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Stereoselective Direct Glycosylation with Anomeric Hydroxy Sugars by Activation with Phthalic Anhydride and Trifluoromethanesulfonic Anhydride Involving Glycosyl Phthalate Intermediates

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Abstract: An efficient direct one-pot glycosylation method with anomeric hydroxy sugars as glycosyl donors employing phthalic anhydride and triflic anhydride as activating agents has been developed. Thus, highly stereoselective β-mannopyranosylations were achieved by the reaction of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranose (**2**) with phthalic anhydride in the presence of DBU at room temperature followed by sequential addition of DTBMP and Tf_2O and glycosyl acceptors to the reaction mixture at -78 °C in one-pot. Stereoselective α-glucopyranosylations with 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-glucopyranose (**25**) and other glycosylations with glucopyranoses and mannopyranoses having tetra-*O*-benzyl- and tetra-*O*-benzoyl protecting groups were also possible by utilizing the present one-pot glycosylation protocol. The possible mechanism for the β-mannosylation with **2** was proposed based on the NMR study, in which α-mannosyl phthalate **55**α and α-mannosyl triflate **59** were detected as intermediates. The versatility and efficiency of the present glycosylation methodology, especially those of the β-mannopyranosylation protocol, were readily demonstrated by the efficient synthesis of protected β-(1→4)-D-mannotriose **62** and β-(1→4)-D-mannotetraose **67** with perfect β-stereoselectivity.

Introduction

The development of efficient and stereoselective glycosylation methodologies[1] has been a major concern in synthetic organic chemistry over the past decade due to important roles of complex oligosaccharides in many fundamental life-sustaining processes.[2] The selection of an appropriate glycosyl donor is one of the key processes for the successful glycosylation in terms of efficiency and stereoselectivity and thus, the bulk of the efforts in this area have focused on devising new efficient glycosyl donors. Several glycosyl donors such as glycosyl trichloroacetimidates,[3] thioglycosides,[4] glycosyl sulfoxides,[5] glycals,[6] *n*-pentenyl glycosides,[7] glycosyl fluorides,[8] glycosyl phosphates,[9] and glycosyl phosphites[10] have been successfully used for the synthesis of various important oligosaccharides and glycoconjugates. We have also previously reported 2′-carboxybenzyl (CB) glycosides as a new type of glycosyl donors for efficient stereoselective glycosylations,[11] and their application to the synthesis of complex oligosaccharides and galactosphingolipids.[12] Nevertheless, the stereoselective construction of certain glycosyl linkages such as β-mannopyranosyl,[13] α-glucopyranosyl,[14] β-arabinofuranosyl,[15] and α-sialyl linkages[16] still poses a great challenge. In addition, application of the glycosylation methods with known glycosyl donors to the automated

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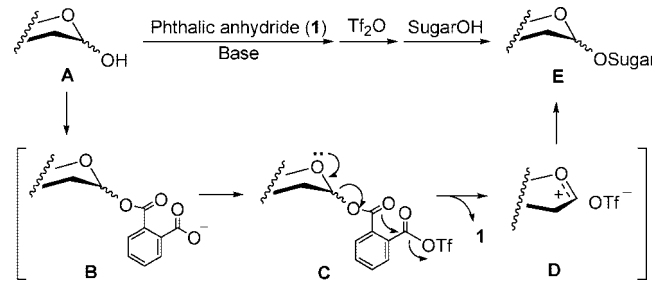
solid-phase synthesis[17] or in the one-pot solution-phase synthesis[18] of oligosaccharides remains a difficult task. In this regard, there is still a need for the development of efficient and stereoselective glycosylation methods by devising new glycosyl donors or modifying known ones. The majority of known glycosylation methodologies consist of the preparation of a glycosyl donor by conversion of an anomeric substituent into a latent leaving group in the first step and activation of the isolated glycosyl donor by a promoter followed by formation of a glycosyl bond by the reaction between the activated donor and a nucleophilic glycosyl acceptor in the second step. On the other hand, a direct glycosylation with anomeric hydroxy glycosyl donors, in which all the operations of anomeric derivatization, activation, and glycosyl bond formation are combined into a one-pot procedure, would offer some advantages in oligosaccharide synthesis over the stepwise glycosylation methods. Although there have been several reports on the direct glycosylation with C1-hydroxy glycosyl donors,[19] they have not attracted much attention. Recently, Gin and co-workers have developed a new method for the glycosylation with anomeric hydroxy sugars involving oxosulfonium intermediates[20] and reported its application to the synthesis of complex oligosaccharides.[21]

As continuation of our search for new glycosyl donors and stereoselective glycosylation methods, we envisaged that treatment of anomeric hydroxy sugar **A** with phthalic anhydride (**1**) in the presence of a base would generate glycosyl phthalate anion **B** (Scheme 1). Then, the addition of Tf_2O to the phthalate

Scheme 1



C followed by cyclization of the resulting triflate mixed anhydride **C** would afford oxocarbenium ion **D** by extrusion of stable nonnucleophilic **1**. Subsequent reaction of **D** with a nucleophilic glycosyl acceptor (SugarOH) would provide glycoside **E**. Herein we report a new stereoselective direct glycosylation with anomeric hydroxy sugars, in which to the anomeric hydroxy donor are sequentially added phthalic anhydride, triflic anhydride, and an acceptor alcohol in the presence of an appropriate base in one-pot without isolation of intermediates as shown in Scheme 1. We also report the NMR study for detection of glycosylation intermediates and the application of the present method to the synthesis of β-(1→4)-mannotetraose of β-(1→4)-mannan.

Results and Discussion

Stereoselective β-Mannopyranosylation. The stereoselective construction of 1,2-*cis*-β-mannopyranosyl linkages still remains one of major challenges in oligosaccharide synthesis.[13] In our initial study, 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranose (**2**) was chosen as a glycosyl donor for the mannopyranosylation. The reason for choosing compound **2** as an mannosyl donor was based on the important discovery by Crich and co-workers[22] that the 4,6-*O*-benzylidene protective group facilitates the high stereoselectivity in the β-mannopyranosylation. In fact, Seeberger and co-workers,[23] utilized compound **2** for the β-mannopyranosylation employing Gin's dehydrative glycosylation method.[20] The present mannosylation with compound **2** requires a sequence of three steps in one-pot: (i) reaction of **2** and **1** in the presence of a base, then (ii) addition of Tf_2O, and finally, (iii) addition of a glycosyl acceptor. Selection of an appropriate base or a combination of bases in the first step was found to be crucial for the success of the present one-pot glycosylation. When triethylamine (3.3 equiv) was used in refluxing CH_2Cl_2, the reaction between **2** (α/β = 2.1:1) and **1** in the first step completed in 5 h to afford an intermediate based on TLC but further addition of Tf_2O and acceptor **3** sequentially to the reaction mixture at −78 °C failed to provide the desired disaccharide **5** (entry 1 in Table 1). We speculated that triethylamine interfered with the second step of the glycosylation by deactivation of Tf_2O. With a weaker and hindered base, di-*tert*-butylmethylpyridine (DTBMP) in refluxing CH_2Cl_2, the glycosylation failed again and compound **2** appeared to be decomposed in the first step based on TLC (entry 2). With a combination of Et_3N (1.1 equiv) and DTBMP (2.2 equiv) in refluxing CH_2Cl_2, the first step proceeded smoothly to give the

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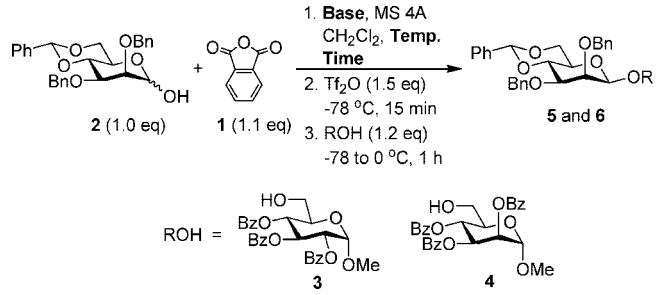
Table 1. Screening of Bases for One-Pot β-Mannopyranosylation with **2** in CH_2Cl_2



	the 1st step					
entry	base	reaction temp	reaction time	acceptor ROH	product	yield, %[a] (ratio, β/α)[a]
1	Et_3N (3.3 equiv)	reflux	5 h	**3**	no reaction	
2	DTBMP (3.3 equiv)	reflux	5 h	**3**	no reaction	
3	Et_3N (1.1 equiv) + DTBMP (2.2 equiv)	reflux	2.5 h	**3**	**5**	79 (β only)
4	Et_3N (1.1 equiv) + DTBMP (2.2 equiv)	reflux	2.5 h	**4**	**6**	73 (β only)
5	DBU (1.1 equiv) + DTBMP (2.2 equiv)	room temp	15 min	**3**	**5**	89 (β only)
6	DBU (1.1 equiv) + DTBMP (2.2 equiv)	room temp	15 min	**4**	**6**	88 (β only)

[a] Determined after isolation.

intermediate in 2.5 h based on TLC, and then sequential addition of Tf_2O and acceptor **3** to the reaction mixture at −78 °C provided β-mannoside **5** exclusively in 79% yield (entry 3). Similarly, the reaction of **2** with acceptor **4** employing Et_3N (1.1 equiv) and DTBMP (2.2 equiv) afforded β-mannoside **6** exclusively 73% yield (entry 4). The best result was obtained with a combination of 1,8-diazobicyclo[5,4,0]undec-7-ene (DBU) and DTBMP as bases. Thus, reaction of **2** and **1** in the first step proceeded rapidly with DBU (1.1 equiv) at room temperature in 15 min and then sequential addition of DTBMP (2.2 equiv), Tf_2O, and acceptor **3** to the reaction mixture at −78 °C afforded β-mannoside **5** exclusively in 89% yield (entry 5). Similarly, reaction of the mannose **2** with the acceptor **4** employing DBU/DTBMP afforded β-mannoside **6** exclusively in 88% yield (entry 6).

