EurJOC
European Journal of Organic Chemistry

Full Paper
doi.org/10.1002/ejoc.202000313

Chemistry Europe
European Chemical Societies Publishing

Glycosylation

β-Mannosylation through *O*-Alkylation of Anomeric Cesium Alkoxides: Mechanistic Studies and Synthesis of the Hexasaccharide Core of Complex Fucosylated N-Linked Glycans

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Abstract: Several structurally diverse D-mannose-derived lactols, including various deoxy-D-mannoses and conformationally restricted bicyclic D-mannoses, have been synthesized and investigated in mechanistic studies of β-mannosylation through Cs_2CO_3-mediated anomeric *O*-alkylation. It was found that deoxy mannoses or conformationally restricted bicyclic D-mannoses are not as reactive as their corresponding parent mannose. This type of β-mannosylation proceeds efficiently when the C2-OH is left free, and protection of that leads to inferior results. NMR studies of D-mannose-derived anomeric cesium alkoxides indicated the predominance of the equatorial β-anomer after deprotonation. Reaction progress kinetic analysis suggested that monomeric cesium alkoxides be the key reactive species for alkylation with electrophiles. DFT calculations supported that oxygen atoms at C2, C3, and C6 of mannose promote the deprotonation of the anomeric hydroxyl group by Cs_2CO_3 and chelating interactions between Cs and these oxygen atoms favor the formation of equatorial anomeric alkoxides, leading to the highly β-selective anomeric *O*-alkylation. Based on experimental data and computational results, a revised mechanism for this β-mannosylation is proposed. The utilization of this β-mannosylation was demonstrated by an efficient synthesis of the hexasaccharide core of complex fucosylated *N*-linked glycans.

Introduction

Tremendous glycobiological studies have demonstrated oligosaccharides and glycoconjugates play essential roles in numerous biological processes.[1] In addition, carbohydrate molecules have been developed as effective therapeutic agents for treating various diseases. Sugar moieties, i.e. glycans, are also known to influence the physical, chemical, and biological properties of their carrier molecules, including proteins, peptides, and small molecules.[2] In order to study their biological functions, access to sufficient amounts of pure and structurally well-defined carbohydrate molecules need to be available to researchers. Isolation from natural sources sometimes has been successful, but the highly heterogeneous nature of glycoforms limits the practicality of this approach. In addition, the highly intrinsic complexity of the diverse carbohydrate structures poses a great challenge to the synthetic community, albeit great progress has been achieved in their chemical[3] and chemo-enzymatic synthesis.[4]

Among various glycosidic linkages, β-mannopyranosides (oftentimes referred to as β-mannosides) exist in a wide range of biologically significant molecules including *N*-linked glycans, bacterial capsular polysaccharides, fungal metabolites, glycolipids, antimicrobial antibiotics, and lipopolysaccharides.[5] As a class of 1,2-*cis* glycosides,[6] β-mannopyranosides are exceptionally difficult to construct due to the steric effect of the axial C2-substituent as well as the absence of anomeric effect and neighboring group participation. Even though remarkable success has been achieved in the stereoselective synthesis of β-mannosides,[7] development of a mild and easily operable β-mannosylation method is desirable and of great interest to the carbohydrate community.

Initially developed by Schmidt[8] and later by others,[9] anomeric *O*-alkylation has been demonstrated as a viable alternative to traditional glycosylation for the successful stereoselective synthesis of oligosaccharides and glycoconjugates. Concerning β-mannosides, Schmidt previously disclosed some studies on anomeric *O*-alkylation of partially or fully protected D-mannopyranoses with simple primary electrophiles in the presence of NaH or KO*t*Bu as the base.[10] Oftentimes, poor to moderate yields[10c] and moderate selectivity[10d] were observed. In addition, over-

