

The solution of compound **15** (50 mg, 182.2 μmol , 1 equiv) in aq. H_2SO_4 (10 mL, 0.1% v/v) was stirred at 80 $^\circ\text{C}$ overnight. After cooled with ice-water bath, the reaction was neutralized with NaHCO_3 to pH 7 and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (chloroform : methanol = 5:1) to give 25 mg of compound **3** as a white solid in 79% yield. ^1H NMR (500 MHz, D_2O) δ 5.08 (s, 1H), 4.82 (s, 0.4 H), 4.41 (d, J = 9.3 Hz, 1H), 4.00 (dd, J = 9.5, 1.5 Hz, 0.4 H), 3.84 (d, J = 8.5 Hz, 1.4 H), 3.75 – 3.63 (m, 2H), 3.60 (t, J = 9.7 Hz, 0.4 H), 3.53 (dd, J = 9.7, 2.8 Hz, 0.4 H), 2.91 – 2.77 (m, 1.4 H); ^{13}C NMR (126 MHz, D_2O) δ = 94.29, 93.94, 80.07, 79.35, 75.57, 75.33, 72.14, 70.84, 70.30, 70.25, 69.89, 69.54, 66.62, 63.19; **HRMS**: calculated for $\text{C}_7\text{H}_9\text{O}_5^-$ [$\text{M}-\text{H}^+$]: 173.0455; found: 173.0464.

4.2.7.6-alkynyl-*D*-mannose tetraacetate (**16**)

To an ice-water cooled solution of compound **3** (17.4 mg, 100 μmol , 1 equiv) in pyridine (2 mL) was added Ac_2O (94 μL , 1.0 mmol, 10 equiv), and the resulting solution was stirred at rt overnight. After completion, the reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 3:1) to give 30mg of compound **16** as a white solid in 88% yield. ^1H NMR (500 MHz, CDCl_3) δ 6.85 (s, 1H), 6.13 (s, 0.7H), 5.60 – 5.34 (m, 3.8H), 5.34 – 5.18 (m, 2.5H), 4.60 (d, J = 9.7 Hz, 0.7H), 2.52 (s, 0.7H), 2.46 (s, 1H), 2.22 – 1.94 (m, 27H); ^{13}C NMR (126 MHz, CDCl_3) δ = 169.85, 169.59, 169.34, 169.25, 168.83, 168.56, 168.27, 90.16, 86.02, 75.37, 75.04, 68.89, 68.25, 68.12, 68.07, 67.17, 66.74, 63.59, 61.04, 20.84, 20.76, 20.69, 20.61, 20.58, 20.54; **HRMS**: calculated for $\text{C}_{15}\text{H}_{18}\text{NaO}_9^+$ [$\text{M}+\text{Na}^+$]: 365.0843; found: 365.0840.

4.3. General procedure for the synthesis of compounds **2**, **3**, and **4** in Scheme 2D and Scheme 3

4.3.1. General procedure for the TBDMS protection of 6-OH of free sugars

To an ice-water cooled solution of D-glucose, L- or D-mannose (5.4 g, 30 mmol, 1 equiv) in dry pyridine (50 mL) was added *tert*-butyldimethylsilyl chloride (5.4 g, 36 mmol, 1.2 equiv) portionwise. The ice-water bath was removed after addition, and the reaction mixture was stirred at rt overnight. The solution was diluted with ethyl acetate (250 mL) and washed thoroughly with aq. HCl (0.5 M) and brine. The residue of concentrated organic layer was purified on flash silica gel chromatography (dichloromethane : methanol = 15 : 1).

6-O-tert-butyldimethylsilyl-D-mannose (17): 50% yield, a white solid. ^1H NMR (500 MHz, MeOD) δ 5.08 (s, 1H), 3.96 (dd, J = 11.0, 2.2 Hz, 1H), 3.85 (dd, J = 11.0, 5.9 Hz, 1H), 3.81 – 3.73 (m, 3H), 3.61 (t, J = 9.4 Hz, 1H), 0.94 (s, 9H), 0.16 – 0.08 (m, 6H); ^{13}C NMR (126 MHz, MeOD) δ = 94.39, 72.97, 71.49, 70.99, 67.66, 63.54, 25.10, 17.98, -6.51; HRMS: calculated for $\text{C}_{12}\text{H}_{26}\text{NaO}_6\text{Si}^+$ [$\text{M}+\text{Na}^+$]: 317.1391; found: 317.1389.

