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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.

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 monomers [1–4]. 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 labeling [5–7]. 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 plants [8–10].

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 cellulose [14]; other glycans grouped as hemicelluloses [15]; acidic gel-forming pectins [16]; and a variety of proteins [17]. 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)

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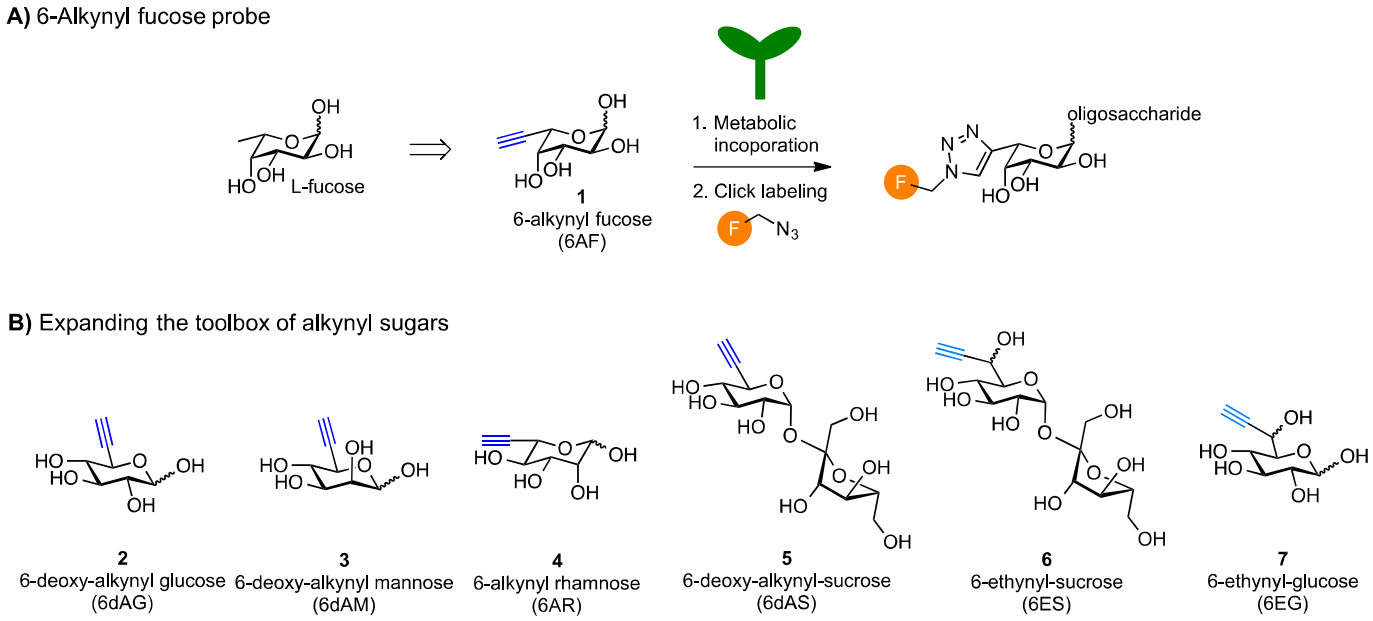

Scheme 1. Expanding the toolbox of 6-deoxy-alkynyl sugar probes for metabolic click labeling of oligosaccharides.

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 pectin [18], 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 hairs [19]. 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 growth [4]. 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 deoxy-aldohexoses, 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-akynyl fucose **1** (Scheme 2B) [5]. However, most of the other hexoses cannot be properly protected by acetonides, so new protecting strategies are necessary.

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 procedure [23] reacted with trimethylsilylethanol to give compound **10**. The OBz groups of **10** were then removed by the treatment of K_2CO_3 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. H_2SO_4 cleanly gave the desired product 6dAM **3** as an anomeric mixture. Treatment of **3** with Ac_2O 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