Therefore, the one-pot β-mannopyranosylation of various glycosyl acceptors with the mannopyranose **2** (α/β = 2.1:1) were carried out by the following sequence as a standard reaction condition: (i) stirring a solution of **2** (1.0 equiv), phthalic anhydride (**1**, 1.1 equiv), and DBU (1.2 equiv) in the presence of 4 Å molecular sieves for 15 min at room temperature in CH_2Cl_2, (ii) sequential addition of DTBMP (2.2 equiv) and Tf_2O (1.5 equiv) to this solution at −78 °C and stirring the resulting solution for 15 min, and then (iii) slow addition of the glycosyl acceptor (1.2 equiv) to the above solution at −78 °C and stirring briefly the reaction mixture at −78 °C and allowing to warm over 1 h to 0 °C. Mannosylations of primary alcohol acceptors **3** and **4** having benzoyl-protective groups with **2** afforded exclusively β-disaccharides **5** and **6** in 89% and 88% yield, respectively, after chromatographic separation (entries 1 and 2 in Table 2), while the same mannosylations of primary alcohol acceptors **7** and **8** having benzyl-protective groups gave predominantly corresponding β-mannosyl disaccharides **16** (β/α = 29:1) and **17** (β/α = 12:1) (entries 3 and 4 in Table 2). Completely β-selective mannosylations of secondary alcohol acceptors **9**−**11** with **2** were also achieved in one-pot to provide corresponding β-mannosyl disaccharides **18**−**20**, respectively, in high yields (entries 5−7). The mannosylation of diacetone galactose **12**, azido sugar **13**, and 1-octanol (**15**) with **2** afforded predominantly β-disaccharides **21** (β/α = 11:1), **22** (β/α = 16:1), and **24** (β/α = 10:1), respectively in high yields (entries 8, 9, and 11). On the other hand, the mannosylation of *N*-phthalimido sugar **14** with **2** gave a mixture of α- and β-disaccharides **23** (β/α = 5.6:1) in 91% yield (entry 10). The stereochemistries of the newly generated anomeric centers of unknown mannosides **19** and **23** were determined unequivocally on the basis of their ^1H and ^{13}C NMR spectral data, in particular one-bond C1−H1 coupling constants: $J_{C1'\text{-}H1'}$ = 161 Hz in the β-disaccharide **19** and $J_{C1'\text{-}H1'}$ = 170 Hz in the α anomer and $J_{C1'\text{-}H1'}$ = 164 Hz in the β anomer of compound **23**.[24] The results indicate that the present one-pot direct mannosylation employing 4,6-*O*-benzylidenemannose **2** with phthalic anhydride and Tf_2O is highly efficient and β-stereoselective and thus it appears to be superior to the previously reported method employing the same mannose **2** with diphenyl sulfoxide and Tf_2O.[23]

α-Glucopyranosylation and Other Glycosylations. We then applied the present one-pot mannosylation protocol to the glucosylation with 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-glucopyranose (**25**). It has been previously reported that the 4,6-*O*-benzylidene protective group promoted the stereoselective α-glucopyranosylation by Crich and co-workers[25] and by us.[26] Reaction of **25** with the glycosyl acceptor **3** by the same procedure used for the β-mannosylation gave not only desired α-disaccharide **26** in 38% yield but also unexpectedly self-condensed ester **27** (Figure 1) in 52% yield. The undesired **27** probably resulted from the coupling between the oxocarbenium ion **D** and the carboxylate anion **B** in Scheme 1. Unlike in the mannopyranosylation discussed above, the conversion of the carboxylate **B** into the triflate **C** in the glucopyranosylation might be slower than the conversion of the triflate **C** into the oxocarbenium ion **D** (Scheme 1) so that a substantial amount of **B** might remain even after generation of the oxocarbenium ion **D**. We envisaged that if the carboxylate anion **B** is protonated and if the resulting protonated carboxylic acid is still readily triflated by Tf_2O in the presence of a weaker base than DBU, the oxocarbenium ion **D** would preferentially react with the glycosyl acceptor alcohol over the less nucleophilic carboxylic acid. We therefore ran the glucosylation reaction under the modified condition, in which TfOH was added as the proton

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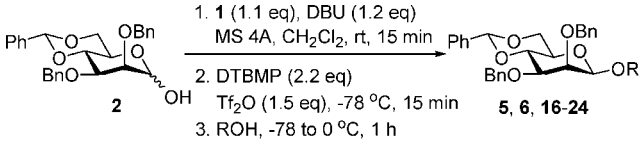
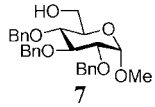
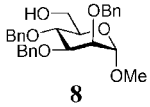
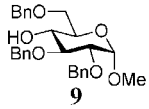
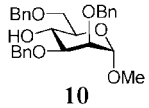
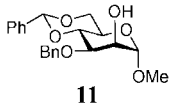
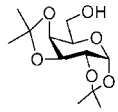
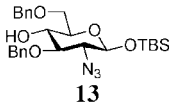
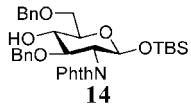
Table 2. Mannosylations of Various Acceptors with Benzylidene-Protected Donor **2**



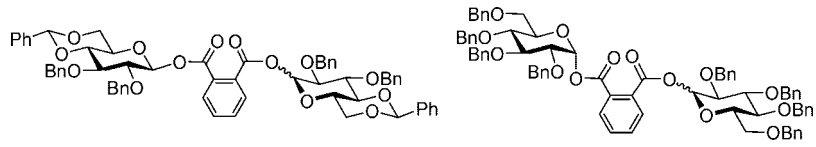
Entry	Acceptor ROH	Product	Yield %[a]	Ratio β/α[b]
1	**3**	**5**	89	β only (20:1)
2	**4**	**6**	88	β only (21:1)
3	 **7**	**16**	90	29:1[c] (17:1)
4	 **8**	**17**	91	12:1[c]
5	 **9**	**18**	88	β only (β only)
6	 **10**	**19**	90	β only (22:1)
7	 **11**	**20**	87	β only (β only)
8	 **12**	**21**	93	11:1[c]
9	 **13**	**22**	88	(16:1)
10	 **14**	**23**	91	5.6:1[c]
	1-Octanol			
11	**15**	**24**	81	(10:1)

[a] Isolated yields. [b]Determined after isolation. Number in parentheses is the ratio from the crude product determined by LC-Mass. [c]After isolation of most of the β-anomer, the ratio of the remaining α/β mixture was determined by ^{1}H NMR.

source together with DTBMP just before addition of Tf_2O. Thus, the glucosylation with compound **25** was carried out by a sequence of four steps in one-pot in CH_2Cl_2: (i) stirring the solution of **25** (1.0 equiv), phthalic anhydride (**1**, 1.1 equiv), and DBU (1.2 equiv) in the presence of 4 Å molecular sieves for 15 min at room temperature in CH_2Cl_2, (ii) sequential addition of DTBMP (3.3 equiv) and TfOH (1.1 equiv) to this solution at −78 °C and stirring the resulting solution for 15 min, (iii) addition of Tf_2O (1.5 equiv) to the above solution at −78 °C and stirring the resulting solution for 15 min, and (iv) slow addition of a glycosyl acceptor (1.2 equiv) at −78 °C and stirring the reaction mixture at −78 °C for 15 min and allowing to warm over 1 h to 0 °C. Indeed, the reaction of **25** with the primary alcohol acceptor **3** having benzoyl protective groups under the modified condition afforded exclusively α-disaccharide **26** in 74% yield along with the self-condensed ester **27** ($\alpha,\beta/\beta,\beta$ = 4:1) in much reduced yield, 20% (entry 1 in Table 3). With this modified protocol, we examined the glucosylation of various other glycosyl acceptors with **25**. Glucosylation of another primary alcohol acceptor **4** having benzoyl protective groups with **25** afforded predominantly α-disaccharide **28** (α/β = 18:1) in 87% yield along with a small amount (5%) of the self-condensed ester **27** (entry 2). However, glucosylations of primary alcohol acceptors **7** and **8** having benzyl protective groups with **25** provided mixtures of α- and β-disaccharides **29** (α/β = 1.6:1) and **30** (α/β = 1.5:1), respectively, in high yields without generation of the self-condensed ester (entries 3 and 4). On the other hand, glucosylations of secondary alcohol acceptors **9** and **11** with **25** provided exclusively α-disaccharides **31** and **32** in 80% and 85% yields, respectively (entries 5 and 6).