6-O-tert-butyldimethylsilyl-D-glucose: 46% yield, a white foam. ^1H NMR (MeOH- d_4 , 360 MHz, ppm): δ 0.08 (s, 6H), 0.91 (s, 9H), 3.09-3.11 (m, 0.45 H), 3.31-3.37 (m, 4H, solvent residue peak included), 3.64-3.69 (t, J = 9.0, 9.4 Hz, 0.57 H), 3.74-3.85 (m, 2H), 3.93-3.97 (d, J = 11.1 Hz, 0.50 H), 4.43-4.45 (d, J = 7.6 Hz, 0.46 H), 5.08-5.09 (d, J = 3.2 Hz, 0.49 H); ^{13}C NMR (MeOH- d_4 , 90 MHz, ppm) δ -5.14, -5.02, 19.34, 26.45, 64.13, 64.30, 71.50, 71.56, 73.20, 73.84, 74.91, 76.23, 78.23, 93.92, 98.12, 101.37; HRMS: calculated for $\text{C}_{12}\text{H}_{26}\text{NaO}_6\text{Si}^+$ [$\text{M}+\text{Na}^+$]: 317.1391; found: 317.1392.

6-O-tert-butyldimethylsilyl-L-mannose: 54% yield, a white solid in. ^1H NMR (500 MHz, MeOD) δ 5.08 (s, 1H), 3.96 (dd, J = 11.0, 2.2 Hz, 1H), 3.85 (dd, J = 11.0, 5.9 Hz, 1H), 3.81 – 3.73 (m,

3H), 3.61 (t, $J = 9.4$ Hz, 1H), 0.94 (s, 9H), 0.16 – 0.08 (m, 6H); ^{13}C NMR (126 MHz, MeOD) $\delta = 94.39, 72.97, 71.49, 70.99, 67.66, 63.54, 25.10, 17.98, -6.51$; **HRMS**: calculated for $\text{C}_{12}\text{H}_{26}\text{NaO}_6\text{Si}^+ [\text{M}+\text{Na}^+]$: 317.1391; found: 317.1389.

4.3.2. General procedure for the synthesis of compounds **18**, **21** and **24**

To an ice-water cooled solution of 6-O-TBDMS sugars (0.40 g, 1.4 mmol, 1 equiv), DIPEA (1.4 mL, 8.2 mmol, 6 equiv) and TBAI (103 mg, 0.28 mmol, 0.2 equiv) in dry THF (20 mL) was added methoxymethyl chloride (520 μL , 6.8 mmol, 5 equiv) dropwise. The ice-water bath was removed after addition, and the reaction mixture was refluxed overnight. After completion, the reaction mixture was diluted with ethyl acetate (100 mL) and washed thoroughly with diluted hydrochloric acid (0.5 M) and brine. The residue of concentrated organic layer was used for next step without further purification. To the solution of the residue in THF (20 mL) was added TBAF (530 mg, 2.0 mmol, 1.5 equiv), and the resulting reaction mixture was stirred at room temperature for 1 hour. After completion, the reaction was diluted with ethyl acetate (100 mL) and washed thoroughly with water and brine. After concentration *in vacuo*, the resulting residue was purified on silica gel flash chromatography (hexanes : acetone = 3:1).

1,2,3,4-tetra-O-methoxymethyl-D-mannose (18): 83% yield, a white foam. ^1H NMR (500 MHz, CDCl_3) δ 5.15 (d, $J = 1.5$ Hz, 1H), 4.93 – 4.85 (m, 2H), 4.84 – 4.71 (m, 4H), 4.69 (d, $J = 6.3$ Hz, 1H), 4.58 (d, $J = 6.5$ Hz, 1H), 4.03 (dd, $J = 9.6, 3.1$ Hz, 1H), 3.99 – 3.91 (m, 2H), 3.90 – 3.80 (m, 2H), 3.73 – 3.65 (m, 1H), 3.46 – 3.40 (m, 12H); ^{13}C NMR (126 MHz, CDCl_3) $\delta = 98.54, 96.95, 96.13, 95.51, 92.98, 76.25, 75.28, 73.29, 72.44, 62.18, 56.29, 56.22, 55.69, 55.64$; **HRMS**: calculated for $\text{C}_{14}\text{H}_{28}\text{NaO}_{10}^+ [\text{M}+\text{Na}^+]$: 379.1575; found: 379.1581.

