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Synthesis of Di-Branched Heptasaccharide by One-Pot Glycosylation Using Seven Independent Building Blocks

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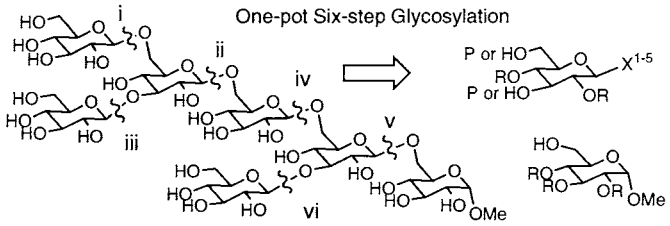
ABSTRACT



We describe an efficient synthesis of di-branched heptasaccharide 1 having phytoalexin elicitor activity in soybeans by one-pot glycosylation. The synthesis involves chemo- and regioselective sequential six-step glycosylations using seven independent building blocks and sequential removal of acyl- and benzyl ether-type protecting groups. The coupling of seven building blocks requires only four chemoselective activitable leaving groups of glycosyl donors. Both the glycosylation and deprotection reactions can be achieved utilizing a parallel manual synthesizer.

Recent recognition of the important biological roles of oligosaccharides[1] has driven the development of new methodologies for the synthesis of such compounds on solid phase and in solution.[2,9e] These methodologies have already allowed the synthesis of a combinatorial library[3−9,10j,l,13] of tri- and tetrasaccharides. However library synthesis of more complex oligosaccharides such as penta- or hexasaccharides is still difficult. Therefore efficient methodology for the such oligosaccharides would be required.

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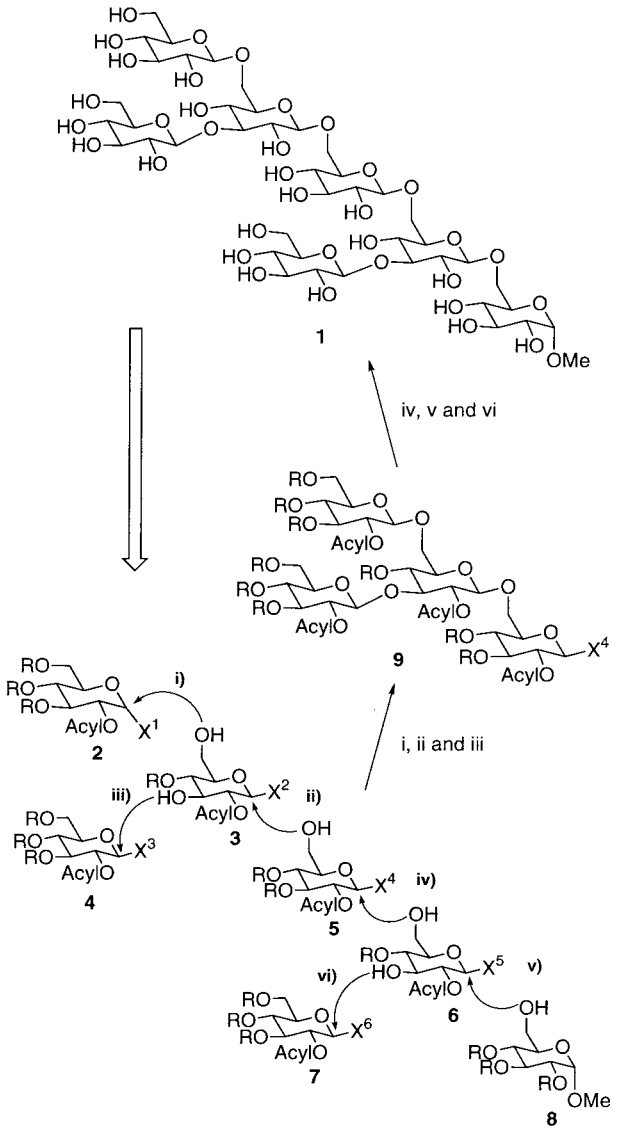
One-pot glycosylation[10−13] to form two or more glycosidic bonds by chemoselective activation of glycosyl donors is an attractive way in solution-phase methodology for the synthesis of an oligosaccharide library because it does not requires purification of their synthetic intermediates. Wong and co-workers have reported an optimer method involving selective activation of thioglycosides whose reactivity depended on their protecting groups.[10j,l] We have investigated one-pot glycosylation based on chemoselective activation of glycosyl donors having different leaving groups[10a,c,k,11−13] with appropriate activators. The order of activation of glycosyl donors relies not only on their protecting groups but also on the combination of their leaving groups and activators.

Heptasaccharide **1** shows phytoalexin elicitor activity in soybeans and contains a β-(1,6)-linked pentaglucosyl backbone with two β-(1,3)-branched glucoses (Scheme 1).[14] The branched structure type of **1** has often been used as a novel target to demonstrate the feasibility of new methodologies for oligosaccharide synthesis.[11a,12b,15,16] We have already reported one-pot synthesis of the di-branched heptasaccharide **1** by two one-pot glycosylations.[12b] However, one-pot synthesis from seven independent building blocks is not accomplished. Here we describe the efficient synthesis of heptasaccharide by one-pot sequential six-step glycosylation.