To explore the scope of the present one-pot glycosylation, we further examined glycosylation reactions with 1-hydroxy sugars having other protective groups rather than the benzylidene group. Glycosylations of the acceptor **3** with benzyl-protected glucose **33** and benzyl-protected mannose **34** under the same reaction condition as that employed for the β-mannosylation were, however, not satisfactory so that the original condition was modified by changing the solvent, the reaction temperature, and the order of addition of reagents. The solvent was changed to CH_2Cl_2/CH_3CN (10:1) from CH_2Cl_2, the reaction temperature was raised to 0 °C from −78 °C in the second and the third steps, and the acceptor was added in the second step before the slow addition of Tf_2O in the final third step. Thus, the glycosylations with the tetrabenzylglucose **33** or tetrabenzylmannose **34** were carried out by the following sequence: (i) stirring the solution of **33** or **34** (1.0 equiv), phthalic anhydride (**1**, 1.1 equiv), and DBU (1.2 equiv) in the presence of 4 Å molecular sieves for 15 min at room temperature in CH_2Cl_2/CH_3CN (10:1), (ii) sequential addition of DTBMP (2.2 equiv) and the glycosyl acceptor (1.2 equiv) to this solution at 0 °C and stirring the resulting solution for 15 min, and (iii) slow addition of Tf_2O (1.5 equiv) to the above solution at 0 °C, stirring the reaction mixture for 15 min at 0 °C, and allowing it to warm over 30 min to room temperature. Under this new standard reaction condition, the reaction of the glucosyl donor **33** and the acceptor **3** afforded disaccharide **35** (α/β = 1.6:1) in 73% yield and self-condensed ester **36** ($\alpha,\beta/\alpha,\alpha$ = 2:1) in 10% yield (entry 1 in Table 4).

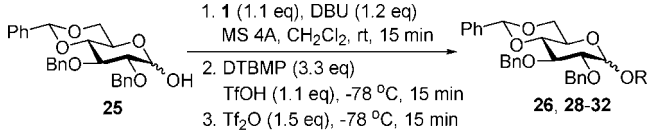
Glucosylations of other primary alcohol acceptors **7** and **12** with **33** also gave mixtures of α- and β-disaccharides **37** (α/β = 1.2:1) and **40** (α/β = 1:1.3), respectively (entries 2 and 5) along with the self-condensed ester **36**, which was generated about in 10% yield in all glucosylation reactions with **33**. Glucosylations of secondary alcohol acceptors **10** and **11** with **33** also afforded mixtures of α- and β-disaccharides **38** (α/β = 1:1.5) and **39** (α/β = 1:7.5), respectively (entries 3 and 4). On the other hand, reactions of tetrabenzylmannose **34** as the mannosyl donor with various acceptors provided only desired mannosides without formation of the undesired self-condensed



27 36

Figure 1. Self-condensed esters **27** and **36**.

Table 3. Glucosylations of Various Acceptors with Benzylidene-Protected Donor **25**

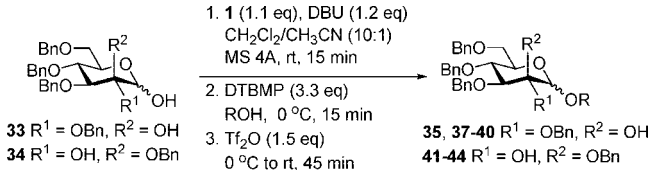


25 **26**, **28**-**32**

entry	acceptor ROH	product	yield, %[a]	ratio α/β[b]
1	**3**	**26** + **27**	74 + 20	α only (α only)
2	**4**	**28** + **27**	87 + 5	(18:1)
3	**7**	**29**	87	1.6:1[c]
4	**8**	**30**	85	1.5:1[c]
5	**9**	**31**	80	α only (α only)
6	**11**	**32**	85	α only (α only)

[a] Isolated yields. [b] Determined after isolation. Number in parentheses is the ratio from the crude product determined by LC–MS. [c] The ratio was determined by ^{1}H NMR.

Table 4. Glycosylations of Various Acceptors with Benzyl-Protected Donors **33** and **34**



entry	glycosyl donor	glycosyl acceptor (ROH)	product	yield, %[a] (ratio, α/β)[b]
1	**33**	**3**	**35** + **36**	73 (1.6:1) + 10
2	**33**	**7**	**37** + **36**	78 (1.2:1) + 10
3	**33**	**10**	**38** + **36**	82 (1:1.5) + 10
4	**33**	**11**	**39** + **36**	70 (1:7.5) + 10
5	**33**	**12**	**40** + **36**	78 (1:1.3) + 10
6	**34**	**3**	**41**	86 (1:1.6)
7	**34**	**9**	**42**	85 (α only)
8	**34**	**11**	**43**	81 (α only)
9	**34**	**12**	**44**	82 (1.8:1)[c]

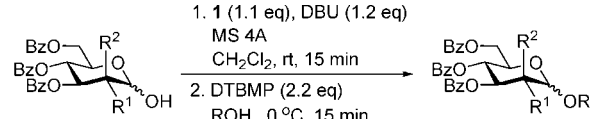
[a] Isolated yields. [b] The ratio determined by ^{1}H NMR. [c] The ratio after isolation.

ester at all. Glycosylations of primary alcohol acceptors **3** and **12** with the mannosyl donor **34** gave mixtures of α- and β-disaccharides **41** (α/β = 1:1.6) and **44** (α/β = 1.8:1), respectively, in good yields (entries 6 and 9). Interestingly, complete α-selective mannosylations of secondary alcohol acceptors **9** and **11** with **34** were achieved to afford corresponding α-disaccharides **42** and **43**, respectively, in good yields (entries 7 and 8).

Glycosylations of various glycosyl acceptors with disarmed[27] tetrabenzoylglucose **45** and tetrabenzoylmannose **46** as glycosyl donors were also examined under the same reaction condition as that described above for the tetrabenzylglucose **33** and the tetrabenzylmannose **34** except using CH_2Cl_2 solvent (Table 5).

(27) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583–5584.

Table 5. Glycosylations of Various Acceptors with Benzoyl-Protected Donors **45** and **46**



entry	glycosyl donor	glycosyl acceptor (ROH)	product	yield, %[a] (ratio, α/β)[a]
1	**45**	**3**	**47**	83 (β only)
2	**45**	**4**	**48**	82 (β only)
3	**45**	**10**	**49**	80 (β only)
4	**45**	**11**	**50**	81 (β only)
5	**46**	**3**	**51**	82 (α only)
6	**46**	**9**	**52**	83 (α only)
7	**46**	**10**	**53**	81 (α only)
8	**46**	**12**	**54**	82 (α only)

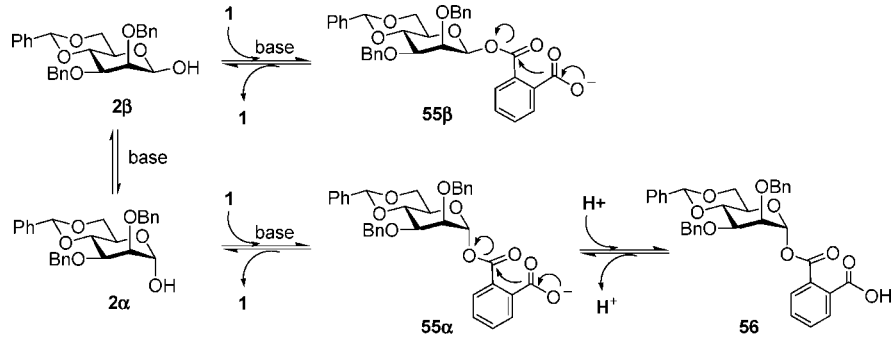
[a] Determined after isolation.

Glucosylations of all primary and secondary alcohol acceptors **3**, **4**, **10**, and **11** with the benzoyl-protected glucose **45** afforded exclusively corresponding β-disaccharides **47**−**50**, respectively, in high yields (entries 1−4 in Table 5), and mannosylations of all primary and secondary alcohol acceptors **3**, **9**, **10**, and **12** with the benzoyl-protected mannose **46** gave exclusively corresponding α-disaccharides **51**−**54**, respectively, in high yields (entries 5−8). This result indicates that less reactive disarmed benzoyl-protected glycosyl donors are also very effective and the neighboring group participation by the benzoate at the C-2 position is operative in the present one-pot glycosylation method.