1,2,3,4-tetra-O-methoxymethyl-D-glucose (21): 88% yield, a white foam. ^1H NMR (CDCl_3 , 360 MHz, ppm): δ 2.72-2.76 (m, 1 H), 3.27-3.38 (m, 13 H), 3.52-3.63 (m, 2 H), 3.76-3.78 (m, 1.8 H), 4.52-4.56 (m, 1.6 H), 4.65-4.77 (m, 3.2 H), 4.83-4.87 (m, 2.8 H), 4.93-4.95 (m, 1 H); ^{13}C NMR

(CDCl₃, 90 MHz, ppm) δ 55.66, 56.11, 56.18, 56.30, 56.38, 56.46, 61.59, 71.34, 75.17, 76.36, 76.57, 76.80, 77.16, 77.36, 77.51, 77.61, 77.94, 78.24, 80.91, 93.60, 94.83, 97.10, 97.42, 98.22, 98.41, 98.93; **HRMS**: calculated for C₁₄H₂₈NaO₁₀⁺ [M+Na⁺]: 379.1575; found: 379.1581.

1,2,3,4-tetra-O-methoxymethyl-L-mannose (24): 76% yield, a white foam. **¹H NMR** (500 MHz, CDCl₃) δ 5.15 (d, *J* = 1.5 Hz, 1H), 4.93 – 4.85 (m, 2H), 4.84 – 4.71 (m, 4H), 4.69 (d, *J* = 6.3 Hz, 1H), 4.58 (d, *J* = 6.5 Hz, 1H), 4.03 (dd, *J* = 9.6, 3.1 Hz, 1H), 3.99 – 3.91 (m, 2H), 3.90 – 3.80 (m, 2H), 3.73 – 3.65 (m, 1H), 3.46 – 3.40 (m, 12H); **¹³C NMR** (126 MHz, CDCl₃) δ = 98.54, 96.95, 96.13, 95.51, 92.98, 76.25, 75.28, 73.29, 72.44, 62.18, 56.29, 56.22, 55.69, 55.64; **HRMS**: calculated for C₁₄H₂₈NaO₁₀⁺ [M+Na⁺]: 379.1575; found: 379.1580.

4.3.3. General procedure for the synthesis of compounds **20**, **22** and **25**

To an ice-water cooled solution of compound **18**, **21** or **24** (356 mg, 1.0 mmol, 1 equiv) in DCM (35 mL) was added Dess-Martin periodinane (636 mg, 1.5 mmol, 1.5 equiv). The reaction mixture was stirred at rt for 5h. After completion, the milky solution was washed thoroughly with saturated aq. solution of sodium thiosulfate and brine. The organic layer was dried over sodium sulfate and concentrated. The resulting residue was used for next step without further purification. To an ice-water cooled solution of the residue and Bestmann's reagent **8** (288 mg, 1.5 mmol, 1.5 equiv) in MeOH (20 mL) was added K₂CO₃ (276 mg, 2.0 mol, 2 equiv), and the resulting mixture was gradually warmed up to rt and stirred overnight. The reaction was diluted with EtOAc (50 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 2:1).

1,2,3,4-tetra-O-methoxymethyl-D-manno-hept-6-ynopyranose (20): 60% yield, a white foam. **¹H NMR** (500 MHz, CDCl₃) δ 5.17 (d, *J* = 1.5 Hz, 1H), 4.94 (d, *J* = 6.4 Hz, 1H), 4.90 (q, *J* = 6.4 Hz, 2H), 4.84 – 4.74 (m, 4H), 4.60 (d, *J* = 6.4 Hz, 1H), 4.39 (dd, *J* = 9.4, 1.7 Hz, 1H), 4.02 (t, *J* = 9.4

Hz, 1H), 3.96 (dd, $J = 9.4, 2.9$ Hz, 1H), 3.93 – 3.89 (m, 1H), 3.50 – 3.41 (m, 12H), 2.49 (d, $J = 2.1$ Hz, 1H); ^{13}C NMR (126 MHz, CDCl_3) $\delta = 97.79, 97.07, 96.54, 95.44, 93.29, 80.95, 75.54, 75.25, 75.21, 73.77, 63.38, 56.54, 56.29, 55.78, 55.72$; **HRMS**: calculated for $\text{C}_{15}\text{H}_{26}\text{NaO}_9^+$ [$\text{M}+\text{Na}^+$]: 373.1469; found: 374.1467.

