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Automated Synthesis of a Protected *N*-Linked Glycoprotein Core Pentasaccharide

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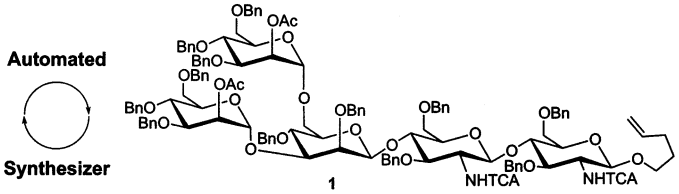
ABSTRACT



Described is the first automated solid-phase synthesis of the core *N*-linked pentasaccharide, common to all *N*-linked glycoproteins via stepwise assembly from mono- and disaccharide building blocks. The challenging β-mannosidic linkage was incorporated by the inclusion of a disaccharide trichloroacetimidate. This automated synthesis provides rapid access to an oligosaccharide common to an entire class of glycoconjugates.

Co-translational modification of proteins by glycosylation of asparagine residues includes three classes of *N*-linked oligosaccharides: high-mannose, hybrid, and complex-type mannans.[1] In addition to the many functions of these branched glycans in mammalian cells, they are found on the glycoproteins of a variety of pathogens, including the viral envelope of HIV,[2] Ebola,[3] and some coronaviruses.[4] Rapid and reliable access to these branched glycans by automated synthesis would facilitate further investigation into the biological role of these glycoconjugates and their potential application as carbohydrate-based vaccines.[5] Currently, synthetic *N*-glycans are used to study carbohydrate/protein interactions using isothermal calorimetry,[6] carbohydrate arrays,[7] and the structural analysis of such complexes (X-ray, NMR).[8]

The three major classes of *N*-linked glycans contain a common core pentasaccharide (Figure 1) that has been a target of several recent syntheses in solution[9] and on solid support.[10] This pentasaccharide contains a number of synthetic challenges, including branching, β-(1→4) glucosamine linkages, and most notably, the daunting β-mannoside.

Described is the first automated solid-phase synthesis of the *N*-linked core pentasaccharide **1**. Retrosynthetic analysis of **1** revealed that the target could be accessed using just three distinct building blocks, two monosaccharides **2**, **3**,[11] and one disaccharide **4** (Figure 2). To avoid anomeric mixtures on the solid support, the β-mannosidic linkage was

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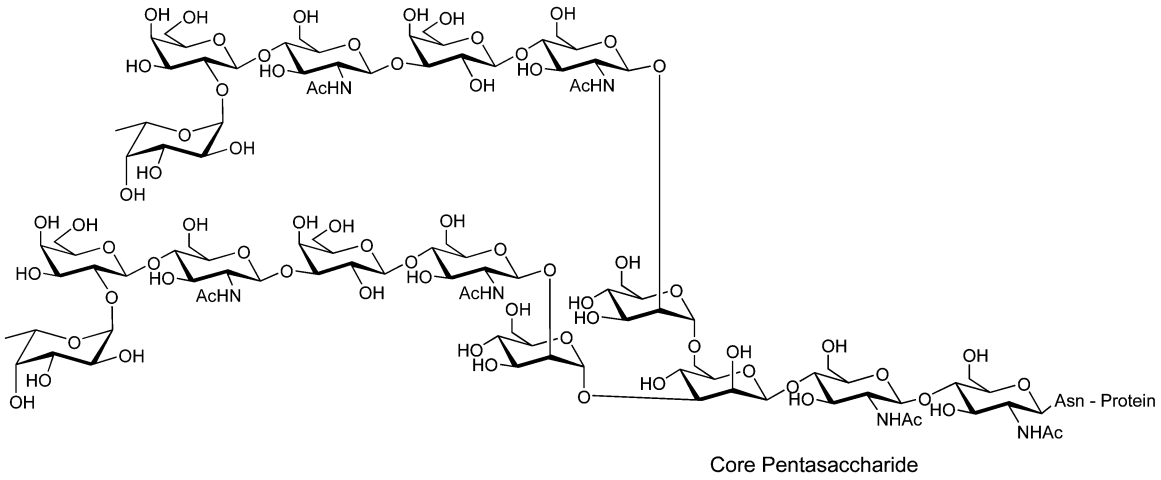


Figure 1. Complex-type *N*-glycan containing the core-pentasaccharide.

to be incorporated during the preparation of disaccharide **4**. Branching would be achieved via the simultaneous di-mannosylation of the trisaccharide core by addition of mannosyl trichloroacetimidate **3**.