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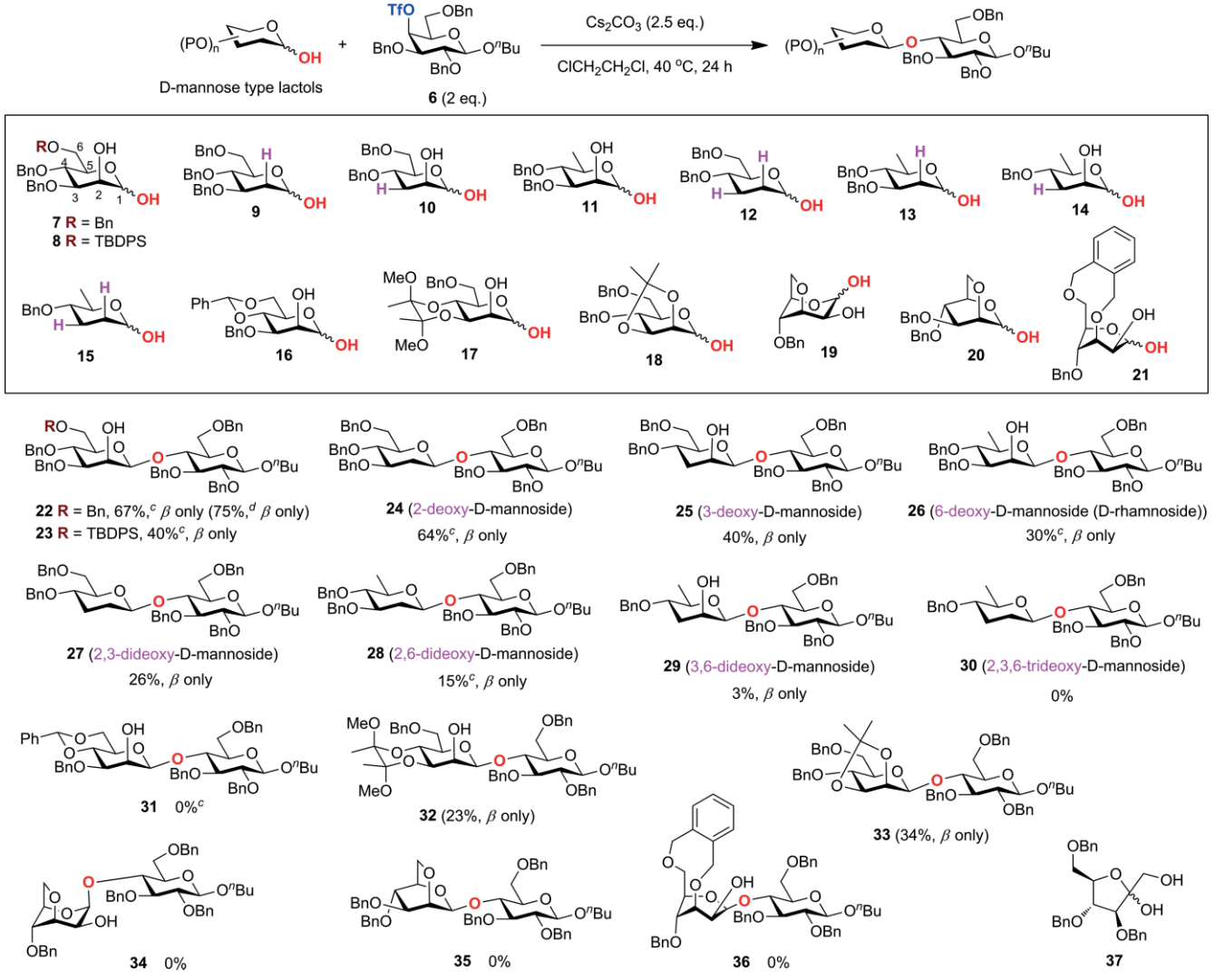
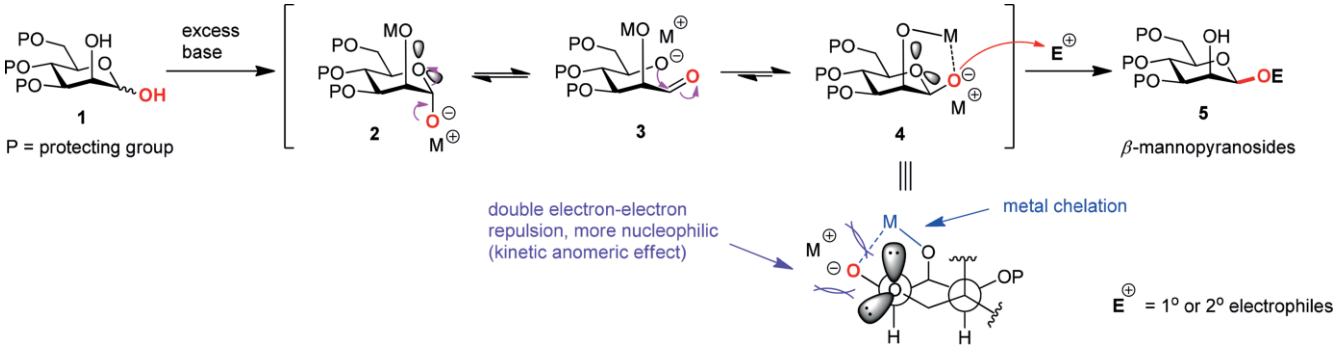
alkylation was found to be a problem when 3,4,6-tri-*O*-benzyl-D-mannopyranose (cf. **7**, Table 1) was employed.[10c] Based on our success in stereoselective synthesis of 2-deoxy sugars,[11,12] we recently reported a stereoselective synthesis of β-mannopyranosides by cesium carbonate (Cs_2CO_3)-mediated anomeric *O*-alkylation of D-mannose-derived 1,2-diols with primary or secondary

Table 1. Anomeric *O*-alkylation of various deoxy-D-mannoses and bicyclic D-mannose-derived lactols with D-galactose-derived secondary triflate **6** in the presence of Cs_2CO_3.[a,b]



[a] General conditions: D-mannose-derived lactols (1.0 equiv.), triflate **6** (2.0 equiv.), Cs_2CO_3 (2.5 equiv.), $ClCH_2CH_2Cl$, 40 °C, 24 h. [b] Isolated yield. [c] See ref.[13] [d] Disaccharide **22** was obtained in 75 % yield when 2.5 equiv. of triflate **6** and 3.0 equiv. of Cs_2CO_3 were used, see ref.[13].



Scheme 1. Initially proposed β-mannosylation via anomeric *O*-alkylation under dual control of kinetic anomeric effect and metal chelation.

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electrophiles.[13,14] It is worth noting that this mild and easily operable β-mannosylation method directly affords the desired β-mannosides with a free C2-OH at the mannose residue. The free C2-alcohol can be directly subjected to subsequent chemical transformations as demonstrated in a formal synthesis of the potent calcium signal modulator acremomannolipin A[15] and a concise synthesis of a trisaccharide oligomer of the *Hyriopsis schlegelii* glycosphingolipid, respectively.[16]

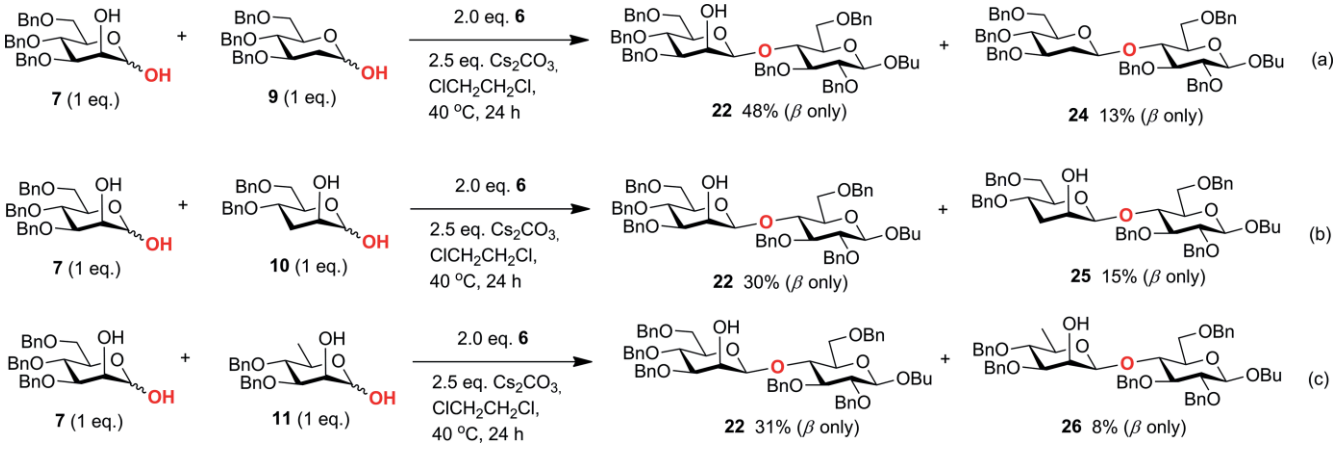
In our originally proposed hypothesis,[13] after deprotonation of D-mannose **1** with excess amounts of base, a mixture of dianions **2** and **4** may be produced and interconvert into each other via open intermediate **3** (Scheme 1). Due to the chelation effect,[10] equatorial anomeric alkoxide **4** would be preferentially formed over the axial counterpart **2**. In addition, equatorial anomeric alkoxide **4** was believed to be more nucleophilic than the axial counterpart **2** because of the double electron-electron repulsion (also known as the kinetic anomeric effect, colored in purple).[8,17] Subsequent S_N2 reaction of equatorial anomeric alkoxide **4** with suitable electrophiles may afford desired β-mannopyranosides **5**. It is believed that the synergy between kinetic anomeric effect and the chelation of cesium ion with oxygen atoms at C1 and C2 was the key for this β-selective anomeric *O*-alkylation to succeed (cf. Newman projection, Scheme 1).