1,2,3,4-tetra-O-methoxymethyl-D-gluco-hept-6-ynopyranose (22): 67% yield, a white foam. ^1H NMR (CDCl_3 , 360 MHz, ppm): δ 2.46-2.49 (m, 1.5 H), 3.35-3.67 (m, 23.5 H), 3.85-3.90 (m, 0.5 H), 4.02-4.06 (m, 1H), 4.38-4.41 (m, 0.5 H), 4.56-5.13 (m, 15 H); ^{13}C NMR (CDCl_3 , 90 MHz, ppm) δ 55.79, 56.27, 56.37, 56.44, 56.49, 56.57, 56.81, 56.85, 62.34, 66.10, 74.15, 74.58, 78.40, 78.71, 79.69, 80.09, 80.82, 93.61, 93.97, 94.89, 97.31, 97.51, 97.84, 98.39, 98.45; **HRMS**: calculated for $\text{C}_{15}\text{H}_{26}\text{NaO}_9^+$ [$\text{M}+\text{Na}^+$]: 373.1469; found: 374.1467.

1,2,3,4-tetra-O-methoxymethyl-L-manno-hept-6-ynopyranose (25): 55% yield, a white foam. ^1H NMR (500 MHz, CDCl_3) δ 5.17 (d, $J = 1.5$ Hz, 1H), 4.94 (d, $J = 6.4$ Hz, 1H), 4.90 (q, $J = 6.4$ Hz, 2H), 4.84 – 4.74 (m, 4H), 4.60 (d, $J = 6.4$ Hz, 1H), 4.39 (dd, $J = 9.4, 1.7$ Hz, 1H), 4.02 (t, $J = 9.4$ Hz, 1H), 3.96 (dd, $J = 9.4, 2.9$ Hz, 1H), 3.93 – 3.89 (m, 1H), 3.50 – 3.41 (m, 12H), 2.49 (d, $J = 2.1$ Hz, 1H); ^{13}C NMR (126 MHz, CDCl_3) $\delta = 97.79, 97.07, 96.54, 95.44, 93.29, 80.95, 75.54, 75.25, 75.21, 73.77, 63.38, 56.54, 56.29, 55.78, 55.72$; **HRMS**: calculated for $\text{C}_{15}\text{H}_{26}\text{NaO}_9^+$ [$\text{M}+\text{Na}^+$]: 373.1469; found: 374.1467.

4.3.4. General procedure for the synthesis of compound 6dAM (3), 6dAG (2) and 6AR (4)

The solution of compound **20**, **22** or **25** (100 mg, 285 μmol , 1 equiv) in aq. sulfuric acid (10 mL, 0.1% v/v) was stirred at 80 $^\circ\text{C}$ overnight. After cooled with ice-water bath, the reaction was neutralized with sodium bicarbonate to pH 7 and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (chloroform : methanol = 5:1).

6-alkynyl-D-mannose (6dAM, 3): 68% yield, a white solid in. ^1H NMR (500 MHz, D_2O) δ 5.08 (s,

1H), 4.82 (s, 0.4 H), 4.41 (d, $J = 9.3$ Hz, 1H), 4.00 (dd, $J = 9.5, 1.5$ Hz, 0.4 H), 3.84 (d, $J = 8.5$ Hz, 1.4 H), 3.75 – 3.63 (m, 2H), 3.60 (t, $J = 9.7$ Hz, 0.4 H), 3.53 (dd, $J = 9.7, 2.8$ Hz, 0.4 H), 2.91 – 2.77 (m, 1.4 H); ^{13}C NMR (126 MHz, D_2O) $\delta = 94.29, 93.94, 80.07, 79.35, 75.57, 75.33, 72.14, 70.84, 70.30, 70.25, 69.89, 69.54, 66.62, 63.19$; **HRMS**: calculated for $\text{C}_7\text{H}_9\text{O}_5^-$ $[\text{M}-\text{H}^+]$: 173.0455; found: 173.0464.

