Carbohydrate Synthesis (2)

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Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside**

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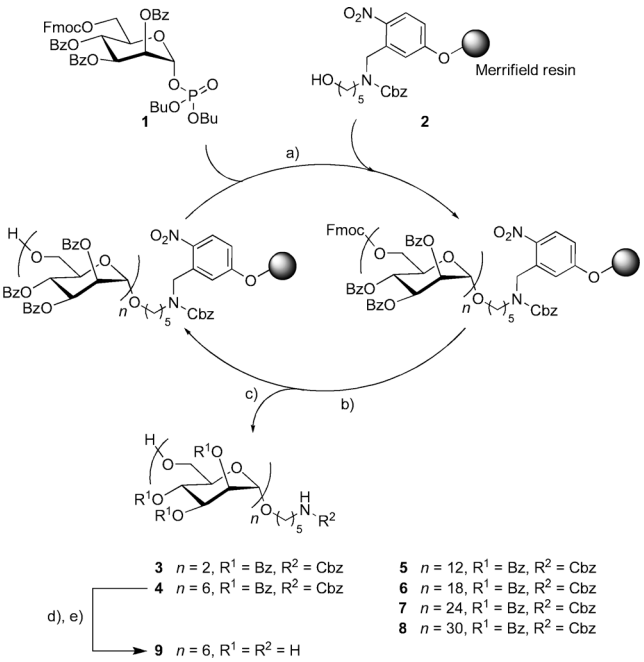
Carbohydrates are structurally highly complex and diverse biopolymers[1] that serve a host of biological functions.[2] Chemical synthesis provides access to well-defined oligosaccharides, as the purification of carbohydrates from natural sources is often difficult or even impossible. While peptides[3] and oligonucleotides[4] are now routinely assembled by automated solid-phase synthesis, methods for automated oligosaccharide synthesis have been evolving more slowly. The need to exercise both regio- and stereocontrol during glycosidic linkage formation poses significantly higher synthetic challenges. Automated solid-phase oligosaccharide synthesis has dramatically accelerated the assembly of increasingly complex carbohydrates.[5] While polysaccharides are common in nature, the synthesis of oligosaccharide sequences past 20 units has been very rare. Automated oligonucleotide synthesis, which can now routinely reach 200mers paving the way to assemble entire genes, serves as an inspiration for polysaccharide assembly.[6]

Herein, we report the automated synthesis of a 30mer α-(1,6)-oligomannoside as a proof-of-principle that automated oligosaccharide synthesis can provide access to long carbohydrate chains. To facilitate purification, a catch-and-release strategy is employed whereby the full-length oligosaccharide was tagged and immobilized on magnetic beads. Following separation from deletion sequences by magnet-assisted decanting, the desired product is released from the beads.

Glycosyl phosphate building block **1** carries permanent benzoyl protecting groups that ensure the formation of trans-glycosidic linkages and can be readily removed with strong base. The temporary fluorenylmethoxycarbonyl protection of the C6 hydroxy group is readily removed by piperidine in anticipation of chain elongation. The anomeric dibutyl phosphate leaving group ensures fast and efficient glycosylation by Lewis acid activation.[7] Merrifield resin **2** equipped with photolabile *o*-nitrobenzyl alcohol linker[8] served as solid support for the automated syntheses (Scheme 1).



Scheme 1. Automated synthesis of α-(1,6)-polysaccharides. Reactions and conditions: a) glycosylation: **1**, TMSOTf, CH_2Cl_2, −15 °C (45 min)–0 °C (15 min); b) Fmoc deprotection: piperidine, DMF, 25 °C (5 min); c) cleavage from solid support: *hν*, CH_2Cl_2; d) NaOMe, MeOH; e) Pd/C, H_2, H_2O. Bu = butyl, Bz = benzoyl, Cbz = benzyloxycarbonyl, DMF = dimethylformamide, Fmoc = fluorenylmethyloxycarbonyl, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

With mannosyl phosphate **1** and functionalized Merrifield resin **2** in hand, α-(1,6)-oligomannosides were assembled using an automated oligosaccharide synthesizer (Scheme 1).[5b] Each glycosylation using three equivalents of mannosyl building block **1** was repeated three times at −15 °C, while trimethylsilyl triflate served as a promoter. Removal of the temporary Fmoc protecting group with piperidine freed the C6 hydroxy group for chain elongation. The coupling efficiency was assessed by UV/Vis measurement of the piperidine–dibenzofulvene adduct released during Fmoc cleavage.[9] α-(1,6)-Oligomannosides ranging in length from disaccharide **3** to 30mer **8** were prepared using this automated method.