Investigation of Intermediates and the Mechanism of the One-Pot *β*-Mannosylation. To identify intermediates generated during the present glycosylation process, we have attempted to isolate them and performed a NMR study to detect them in the β-mannosylation with 4,6-*O*-benzylidenemannose **2**. On the basis of our working hypothesis, the intermediates in the reaction of **2** with **1** in the first step of the present mannosylation would be mannosyl phthalate anions **55**α and **55**β as shown in Scheme 2. Reaction of **2** (α/β = 2.1:1) with **1** in the presence of DBU in CH_2Cl_2 at room temperature provided exclusively the α-mannosyl phthalate **55**α within a few minutes. Although we were able to isolate mannosyl hydrogen phthalate **56**, the protonated form of **55**α, and obtain its NMR spectra, it slowly decomposed back to the starting materials, **2** and **1**, during isolation and sampling for NMR.[28] Reaction of **2** and **1** in the presence of DBU was so fast that tracking the progress of the reaction was difficult by NMR either at 25 °C or at −60 °C. Triethylamine, instead of DBU, was found to be the proper base for the purpose of tracking the progress of the reaction of **2**

(28) (a) Other glycosyl hydrogen phthalates were found to be more labile than **56**. We have previously observed the instability of glycosyl hydrogen phthalates. See : Kim, K. S.; Lee, Y. J.; Kim, H. Y.; Kang, S. S; Kwon, S. Y. *Org. Biomol. Chem.* **2004**, *2*, 2408–2410. (b) Kwon, S. Y.; Lee, B.-Y.; Jeon, H. B.; Kim, K. S. *Bull. Korean Chem. Soc.* **2005**, *26*, 815–818.

Scheme 2. Proposed Mechanism for the Formation of α-Mannopyranosyl Phthalate Anion **55**α from **2** and Phthalic Anhydride (**1**)



and **1**. When a mixture of **2** (α/β = 2.1:1) (1.0 equiv) and **1** (1.1 equiv) in CD_2Cl_2 at 25 °C in the NMR tube was treated with triethylamine (4.0 equiv), ^{1}H NMR spectrum after 5 min showed the anomeric proton resonance at δ 5.90 for β-mannosyl phthalate **55**β along with a small peak at δ 6.29 for the corresponding α-anomer **55**α (Figure 2a). ^{1}H NMR spectra after 1 h at room temperature (Figure 2b) and then at 10 min after raising temperature of the same sample to 35 °C (Figure 2c) clearly exhibited slow increase of the anomeric signal of **55**α over **55**β. After 3 h at 35 °C, ^{1}H NMR indicated that almost all **55**β were converted into **55**α (Figure 2d). Finally, prolonged reaction time at 35 °C, the anomeric signal of **55**α remained

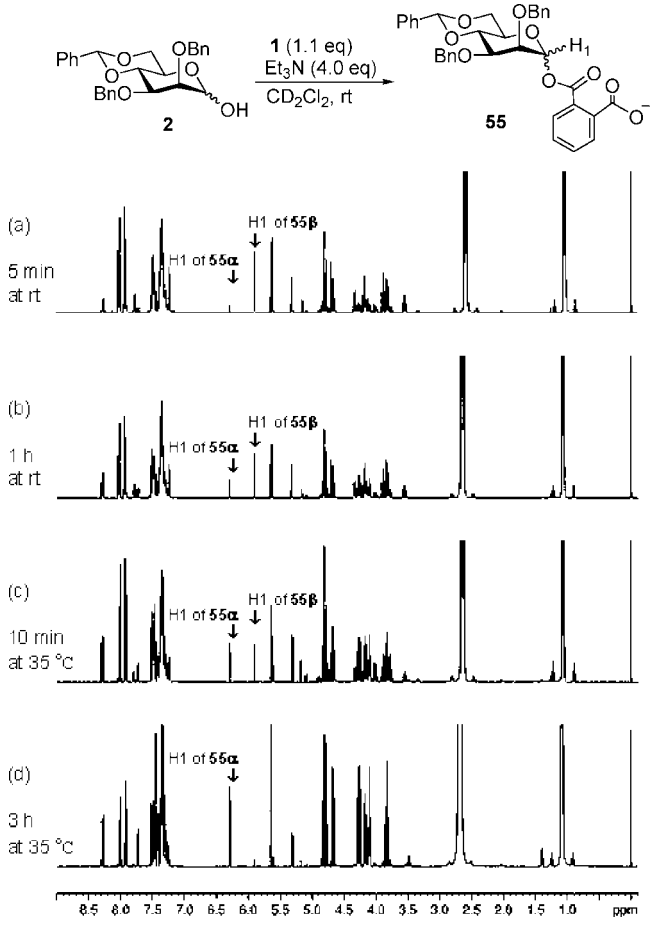


Figure 2. Reaction of **2** (α/β = 2.1:1) and phthalic anhydride monitored by ^{1}H NMR in CD_2Cl_2: (a) mannosyl phthalates **55**α and **55**β, room temperature, 5 min after addition of Et_3N; (b) room temperature, 1 h after addition of Et_3N; (c) 35 °C, 10 min after raising temperature; (d) 35 °C, 3 h after raising temperature.

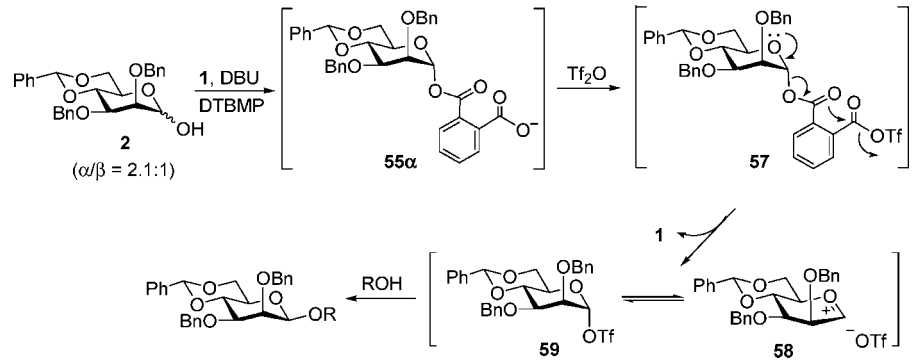
with complete disappearance of the **55**β signal. NMR analysis and the isolation of the α-mannosyl hydrogen phthalate **56** indicate that β-hemiacetal **2**β reacts preferentially with **1** over **2**α in the presence of a base to produce initially **55**β as observed in the trichloroacetimidate formation by Schmidt et al.[29] Then, decomposition of the kinetic product **55**β back to **2**β and **1** would make the reaction reversible and, consequently, the equilibrium could be established not only between **55**β and **2**β but also between**55**β and **55**α through **2**β and **2**α so that the thermodynamic product **55**α is produced exclusively under the present mannosylation condition as shown in Scheme 2. On the other hand, NMR study showed that the intermediates in the first step of the glycosylation with other anomeric hydroxy sugars were predominantly α-glycosyl phthalates for 4,6-benzylideneglucose **25** and tetrabenzylglucose **33** along with a small amount of corresponding β-anomers.

Possible intermediates in the second step of the present one-pot mannosylation would be triflate mixed anhydride **57**, mannosyl oxocarbenium ion **58**, and/or mannosyl triflate **59** as shown in Scheme 3. To detect those intermediates, we first ran the NMR tube-scale reaction of mannose **2** (1.0 equiv) with phthalic anhydride (**1**, 1.1 equiv) in CD_2Cl_2 in the presence of DBU (1.2 equiv) and DTBMP (2.2 equiv) at room temperature. Within a few min, the ^{1}H NMR spectrum of the reaction mixture showed the anomeric proton peak at δ 6.31 for α-mannosyl phthalate anion **55**α (Figure 3b). Then, the reaction mixture in the NMR tube was cooled down to −78 °C and Tf_2O (1.5 equiv) was added. At 15 min after addition of Tf_2O, the ^{1}H NMR spectrum at −60 °C showed the anomeric proton peak at δ 6.03 for α-mannopyranosyl triflate **59** (Figure 3c), which turned out to be the same species as that produced from a thioglycoside by Crich and Sun.[30] The ^{13}C NMR spectrum at −60 °C also indicated the formation of **59** with an anomeric carbon peak at δ 105.4. Then, upon addition of isopropanol to the reaction mixture, ^{1}H and ^{13}C NMR spectra at −60 °C showed immediate consumption of the triflate **59** and appearance of the anomeric carbon peak at δ 100.2 for isopropyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside.