6-alkynyl-D-glucose (6dAG, 2): 80% yield, a white solid. ^1H NMR ($\text{MeOH}-d_4$, 400 MHz, ppm): δ 2.74-2.75 (d, $J = 1.8$ Hz, 1H), 2.82-2.83 (d, $J = 1.8$ Hz, 0.62 H), 3.08-3.12 (t, $J = 8.4, 8.4$ Hz, 0.7 H), 3.23-3.34 (m, 8H), 3.54-3.58 (t, $J = 9.2, 9.2$ Hz, 1H), 3.92-3.94 (d, $J = 7.6$ Hz, 0.7 H), 4.39-4.43 (m, 1.7 H), 5.02-5.03 (d, $J = 3.6$ Hz, 1H); ^{13}C NMR ($\text{MeOH}-d_4$, 100 MHz, ppm) δ 62.41, 67.01, 72.14, 72.87, 73.09, 73.76, 73.94, 74.26, 74.52, 75.89, 80.02, 81.06, 92.71, 96.83; **HRMS**: calculated for $\text{C}_7\text{H}_9\text{O}_5^-$ $[\text{M}-\text{H}^+]$: 173.0455; found: 173.0464.

6-alkynyl-L-mannose (6AR, 4): 70% yield, a white solid. ^1H NMR (500 MHz, D_2O) δ 5.08 (s, 1H), 4.82 (s, 0.4 H), 4.41 (d, $J = 9.3$ Hz, 1H), 4.00 (dd, $J = 9.5, 1.5$ Hz, 0.4 H), 3.84 (d, $J = 8.5$ Hz, 1.4 H), 3.75 – 3.63 (m, 2H), 3.60 (t, $J = 9.7$ Hz, 0.4 H), 3.53 (dd, $J = 9.7, 2.8$ Hz, 0.4 H), 2.91 – 2.77 (m, 1.4 H); ^{13}C NMR (126 MHz, D_2O) $\delta = 94.29, 93.94, 80.07, 79.35, 75.57, 75.33, 72.14, 70.84, 70.30, 70.25, 69.89, 69.54, 66.62, 63.19$; **HRMS**: calculated for $\text{C}_7\text{H}_9\text{O}_5^-$ $[\text{M}-\text{H}^+]$: 173.0455; found: 173.0464.

4.4. Synthesis of compound 5 in Scheme 4

4.4.1. 6-O-acetylsucrose (29)

To a solution of sucrose (10.0 g, 29.2 mmol, 1 equiv) and triethylorthoacetate (7.0 mL, 38.1 mmol, 1.3 equiv) in DMF (50 mL) at rt was added 4-methylbenzenesulfonic acid (60 mg, 0.3 mmol, 0.01 equiv), and the resulting reaction mixture was stirred at rt for 3h. To the reaction mixture was added water (50 mL), and the resulting mixture was stirred at rt for 1 h followed by

the addition of *tert*-butylamine (1mL). After stirred at rt for 1.5h, the reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (dichloromethane : methanol = 5:1) to give 4.9 g of compound **29** as a white foam in 44% yield. ¹H NMR (500 MHz, DMSO) δ 5.27 – 5.08 (m, 3H), 5.03 (d, *J* = 5.7 Hz, 1H), 4.90 (d, *J* = 4.8 Hz, 1H), 4.82 (t, *J* = 6.1 Hz, 1H), 4.58 (d, *J* = 7.8 Hz, 1H), 4.46 – 4.34 (m, 1H), 4.23 (d, *J* = 11.3 Hz, 1H), 4.14 – 4.05 (m, 1H), 4.00 (dd, *J* = 11.4, 6.3 Hz, 1H), 3.95 – 3.80 (m, 2H), 3.79 – 3.67 (m, 1H), 3.64 – 3.44 (m, 4H), 3.44 – 3.26 (m, 3H), 3.25 – 3.10 (m, 3H), 3.09 – 2.98 (m, 1H), 2.03 (s, 3H); ¹³C NMR (126 MHz, DMSO) δ = 170.90, 104.31, 91.84, 83.20, 77.31, 74.93, 73.13, 71.97, 70.66, 70.40, 64.27, 63.07, 62.67, 49.07, 21.16; **HRMS**: calculated for C₁₄H₂₄NaO₁₂⁺ [M+Na⁺]: 407.1160; found: 407.1161.