Disaccharide **3** and hexasaccharide **4** were readily purified by separation of the deletion sequences using silica flash column chromatography. Global deprotection using base to remove benzoate esters and subsequent hydrogenolysis on Pd/C afforded fully deprotected hexasaccharide **9** in 25 %

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yield over 15 steps. The purification of polymannosides longer than 12mers (**5**, **6**, **7**, and **8**) was more challenging owing to their changing solubility. As the crude products dissolved only in chlorinated solvents, reverse-phase, normal phase, and size exclusion HPLC became very challenging.

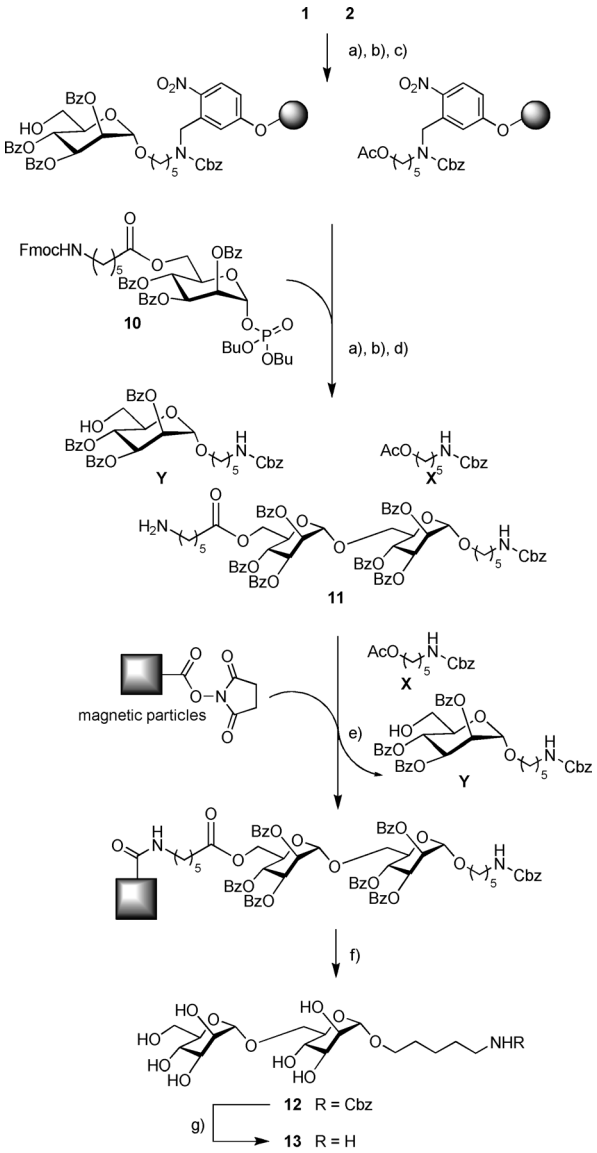
A cap-and-tag strategy was adopted to facilitate polysaccharide purification. Peptides, oligonucleotides, and oligosaccharides have been purified using affinity chromatography that relies on labels such as biotin[10] and oligohistidine.[11] This strategy cannot be adopted for the purification of protected oligomannosides since aqueous solvents are required as eluents. Fluorous tags have helped to separate oligonucleotides up to 100mers[12] using fluorous solid-phase extraction and liquid–liquid extraction.[13] Solubility issues associated with fluorous labels rendered them useless in the context of the isolation of longer α-(1,6)-oligomannosides. Therefore, we considered capture–release techniques[14] that rely on the covalent attachment of the labeled target molecule to a solid support to separate the desired oligosaccharide from any deletion sequences. A capping step was included in the synthesis cycle to block any unreacted hydroxy groups prior to the following glycosylation reaction. After completing the oligosaccharide sequence, the tag was attached to the C6 hydroxy group, thus allowing facile separation of the desired product from the deletion sequences and byproducts.

