SHORT COMMUNICATION

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Thermodynamically Controlled Regioselective Glycosylation of Fully Unprotected Sugars through Bis(boronate) Intermediates

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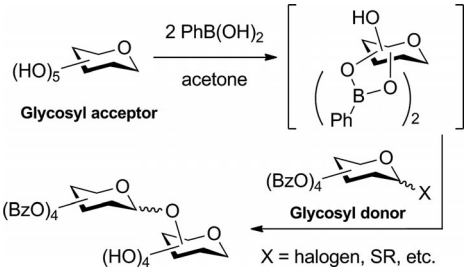
Fully unprotected D-glucose, D-mannose, and D-fructose were regioselectively glycosylated with several phenylthioglycosides to afford glycosyl-β(1→6)-α/β-D-glucopyranose, glycosyl-β(1→1)-α-D-mannofuranoside, and glycosyl-β(1→1)-β-D-fructopyranose in good yields. The regioselectivity might have arisen from a thermodynamically controlled bis(phenylboronate) intermediate, which leaves only one free hydroxy group to be glycosylated in complete regioselectivity.

Introduction

Carbohydrates are considered to be target molecules of great importance owing to their biological functions.[1] Their elucidation requires the chemical synthesis of oligosaccharides, which are indispensable for providing diverse substances in pure form in enough quantity, especially if isolation from natural sources is limited. An extensive arsenal of methods for oligosaccharide synthesis is currently available.[2] Conventional methods based on a protection–deprotection processes typically involve several synthetic steps, expensive reagents, and extensive manipulation of the protecting group. To minimize these drawbacks, several methods for the regioselective protection of carbohydrates have been developed and are used for oligosaccharide synthesis, which thereby reduces the number of reaction steps for the preparation of partially protected sugars.[3] However, more straightforward methodology is required for the regioselective glycosylation of fully unprotected sugars without the need for the preparation of the protected sugars.

In this context, very few precedents have been reported; however, stannylene-mediated regioselective glycosylation of unprotected hexopyranosides is reported to produce a one-pot assembly of some oligosaccharides.[4] In addition, boronate-mediated regioselective glycosylation has also been reported.[5] Recently, we developed a novel method for the regioselective glycosylation of fully unprotected methyl hexopyranosides by means of "transient masking" of the hydroxy groups with arylboronic acids.[6] All previous methods were limited to alkyl glycoside acceptors, for example, methyl α/β-D-glucopyranoside, methyl α/β-D-galactopyranoside, methyl α/β-D-rhamnopyranoside, and so on, and the yields and regioselectivity were not always high. Therefore, it is of special interest to develop a novel regioselective glycosylation method applicable to fully unprotected 1-OH free hexoses. To realize our goal, we noted the bis(arylboronate)s of hexoses, which leave only one free hydroxy group feasible for perfect regioselective glycosylation (cf. Scheme 1). Regioselectivity might be expected owing to the bis(phenylboronate) structures, which are capable of masking four hydroxy groups of the hexoses in the most stable thermodynamic forms. Thus, D-glucose, D-galactose, and D-mannose as aldohexoses as well as D-fructose as a ketohexose were employed as glycosyl acceptors. Several *O*-acylated thioglycosides were used as glycosyl donors for glycosylation of these acceptors under the conditions employed in our "transient masking" method, which was used for the regioselective glycosylation of several alkyl glycosides.[6]



Scheme 1. Regioselective glycosylation of fully unprotected hexoses by transient masking method; Bz = benzoyl.

Results and Discussion

First, we tested our method by using D-glucose as a fully unprotected acceptor. Molecular recognition between gluc-

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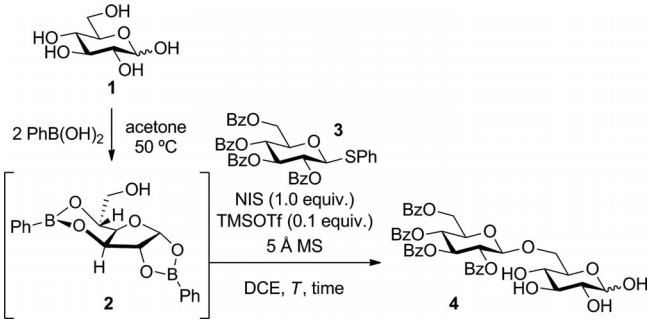
ose and arylboronic acid in aqueous media has been widely investigated in the detection of glucose for biological purposes.[7] In non-aqueous media, however, the structures of the boronate complexes of glucose are poorly understood in most cases. Eggert et al. reported a furanose-type complex of 1,2:3,5-bis(boronate) **2** of glucose (Table 1) prepared in DMF, the structure of which was elucidated on the basis of NMR spectroscopy.[8] We screened various non-aqueous solvents that might be suitable for our purpose. Among the low-boiling solvents tested, acetone was found to be a good solvent for generating the bis(arylboronate)s complex of D-glucose. The structure was identified as bis(boronate) complex **2**.

