

Orthogonal One-Pot Synthesis of Oligosaccharides Based on Glycosyl ortho-Alkynylbenzoates

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ABSTRACT: One of the most popular one-pot glycosylation strategies is orthogonal one-pot synthesis, which was mainly based on thioglycosides. Despite its successful application, shortcomings of thioglycosides including aglycon transfers, interference of departing species and unpleasant odor restrict its application scope. Herein, we report a new and efficient orthogonal one-pot synthesis of oligosaccahrides based on glycosyl *ortho*-alkynylbenzoate, which solves the issues of thioglycoside-based orthogonal one-pot synthesis. Over a dozen of oligosaccharides have been efficiently synthesized by this method.

arbohydrates are essential biomolecules in living → organisms, which play such important roles in numerous biological processes as immune response, viral and bacterial infection, and cell growth and proliferation.¹ In comparison with the gene-regulated synthesis of DNA and proteins, biosynthesis of oligosaccharides is a nontemplate-driven and stepwise process via post-translational modification in the Golgi apparatus and endoplasmic reticulum, which results in highly heterogeneous and extremely diverse carbohydrate structures.² It is a formidable task to isolate pure and homogeneous glycans from natural resources. Chemical synthesis is an effective means to obtain well-defined glycans for deciphering their functions and developing new therapeutic reagents.³ During the past three decades, many novel methods and strategies have been developed to streamline the chemical synthesis of oligosaccharides, such as automated chemical synthesis,⁴ one-pot glycosylation strategies₂⁵ latent-active glycosylation,⁶ and orthogonal glycosylation,⁷ among which one-pot glycosylation strategies are particularly attractive. Unlike traditional glycosylation methods, which require tedious protection and deprotection manipulations during assembly of oligosaccharides, one-pot glycosylation strategies not only omit glycosylation-interval workups and intermediates purifications but also greatly reduce chemical wastes and highly accelerate oligosaccharide synthesis. Nevertheless, it is still highly challenging to perform one-pot synthesis of oligosaccharides due to the regio- and stereochemical issues during oligosaccharide synthesis.5,8

In general, one-pot glycosylation strategies mainly include reactivity-based one-pot glycosylation,⁹ orthogonal one-pot

glycosylation¹⁰ and preactivation-based iterative one-pot glycosylation,¹¹ among which orthogonal one-pot glycosylation is particularly noteworthy. Glycosyl trichloroacetimidate (TCAI) and thioglycoside pair,¹² glycosyl N-phenyltrifluoroacetimidate (PTFAI) and thioglycoside pair,¹³ S-benzoxazolyl (SBox) glycoside and thioglycoside pair,¹⁴ glycosyl bromide and thioglycoside pair,¹⁵ together with glycosyl phosphites and phosphates with thioglycoside pair¹⁶ had been utilized for twostep, orthogonal one-pot synthesis of several oligosaccharides and natural products.(Scheme 1) Despite its successful application, the shortcomings inherent to thioglycosides including the propensity of aglycon transfer,¹⁷ the high electrophilic character of departing species¹⁸ and unpleasant odor restrict its application scope. Furthermore, the number of leaving groups that could be employed for multistep orthogonal one-pot synthesis is still limited.¹⁹

In 2008, Yu's group reported that a novel leaving group *ortho*-alkynylbenzoate (ABz) could be activated by a catalytic amount of gold(I) complexes.^{20a} This venerable Yu glyco-sylation enjoys mild and neutral glycosylation reaction conditions and has been extensively utilized in the total synthesis of complex glycosylated natural products as well as assembly of complex oligosaccharides.²⁰ We envisioned that orthogonal one-pot synthesis of oligosaccharides based on glycosyl ABz, with much less nucleophilicity compared with *S*-glycosides as donor, stable isocoumarin as the departing species of the leaving group, and odor-free *ortho*-alkynylben-

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Scheme 1. Strategies of Orthogonal One-Pot Synthesis of Oligosaccharides





zoic acid as starting material may solve the issues associated with thioglycoside-based two-step orthogonal one-pot synthesis (Scheme 1).

Herein, we report a new and highly efficient orthogonal onepot method for synthesis of oligosaccharides on the basis of glycosyl ABz, which overcomes the limitations of thioglycoside-based orthogonal one-pot synthesis. Over a dozen of oligosaccharides had been highly efficiently synthesized by this method, which highly streamlines the chemical synthesis of oligosaccharides.