One-pot synthesis of the oligosaccharide **1** involving formation of six β-glycosidic bonds requires sequential activation of six glucose units attached with an acyl protecting group at each 2 position. The C2 acyl protecting group allows formation of a 1,2 *trans* glycosidic bond by neighboring group participation but reduces reactivity of the glycosyl

Scheme 1. Strategy for Synthesis of Di-Branched Heptasaccharide **1**



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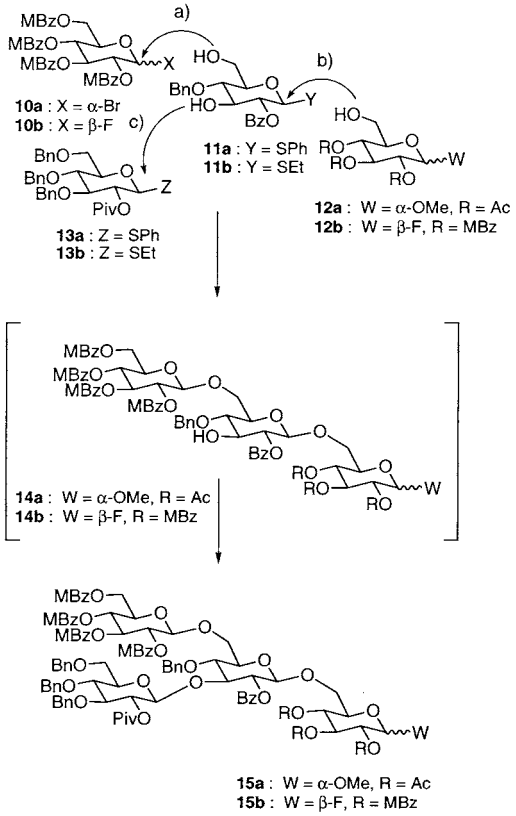
donors. Our strategy for the synthesis of **1** involving six sequential glycosylations (i−vi) based on chemoselective activation of glycosyl donors **2**−**7** with a leaving group X^1−X^6 is shown in Scheme 1. The reaction sequence involves (i) regioselective glycosylation of the primary alcohol of **3** in the presence of the secondary one, followed by (ii) glycosylation of acceptor **5** without self-condensation and (iii) glycosylation of the remaining secondary alcohol of **3** with donor **4** to give tetrasaccharide **9**.[5b,17] The branched tetrasaccharide formation, which is different from our previously reported method,[12b] requires only two different leaving groups to couple with four independent building blocks because the same leaving group was used at the second and third steps. A successive one-pot glycosylation initiated from tetrasaccharide **9** using three saccharide building blocks **6**, **7** and **8** (iv, v, and vi) allows the one-pot heptasaccharide synthesis using only four different leaving groups. We selected glycosyl bromide **2**, ethylthioglycosides **3** and **4**, glycosyl fluoride **5**, and phenylthioglycosides **6** and **7** as glycosyl donors, which would be sequentially activated by addition of AgOTf,[18] MeOTf,[19] $HfCp_2Cl_2$/AgOTf,[20b] and DMTST,[21] in that order.

To accomplish the one-pot six-step glycosylation we conducted the first part of the one-pot synthesis of a tetraglycosyl fluoride **15b** using the four building blocks **10a**, **11b**, **12b**, and **13b** (Scheme 2). Glycosylation of thioglycoside **11b** (1.00 equiv) with glycosyl bromide **10a** (1.10 equiv) in the presence of AgOTf, followed by coupling of acceptor **12b** (1.10 equiv) with excess amount of MeOTf provided trisaccharide **14b**. This is followed by activation of thioglycoside **13b** (1.80 equiv) with the remaining MeOTf to give the branched tetrasaccharide **15b** in 48% yield. The addition of the excess activator in the second glycosylation means that no activator must be added for the third.

Coupling of the remaining three building blocks **11a**, **12a**, and **13a** was examined using glycosyl fluoride **10b** instead of **15b**. Coupling of thioglycoside **11a** (1.00 equiv), glycosyl fluoride **10b** (1.10 equiv), and acceptor **12a** (1.10 equiv) was achieved by $ZrCp_2Cl_2$/AgOTf[20a] and excess amount of DMTST. Successive addition of thioglycoside **13a** (3.00 equiv) resulted in the 53% yield of branched tetrasaccharide **15a**.

Scheme 2. Synthesis of Trisaccharide **15a,b** by One-Pot Glycosytlation[a]



[a] Reagents and conditions. Reaction sequence for **15b**: (a) **10a**, **11b**, AgOTf, CH_2Cl_2, MS4A, −40 °C; (b) **12b,** MeOTf, CH_2Cl_2, rt; (c) **13b**, CH_2Cl_2, 48% yield based on **11b**. For **15a**: (a) **10b**, **11a**, $ZrCp_2Cl_2$, AgOTf, MS4A, CH_2Cl_2, 0 °C; (b) **12a**, DMTST, CH_2Cl_2, 0 °C; (c) **13a**, CH_2Cl_2, 0 °C, 53% yield based on **11a**.