On the basis of these results, we propose the mechanism for the present β-mannosylation employing **2** as the mannosyl donor (Scheme 3). Treatment of **2** with **1** in the presence of DBU and DTBMP provides the α-mannosyl phthalate anion **55**α, which then reacts with Tf_2O to afford triflate mixed anhydride **57**. The instantaneous lactonization of **57** by extrusion of stable **1** would generate oxocarbenium ion **58**, which might be in equilibrium with α-mannosyl triflate **59**. Subsequent reaction of **58** or **59**

(29) Schmidt, R. R.; Michel, J. *Tetrahedron Lett.* **1984**, *25*, 821–824.
(30) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.

Scheme 3. Proposed Mechanism of the β-Mannopyranosylation with **2** Employing Phthalic Anhydride (**1**) and Tf_2O as Activating Agents



with a glycosyl acceptor (ROH) would provide the desired β-mannopyranoside.[31] We also confirmed by NMR study that the intermediate in the first step of this one-pot mannosylation is the carboxylate anion **55**α but not carboxylic acid **56**.[32]

Synthesis of β-(1→4)-Linked D-Manno-oligosaccahrides. We applied this new one-pot glycosylation method to the synthesis of β-(1→4)-D-mannotriose **62** and β-(1→4)-D-mannotetraose **67** to show its effectiveness for the stereoselective synthesis of oligosaccharides containing β-mannopyranosyl linkages. β-(1→4)-linked D-manno-oligosaccharides are integral parts of β-(1→4)-mannans, which are polysaccharides found in wood and plant seeds and have both structure and energy-reserve functions.[33] Our synthesis as shown in Scheme 4 commenced with acid hydrolysis of the benzylidene group of the protected β-(1→4)-D-mannobiose **19**, which was prepared from the reaction of the donor **2** with the acceptor **10** (entry 6 in Table 2). Selective benzoylation of resulting disaccharide diol **60** affords 6-*O*-benzoate **61**. The mannosylation of the mannobiose acceptor

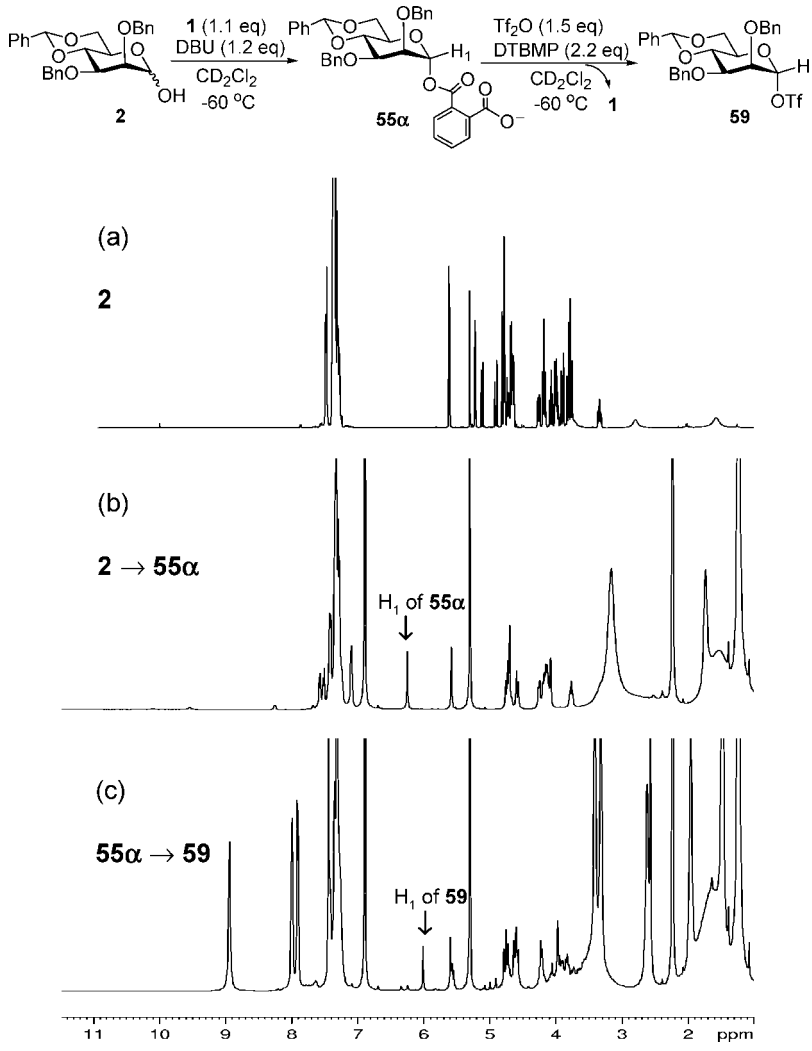
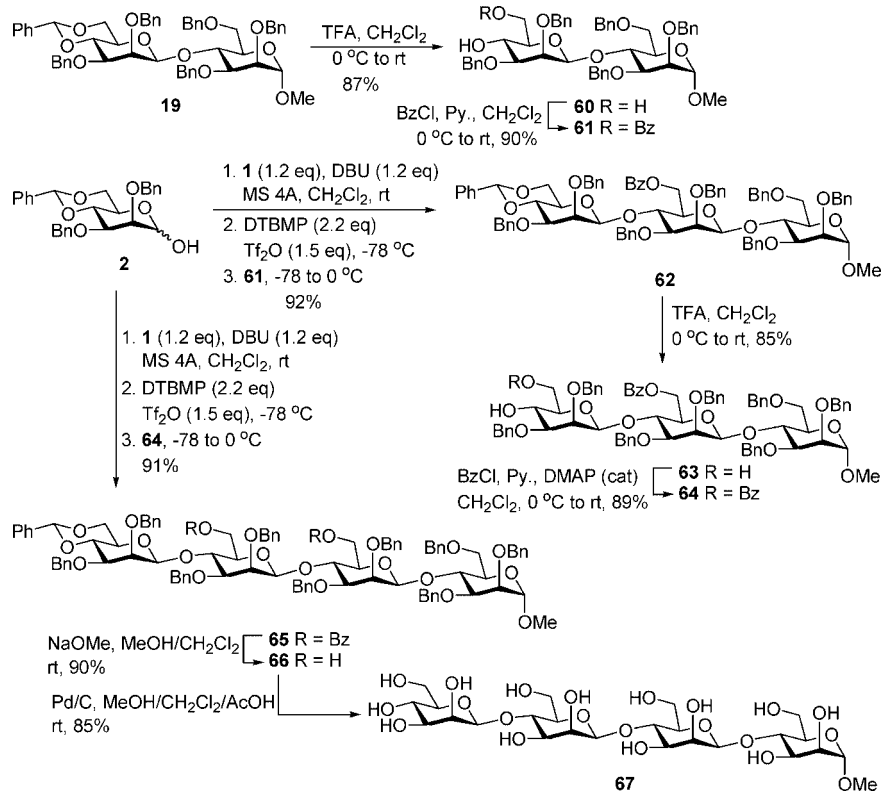


Figure 3. ^{1}H NMR spectra in CD_2Cl_2 for (a) mannose **2**, (b) α-mannosyl phthalate **55**α (δ 6.31) generated after addition of **1** and DBU to **2** at room temperature, and (c) α-mannosyl triflate **59** (δ 6.03) generated after addition of Tf_2O to **55**α at −60 °C.

Scheme 4. Synthesis of β-(1→4)-D-Mannotetraose **67**



61 with the mannosyl donor **2** under the standard β-mannosylation condition provided protected β-(1→4)-mannotriose **62** in 92% yield with complete β-stereoselectivity. Hydrolysis of the benzylidene group of the mannotriose **62** followed by selective benzoylation of resulting diol **63** gave trisaccharide acceptor **64** without any problem. Then, the repetitive glycosylation of the acceptor **64** with the donor **2** gave β-tetrasaccharide **65** exclusively in 91% yield. Saponification of the benzoyl ester functionality of **65** with sodium methoxide and subsequent hydrogenolysis of a benzylidene and nine benzyl protective groups of resulting tetrasaccharide **66** afforded fully deprotected β-(1→4)-mannotetraose **67** as a methyl glycoside in high yield. One-bond C1−H1 coupling constants of the tetrasaccharide **65**, 158.4, 155.0, and 154.7, clearly indicated that its three newly generated glycosyl linkages are all in β-configurations. Although syntheses of β-(1→4)-manno-oligosaccharides have been reported before,[34] our syntheses appear to be comparable to and maybe even better than earlier syntheses in terms of the efficiency and the stereoselectivity; all three mannosylation steps produced desired β-mannosyl linkages in higher than 90% yields with perfect stereoselectivities.