Table 1. Regioselective glycosylation of D-glucose.[a]



Entry	Donor [equiv.]	*T* [°C]	Time [h]	Yield[b] [%]
1	2.0	–30	10	79
2	3.0	–30	5	90
3	4.0	–30	6	94
4	2.0	–10	1.5	79
5	2.0	0	0.3	86
6	2.0	r.t.	0.2	79

[a] NIS = *N*-iodosuccinimide, TMSOTf = trimethylsilyl trifluoromethanesulfonate, MS = molecular sieves. [b] Yield of isolated product.

Although boronate is not stable enough to store at ambient temperatures,[9] it is acceptable for the "transient masking" of hydroxy groups. Accordingly, bis(boronate) **2** seemed to be an appropriate intermediate for the glycosyl-(1→6)-linked glucose structure, and the glycosylation conditions required for **2** were investigated after evaporation of acetone with glycosyl donor **3** in 1,2-dichloroethane (DCE, cf. Table 1).

Under the reaction conditions employed (Table 1), glycosylation of **2** with tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3**) provided disaccharide **4** in 79% yield. The yield of disaccharide **4** was increased to 94% if the number of equivalents of donor **3** (2–4 equiv.) was also increased (Table 1, entries 1–3).

The effects of the reaction temperature were also examined with the aim of obtaining sizeable improvements in the yield as well as improved reaction times (Table 1, entries 1, 4–6). Among those examined, entry 5 could be recommended owing to a good yield (86%), short reaction time (20 min), and lower number of equivalents of the donor (2 equiv.).

Because of the complexity of the NMR spectra of disaccharide **4** obtained from the all-hydroxy-free D-glucose acceptor, acetylation of the crude product was performed to furnish an anomeric mixture of 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-α,β-D-glucopyranose (**5**),[10] namely a gentiobiose derivative. Alternatively, to avoid formation of anomeric isomers of **5**, we tried to lead **4** into bis(boronate) derivative **6** by treatment of **4** with phenylboronic acid (2 equiv.), which afforded a single isomer of **6** in pure form (Figure 1). The structure of **6** was reasonably elucidated by the HMBC spectra designated in Figure 1. Previous access to gentiobiose by the conventional protection–deprotection method required six steps from D-glucose to give a combined yield of less than 25%.[11]

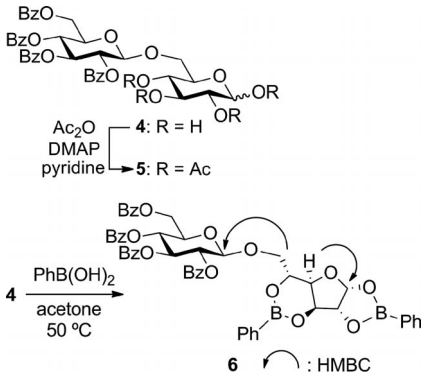
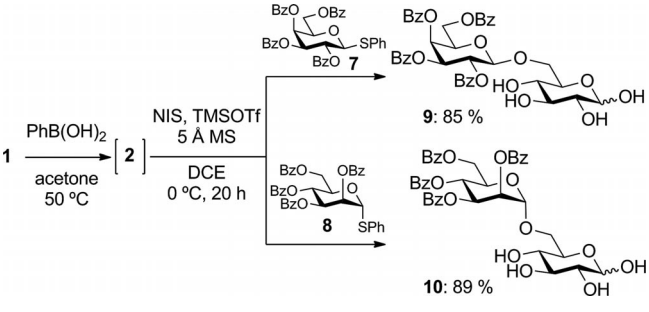


Figure 1. Acetyl and bis(boronate) derivatives of disaccharide **4**. DMAP = 4-(*N*,*N*-dimethylamino)pyridine.

As the disaccharide (a gentiobiose derivative) was obtained from D-glucose as expected, we attempted our method on glycosyl donors **7** and **8**. A one-pot reaction of D-glucose with phenylboronic acid (2 equiv.) followed by glycosylation with phenylthiogalactoside **7** and mannoside **8** under the reaction conditions employed in entry 5 of Table 1, except for the reaction time (20 h), readily gave desired disaccharides **9** and **10** in good yields as the sole products (Scheme 2). The structures of the disaccharides were unambiguously elucidated on the basis of *O*-acetylated derivatives **11** and **12**, as described for **4**.[12] The anomeric configuration of the nonreducing end of **9** and **10** was deduced to be β and α, respectively, as expected by anchimeric assistance of the 2′-*O*-benzoyl group.