Results and Discussion

In order to gain more insight into this β-mannosylation, we prepared additional deoxy D-mannose-derived lactol substrates including 4,6-di-*O*-benzyl-3-deoxy-D-mannose **10**, 4,6-di-*O*-benzyl-2,3-dideoxy-D-mannose **12**,[18] 4-*O*-benzyl-3,6-dideoxy-D-mannose **14**, and 4-*O*-benzyl-2,3,6-trideoxy-D-mannose **15**[19] (Table 1).[20] It was found that Cs_2CO_3-mediated anomeric *O*-alkylation of 4,6-di-*O*-benzyl-3-deoxy-D-mannose **10**, 4,6-di-*O*-benzyl-2,3-dideoxy-D-mannose **12**, and 4-*O*-benzyl-3,6-dideoxy-D-mannose **14** with secondary triflate **6** (2.0 equiv.) in the presence of Cs_2CO_3 (2.5 equiv.) under the same conditions afforded 4,6-di-*O*-benzyl-3-deoxy-β-D-mannoside **25**, 4,6-di-*O*-benzyl-2,3-dideoxy-β-D-mannoside **27**, 4-*O*-benzyl-3,6-dideoxy-β-D-mannoside **29** in 40 %, 26 %, and 3 % yields (β only), respectively. However, no 4-*O*-benzyl-2,3,6-trideoxy-β-D-mannoside **30** was detected when 4-*O*-benzyl-2,3,6-trideoxy-D-mannose **15** reacted with triflate **6** under the same conditions.

Next, direct competition experiments were carried out for comparative studies of the reactivity of the parent D-mannose **7** and its deoxy derivatives **9**, **10**, and **11**. When a mixture of D-mannose **7** (1.0 equiv.), 2-deoxy-D-mannose **9** (1.0 equiv.), and triflate **6** (2.0 equiv.) in 1,2-dichloroethane was warmed at 40 °C for 24 hours in the presence of 2.5 equivalents of Cs_2CO_3, β-mannoside **22** and 2-deoxy disaccharide **24** were obtained in 48 % and 13 %, respectively (β only) (a, Scheme 2). This interesting result demonstrates that D-mannose **7** is much more reactive than 2-deoxy-D-mannose **9** in this type of anomeric *O*-alkylation and suggests the important role of C2-OH of the D-mannose, even though previous experiments showed that anomeric *O*-alkylation of parent D-mannose **7** or 2-deoxy-D-mannose **9** with triflate **6** afforded **22** or **24** in similar yields and anomeric selectivity (Table 1, vide supra). Under exactly the same conditions, β-mannoside **22** and 3-deoxy disaccharide **25** were obtained in 30 % and 15 %, respectively (β only) (b, Scheme 2). Another competition experiment involving parent mannose **7** and 3,4-di-*O*-benzyl-6-deoxy-D-mannose **11** afforded β-mannoside **22** and 6-deoxy disaccharide **26** in 31 % and 8 %, respectively (β only) (c, Scheme 2). These results indicate that deoxy mannoses do not react as efficiently as their parent mannose in this type of Cs_2CO_3-mediated anomeric *O*-alkylation and suggest that all of the oxygen atoms at C2, C3, and C6 of mannose contribute to the reactivity of the mannose lactols.[21]

Electronically, anomeric alkoxides derived from deoxy sugars should be more electron-rich than those derived from their corresponding parent sugars due to the absence of electron-withdrawing oxygen atom(s). In addition, deoxy sugars are less sterically hindered in comparison with their parent sugar molecules. Therefore, deoxy sugar-derived anomeric alkoxides should be more nucleophilic than those derived from their parent sugar molecules and afford the corresponding deoxy glycosides in higher yields via anomeric *O*-alkylation, which is consistent with our previous observations.[11] However, our experimental results demonstrated that parent 3,4,6-tri-*O*-benzyl-D-mannose **7** was superior to those deoxy-D-mannoses (**9–15**) in this cesium carbonate-promoted anomeric *O*-alkylation (Table 1), which seemed

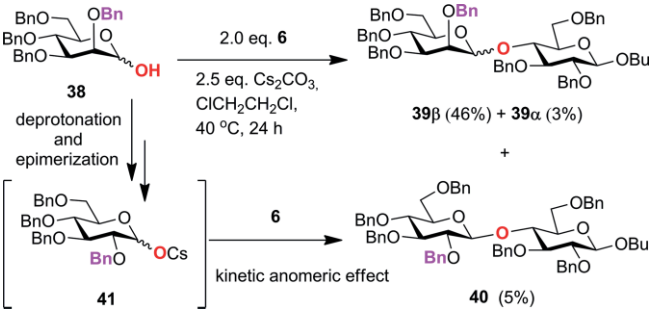


Scheme 2. Competition anomeric *O*-alkylations between parent D-mannose and deoxy-D-mannoses.

somewhat contradictory to what we had observed previously for the stereoselective synthesis of 2-deoxy-β-glycosides.[11] Such findings encouraged us to further investigate the mechanistic aspects of this β-selective mannosylation.