4.4.2.1',2,3,3',4,4',6'-hepta-*O*-triethylsilylsucrose (**30**)

To an ice-water cooled solution of compound **29** (384.3 mg, 1.0 mmol, 1 equiv) and triethylamine (5.6 mL, 40.0 mmol, 40 equiv) in DCM (100 mL) was added TESOTf (4.5 mL, 20.0 mmol, 20 equiv) dropwise. The reaction mixture was stirred at room temperature overnight, and quenched by adding methanol (10 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was used for next step without further purification. To a solution of the residue in DCM (50 mL) at -78 °C was added DIBAL-H (3.1 mL, 5.0 mmol, 5 equiv, 1.6 M solution in hexanes) dropwise. The reaction was stirred at -78 °C for 1 hour, and then quenched by adding saturated aqueous NH₄Cl solution (10 mL). The reaction was diluted with DCM (50 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (hexanes : EtOAc = 10:1) to give 920 mg of compound **30** as a colorless oil in 88% yield. ¹H NMR (500 MHz, CDCl₃) δ 5.29 (d, *J* = 2.2 Hz, 1H), 4.42 (d, *J* = 8.0 Hz, 1H), 4.07 – 3.91 (m, 2H), 3.91 – 3.77 (m, 2H), 3.77 – 3.60 (m, 4H), 3.60 – 3.51 (m, 1H), 3.51 – 3.33 (m, 3H), 1.87 (s, 1H), 1.09 – 0.81 (m, 63H), 0.79 – 0.50 (m, 42H); ¹³C NMR (126 MHz, CDCl₃) δ = 103.42, 91.07, 82.29, 76.50, 75.49, 74.22, 73.56, 72.73, 72.08, 64.15, 63.55, 61.57, 7.22, 7.09,

6.99, 6.91, 6.90, 6.75, 6.62, 5.69, 5.33, 5.20, 5.12, 4.52, 4.43; **HRMS**: calculated for $C_{54}H_{120}NaO_{11}Si_7^+$ [$M+Na^+$]: 1163.7108; found: 1163.7110.

4.4.3.6-alkynylsucrose heptaacetate (**32**)

To a solution of compound **30** (523 mg, 0.5 mmol, 1 equiv) in DCM (35 mL) at room temperature was added Dess–Martin periodinane (636 mg, 1.5 mmol, 3 equiv), and the resulting reaction mixture was stirred at room temperature for 5h. After completion, the reaction was diluted with DCM (50 mL), and washed thoroughly with saturated aqueous sodium thiosulfate solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was used for next step without further purification. To an ice-water cooled solution of the residue and Bestmann's reagent (144 mg, 750 μ mol, 1.5 equiv) in methanol (15 mL) was added potassium carbonate (138 mg, 1.0 μ mol, 2 equiv), and the resulting mixture was gradually warmed up to room temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was used for next step without further purification. To a solution of the residue in THF (10 mL) was added TBAF (5.0 mL, 5.0 mmol, 10 equiv, 1M solution in THF), and the resulting solution was stirred at room temperature for 1 hour. After completion, the reaction concentrated *in vacuo*, and the resulting residue was used for next step without further purification. To an ice-water cooled solution of the residue in pyridine (10 mL) was added acetic anhydride (943 μ L, 10.0 mmol, 20 equiv), and the resulting solution was stirred at room temperature overnight. After completion, the reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 2:1) to give 125 mg of compound **32** as a white foam in 40% yield over four steps. 1H NMR (500 MHz, $CDCl_3$) δ 5.73 (s, 1H), 5.51 – 5.26 (m, 4H), 5.19 (t, J = 9.9 Hz, 1H), 4.87 – 4.72 (m, 2H), 4.44 – 4.07 (m, 5H), 2.47 (s, 1H), 2.25 – 1.94 (m, 21H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 171.04, 170.56, 170.18, 170.02, 169.94, 169.81, 169.73, 169.46, 169.34, 168.72, 104.54, 89.90, 79.26, 78.09, 75.93,

75.11, 75.04, 71.16, 70.33, 68.97, 63.21, 63.08, 61.35, 20.73, 20.68; **HRMS**: calculated for $C_{27}H_{34}NaO_{17}^+$ [$M+Na^+$]: 653.1688; found: 653.1688.