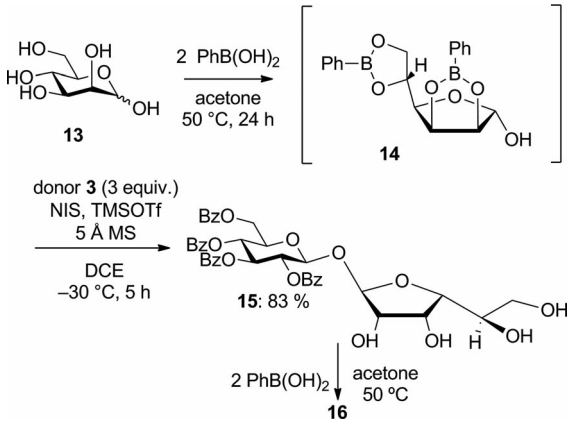


Scheme 2. Regioselective glycosylation of fully unprotected glucose with galactosyl and mannosyl donors.

Next, we applied our method to other acceptors, D-galactose and D-mannose, as aldohexoses other than D-glucose. We observed that the bis(boronate)s of D-galactose that were formed in acetone at 50 °C gave a complex mixture of isomers of boronates, the structures of which were not elucidated at this stage on the basis of NMR spectroscopy.

Subsequently, we tested the bis(boronate) formation of D-mannose, which was reported to form an α-D-mannofuranose cyclic 2,3:5,6-bis(*n*-butylboronate).[8b] As expected, hydroxy-free D-mannose **13** reacted with phenylboronic acid (2 equiv.) in acetone to generate 2,3:5,6-bis(phenylboronate) complex **14** of the furanose form possessing a free hemiacetal function at the anomeric center.

Glycosylation readily occurred with glucosyl donor **3** under the conditions described above to afford β-D-glucopyranosyl-(1→1)-α-D-mannofuranoside **15** in 83% yield (Scheme 3). Structural identification was performed by analysis of bis(boronate) derivative **16** by NMR spectroscopy, as depicted in Figure 2. The newly formed 1→1-linked intersaccharide linkage of **16** was identified by HMBC spectroscopy, which clearly showed cross signals between H-1′ and C-1, H-1′ and C-5′, H-1 and C-1′, as well as H-1 and C-4. From the anomeric configurations, the β-D-glucopyranosyl form was demonstrated from the coupling constant of $J_{H1',H2'}$ = 8.0 Hz, whereas the NOE between H-1 and H-1′ suggested an α-configuration of the mannofuranosyl moiety (Figure 2).



Scheme 3. Glycosylation of D-mannose **13** with glucosyl donor **3**.

Finally, we applied our method to D-fructose (**17**), a ketohexose. Although the bis(boronate) of D-fructose has been reported,[13] we tried to obtain it by using our own method to yield β-D-fructopyranose cyclic 2,3:4,5-bis(phenylboronate) (**18**, Scheme 4). Glycosylation of **18** in situ with glucosyl donor **3** readily gave β-D-glucopyranosyl-(1→1)-β-D-fructopyranose **19** in 97% yield. The regio- and stereoselectivity of the newly formed intersaccharide linkages were unambiguously determined on the basis of their NMR spectra (HMBC and ROESY). An anomeric configuration of the β-D-glucopyranosyl portion was identified by the $J_{H1',H2'}$ coupling constant (8.0 Hz, 5.04 ppm). However, the second-order HMBC spectrum showed cross signals between H-1′ and C-1 and between H-1 and C-5, and ROESY

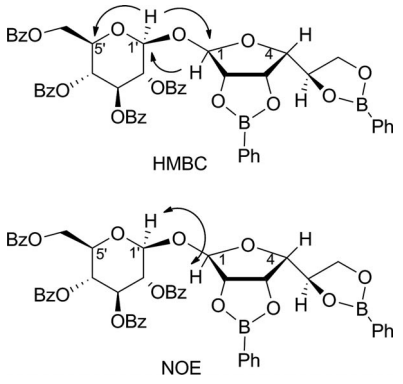
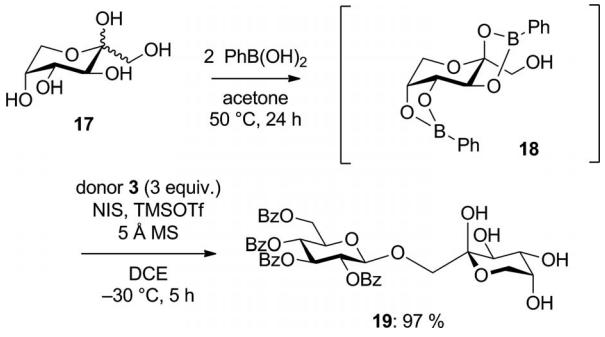