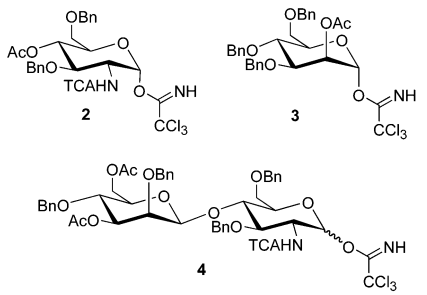


Figure 2. Building blocks used for the automated synthesis of **1**.

Upon identification of the building blocks necessary for the synthesis, glycosyl trichloroacetimidate **2** was prepared from known differentially protected glucosamine **5**[12] (Scheme 1). The 4,6-*O*-benzylidene was opened selectively by treatment with TES/TFA/TFAA to afford **6** in 85% yield. Subsequent acetylation of the C4 hydroxyl (99% yield), followed by desilylation and treatment with DBU and trichloroacetonitrile, furnished glycosyl trichloroacetimidate **2** in 76% yield.

Disaccharide trichloroacetimidate **4** was prepared via direct β-mannosylation using the Crich method[13] (Scheme 2). Mannosylation of **6** by treatment of sulfoxide **7**[14] with triflic anhydride and di-*tert*-butyl pyridine furnished the β-linked disaccharide in 68% yield. This procedure efficiently installed the β-mannosidic linkage, without the need for tedious chromatographic separation of an anomeric mixture.

The C3 *p*-methoxy benzyl ether was replaced with the base-labile acetate ester to yield **8** in 79% over two steps. Selective opening of the 4,6-*O*-benylidene to expose the primary C6 hydroxyl was achieved by treatment with dichlorophenylborane and triethylsilane.[15] Subsequent acetylation yielded differentially protected disaccharide **9** (82%, two steps). Access to the disaccharide glycosyl trichloroacetimidate was readily achieved by desilylation followed by treatment with trichloroacetonitrile and DBU to give donor **4** in 89% yield.

With the necessary building blocks **2**, **3**, and **4** in hand, we proceeded to the automated synthesis using octenediol functionalized Merrifields resin **10** and an automated oligosaccharide synthesizer (Scheme 3).[16] The automated assembly made use of five programmed modules (Table 1): (A) glycosylation, consisting of the addition of 3.5 equiv of

Scheme 1 Synthesis of Glycosyl Trichloroacetimidate **2**

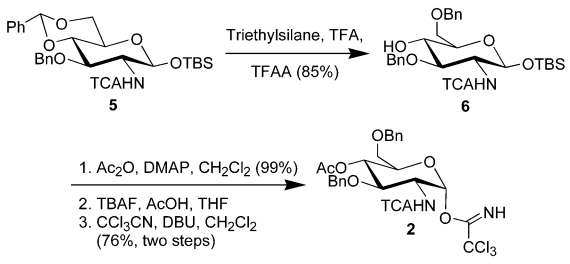
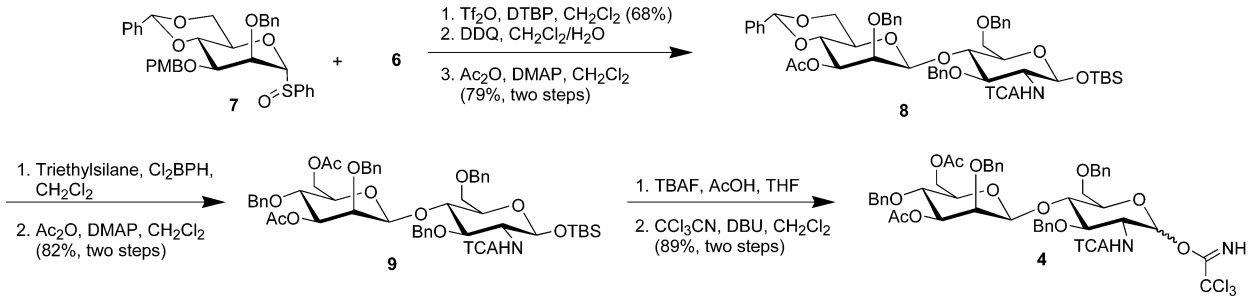