Conclusion

We have described a new efficient one-pot direct glycosylation method with anomeric hydroxy sugars as glycosyl donors. Highly stereoselective β-mannopyranosylation of various glycosyl acceptors with 4,6-*O*-benzylidene-protected mannopyranose **2** has been achieved by a sequence of three steps in one-pot without isolation of intermediates: (i) reaction of **2** and phthalic anhydride in the presence of DBU at room temperature, (ii) addition of DTBMP and Tf_2O to this solution at −78 °C, and (iii) addition of the glycosyl acceptor at −78 °C. The α-glucopyranosylation with 4,6-*O*-benzylidene-protected glucopyranose **25** and glycosylations with other types of glycosyl donors having tetra-*O*-benzyl and tetra-*O*-benzoyl protective groups were also possible by utilizing the present one-pot glycosylation protocol. Glycosyl phthalates including α-mannosyl phthalate **55**α were detected by NMR as the intermediates in the first step of the glycosylation, and unstable α-mannosyl hydrogen phthalate **56**, the protonated form of **55**α, was isolated. We have also detected α-mannosyl triflate **59** as the intermediate in the second step of the β-mannosylation by low temperature NMR. On the basis of the investigation of the intermediates,

(31) For discussions on the mechanism of the 4,6-*O*-benzylidene directedβ-mannosylations, see: (a) Crich, D.; Chandrasekera, N. S. *Angew. Chem., Int. Ed.* **2004**, *43*, 5386−5389. (b) Weingart, R.; Schmidt, R. R. *Tetrahedron Lett.* **2000**, *41*, 8753–8758. (c) Nukuda, T.; Berces, A.; Whitfield, D. M. *Carbohydr. Res.* **2002**, *337*, 765–774. For discussions on the mechanism of the 4,6-*O*-benzylidene directed α-glucosylations, see references 14c, 25, and 31c.

(32) On the basis of HMBC NMR data of the carboxylic acid **56** in the presence of DTBMP in CD_2Cl_2, a peak at δ 170.5 was assigned as the carbon-13 resonance in the 2′-carboxy group of **56**. And, upon addition of DBU to this solution, the carboxy carbon peak moved toward a little down field to appear at δ 172.2, which was assigned as the 2′-carboxy carbon peak for the carboxylate anion **55**α. The ^{13}C NMR spectrum of the product mixture directly obtained from the reaction of the hydroxy sugar **2** and phthalic anhydride (**1**) in the presence of DTBMP, and DBU in CD_2Cl_2 also showed the 2′-carboxy carbon peak at δ 172.3 for the carboxylate anion **55**α. ^1H NMR spectra also indicated that DTBMP was not protonated when it was mixed with **56** but showed protonated DBU peaks when DBU was added to the mixture of **56** and DTBMP. It is known that the carboxy carbon of the carboxylate anion resonates at lower field than that of the corresponding carboxylic acid. See: Hagen, R.; Roberts, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 4504–4506.

(33) Aspinall, G. O. *The Polysaccharides*; Academic Press: London, 1982; Vol. 1.

(34) (a) Twaddle, G. W. J.; Yashunsky, D. V.; Nikolaev, A. V. *Org. Biomol. Chem.* **2003**, *1*, 623–628. (b) Crich, D.; Banerjee, A.; Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 14930–14934.

the possible mechanism of the β-mannopyranosylation with **2** has been proposed. The present glycosylation methodology, especially the β-mannopyranosylation protocol, was successfully applied to the efficient synthesis of β-(1→4)-D-mannotriose **62** and β-(1→4)-D-mannotetraose **67** with complete β-stereoselectivity.

Experimental Section

General Procedure for the β-Mannopyranosylation with 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranose (2) (Table 2). A solution of **2** (0.15 mmol, 1.0 equiv, α/β = 2.1:1), phthalic anhydride (**1**, 1.1 equiv), and DBU (1.2 equiv) in CH_2Cl_2 (15 mL) in the presence of 4 Å molecular sieves was stirred for 15 min at room temperature and cooled down to −78 °C. Then DTBMP (2.2 equiv) and Tf_2O (1.5 equiv) were added sequentially at −78 °C and the resulting solution was stirred for further 15 min at −78 °C. After dropwise addition of a solution of a glycosyl acceptor (1.2 equiv) in CH_2Cl_2 (2 mL) to the above solution via cannula, the reaction mixture was stirred at −78 °C for 15 min, allowed to warm up over 1 h to 0 °C, quenched with saturated aqueous $NaHCO_3$, and then extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography.

General Procedure for the α-Glucopyranosylation with 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-D-glucopyranose (25) (Table 3). A solution of **25** (0.15 mmol, 1.0 equiv, α/β = 1.3:1), phthalic anhydride (**1**, 1.1 equiv), and DBU (1.2 equiv) in CH_2Cl_2 (15 mL) in the presence of 4 Å molecular sieves was stirred for 15 min at room temperature and cooled down to −78 °C. DTBMP (3.3 equiv) and TfOH (1.1 equiv) were added sequentially and the resulting solution was stirred at −78 °C for 15 min. Then Tf_2O (1.5 equiv) was added and the resulting solution was stirred at −78 °C for 15 min. After dropwise addition of a solution of a glycosyl acceptor (1.2 equiv) in CH_2Cl_2 (2 mL) to the above solution via cannula, the reaction mixture was stirred at −78 °C for 15 min, allowed to warm up over 1 h to 0 °C, quenched with saturated aqueous $NaHCO_3$, and then extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography.

General Procedure for the Glycosylation with 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (33) and with 2,3,4,6-Tetra-*O*-benzyl-D-mannopyranose (34) (Table 4). A solution of **33** or **34** (0.1 mmol, 1.0 equiv), phthalic anhydride (**1**, 1.1 equiv), and DBU (1.2 equiv) in CH_2Cl_2/CH_3CN (10:1, 3 mL) in the presence of 4 Å molecular sieves was stirred for 15 min at room temperature and cooled down to 0 °C. Then DTBMP (2.2 equiv) and a glycosyl acceptor (1.2 equiv) were added sequentially and the resulting solution was stirred at 0 °C for 15 min. After dropwise addition of a solution of Tf_2O (1.5 equiv) in CH_2Cl_2 (2 mL) to the above solution via cannula, the reaction mixture was stirred at 0 °C for 15 min, allowed to warm up over 30 min to room temperature, quenched with saturated aqueous $NaHCO_3$, and then extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography.

General Procedure for the Glycosylation with 2,3,4,6-Tetra-*O*-benzoyl-D-glucopyranose (45) and with 2,3,4,6-Tetra-*O*-benzoyl-D-mannopyranose (46) (Table 5). The exactly same procedure was employed as that for the glycosylation with **33** and with **34** except the solvent. Only CH_2Cl_2 was used as the solvent instead of CH_2Cl_2/CH_3CN (10:1).

Methyl (2,3-Di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (60). A solution of the compound **19** (500 mg, 0.56 mmol) and trifluoroacetic acid (0.22 mL, 2.79 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 10 min and at room temperature for 2 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The combined organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 1:1) to afford the compound **60** (393 mg, 87%): colorless oil, R_f = 0.38 (hexane/EtOAc, 1:1, v/v); $[\alpha]^{20}_D$ −23.6 (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (brs, 2H), 3.05−3.12 (m, 2H), 3.37 (s, 3H), 3.45 (dd, *J* = 11.6, 6.8 Hz, 1H), 3.68−3.86 (m, 8H), 4.24 (t, *J* = 8.8 Hz, 1H), 4.25 (d, *J* = 12.0 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.44 (d, *J* = 6.0 Hz, 1H), 4.48 (d, *J* = 12.4 Hz, 1H), 4.56 (d, *J* = 11.6 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.70−4.78 (m, 3H), 4.71 (d, *J* = 12.4 Hz, 1H), 4.80 (d, *J* = 12.0 Hz, 1H), 4.82 (d, *J* = 11.6 Hz, 1H), 7.20−7.40 (m, 25H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.1, 62.9, 67.5, 69.5, 71.2, 71.7, 72.9, 73.2, 73.7, 74.2, 74.4, 75.6, 75.7, 75.9, 77.8, 82.1, 99.7, 101.2, 127.5, 127.59, 127.60, 127.75, 127.80, 127.9, 128.00, 128.04, 128.08, 128.10, 128.3, 128.4, 128.5, 128.6, 128.7, 137.9, 138.3, 138.5, 138.8, 139.0. Anal. Calcd for $C_{48}H_{54}O_{11}$: C, 71.44; H, 6.75. Found: C, 71.48; H, 6.81.