As shown in Scheme 1, orthogonal one-pot glycosylation that involves sequential and selective activation of different leaving groups LG₁, LG₂, and LG₃ in the same reaction vessel allows the expeditious synthesis of oligosaccharides, which is independent of reactivities of the building blocks. We reasoned that two criteria must be met for this successful orthogonal one-pot glycosylation: (1) the leaving group LG_{*n*+1} must be stable in the activation of the previous leaving group LG_{*n*} with the promoter *n*; (2) byproducts obtained from the activation should not interfere with glycosylations.

To this end, we embarked on the investigation of the orthogonality of glycosyl ABz with the other leaving groups. First, we fixed glycosyl ABz as the bifunctional acceptor, which bears the free hydroxyl group and the activatable leaving group (Table 1). A variety of glycosyl donors were screened, including glycosyl TCAI,²¹ glycosyl PTFAI,²² *p*-toluene glycosyl PTFAI,²² p-toluene thioglycoside (STol),²³ SBox glycoside,²⁴ STaz glycoside,²⁵ and glycosyl isoquinoline-1-carboxylate (IQC).²⁶ The results showed that the bifunctional acceptor glycosyl ABz 2 was stable under the activation conditions for TCAI 1a and PTFAI 1b (cat. TMSOTf), SBox 1c (AgOTf), STaz 1d (MeOTf), and IQC 1e $[Cu(OTf)_2]$, providing the ABz disaccharides 3a-3d in excellent yields. No self-condensation products were detected. However, coupling of STol thioglycoside with the glycosyl ABz 2 under the activation conditions such as NIS/ TMSOTf and Ph₃Bi(OTf)₂ failed to provide the desired disaccharide.

Next, we investigated whether glycosyl ABz could be coupled under the catalysis of a gold(I) complex with the other bifunctional acceptors (Table 2). Glycosylation of glucosyl ABz 4 with the disarmed thioglycoside 5a under the activation of PPh₃AuOTf at -15 °C afforded thioglycoside 6a in an excellent yield (85%). However, when the other
 Table 1. Selective Activation of Other Leaving Groups over

 the ABz Leaving Group







thioglycosides such as armed thioglycosides were used as the bifunctional acceptor, unsatisfactory results were obtained (Supporting Information), which indicated the issues inherent to thioglycosides such as undesired intermolecular aglycon transfers.¹⁷ Both disarmed *n*-Pen **5b** and armed *n*-Pen **5c** were glycosylated with glucosyl ABz 4 smoothly under the catalysis of PPh₃AuOTf at room temperature, producing *n*-Pen disaccharide **6b** and **6c**, respectively, in excellent yields (entries 2-3).²⁷

Having examined the orthogonality of glycosyl ABz with the other leaving groups, we next focused on the orthogonal onepot synthesis of oligosaccharides (Scheme 2). For the glycosyl TCAI and ABz pair, glycosylation of TCAI **1a** (1.2 equiv) with ABz 7 (1.0 equiv) under the activation of TMSOTf afforded the intermediate, which was further coupled with the acceptor Scheme 2. Orthogonal One-Pot Synthesis of Oligosaccharides Based on Glycosyl ABz



8 (0.9 equiv) under the catalysis of PPh₃AuOTf, affording trisaccharide 21 in 89% yield in one pot. For the glycosyl PTFAI and ABz pair, using the above similar procedures, trisaccharide 22 was obtained in 95% yield by successive coupling of PTFAI 9, ABz 7, and acceptor 10 in one pot. Trisaccharide 24 is the glycosphingolipid analogue of isoGb₃, which was discovered as a key endogenous human NKT cell's antigen.²⁸ Sequential glycosylation of PTFAI 1b, ABz 11, and 4-OH acceptor 12 in the same pot generated 24 in 70% yield with excellent stereoselectivity. Replacing the above acceptor 12 with the poor 4-OH glucosaminyl acceptor 13 generated trisaccharide 25 in one pot with a 63% yield, which was identified as an α -Gal epitope that could induce acute rejections during xenotransplantations.²⁹ For the SBox glycoside and glycosyl ABz pair, AgOTf was used to activate SBox 1c (1.2 equiv) over ABz 2 (1.0 equiv), followed by the

addition of acceptor 14 (0.9 equiv) and the catalyst PPh₃AuOTf, affording trisaccharide 26 efficiently in one pot in 86% yield. The above procedure was employed to sequentially assemble building blocks 15, 7, and 5b, generating trisaccharide 27 in one pot with an 84% yield. The key intermediate of S. pneumonia type 3 trisaccharide 28^{30} was obtained in 62% yield in one pot by exchanging the above acceptor 5b for 4-OH acceptor 12. For the STaz glycoside and glycosyl ABz pair, coupling of STaz 1d (1.2 equiv) with ABz 2 (1.0 equiv) under the activation of MeOTf, followed by Yu glycosylation with the acceptor 16 (0.9 equiv) in the same pot, produced trisacchairde 29 in 70% yield. For the glycosyl IQC and ABz pair, although ABz 2 was stable in the activation of IQC 1e with $Cu(OTf)_{2}$, one-pot synthesis was unsuccessful due to the interference of the departing species IQC copper salt in the gold-catalyzed glycosylation reaction. For the