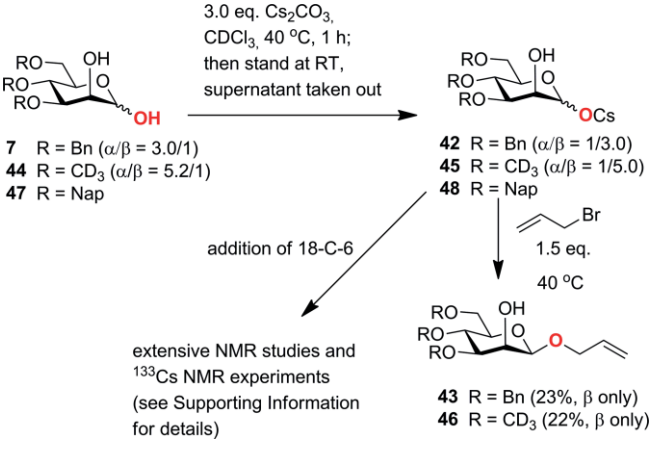
Previous studies also indicated that conformationally restricted bicyclic 3-*O*-benzyl-4,6-*O*-benzylidene-D-mannose **16** did not react with triflate **6** in the presence of cesium carbonate to afford corresponding disaccharide **31**,[13] which suggests that a conformationally flexible sugar ring may be critical for this type of anomeric *O*-alkylation. To further study the impact of the conformation and ring strain to the reactivity of the D-mannose-derived anomeric alkoxide in the β-mannosylation reaction, we prepared additional bicyclic D-mannose-derived lactol substrates. They include 3,4-*O*-bisketal-protected mannose **17**, 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-D-mannose **18**,[22] 3,6-anhydro-4-*O*-benzyl-D-mannose **19**, 2,6-anhydro-3,4-di-*O*-benzyl-D-mannose **20**, and 4-*O*-benzyl-3,6-*O*-(*o*-xylylene)-D-mannose **21**.[20,23] As shown in Table 1, Cs_2CO_3-mediated anomeric *O*-alkylation of 3,4-*O*-bisketal-protected mannose **17** and 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-D-mannose **18** bearing conformationally flexible C6-oxygen atom with secondary triflate **6** under standard conditions afforded 3,4-*O*-bisketal-protected mannoside **32** and 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-D-mannoside **33** in 23 % and 34 % yields (β only), respectively. However, none of 3,6-anhydro-4-*O*-benzyl-D-mannose **19**, 2,6-anhydro-3,4-di-*O*-benzyl-D-mannose **20**, and 4-*O*-benzyl-3,6-*O*-(*o*-xylylene)mannose **21** reacted with triflate **6** to afford detectable amounts of corresponding disaccharides **34**, **35** or **36**, respectively.[24] These results suggested the relatively poor nucleophilicity of these bicyclic mannose-derived anomeric alkoxides. After careful analysis of the reaction mixtures, none of the bicyclic lactol donors (**16**, **17**, **19–21**) was detected except that 29 % of the relatively more flexible 6,5-*cis*-fused lactol **18** was recovered. The decomposition of those strained bicyclic lactols may indicate their instability in addition to their low reactivity.[25]

Furthermore, we prepared 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose **38** and studied this substrate in this Cs_2CO_3-mediated anomeric *O*-alkylation. As shown in Scheme 3, 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose **38** was able to react with C4-triflate **6** (2.0 equiv.) in the presence of cesium carbonate (2.5 equiv.) to afford desired β-mannopyranoside **39**β in 46 % isolated yield and α-mannopyranoside **39**α in 3 % yield, respectively (β/α = 15.3:1). In addition, a small amount (5 %) of β-glucoside **40** was also isolated from the reaction mixture. Presumably, deprotonation of the anomeric hydroxyl group of **38** followed by a sequential ring-opening, epimerization of the C2 stereocenter through enolization of the aldehyde and re-protonation, and cyclization afforded a small amount of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose-derived anomeric alkoxide **41**. Anomeric *O*-alkylation of this alkoxide **41** gave rise to β-glucoside **40** under the kinetic anomeric effect.[8,17] This result indicated that 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose **38** is less reactive than 3,4,6-tri-*O*-benzyl-D-mannopyranose **7**, probably due to more severe steric effects. Despite that the presence of a free hydroxyl group at C2 of the mannose is not essential for this β-mannosylation, leaving this C2 hydroxyl group unprotected helps achieve superior yields and excellent β-selectivity.



Scheme 3. Anomeric *O*-alkylation of 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose.

We also carried out NMR studies for studying lactol **7** and its corresponding anomeric cesium alkoxide (**42**). In the event, to a 0.1 M solution of 3,4,6-tri-*O*-benzyl-D-mannose (**7**, α/β = 3.0:1) in $CDCl_3$[26] was added 3 equiv. of Cs_2CO_3 and the reaction mixture was stirred at 40 °C for 1 hour. The resulting mixture was allowed to stand for 0.5 h and the supernatant, presumably containing cesium alkoxide (cf. **42**, Scheme 4) in $CDCl_3$, was taken out for NMR studies. It was found that cesium alkoxide **42** exists as a mixture of α and β anomers (α/β = 1:3.0).[20] Next, 1.5 equiv. of allyl bromide was added to this supernatant containing cesium alkoxide **42** in $CDCl_3$ and the resulting mixture was stirred at 40 °C for 24 hours to afford desired allyl 3,4,6-tri-*O*-benzyl-β-D-mannoside (cf. **43**,[15] Scheme 4) in 23 % yield. This result indicated that cesium alkoxide **42**β is the reactive species in the anomeric *O*-alkylation.



Scheme 4. Preparation of anomeric cesium alkoxides in $CDCl_3$ and subsequent alkylation with allyl bromide.

We also prepared 3,4,6-tri-*O*-CD_3-D-mannose (**44**) and its corresponding anomeric cesium alkoxide (**45**) for extensive NMR studies. The use of CD_3 ether instead of benzyl ether protecting group (cf. **7**) was to simplify the NMR spectra. As shown in Figure 1, ^{1}H NMR spectrum of 3,4,6-tri-*O*-CD_3-D-mannose (**44**) (0.1 M in $CDCl_3$) showed that the more thermodynamically stable axial α-anomer was predominant before deprotonation due to the anomeric effect (α/β = 5.2:1).[20] Next, 3 equiv. of Cs_2CO_3 was added to this 0.1 M solution of **44** in $CDCl_3$ and the reaction mixture was stirred at 40 °C for 1 hour. The resulting mixture was allowed to stand for 0.5 h and the supernatant containing cesium alkoxide **45** was taken out and subjected to extensive NMR ex-