4.5. Synthesis of compounds **6** and **7** in Scheme 5

4.5.1. 1',2,3,3',4,4',6'-hepta-*O*-triethylsilyl-6-trimethylsilylethynyl-sucrose (**33**)

To a solution of trimethylsilylacetylene (29.5 mg, 0.3 mmol, 3 equiv) in THF (5 mL) under argon atmosphere at -78 °C was added *n*-BuLi (187.5 μ L, 0.3 mmol, 3 equiv, 1.6 M solution in hexanes), and the resulting reaction mixture was gradually warmed up to 0 °C. To the reaction mixture was added the solution of compound **31** (114.0 mg, 0.1 mmol, 1 equiv) in THF (5 mL) dropwise, and the resulting reaction mixture was stirred at 0 °C for 2 hours. After quenching by the addition of saturated aqueous solution of ammonium chloride (10 mL), the reaction mixture was diluted with EtOAc (30 mL), and washed thoroughly with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by silica gel flash chromatography (hexanes : EtOAc = 5:1) to give 105 mg of compound **33** as a colorless oil in 85% yield; 1H NMR (500 MHz, $CDCl_3$) δ 5.31 (s, 1H), 4.50 (d, J = 6.7 Hz, 1H), 4.41 (d, J = 7.6 Hz, 1H), 4.17 – 4.00 (m, 2H), 3.91 – 3.65 (m, 3H), 3.67 – 3.33 (m, 5H), 3.04 (s, 1H), 0.99 (dd, J = 9.5, 5.3 Hz, 63H), 0.82 – 0.50 (m, 42H), 0.18 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 104.35, 103.19, 91.00, 82.36, 74.99, 74.50, 74.25, 74.04, 73.38, 64.31, 63.63, 62.74, 7.18, 7.12, 7.00, 6.95, 6.93, 6.82, 6.63, 5.65, 5.37, 5.21, 5.19, 5.14, 4.52, 4.37, -0.23; **HRMS**: calculated for $C_{59}H_{128}NaO_{11}Si_8^+$ [$M+Na^+$]: 1259.7503; found: 1259.7505.

4.5.2. 6-ethynyl-sucrose (6-ES, **6**)

To the solution of compound **33** (124 mg, 0.1 mmol, 1 equiv) in THF (10 mL) was added TBAF (1.6 mL, 1.6 mmol, 16 equiv, 1M solution in THF), and the reaction was stirred at rt overnight. After completion, the reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (chloroform : methanol = 5:1) to give 25

mg of compound **6** as a gum in 68% yield. ^1H NMR (500 MHz, D_2O) δ 5.36 (d, J = 3.6 Hz, 1H), 4.12 (d, J = 8.8 Hz, 1H), 3.99 (t, J = 8.5 Hz, 1H), 3.90 (dd, J = 10.0, 2.0 Hz, 1H), 3.85 – 3.74 (m, 2H), 3.74 – 3.64 (m, 2H), 3.59 (s, 2H), 3.49 (dd, J = 9.9, 3.7 Hz, 1H), 3.42 (t, J = 9.6 Hz, 1H), 3.26 (s, 2H); ^{13}C NMR (126 MHz, D_2O) δ = 103.68, 91.97, 81.26, 76.37, 74.08, 73.87, 72.28, 70.92, 70.64, 62.35, 61.53, 61.27, 48.83; **HRMS**: calculated for $\text{C}_{14}\text{H}_{22}\text{NaO}_{11}^+$ [$\text{M}+\text{Na}^+$]: 389.1054; found: 389.1060.