Figure 2. HMBC and NOE spectra of β-D-glucopyranosyl-(1→1)-α-D-mannofuranoside 2,3:5,6-bis(phenylboronate) **16**.

showed a cross signal between H-1 and H-3. The β-D-fructopyranosyl structure should be estimated from the above data as well as intermediary 2,3:4,5-bis(boronate) structure **18**, with which glycosyl donor **3** attacks a free 1-OH function of **18** at the glycosylation stage.



Scheme 4. Glycosylation of D-fructose (**17**) with glucosyl donor **3**.

Conclusions

In summary, straightforward and novel regioselective glycosylation of fully unprotected hexoses was developed for the following. 1) Hexose structure-dependent bis(boronate)s were formed in situ and glycosylated with several glycosyl donors to afford disaccharides with complete regioselectivity. 2) Among the aldohexoses employed, D-glucose was glycosylated to 6-OH of the acceptors to provide 1→6-linked disaccharides, whereas D-mannose was glycosylated to 1-OH to afford a 1→1-linked disaccharide comprising a mannofuranoside. 3) A ketohexose, D-fructose, was glycosylated to give 1→1-linked disaccharide comprising a fructopyranose. Our method might significantly improve the synthesis of disaccharides included in the above categories with regard to the reaction steps and manipulations required.

Supporting Information (see footnote on the first page of this article): Experimental procedures and characterization data for all new compounds associated with this article.

Acknowledgments

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[1] a) P. H. Seeberger, D. B. Werz, *Nature* **2007**, *446*, 1046–1051; b) P. Stalforth, B. Lepenies, A. Adibekiana, P. H. Seeberger, *J. Med. Chem.* **2009**, *52*, 5561–5577; c) B. La Ferla, C. Airoldi, C. Zona, A. Orsato, F. Cardona, S. Merlo, E. Sironi, G. D'Orazio, F. Nicotra, *Nat. Prod. Rep.* **2011**, *28*, 630–648; d) J. S. Thorson, T. Vogt, *Glycosylated Natural Products*, in: *Carbohydrate-Based Drug Discovery* (Ed.: C. H. Wong), Wiley-VCH, Weinheim, Germany, **2003**; pp 685–711; e) J. W. Grate, G. C. Frye, *Sensors Update* (Eds.: H. Baltes, W. Göpel, J. Hesse), Wiley-VCH, Weinheim, Germany, **1996**, vol. 2, p. 10–20.

[2] a) F. W. Lichtenthaler, *Chem. Rev.* **2011**, *111*, 5569–5609; b) A. Liptak, Borbas, A. I. Bajza, *Protective Group Manipulation in Carbohydrate Synthesis* in: *Comprehensive Glycoscience* (Eds.: J. P. Kamering, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. Vorangen), Elsevier, Oxford, UK, **2007**, vol. 1, p. 203–259; c) K. Toshima, K. Sasaki, *O-Glycosidation Methods*, in: *Comprehensive Glycoscience* (Eds.: J. P. Kamering, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. Vorangen), Elsevier, Oxford, UK, **2007**, vol. 1, p. 203–259; d) H. Tanaka, H. Yamada, T. Takahashi, *Trends Glycosci. Glycotechnol.* **2007**, *19*, 183–193; e) K. Toshima, *Glycosyl Halides and Anomeric Esters as Donors*, in: *Glycoscience* (Eds.: B. O. Fraser-Reid, K. Tatsuta, J. Thiem) Springer, Berlin, **2001**, vol. 1, p. 583–626; f) D. Crich, M. Smith, *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020; g) P. H. Seeberger, W. C. Haase, *Chem. Rev.* **2000**, *100*, 4349–7393; h) K. Toshima, K. Tatsuta, *Chem. Rev.* **1993**, *93*, 1503–1531.