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periments (Scheme 5) at room temperature.[20,27] The ^{1}H NMR spectrum indicated that deprotonation of anomeric hydroxyl groups of **44** with Cs_2CO_3 cleanly afforded the corresponding anomeric cesium alkoxides (**45**) as a mixture of anomers (α/β = 1:5.0, Figure 1). Both signals of H-4 from cesium alkoxides **45**β (major) and **45**α (minor) were shown as triplets ($J_{(H\text{-}3,H\text{-}4)}$ = $J_{(H\text{-}4,H\text{-}5)}$ = 9.6 Hz) indicating a *trans*-diaxial relationship between H-3 and H-4 as well as H-4 and H-5.[20] These data indicate that both anomeric cesium alkoxides (**45**α and **45**β) mainly adopt a stable chair conformation at room temperature or 40 °C.[27] In addition, proton signal broadening (except H4) may indicate the possible chelation of cesium with various oxygen atoms. The predominance of the equatorial β-anomer after deprotonation (α/β = 1:5.0) was probably due to the chelation of the cesium ion to C2-oxygen at room temperature, while in the axial α-anomer such chelation would not be possible due to the 1,2-*trans* relationship. In addition, this solution of anomeric cesium alkoxide **45** in $CDCl_3$ was treated with 1.5 equiv. of allyl bromide and stirred at 40 °C for 24 hours to afford the desired allyl 3,4,6-tri-*O*-CD_3-β-D-mannoside (**46**) in 22 % yield (β only, Scheme 5). This result was comparable to that involving 3,4,6-tri-*O*-benzyl-D-mannose (**7**), which indicates that the benzyl protecting group is not required for this reaction. In addition, we also prepared 3,4,6-tri-*O*-(2-naphthylmethyl(NAP))-D-mannopyranose (**47**) and the corresponding cesium alkoxide **48** following the same procedure.[20] Unfortunately, various attempts to crystallize D-mannose-derived anomeric alkoxides **42**, **45**, and **48** suitable for *X*-ray crystallographic analysis were unsuccessful.[28]

Previously, anomeric *O*-alkylation of 3,4,6-tri-*O*-benzyl-D-mannose **7** with allyl bromide in the presence of Cs_2CO_3 was found to afford allyl 3,4,6-tri-*O*-benzyl-β-D-mannoside **43** in 94 % yield (β only).[15] Based on that, we conducted reaction progress kinetic analysis involving two different initial concentrations of mannose **7**, in order to establish whether monomeric cesium alkoxide **42**β or its dimeric form is the key reactive intermediate for this anomeric *O*-alkylation (Figure 2).[20] The measured concentration of mannose **7** and allyl β-D-mannoside **43** dependence on time is depicted in Figure 2 (a, 0.1 M of initial concentration of mannose **7**), while kinetic data corresponding to 0.2 M of initial concentration of mannose **7** are provided in the Supporting Information.[20] Next, kinetic analysis proved that this reaction exhibits a strict first-order dependence on mannose **7** at both 0.1 M and 0.2 M initial concentrations (b, Figure 2). As the deprotonation is complete in a relatively short period (Scheme 4) and the subsequent S_N2 reaction is believed to be the rate-determining step, our kinetic studies indicate that monomeric cesium alkoxide **42**β should be the real reactive species for this anomeric *O*-alkylation.[29]

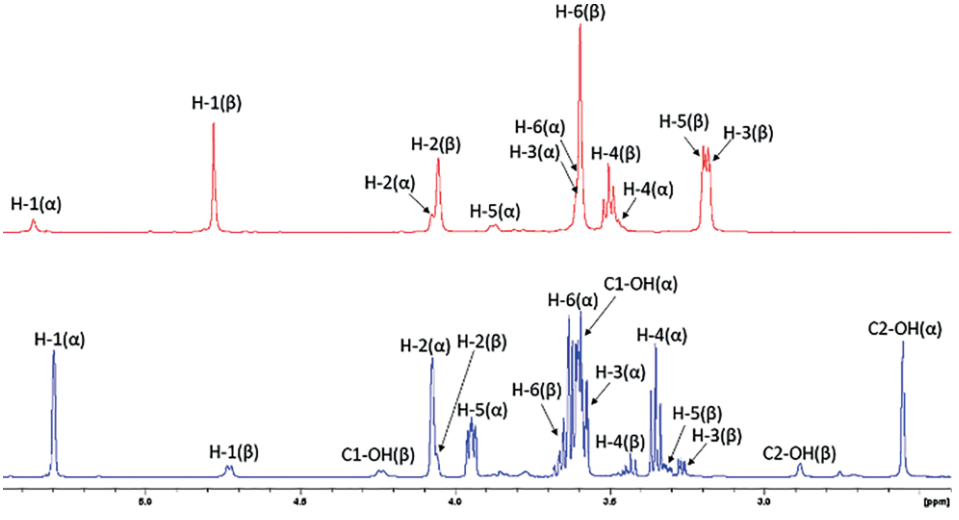
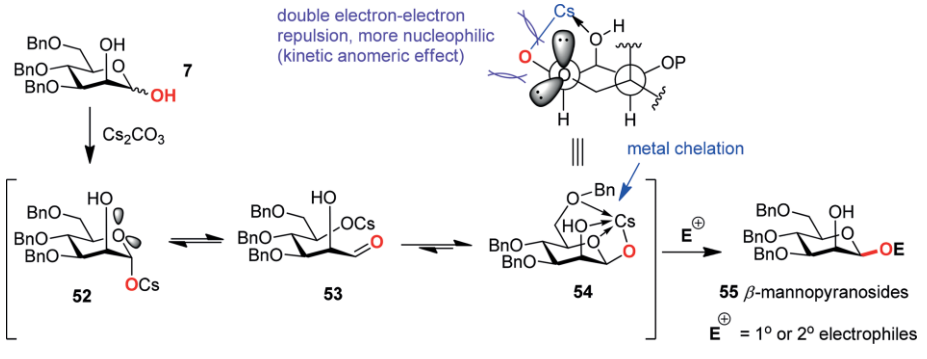


Figure 1. Comparison of ^{1}H NMR spectra of 3,4,6-tri-*O*-CD_3-D-mannose (**44**, α/β = 5.2:1, colored in blue, bottom spectrum) and its corresponding cesium alkoxide **45** (α/β = 1:5.0, colored in red, top spectrum).



Scheme 5. Revised mechanism for stereoselective β-mannosylation via Cs_2CO_3-mediated anomeric *O*-alkylation.