Methyl (6-*O*-Benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (61). A solution of **60** (766 mg, 0.95 mmol), pyridine (0.15 mL, 1.90 mmol), and benzoyl chloride (0.11 mL, 0.95 mmol) in CH_2Cl_2 (15 mL) was stirred at 0 °C for 10 min and at room temperature for 4 h. The reaction mixture was washed with saturated aqueous NH_4Cl and brine and extracted with CH_2Cl_2. The combined organic layer was dried with $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 2:1) to afford compound **61** (780 mg, 90%): colorless oil, R_f = 0.20 (hexane/EtOAc, 2:1, v/v); $[\alpha]^{20}_D$ −18.6 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (brs, 1H), 3.17 (dd, *J* = 9.6, 2.8 Hz, 1H), 3.25−3.31 (m, 1H), 3.31 (s, 3H), 3.64−3.70 (m, 1H), 3.71−3.80 (m, 4H), 3.91 (dd, *J* = 8.4, 3.2 Hz, 1H), 3.99 (t, *J* = 9.6 Hz, 1H), 4.25 (t, *J* = 8.8 Hz, 1H), 4.35 (d, *J* = 11.6 Hz, 1H), 4.41−4.52 (m, 4H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.61 (d, *J* = 15.2 Hz, 1H), 4.64−4.71 (m, 4H), 4.73 (d, *J* = 2.0 Hz, 1H), 4.80 (d, *J* = 11.6 Hz, 1H), 4.83 (d, *J* = 12.0 Hz, 1H), 7.16−7.37 (m, 28H), 7.92−7.97 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.0, 64.0, 66.4, 69.6, 71.3, 71.5, 72.7, 72.8, 73.6, 74.2, 74.5, 74.6, 75.7, 75.8, 78.0, 81.8, 99.6, 101.7, 127.35, 127.40, 127.6, 127.7, 127.76, 127.80, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 128.7, 129.9, 133.0, 137.9, 138.5, 138.6, 139.1, 139.2, 167.0. Anal. Calcd for $C_{55}H_{58}O_{12}$: C, 72.51; H, 6.42. Found: C, 72.55; H, 6.43.

Methyl (2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (62). A solution of **2** (94 mg, 0.21 mmol), phthalic anhydride (**1**, 37 mg, 0.25 mmol), and DBU (38 μL, 0.25 mmol) in CH_2Cl_2 (3 mL) was stirred in the presence of 4 Å molecular sieves for 15 min at room temperature and cooled down to −78 °C. Then DTBMP (95 mg, 0.46 mmol) and Tf_2O (53 μL, 0.31 mmol) were added sequentially and the resulting solution was stirred at −78 °C for a further 15 min. After dropwise addition of a solution of glycosyl acceptor **61** (286 mg, 0.31 mmol) in CH_2Cl_2 (5 mL) to the above solution via cannula, the reaction mixture was stirred at −78 °C for 15 min, allowed to warm up over 1 h to 0 °C, quenched with saturated aqueous $NaHCO_3$, and then extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 2:1) to afford compound **62** (259 mg, 92%): colorless oil, R_f = 0.25 (hexane/EtOAc, 2:1, v/v); $[\alpha]^{20}_D$ −18.0 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96−3.03 (m, 1H), 3.31 (s, 3H), 3.33−3.37 (m, 1H), 3.39 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.44 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.58 (t, *J* = 10.0 Hz, 1H), 3.66−3.75 (m, 5H), 3.86−3.93 (m, 3H), 4.03 (t, *J* = 9.6 Hz, 1H), 4.15 (t, *J* = 9.2 Hz, 1H), 4.22 (t, *J* = 9.2 Hz, 1H), 4.31 (dd, *J* = 12.0, 2.0 Hz, 1H), 4.36 (dd, *J* = 12.0, 3.6 Hz, 1H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.45−4.60 (m, 3H), 4.61−4.75 (m, 10H), 4.79−4.88 (m, 3H), 5.48 (s, 1H), 7.13−7.42 (m, 43H), 7.91−7.96 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.0, 63.6, 67.5, 68.6, 69.5, 71.3, 72.2, 72.4, 72.7, 72.9, 73.5, 73.6, 74.3, 75.3, 75.7, 75.9, 76.06, 76.10, 77.3, 78.4, 78.46, 78.50, 80.5, 99.5 (J_{C-H} = 170.4 Hz), 101.4 (J_{C-H} = 161.2 Hz), 101.7 (J_{C-H} = 155.2 Hz), 102.4, 126.2, 127.17, 127.20, 127.27, 127.30, 127.55, 127.60, 127.8, 127.9, 128.16, 128.20, 128.27, 128.30, 128.4, 128.5, 133.1, 137.7, 138.5, 138.6, 138.8, 138.9, 139.3, 166.4. HRMS: Calcd for $C_{82}H_{84}O_{17}Na$ [M + Na]$^+$, 1363.5606; found, 1363.5602.

Methyl (2,3-Di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (63). Compound **62** (430 mg, 0.32 mmol) was hydrolyzed under the same reaction condition as that for the preparation of **60** from **19**. The reaction mixture was purified by flash column chromatography (hexane/EtOAc, 1:1) to give title compound **63** (342 mg, 85%): colorless oil, R_f = 0.25 (hexane/EtOAc, 1:1, v/v); $[\alpha]^{20}_D$ −21.0 (*c* 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.95−3.01 (m, 1H), 3.12 (dd, *J* = 9.2, 2.4 Hz, 1H), 3.32 (s, 3H), 3.33 (t, *J* = 2.8 Hz, 1H), 3.34−3.39 (m, 1H), 3.41 (t, *J* = 6.0 Hz, 1H), 3.62 (t, *J* = 3.2 Hz, 1H), 3.64 (t, *J* = 2.8 Hz, 1H), 3.67−3.84 (m, 4H), 3.86−3.91 (m, 2H), 4.14−4.27 (m, 3H), 4.33 (dd, *J* = 12.0, 4.4 Hz, 1H), 4.38−4.52 (m, 6H), 4.58−4.78 (m, 10H), 4.82 (d, *J* = 4.0 Hz, 1H), 4.85 (d, *J* = 4.4 Hz, 1H), 7.15−7.40 (m, 38H), 7.92−7.98 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.0, 62.8, 63.8, 67.2, 69.5, 71.2, 71.3, 72.6, 72.7, 72.9, 73.60, 73.63, 74.2, 74.5, 75.6, 75.7, 75.8, 76.0, 76.5, 78.3, 80.3, 82.0, 99.5, 101.6, 101.8, 127.85, 127.88, 127.90, 128.1, 128.2, 128.27, 128.33, 128.4, 128.5, 128.6, 128.7, 129.8, 130.0, 133.3, 137.7, 138.6, 138.7, 139.2, 139.3, 166.5. HRMS: Calcd for $C_{75}H_{80}O_{17}Na$ [M + Na]$^+$, 1275.5293; found, 1275.5289.

Methyl (6-*O*-Benzoyl-2,3-di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (64). A solution of **63** (292 mg, 0.23 mmol), pyridine (47 μL, 0.58 mmol), and benzoyl chloride (33 μL, 0.28 mmol) in the presence of a catalytic amount of DMAP in CH_2Cl_2 (15 mL) was stirred at 0 °C for 10 min and at room temperature for further 3 h. The reaction mixture was washed with saturated aqueous NH_4Cl and brine, and then extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 2:1) to afford compound **64** (281 mg, 89%): colorless oil, R_f = 0.15 (hexane/EtOAc, 2:1, v/v); $[\alpha]^{20}_D$ −24.4 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.15−3.21 (m, 2H), 3.31 (s, 3H), 3.36−3.43 (m, 2H), 3.61−3.73 (m, 5H), 3.87 (dd, *J* = 8.8, 3.2 Hz, 1H), 3.90 (d, *J* = 2.0 Hz, 1H), 4.00 (t, *J* = 9.2 Hz, 1H), 4.18 (t, *J* = 8.8 Hz, 1H), 4.19 (t, *J* = 8.8 Hz, 1H), 4.32−4.37 (m, 3H), 4.38 (d, *J* = 12.8 Hz, 1H), 4.41−4.53 (m, 6H), 4.55 (d, *J* = 8.8 Hz, 1H), 4.58 (d, *J* = 5.6 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.68 (d, *J* = 12.8 Hz, 1H), 4.69−4.77 (m, 5H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.85 (d, *J* = 7.2 Hz, 1H), 7.12−7.49 (m, 41H), 7.90−7.99 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.0, 63.7, 66.2, 69.5, 71.3, 71.4, 72.3, 72.7, 72.9, 73.49, 73.54, 74.3, 74.4, 74.5, 74.7, 75.67, 75.73, 76.07, 76.12, 78.3, 80.3, 81.6, 99.5, 101.6, 102.2, 127.2, 127.3, 127.4, 127.55, 127.63, 127.7, 127.75, 127.79, 127.9, 128.0, 128.1, 128.2, 128.3, 128.36, 128.39, 128.42, 128.5, 128.55, 128.60, 128.7, 129.86, 129.91, 130.0, 130.3, 133.0, 133.2, 133.7, 137.8, 138.4, 138.6, 138.8, 139.0, 139.3, 166.5, 166.9. HRMS: Calcd for $C_{82}H_{84}O_{18}Na$ [M + Na]$^+$, 1379.5555; found, 1379.5557.