A successful catch–release approach applied to automated solid phase oligosaccharide synthesis requires a fast and efficient capping reaction. The caps introduced after each glycosylation have to be stable during subsequent synthetic steps and the tag has to contain a unique handle for facile separation of the product. Based on these considerations, acetylation was used for capping and the full length oligosaccharide was tagged as a 6-amino caproic acid ester. The unique amino group facilitates isolation of the desired oligosaccharide by attachment to magnetic beads decorated with carboxylic acid moieties.

This cap–tag strategy was first evaluated in the context of the automated synthesis of α-(1,6)-dimannoside **13** (Scheme 2). Utilizing the automated procedure, a capping step employing acetic anhydride in pyridine was incorporated into the synthetic cycle. Following completion of the automated synthesis, building block **10**, which is equipped with an amino caproic ester at the C6 position, was used. The disaccharide product was cleaved from the resin in continuous flow by exposure to UV light before the crude product mixture was reacted with magnetic beads functionalized with NHS-activated carboxylic acid.[15]

The unique amine group on the disaccharide resulted in covalent attachment to the beads before washing with dichloromethane and methanol. Treatment of the magnetic beads with sodium methoxide in methanol released deprotected disaccharide **12** that was purified by gel filtration chromatography to afford **12** in 22% yield over nine steps. Cleavage of the benzyl carbamate by hydrogenation provided disaccharide **13** in 74% yield.

To improve the coupling efficiency of the target saccharide to the activated magnetic beads and thus the overall yield, a second coupling step was introduced. Any oligosaccharides remaining after the first coupling were reacted with

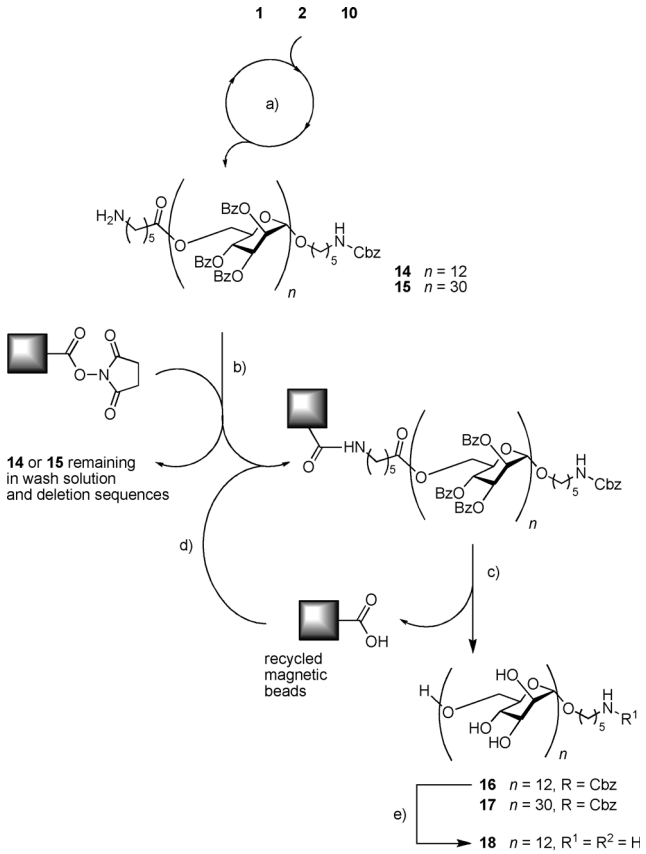


Scheme 2. Catch–release purification applied to the automated solid-phase synthesis of α-(1,6)-dimannoside **13**. Reactions and conditions: a) glycosylation: **1** or **10**, TMSOTf, CH_2Cl_2, −15 °C (45 min)–0 °C (15 min); b) capping: Ac_2O, pyridine 25 °C (60 min); c) Fmoc deprotection: piperidine, DMF, 25 °C (5 min); d) cleavage from solid support: hv, CH_2Cl_2; e) immobilization on magnetic beads: 1. NEt_3, CH_2Cl_2, 25 °C; 2. washing with CH_2Cl_2 to remove deletion sequences of the type **X** and **Y**; f) release from magnetic beads: NaOMe, MeOH; g) Pd/C, H_2, H_2O. Ac = acetyl.