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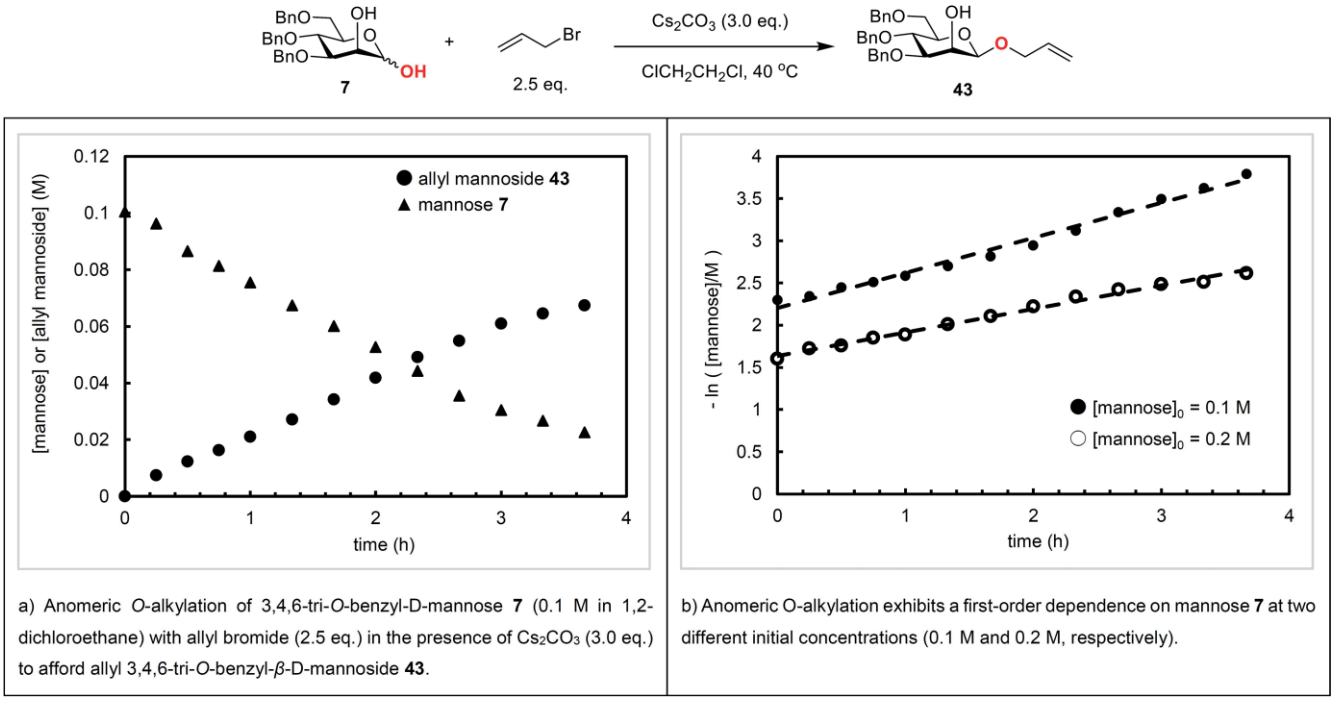

a) Anomeric *O*-alkylation of 3,4,6-tri-*O*-benzyl-D-mannose **7** (0.1 M in 1,2-dichloroethane) with allyl bromide (2.5 eq.) in the presence of Cs_2CO_3 (3.0 eq.) to afford allyl 3,4,6-tri-*O*-benzyl-*β*-D-mannoside **43**.

b) Anomeric O-alkylation exhibits a first-order dependence on mannose **7** at two different initial concentrations (0.1 M and 0.2 M, respectively).

Figure 2. Kinetic studies of anomeric *O*-alkylation of mannose 7 with allyl bromide (2.5 equiv.) in the presence of Cs_2CO_3 (3.0 equiv.) in 1,2-dichloroethane at 40 °C.

With all of the experimental results in hand, we next conducted computational studies to explore the role of Cs_2CO_3 in the anomeric *O*-alkylation and the origin of the decreased reactivities of deoxy-D-mannoses **9**, **10**, and **11**.[30] Based on the experimental studies discussed above, we surmised that the cesium salt may either promote the deprotonation of the anomeric OH group to form anomeric cesium alkoxides (e.g. **44**) or enhance the nucleophilicity of the anomeric cesium alkoxides in the subsequent alkylation step. These two potential effects were computationally analyzed using density functional theory (DFT) calculations. All calculations were performed at the M06-2X/def2-QZVP/SMD(dichloroethane)//M06-2X/SDD-6-31G(d) level of theory (see SI for computational details).

We first investigated the relative acidities of the anomeric OH groups in tri-*O*-benzyl-D-mannose **7** and deoxy-D-mannoses **9**, **10**, and **11**. We computed the reaction energies of the deprotonation of these substrates using anomeric cesium alkoxide **7-Cs** as the base.[31] All three deoxy-D-mannoses require much higher