We next examined one-pot six-step glycosylation using a manual synthesizer (Quest 210),[22] whose utility for the synthesis of an oligosaccharide library we have already reported.[13] The reaction requires the sequential addition of the seven reaction components with six appropriate activators (Scheme 3). The synthesis of tetrasaccharide **15b** was achieved by the above-described manipulation. Accomplishment of each glycosylation reaction was checked by TLC analysis. Sequential addition of thioglycoside **11a** (1.00 equiv) with $HfCp_2Cl_2$/AgOTf, acceptor **12a** (1.10 equiv) with excess amount of DMTST, and thioglycoside **13a** (6.00 equiv) gave protected heptasaccharide **16**. The reactions are finally quenched by the addition of triethylamine, followed by filtration to remove MS4A. After concentration of the filtrate, the crude product is purified by silica gel column chromatography following by gel permeation chromatography (GPC) to give **16** in 24% yield based on **11a**, the largest oligosaccharide among the reaction products. The structure of the heptasaccharide **16** was confirmed using an authentic sample prepared by a stepwise synthesis. To our knowledge, this one-pot glycosylation couples the highest number of

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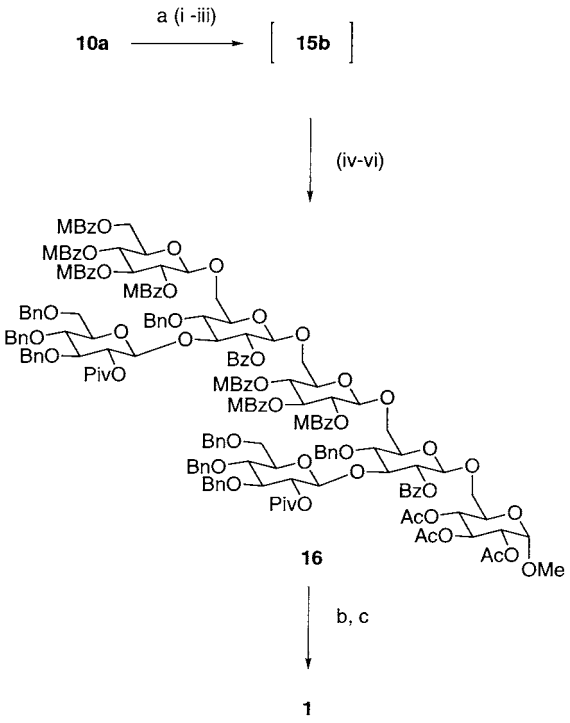
(22) Quest 210 was purchased from Argonaut Technologies, USA.

Scheme 3. Syntehsis of Phytoalexin Elicitor Active Heptasaccharide by One-Pot Glycosylation Using a Manual Synthesizer[a]



[a] Reagents and conditions: (a) (i) **10a** (1.16 equiv), **11b** (1.10 equiv), AgOTf (2.00 equiv), CH_2Cl_2, MS-4A, −20 °C, (ii) **12b** (1.10 equiv), MeOTf (10.0 equiv), CH_2Cl_2, rt, (iii) **13b** (1.80 equiv), CH_2Cl_2, rt, (iv) **11a** (1.00 equiv), $HfCp_2Cl_2$/AgOTf (2.00 equiv/4.00 equiv), CH_2Cl_2, 0 °C, (v) **12a** (1.25 equiv), DMTST (12.0 equiv), CH_2Cl_2, 0 °C, (vi) **13a** (6.00 equiv), CH_2Cl_2, 0 °C, 24% based on **11a**; b) H_2, $Pd(OH)_2$, MeOH−H_2O, 8 h; (c) NaOMe, MeOH−H_2O, 12 h, 52% based on **16**.

independent building blocks using chemoselective activation with selected activators.

Complete deprotection of **16** was effected with the manual synthesizer. Hydrogenation of benzyl ethers with $Pd(OH)_2$ was achieved under H_2 (1 atm) in the first reaction vessel. The reaction mixture was sent to a second reaction vessel through a filter to remove Pd catalyst. Hydrolysis of ester groups using NaOMe in MeOH/H_2O gave **1** in 52% yield from **16**, and its structure was established by NMR and mass spectra, which were shown to be identical with that previously prepared by van Boom et al.[15g] The heptasaccharide synthesis using seven building blocks involved only two workups and two purification operations.

In conclusion, we have described a one-pot synthesis of protected dibranched heptasaccharides **16** from seven building blocks using four different leaving groups with a manual synthesizer. Sequential activation of glycosyl donors such as glycosyl bromide, ethylthioglycoside, glycosyl fluoride, and phenylthioglycoside, in that order, is effective for the synthesis of oligosaccharides by multicomponent coupling. Library synthesis of the heptasaccharides and the application of this one-pot methodology to an automated synthesizer are in progress.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds**15a**,**b**, **16**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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