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Solid-Phase Oligosaccharide Synthesis: Preparation of Complex Structures Using a Novel Linker and Different Glycosylating Agents

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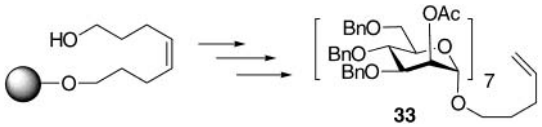
ABSTRACT



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A β-(1→4)-linked trisaccharide was prepared in 53% yield on a polymer support using glycosyl phosphates and released by cross-metathesis of a novel linker to reveal the anomeric *n*-pentenyl glycoside. Heptasaccharide 33 was prepared in 9% yield in 14 steps.

Three major classes of repeating biopolymers are responsible for the transfer of information in biological systems. While the structure and function of nucleic acids and proteins have been studied in great detail, oligosaccharides in nature encountered as glycoconjugates are less well understood. Efficient and rapid access to oligopeptides[1] and oligonucleotides[2] is now routinely achieved on automated synthesizers using solid-phase synthesis. The procurement of pure oligosaccharides, on the other hand, has proven much more difficult and has greatly hampered biochemical and biophysical studies of such polymers. Spurred by the quest for structurally defined carbohydrates,[3] the past 15 years have seen important advances in the chemical synthesis of oligosaccharides.[4,5] Still, access to complex carbohydrates remains difficult, time-consuming, and limited to a few specialized laboratories. A general solid-phase method for the assembly of oligosaccharides would hold great promise with regard to efficiency, speed, and eventually automation.

Intense efforts have focused on the development of glycosylation reactions under the solid-phase paradigm.[6] Anhydrosugars,[7] glycosyl sulfoxides,[8] trichloroacetimidates,[9] thioglycosides,[10] glycosyl fluorides,[11] and *n*-pentenyl glycosides[12] have been explored, but no generally applicable

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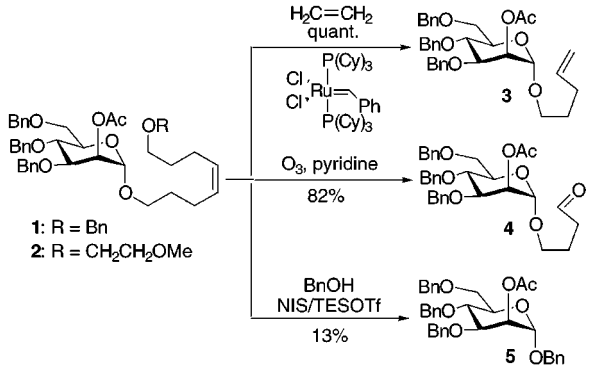
method has yet emerged.[13] Successful solid-phase oligosaccharide synthesis (SOS) mandates a stable linker to connect the first sugar to the polymer matrix. High-yielding, selective glycosylating reactions and efficient deprotection protocols will be required to repeatedly install the desired linkages. Powerful on-resin analytical techniques will be important to monitor the unfolding syntheses.

Recently, we developed a facile one-pot procedure for the preparation of glycosyl phosphates which proved to be excellent glycosylating agents in solution.[14] Now we present a novel, versatile octenediol linker which, in concert with glycosyl phosphate donors, provides rapid access to β-(1→4)- and β-(1→6)-linked trisaccharides in high stepwise coupling yields and short reaction times. We also applied glycosyl trichloroacetimidates to the synthesis of α-(1→2)-linked mannose tri-, penta-, and heptasaccharides. Very high stepwise coupling yields allowed for the straightforward isolation of the desired oligosaccharide products.

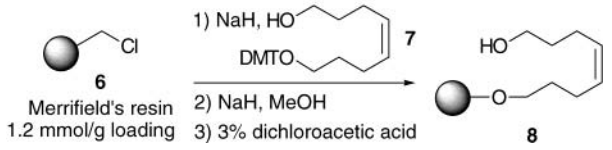
We envisioned a new linker concept which would (a) be readily prepared in high loading capacity; (b) be completely stable to a wide range of reaction conditions; (c) be readily cleaved under mild conditions; and (d) allow for access to different anomeric functionalities and fully protected glycosyl donors as building blocks for larger structures. This new linker, based on an anomeric pentenyl functionality, was first evaluated in solution (Scheme 1). Model compounds containing an octenediol linker connecting the anomeric position of a protected mannose derivative with a benzyl group (**1**, a model for polystyrene) or an ethyl methyl ether (**2**, a model for TentaGel type resins) were readily prepared. Olefin cross-metathesis of **1** by treatment with Grubbs' catalyst under an atmosphere of ethylene[15] provided in quantitative yield the desired mannose *n*-pentenyl glycoside **3** which may function as a glycosyl donor.[16] While ring-closing metathesis had previously been used to cleave molecules off the solid phase, to our knowledge, this linker is the first to be cleaved by olefin cross-metathesis.[17] Ozonolysis of **1** furnished aldehyde **4** which will facilitate functionalization of the reducing end for access to different neoglycoconjugates.[18] Cleavage of model linker **2** with *N*-iodosuccinimide (NIS) and TESOTf in the presence of benzyl alcohol provided benzyl glycoside **5** in low yield (Scheme 1).[16]

Scheme 1. Solution-Phase Cleavage of the Octenediol Linker



Encouraged by these model studies, we functionalized Merrifield's resin **6** (chloromethylated polystyrene cross-linked with 1% divinylbenzene) with the new linker by reaction of mono-DMT-protected octenediol **7** followed by capping of any unreacted resin with methanol (Scheme 2).