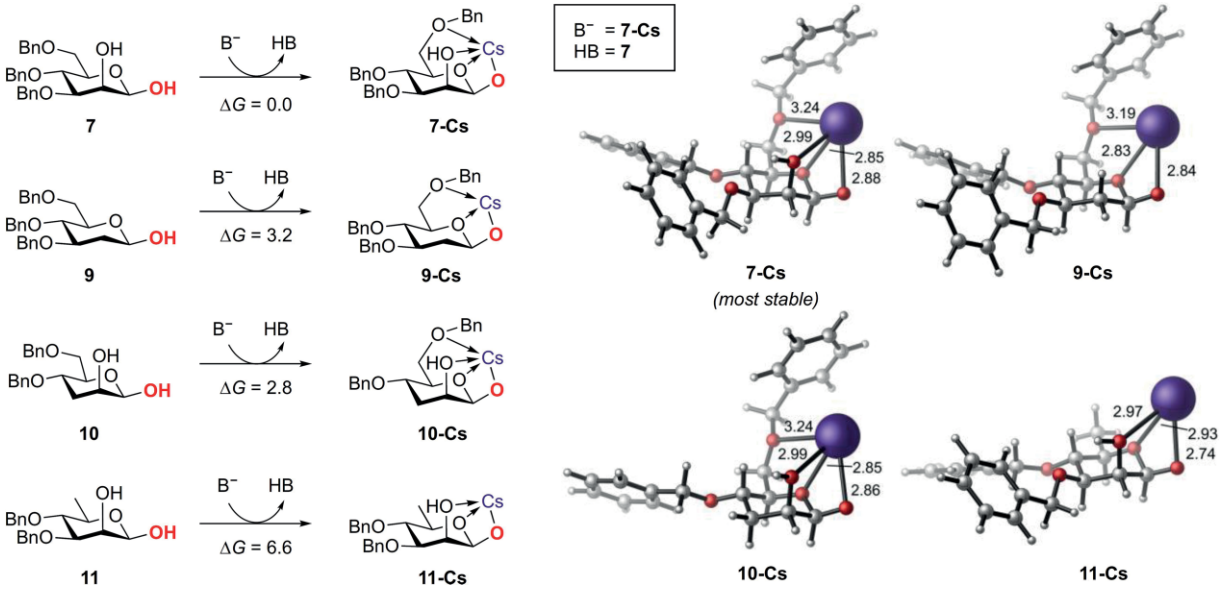

Figure 3. Relative acidity of anomeric OH of D-mannose **7** and deoxy-D-mannoses **9**, **10**, and **11**. Cesium alkoxide **7-Cs** is used as the base to calculate the reaction energies of all deprotonation reactions. All energies are in kcal/mol. Bond lengths are in angstrom.

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energy to deprotonate than D-mannose **7**, indicating cesium alkoxide **7-Cs** is much more stable than cesium alkoxides derived from the deoxy-D-mannoses. Examination of the lowest energy conformers of the deprotonated cesium alkoxides (Figure 3) indicated that the pyranose rings in **7-Cs** adopt a $^4\mathbf{C}_1$ conformation. The $^1\mathbf{C}_4$ conformer of **7-Cs** is 6.4 kcal/mol less stable (see SI for details). Strong chelation of the cesium ion with four oxygen atoms, O1, O2, O6, and O5 (the endocyclic oxygen), was observed.[32] The lower stability of anomeric cesium alkoxides **9-Cs** and **11-Cs** is attributed to the lack of O2 and O6 substituents, respectively, which diminished the chelating Cs–O interactions. On the other hand, **10-Cs** is stabilized by chelation with O1, O2, O6, and O5 atoms, which is similar to that in **7-Cs**. Nonetheless, the inductive effects of the C3-OBn group in **7-Cs** provide additional stabilization of the negative charge in the alkoxide and thus make the anomeric OH of **7** more acidic than that of **10**.

Next, we investigated the relative nucleophilic reactivities of the anomeric cesium alkoxides using allyl bromide as the electrophile. The computed activation energies of the S_N2 transition states with respect to each of the cesium alkoxide intermediates are shown in Figure 4. The allylation of all four cesium alkoxides requires comparable activation energies ($\Delta G^{\ddagger}$ = 19.4–21.6 kcal/mol), suggesting once these alkoxides are formed, their reaction rates in the subsequent *O*-alkylation would be similar.

Taken together, the above computational analyses indicate the Cs ion forms strong chelating interactions with multiple oxygen atoms in anomeric alkoxides. The possible chelation of cesium ion with the oxygen atoms on the sugar is also supported by ^{133}Cs NMR studies as well as 18-crown-6 titration studies.[20] This stabilizing chelation effect promotes the deprotonation of D-mannose. The importance of the Cs–O chelation is evident in the experimentally observed low reactivities of deoxy-D-mannoses **9**, **10**, and **11**. Removal of oxygen substituents at either C2, C3, or C6 positions destabilizes the chelated cesium alkoxides, and thus makes the deprotonation of the anomeric hydroxyl group in deoxy-mannoses more difficult.

Based on the aforementioned discussions, we revised the originally proposed mechanistic hypothesis as shown in Scheme 5. After deprotonation of the anomeric hydroxyl group of D-mannose **7** with cesium carbonate, a mixture of *axial* alkoxide **52** and *equatorial* alkoxide **54** may be produced and interconvert into each other via open aldehyde intermediate **53**. Due to chelation, *equatorial* anomeric cesium alkoxide **54**[33] (cf. **7-Cs**, Figure 3), would be preferentially formed. In addition, effective chelation may also alleviate the steric effect of the C2-hydroxyl group. Subsequent anomeric *O*-alkylation of the cesium alkoxide **54** with the assistance of kinetic anomeric effect, i.e. double electron-electron repulsion, with suitable electrophiles via S_N2 substitution then affords the desired β-mannopyranosides **55** (Scheme 5).[34]

This β-mannosylation via anomeric *O*-alkylation was next utilized in the synthesis of the hexasaccharide core[35] of complex fucosylated *N*-linked glycans.[36] For instance, bi-antennary α-(2,3)-sialylated core-fucosylated *N*-glycan dodecasaccharide was