Methyl (2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-(6-*O*-benzoyl-2,3-di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (65). A solution of **2** (40 mg, 0.089 mmol), phthalic anhydride (**1**, 16 mg, 0.11 mmol), and DBU (16 μL, 0.11 mmol) in CH_2Cl_2 (2 mL) was stirred in the presence of 4 Å molecular sieves for 15 min at room temperature and cooled down to −78 °C. Then DTBMP (40 mg, 0.20 mmol) and Tf_2O (23 μL, 0.13 mmol) were added sequentially and the resulting solution was stirred at −78 °C for 15 min. After dropwise addition of a solution of glycosyl acceptor **64** (182 mg, 0.13 mmol) in CH_2Cl_2 (4 mL) via cannula, the reaction mixture was stirred at −78 °C for 15 min, allowed to warm up over 1 h to 0 °C, quenched with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 2:1) to afford compound **65** (145 mg, 91%): colorless oil, R_f = 0.45 (hexane/EtOAc, 2:1, v/v); $[\alpha]^{20}_D$ −21.8 (*c* 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90−2.98 (m, 1H), 3.25−3.34 (m, 2H), 3.31 (s, 3H), 3.35 (d, *J* = 2.4 Hz, 1H), 3.38 (t, *J* = 3.2 Hz, 1H), 3.41 (t, *J* = 1.2 Hz, 1H), 3.43 (d, *J* = 2.0 Hz, 1H), 3.56 (t, *J* = 10.4 Hz, 1H), 3.63 (d, *J* = 2.8 Hz, 1H), 3.64 (d, *J* = 4.8 Hz, 1H), 3.67−3.73 (m, 4H), 3.83−3.89 (m, 4H), 4.03 (t, *J* = 10.0 Hz, 1H), 4.11−4.21 (m, 4H), 4.24−4.36 (m, 2H), 4.38 (d, *J* = 12.4 Hz, 1H), 4.46 (d, *J* = 6.0 Hz, 1H), 4.48 (d, *J* = 13.6 Hz, 1H), 4.50 (d, *J* = 6.0 Hz, 1H), 4.53−4.65 (m, 7H), 4.68 (d, *J* = 10.0 Hz, 1H), 4.69−4.79 (m, 8H), 4.83 (d, *J* = 12.0 Hz, 1H), 5.47 (s, 1H), 7.13−7.48 (m, 56H), 7.90−7.99 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.0, 63.5, 63.7, 67.5, 68.6, 69.5, 71.3, 72.0, 72.3, 72.4, 72.7, 72.9, 73.45, 73.53, 74.3, 74.5, 75.27, 75.33, 75.7, 75.8, 75.97, 76.00, 76.1, 78.2, 78.4, 78.5, 80.1, 80.6, 99.5 (J_{C-H} = 169.4 Hz), 101.4 (J_{C-H} = 158.4 Hz), 101.6 (J_{C-H} = 155.0 Hz), 101.97 (J_{C-H} = 154.7 Hz), 102.01, 126.2, 127.17, 127.24, 127.30, 127.34, 127.4, 127.5, 127.6, 127.8, 128.9, 129.8, 129.87, 129.92, 130.0, 133.2, 137.8, 138.4, 138.5, 138.6, 138.7, 138.9, 139.2, 139.3, 166.37, 166.41. MALDI-TOF Calcd for $C_{109}H_{110}O_{23}Na$ [M + Na]$^+$, 1809.7336; found, 1809.7303.

Methyl (2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-mannopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-*β*-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (66). A solution of compound **65** (60 mg, 0.034 mmol) and sodium methoxide (2.0 mg, 0.034 mmol) in CH_2Cl_2/MeOH (1:4, v/v, 5 mL) was stirred for overnight at room temperature. The reaction mixture was neutralized with DOWEX CCR-3 (H^+ mode) resin, filtered through Celite, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc, 1:1) to afford compound **66** (48 mg, 90%): amorphous solid, R_f = 0.36 (hexane/EtOAc, 1:1, v/v); $[\alpha]^{20}_D$ −29.3 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99−3.05 (m, 1H), 3.10−3.18 (m, 2H), 3.22 (dd, *J* = 2.4, 9.4 Hz, 1H), 3.29−3.36 (m, 2H), 3.37 (s, 3H), 3.46 (dd, *J* = 2.8, 9.2 Hz, 1H), 3.51−3.63 (m, 4H), 3.65−3.73 (m, 4H), 3.78−3.86 (m, 4H), 3.96−4.05 (m, 3H), 4.09 (t, *J* = 9.6 Hz, 1H), 4.23 (t, *J* = 8.8 Hz, 1H), 4.40−4.63 (m, 7H), 4.68−4.86 (m, 14H), 4.89 (d, *J* = 12.0 Hz, 1H), 5.51 (s, 1H), 7.23−7.46 (m, 50H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.1, 61.9, 62.1, 67.5, 68.7, 69.2, 71.6, 72.4, 72.7, 72.8, 73.2, 73.7, 74.6, 74.8, 75.17, 75.20, 75.4, 75.7, 75.9, 76.0, 76.6, 77.3, 77.4, 78.0, 78.5, 78.8, 80.4, 80.6, 99.6, 101.1, 101.4, 101.5, 102.2, 126.3, 126.9, 127.0, 127.1, 127.3, 127.45, 127.47, 127.52, 127.58, 127.62, 127.67, 127.72, 127.88, 127.94, 128.1, 128.2, 128.26, 128.28, 128.35, 128.44, 128.46, 128.54, 128.9, 137.8, 138.3, 138.56, 138.59, 138.7, 138.9, 138.99, 139.01, 139.3. HRMS Calcd for $C_{95}H_{102}O_{21}Na$ [M + Na]$^+$, 1601.6811; found, 1601.6814.

Methyl (*β*-D-Mannopyranosyl)-(1→4)-(*β*-D-mannopyranosyl)-(1→4)-(*β*-D-mannopyranosyl)-(1→4)-α-D-mannopyranoside (67). A mixture of compound **66** (30 mg, 0.019 mmol) and Pd/C (10%, 20 mg) in MeOH/CH_2Cl_2/AcOH (5:3:2, v/v/v, 5 mL) was stirred under hydrogen atmosphere using a balloon at room temperature for overnight. The reaction mixture was filtered through Celite and concentrated in vacuo. The residue was purified by flash column chromatography on Iatrobeads ($CHCl_3$/MeOH, 1:5, v/v) to afford compound **67** (11 mg, 85%): colorless oil, R_f = 0.08 ($CHCl_3$/MeOH, 1:5, v/v); $[\alpha]_D^{20}$ −7.1 (c 0.4, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 3.14−3.32 (m, 2H), 3.26 (s, 3H), 3.37−3.52 (m, 4H), 3.55−3.86 (m, 15H), 3.90 (brs, 1H), 3.97 (brs, 2H), 4.55−4.65 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 55.7, 61.4, 61.5, 62.0, 67.7, 70.2, 70.5, 70.95, 71.00, 71.5, 72.0, 72.5, 73.8, 76.1, 77.48, 77.52, 77.6, 101.1, 101.15, 101.20, 101.7; HRMS Calcd for $C_{25}H_{44}O_{21}Na$ [M + Na]$^+$: 703.2273, found: 703.2270.

Procedure for the Detection of Intermediates 55α and 59 in the β-Mannopyranosylation with 2 in CD_2Cl_2 by NMR. To a 5 mm NMR tube containing **2** (4.5 mg, 0.010 mmol), phthalic anhydride (**1**, 1.6 mg, 0.011 mmol), and DTBMP (4.5 mg, 0.022 mmol) in CD_2Cl_2 (750 μL) was added DBU (1.7 μL, 0.012 mmol) at room temperature. After being briefly agitated, the tube was placed in the NMR probe at room temperature. The ^{1}H NMR spectrum indicated the conversion of mannose **2** to mannosyl phthalate **55**α was complete within a few minutes and showed the α-anomeric proton peak of **55**α at δ 6.31 (Figure 3b). The reaction mixture in the NMR tube was cooled down to −78 °C and Tf_2O (2.5 μL, 0.015 mmol) was added to this solution. After being briefly agitated, the NMR tube was placed in the precooled NMR probe at −60 °C and the conversion of **55**α to α-mannosyl triflate **59** was almost instantaneous. The ^{1}H and ^{13}C NMR spectra showed the α-anomeric proton peak at δ 6.03 (Figure 3c) and the anomeric carbon peak at δ 105.4 of **59**. Then, 2-propanol (0.9 μL, 0.015 mmol) was added to the reaction mixture in the tube at −78 °C. After being briefly agitated, the NMR tube was placed in the precooled NMR probe at −60 °C. The ^{1}H and ^{13}C NMR spectra indicated immediate consumption of **59** with formation of isopropyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside, which was confirmed by the comparison of its spectral data with those of the authentic sample and by mass spectrometry without isolation.

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Supporting Information Available: Experimental procedure, characterization data, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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