Scheme 2. Synthesis of Linker Functionalized Resin



Removal of the DMT group served two purposes: It furnished the functionalized resin **8** and allowed for determination of the resin loading (0.45−0.55 mmol/g of resin) by a colorimetric assay.[19] The exposed primary hydroxyl functionality then acted as an acceptor in subsequent glycosylations.

The performance of glycosyl phosphates as building blocks for the assembly of oligosaccharides on a solid support was first evaluated on β-(1→4)-linked trisaccharide **15** requiring glycosylation of the hindered C4-hydroxyl moieties (Scheme 3). Reaction of **8** with 3 equiv of donor **9** upon activation with trimethylsilyl triflate (TMSOTf) at −50 °C for 1 h furnished glycosylated resin **10**. Removal of the C4-TBS protecting group exposed the hydroxyl group which then

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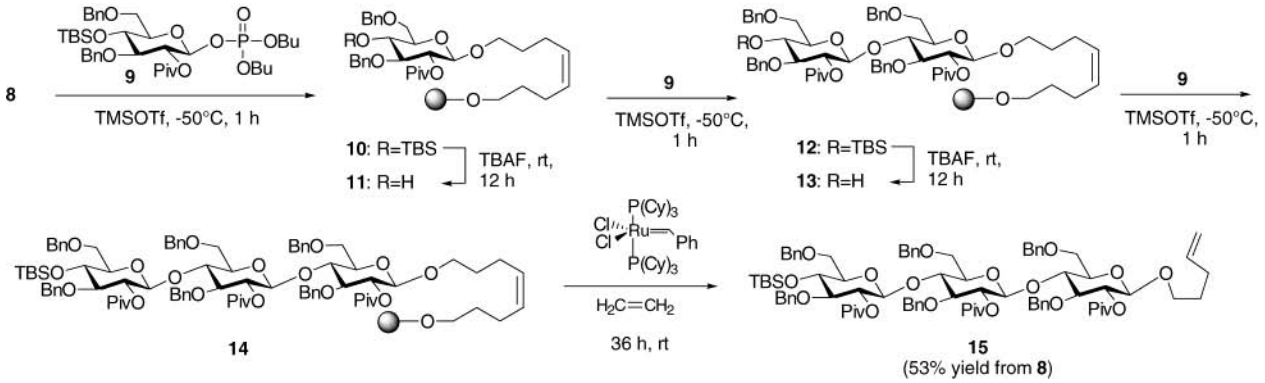
Scheme 3. Solid-Phase Synthesis with Glycosyl Phosphates



functioned as an acceptor in the reaction of **11** with glycosyl donor **9**. To ensure complete glycosylation of this sterically hindered acceptor, the coupling step was repeated (double glycosylation) to produce **12**. Deprotection and coupling were repeated to fashion trisaccharide **14**. Cleavage of the trisaccharide from the solid support by olefin metathesis was accomplished by reaction with 20 mol % of Grubbs' catalyst under an atmosphere of ethylene to yield fully protected *n*-pentenyl glycoside **15** in 53% overall yield from **8** (corresponding to an average yield of 90% per step over six steps). Using this synthetic strategy, the β-(1→6)-linked trisaccharide **16** was prepared in 32% overall yield (Table 1).

Table 1. Preparation of Trisaccharides on Merrifield's Resin Using a Novel Octenediol Linker

Compound	Glycosyl Donor	Activator	Yield
15	glycosyl phosphates	TMSOTf	53%
16[a]	glycosyl phosphates	TMSOTf	32%
16[a]	thioglycosides	MeOTf	15%
23	trichloroacetimidates	TESOTf	76%
23	trichloroacetimidates	TMSOTf	71%

[a] **16** = 4-pentenyl 3,4-di-*O*-benzyl-2-*O*-pivaloyl-6-*O*-triisopropylsilyl-β-D-glucopyranoside-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranoside.

The promising results obtained with glycosyl phosphate donors on the solid support prompted us to investigate the compatibility of the new linker concept with other glycosylating agents. Trichloroacetimidates are the most widely used glycosylating agents for the assembly of oligosaccharides in solution.[20] These donors had also been successfully applied to SOS, but a significant drop in coupling yields at the trisaccharide stage had been reported when the axial 2-hydroxyl group of mannose was used as an acceptor.[9b] Using a two-step coupling−deprotection cycle, trisaccharide **23**, pentasaccharide **28**, and heptasaccharide **33** were prepared (Scheme 4). Glycosylation of support **8** was achieved by reaction with 3 equiv of donor **17**[21] in the presence of 0.05 equiv of TMSOTf at room temperature for 1 h or alternatively by activation with TESOTf at 0 °C.[12b] A first indication of the success of the glycosylation was obtained by gravimetric analysis and FT-IR microspectroscopy.[22] High-resolution magic angle spinning NMR of **18** confirmed the formation of the desired linkage.[23]