glycosyl ABz and *n*-Pen glycoside pair, Yu glycosylation between ABz 4 (1.2 equiv) and *n*-Pen **5b** (1.0 equiv) provided the intermediate, which was further coupled with acceptor 17 (0.9 equiv) under the promotion of NIS and TMSOTf, generating trisaccharide **30** in one pot with an 83% yield. Trisaccharide motif **31** occurs in complex *N*-glycoprotein structures with two challenging Gal- β 1,4-GlcNAc and GlcNAc- β 1,2-Man linkages. Following the above similar procedure, successive glycosylation of ABz **18**, 4-OH *n*-Pen **13**, and 2-OH mannosyl acceptor **19** in the same pot afforded trisaccharide **31** in excellent 80% yield, which had been synthesized previously in moderate 54% overall yield by a preactivationbased one-pot method requiring low reaction temperature (-60 °C to rt) conditions.¹¹

Finally, we investigated the multistep orthogonal one-pot synthesis of oligosaccharides (Scheme 2). Sulfated derivatives of tetrasaccharide 32 show significant proangiogenic activity.³¹ Selective activation of TCAI 1a (1.3 equiv) over ABz 2 (1.0 equiv) under the catalysis of TMSOTf provided the disaccharide intermediate, which was further coupled with disarmed STol 5a (0.9 equiv) under the catalytic amount of PPh₃AuOTf, affording the trisaccharide intermediate. The above intermediate was further coupled with the acceptor 17 (0.9 equiv) under the promotion of NIS and TMSOTf to generate tetrasaccharide 32 in one pot with an excellent 82% yield, which had been synthesized previously in only 39% overall yield by stepwise glycosylation.²⁶ For the SBox glycoside, glycosyl ABz, and n-Pen glycoside triplet, orthogonal glycosylation between SBox 15 (1.2 equiv) and ABz 20 (1.0 equiv) under the activation of AgOTf produced the disaccharide intermediate, which was followed by Yu glycosylation with n-pen 5b (0.9 equiv), providing the trisaccharide intermediate. The above trisaccharide was further assembled with acceptor 17 (0.9 equiv) under the promotion of NIS/TMSOTf, generating tetrasaccharide 33 in 75% yield in one pot, which was comparable to the previous one-pot synthesis of 33 with a 73% overall yield.¹⁹⁶ Hexasaccharide motif 34 occurs in fungal β -glucan oligosaccharide structures, which could induce antibiotic phytoalexins in the soybean plant.³² Selective coupling of PTFAI 9 (1.3 equiv) with ABz 7 (1.0 equiv) in the presence of TMSOTf afforded the disaccharide intermediate, which was further glycosylated with *n*-Pen **5b** (0.9 equiv) under Yu glycosylation conditions to generate a trisaccharide intermediate. Acceptor 23 (0.9 equiv) obtained from trisaccharide 22 was further coupled with the above trisaccharide intermediate under the activation of NIS and TMSOTf, producing hexasaccharide 34 in 67% yield in a one-pot manner. The analogue of hexasaccharide 34 had been synthesized previously by an automated solid-phase method, which required an excess of each building block (15 equiv) for glycosylation reactions.^{4c}

In summary, we systematically investigated the orthogonality of glycosyl ABz with the other leaving groups and developed a new and highly efficient orthogonal one-pot method for synthesis of oligosaccharides based on glycosyl ABz. This new one-pot method solves such issues as aglycon transfers, undesired interference of the departing species, and the unpleasant odor inherent to the previously developed thioglycoside-based orthogonal one-pot synthesis. Over a dozen of oligosaccharides including an isoGb3 trisaccharide analogue, a key intermediate of *S. pneumonia* type 3 trisaccharide, an α -Gal epitope trisaccharide, a Gal- β -1,4-GlcNAc- β -1,2-Man trisaccharide motif in complex *N*-glycoprotein structures, and a hexa- β -glucoside motif in phytoalexin elicitor β -glucan structures had been highly efficiently synthesized by this method, which highly streamlines the chemical synthesis of oligosaccharides.

ASSOCIATED CONTENT

Supporting Information

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Experimental procedures and characterization data (PDF)

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