[3] a) C.-C. Wang, J.-C. Lee, S.-Y. Luo, S. S. Kulkarni, Y.-W. Huang, C.-C. Lee, K.-L. Chang, S.-C. Hung, *Nature* **2007**, *446*, 896–899; b) T. Kawabata, T. Furuta, *Chem. Lett.* **2009**, *38*, 640–647; c) L. Chan, M. S. Taylor, *Org. Lett.* **2011**, *13*, 3090–3093; d) D. Lee, M. S. Taylor, *J. Am. Chem. Soc.* **2011**, *133*, 3724–3727; e) D. Lee, C. L. Williamson, L. Chan, M. S. Taylor, *J. Am. Chem. Soc.* **2012**, *134*, 8260–8267; f) Y. Demizu, Y. Kubo, H. Miyoshi, T. Maki, Y. Matsumura, N. Moriyama, O. Onomura, *Org. Lett.* **2008**, *10*, 5075–5077; g) T. Maki, N. Ushijima, Y. Matsumura, O. Onomura, *Tetrahedron Lett.* **2009**, *50*, 1466–1468; h) D. Lee, M. S. Taylor, *Org. Biomol. Chem.* **2013**, *11*, 5409–5412; i) review: C. A. McClary, M. S. Taylor, *Carbohydr. Res.* **2013**, *381*, 112–122; j) X. Sun, H. Lee, S. Lee, K. L. Tan, *Nature Chem.* **2013**, *5*, 790–795; k) D. Lee, M. S. Taylor, *Synthesis* **2012**, *44*, 3421–3431.

[4] a) P. J. Garegg, J. Maloisel, S. Oscarson, *Synthesis* **1995**, 409–414; b) R. K. P. Kartha, M. Kiso, A. Hasegawa, H. L. Jennings, *J. Chem. Soc. Perkin Trans. 1* **1995**, 3023–3026; c) E. Kaji, N. Harita, *Tetrahedron Lett.* **2000**, *41*, 53–56; d) E. Kaji, K. Shibayama, K. In, *Tetrahedron Lett.* **2003**, *44*, 4881–4885; e) A. Maggi, R. Madsen, *Eur. J. Org. Chem.* **2013**, 2683–2691; f) W. Muramatsu, H. Yoshimatsu, *Adv. Synth. Catal.* **2013**, *355*, 2518–2524.

[5] a) K. Oshima, Y. Aoyama, *J. Am. Chem. Soc.* **1999**, *121*, 2315–2316; b) K. Oshima, T. Yamauchi, M. Shimomura, Y. Aoyama, *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1319–1324; c) C. Gouliaras, D. Lee, L. Chan, M. S. Taylor, *J. Am. Chem. Soc.* **2011**, *133*, 13926–13929; d) T. H. Fenger, R. Madsen, *Eur. J. Org. Chem.* **2013**, 5923–5933.

[6] a) E. Kaji, T. Nishino, K. Ishige, Y. Ohya, Y. Shirai, *Tetrahedron Lett.* **2010**, *51*, 1570–1573; b) T. Nishino, Y. Ohya, R. Murai, T. Shirahata, D. Yamamoto, K. Makino, E. Kaji, *Heterocycles* **2012**, *84*, 1123–1140.

[7] a) M. Takeuchi, H. Kijima, I. Hamachi, S. Shinkai, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 699–705; b) O. Hirata, Y. Kubo, M. Takeuchi, S. Shinkai, *Tetrahedron* **2004**, *60*, 11211–11218; c) R. Nishiyabu, Y. Kubo, T. D. James, J. S. Fossey, *Chem. Commun.* **2011**, *47*, 1106–1123, and references cited therein.

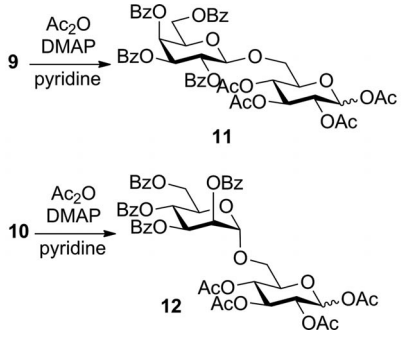
[8] a) J. C. Norrild, H. Eggert, *J. Am. Chem. Soc.* **1995**, *117*, 1479–1484; b) R. Smoum, A. Rubinstein, M. Srebnik, *Magn. Reson. Chem.* **2003**, *41*, 1015–1020.

[9] T. W. Greene, P. G. M. Wuts, *Protective Groups in Organic Synthesis* 3rd ed., Wiley-VCH, New York, **1999**, p. 243–245.

[10] Compound **5**, **11**, and **12** were obtained as anomeric mixtures of α/β ≈ 1:1, for which the spectroscopic data are shown in the Supporting Information.

[11] D. D. Reynolds, W. L. Evans, *J. Am. Chem. Soc.* **1938**, *60*, 2559–2561.

[12] Compounds **11** and **12** were prepared as below. Spectral data of **11** and **12** are described in the Supporting Information.



[13] P. J. Wood, I. R. Siddiqui, *Carbohydr. Res.* **1974**, *36*, 247–256.

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