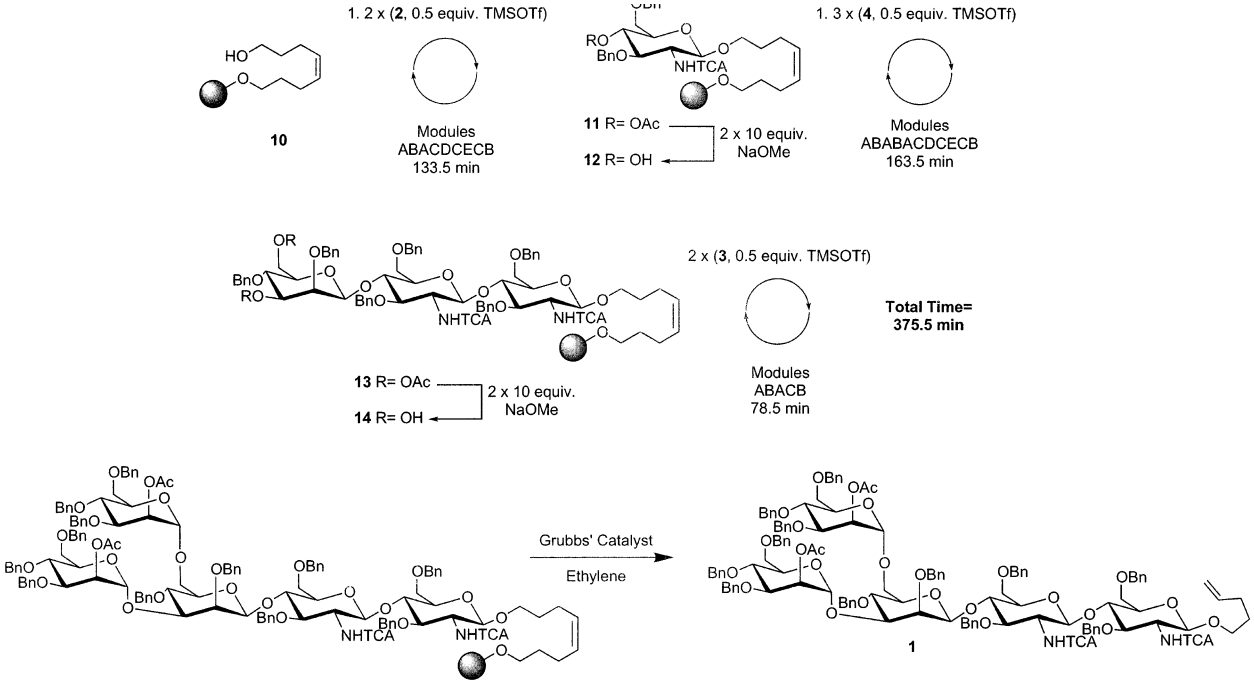
Table 1. Conditions and Reagents Used in the Automated Synthesis of **1**

module	function	reagent	time (min)
A	glycosylation	3.5 equiv of donor and TMSOTf	21
B	wash	CH_2Cl_2	9
C	wash	THF	9.5
D	deprotection	2 × 10 equiv of NaOMe	33
E	wash	0.2 M AcOH/THF	12

Scheme 2. Synthesis of Disaccharide Trichloroacetimidate Building Block **4**



Scheme 3. Automated Synthesis of Pentasaccharide **1**



donor and catalytic amounts of TMSOTf; (B) methylene chloride wash; (C) THF wash; (D) acetate deprotection by the addition of 10 equiv of sodium methoxide in methanol twice; and (E) pH neutralization with 0.2 M acetic acid in THF.

Glycosylation of the linker with **2** (repeated once) utilized C2-trichloroacetamide participation to ensure anomeric selectivity at the reducing end. Glycosylation with disaccharide donor **4**, determined by solution-phase model studies to be the most challenging step, was repeated three times to ensure complete addition to the support-bound acceptor. Finally, branching was introduced by glycosylation with mannosyl donor **3** via a simultaneous dimannosylation of the C3 and C6 hydroxyl groups.

Following the final glycosylation, the resin was thoroughly washed and dried. Cleavage of the octenediol linker by olefin cross-metathesis was performed using Grubbs catalyst in an atmosphere of ethylene to furnish the *n*-pentenyl glycoside.[17] The resulting crude product, core pentasaccharide **1**, was purified by semipreparative HPLC. Relative peak area analysis by HPLC showed 27% desired product **1**, with the remainder of isolated side-products consisting of $(n - 1)$ and $(n - 2)$ deletion sequences.

Starting from the monosaccharide and disaccharide glycosyl donors, the desired pentasaccharide target was assembled and purified in less than 3 days. This and other solid-phase oligosaccharide synthesis studies show that synthetically

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challenging and structurally diverse oligosaccharides can be rapidly prepared. While existing methods for the construction of large oligosaccharides have been immensely successful, access to the mono- and disaccharide glycosyl donors remains one of the most challenging aspects of synthetic carbohydrate chemistry—leaving room for further advancement in the field.

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Supporting Information Available: Spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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