4.5.3. 6-ethynylsucrose octaacetate (**34**)

To an ice-water cooled solution of compound **6** (10 mg, 27.3 μmol , 1 equiv) in pyridine (1 mL) was added acetic anhydride (52 μL , 546 μmol , 20 equiv), and the resulting solution was stirred at rt overnight. After completion, the reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 1:1) to give 17 mg of compound **34** as a white solid in 88% yield. ^1H NMR (500 MHz, CDCl_3) δ 5.75 (s, 1H), 5.58 (s, 1H), 5.53 – 5.41 (m, 2H), 5.38 (t, J = 5.6 Hz, 1H), 5.29 (t, J = 9.8 Hz, 1H), 4.90 (dd, J = 10.3, 3.1 Hz, 1H), 4.42 – 4.27 (m, 3H), 4.22 (s, 3H), 2.56 (s, 1H), 2.24 – 1.96 (m, 24H); ^{13}C NMR (126 MHz, CDCl_3) δ = 170.55, 170.13, 169.89, 169.70, 169.54, 169.37, 104.14, 90.08, 79.21, 75.81, 75.64, 75.07, 70.61, 70.06, 69.55, 68.65, 63.69, 62.76, 62.36, 20.84, 20.75, 20.68, 20.60; **HRMS**: calculated for $\text{C}_{30}\text{H}_{38}\text{NaO}_{19}^+$ [$\text{M}+\text{Na}^+$]: 725.1900; found: 725.1900.

4.5.4. 6-ethynylglucose pentaacetate (**37**)

To a solution of trimethylsilylacetylene (147 mg, 1.5 mmol, 3 equiv) in THF (20 mL) under argon atmosphere at $-78\text{ }^\circ\text{C}$ was added *n*-BuLi (938 μL , 1.5 mmol, 3 equiv, 1.6 M solution in hexanes), and the resulting reaction mixture was gradually warmed up to $0\text{ }^\circ\text{C}$. To the reaction mixture was added the solution of compound **35** (177 mg, 0.5 mmol, 1 equiv) in THF (10 mL) dropwise, and the resulting reaction mixture was stirred at $0\text{ }^\circ\text{C}$ for 2 hours. After quenching by the addition of saturated aqueous solution of ammonium chloride (30 mL), the reaction mixture was diluted with EtOAc (100 mL), and washed thoroughly with water and brine. The organic

layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The resulting residue was used for next step without further purification. To the solution of the residue in THF (10 mL) was added TBAF (1.0 mL, 1.0 mmol, 2 equiv, 1M solution in THF), and the reaction was stirred at room temperature for 1 hour. After completion, the reaction mixture was diluted with EtOAc (50 mL) and washed thoroughly with water and brine. The organic layer was concentrated *in vacuo*, and the resulting residue was used for next step without further purification. The solution of the residue in diluted sulfuric acid (10 mL, 0.1% v/v) was stirred at 80 °C overnight. After cooled with ice-water bath, the reaction was neutralized with sodium bicarbonate to pH 7 and concentrated *in vacuo*. The resulting residue was used for next step without further purification. To an ice-water cooled solution of the residue in pyridine (10 mL) was added acetic anhydride (471 μL, 5.0 mmol, 10 equiv), and the resulting solution was stirred at rt overnight. After completion, the reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel flash chromatography (hexanes : ethyl acetate = 2:1) to give 110 mg of compound **37** as a white solid in 53% yield. ¹H NMR (500 MHz, CDCl₃) δ 6.47 – 6.28 (m, 1H), 5.83 – 5.66 (m, 1H), 5.61 – 5.42 (m, 3H), 5.37 – 5.22 (m, 2H), 5.22 – 5.04 (m, 3H), 4.29 – 4.10 (m, 1H), 3.97 – 3.81 (m, 1H), 2.57 (s, 1H), 2.52 (d, *J* = 1.9 Hz, 1H), 2.25 – 1.88 (m, 32H); ¹³C NMR (126 MHz, CDCl₃) δ = 170.25, 170.17, 169.79, 169.66, 169.60, 169.48, 169.39, 169.23, 168.81, 168.58, 91.88, 91.41, 89.03, 88.97, 76.59, 76.17, 76.14, 76.02, 75.88, 75.50, 75.22, 74.64, 74.38, 72.77, 72.65, 71.88, 71.81, 70.20, 69.91, 69.82, 69.79, 69.08, 68.94, 68.31, 67.58, 67.34, 62.26, 60.56, 60.33, 20.83, 20.78, 20.64, 20.56, 20.53, 20.44; **HRMS**: calculated for C₁₈H₂₂NaO₁₁⁺ [*M*+Na⁺]: 437.1054; found: 437.1055.

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Appendix: Supplementary material

Supplementary data including NMR spectra of all new compounds can be found online.

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