the magnetic beads from the first "release" in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate and *N*,*N*-diisopropylethylamine (Scheme 3).

The automated synthesis of the dodecamanoside provided, after gel filtration chromatography, deprotected 12mer **16** in 13% overall yield without the need for HPLC purification. Removal of the Cbz protecting group by hydrogenolysis on Pd/C in water provided α-(1,6)-dodecamannoside **18** in 62% yield.

The automated synthesis of an α-(1,6)-oligomannoside 30mer presented the next challenge as this polysaccharide is



Scheme 3. Catch–release purification applied to α-(1,6)-oligomannosides **16** and **17** prepared by automated solid-phase assembly. Reactions and conditions: a) automated synthesis; b) immobilization on magnetic beads: 1. NEt_3, CH_2Cl_2, 25 °C; 2. Remove deletion sequences by CH_2Cl_2 wash; c) release from magnetic beads: NaOMe, MeOH, H_2O; d) magnetic bead recycling: **14**, **15**, PyBOP, DIPEA, CH_2Cl_2, 25 °C; e) Pd/C, H_2, H_2O. DIPEA = diisopropylethylamine, PyBOP = benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate.

by far the largest carbohydrate ever made by automation and amongst the largest ever synthesized by any method. Executing the method described above, the crude protected 30mer **15** was obtained in less than one week. Analysis of the crude product by MALDI mass spectroscopy confirmed the presence of the target compound. Catch–release purification separated the deprotected 30mer **17** from any deletion sequences, providing the pure 30mer after gel filtration chromatography in 1% overall yield (96% average per step). The identity of the product was confirmed with the help of NMR and MALDI mass spectroscopy (Figure 1).[16]

In conclusion, we describe the first automated solid-phase assembly of a polysaccharide. To streamline the purification of the product, a catch–release method was developed. Rapid access to synthetic polysaccharides of lengths previously not accessible has now become possible. Applications of these defined polysaccharide products as biological probes and even for the construction of novel materials are currently being pursued.

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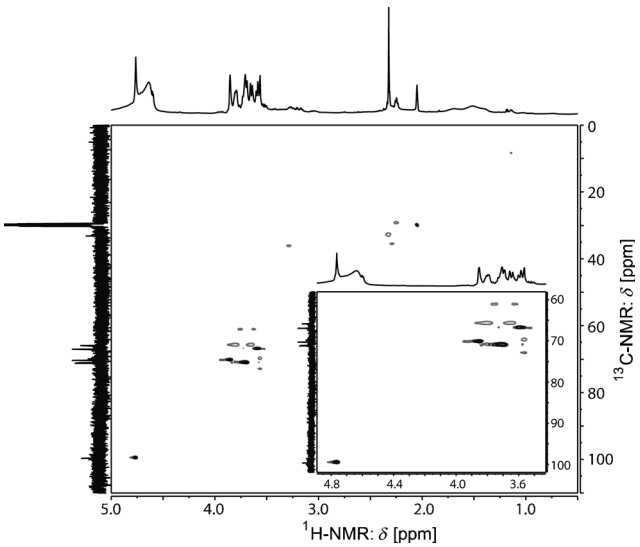

Figure 1. Characterization of polymannoside **17**. HSQC NMR (D_2O, 600 MHz) of α-(1,6)-30mer **17.**

Keywords: automation · catch–release technique · magnetic beads · polysaccharides · solid-phase synthesis

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[16] Owing to the small amount of 30mer **17**, the removal of the Cbz protecting group was not attempted.