Removal of the C2-acetate protecting group by treatment with sodium methoxide in methanol liberated a support-bound acceptor moiety to undergo the sequential coupling and deprotection steps. Trisaccharide **23** was isolated in 76% yield (95% per step) after cleavage by olefin metathesis and purification by simple flash column chromatography (Table 1). Further couplings resulted in the formation of pentasaccharide **28** and heptasaccharide **33** in 41% and 9% overall yields, respectively, after cleavage from the resin. The excellent stepwise yields (84−95%) achieved for couplings involving a relatively poor axial hydroxyl acceptor suggest that complex structures containing other glycosidic linkages are now within reach. The formation of seven glycosidic linkages, the most glycosylations carried out on a solid support to date, resulted in a heptasaccharide as the largest oligosaccharide assembled when relying exclusively on monosaccharide building blocks.[24] The high loading capacity of the resin (~0.5 mmol/g) allows for the synthesis of hundreds of milligrams of oligosaccharide.

In summary, we have developed a novel linker for solid-phase oligosaccharide synthesis which allows access to different anomeric moieties and is stable to a wide range of reaction conditions. We have established a synthetic protocol utilizing this linker together with glycosyl phosphates as glycosylating agents in the efficient assembly of difficult to fashion β-(1→4) glycosidic linkages. Glycosyl trichloroace-

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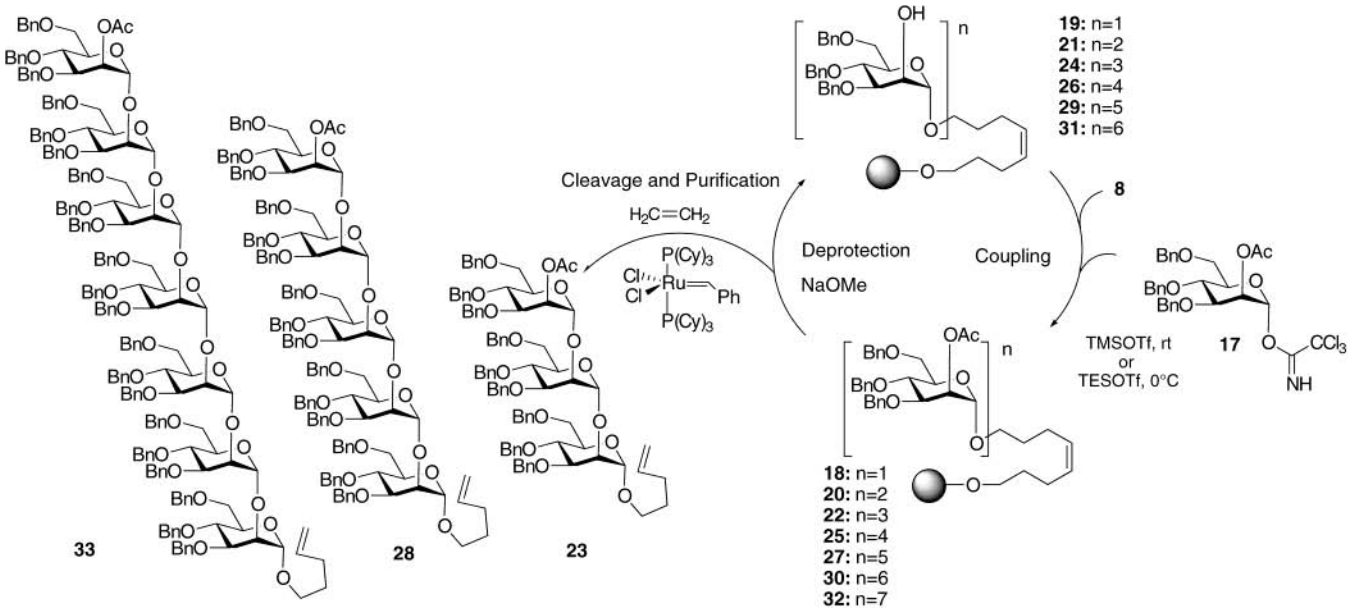
(24) The synthesis of a dodecamer was achieved by coupling four trisaccharides; see ref 10b.

Scheme 4. Solid-Phase Synthesis with Trichloroacetimidates



timidates served as donors in seven consecutive glycosylation reactions to furnish the α-(1→2)-linked heptamannoside **33** in 9% overall yield. Neoglycoconjugates connected via the reducing end can be prepared by oxidative cleavage of the octenediol linker. Currently, we are expanding this synthetic strategy to the assembly of larger, branched oligosaccharides containing a variety of different glycosidic linkages with the goal of comparing the performance of different donors. We have also begun to automate the synthesis process with the long-term goal of developing an oligosaccharide synthesizer.

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Supporting Information Available: Two additional schemes along with detailed experimental procedures and compound characterization data, including ^{1}H, ^{13}C, and ^{31}P NMR spectral data for all described compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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