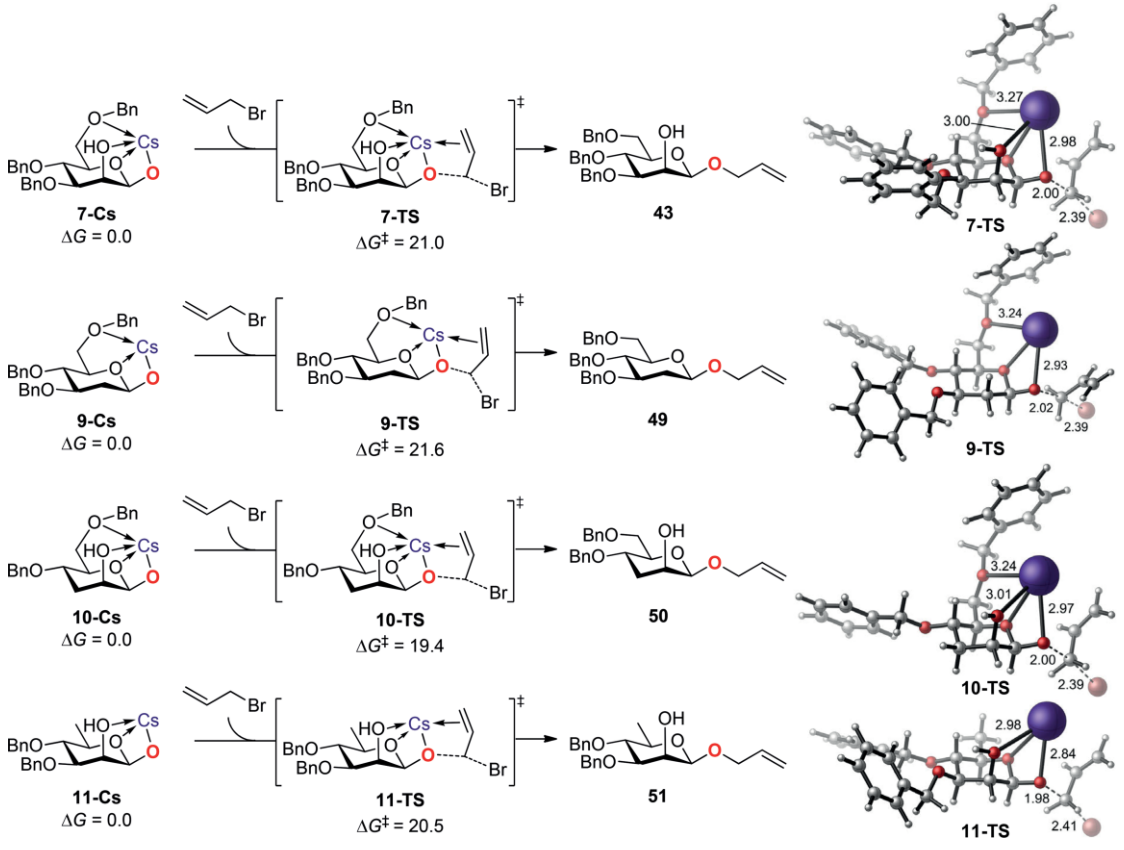
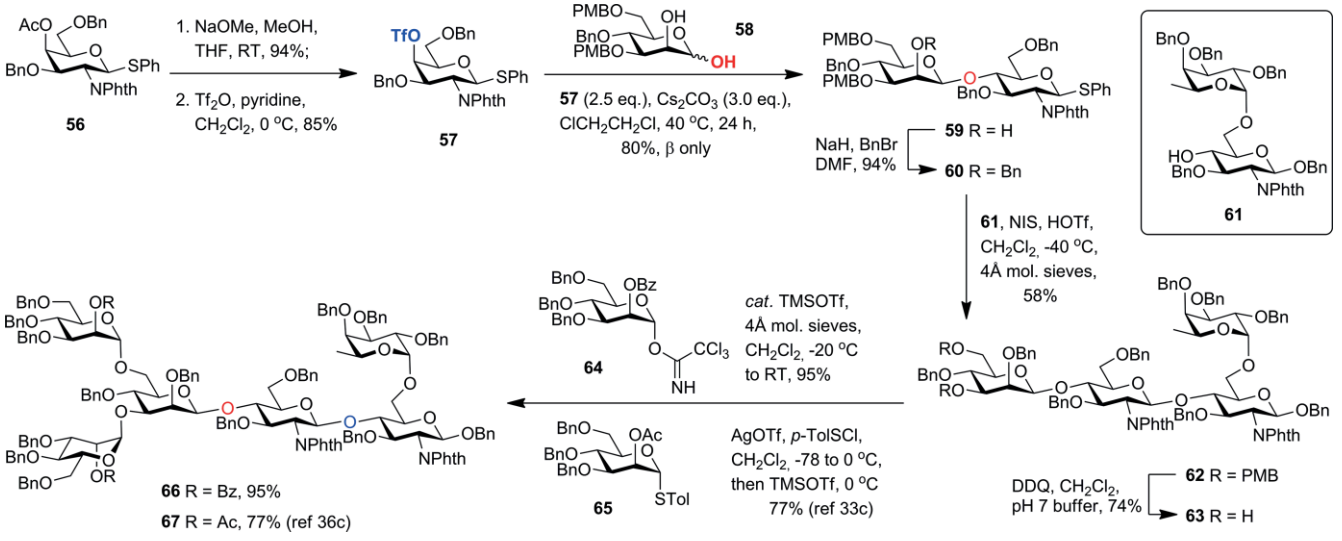


Figure 4. Calculated activation free energies of the *O*-allylation of anomeric cesium alkoxides **7-Cs**, **9-Cs**, **10-Cs**, and **11-Cs** with allyl bromide. All energies are in kcal/mol. Bond lengths are in angstrom.



Scheme 6. Synthesis of the hexasaccharide core of fucosylated *N*-linked glycans.

found on alpha fetoprotein (AFP) isolated from patients with hepatocellular carcinoma. The structure of this *N*-glycan was believed to be a much more reliable marker than AFP to differentiate benign and malignant liver diseases.[37] This dodecasaccharide was also detected on the surface of erythropoietin[38] and is believed to be important for the in vivo activity of erythropoietin.[39] As shown in Scheme 6, known protected β-D-galactosamine-derived thioglycoside **56**[40] underwent standard deacetylation (94 %) followed by triflation of the resulting C4-alcohol to afford triflate **57** (85 %). Next, cesium carbonate-mediated anomeric *O*-alkylation of known 3,6-di-*O*-(4-methoxybenzyl)-4-*O*-benzyl-D-mannopyranose **58**[13] with aforementioned triflate acceptor **57** (2.5 equiv.) under the optimized conditions gave the desired β-disaccharide **59** in 80 % yield (β only). Standard benzylation of the C2′-OH of disaccharide **59** afforded **60** which was subjected to the traditional glycosylation with known disaccharide acceptor **61**[36c] (NIS, cat. TfOH) to produce the corresponding tetrasaccharide **62** in 58 % yield. After DDQ-mediated deprotection of bis-PMB ethers of **62** under neutral conditions, **63** was obtained in 74 % yield whose spectroscopic data were found to be identical to those previously reported.[36c] Previously, double α-mannosylation of tetrasaccharide **63** with thioglycoside donor **65** (AgOTf, *p*-toluenesulfenyl chloride; then catalytic TMSOTf) afforded hexasaccharide **67** in 77 % yield.[36c] In our hands, double α-mannosylation of tetrasaccharide **63** with trichloroacetimidate donor **64** in the presence of the catalytic amount of TMSOTf afforded complex hexasaccharide **66** in 95 % yield.

Conclusions

In conclusion, various structurally diverse deoxy-D-mannoses and conformationally restricted bicyclic D-mannoses have been prepared and subjected to the mechanistic studies of this umpolung-type β-mannosylation via anomeric *O*-alkylation. Deoxy mannoses were found to be not as reactive as parent mannose, and conformationally restricted bicyclic D-mannoses afforded no product or lower yields than conformationally flexible D-mannoses. When the hydroxyl group at C2 was masked, this type of β-mannosylation did not proceed as efficiently, albeit the corresponding β-mannoside was still detected as the major product. Studies of various alkali metal bases indicated that cesium carbonate (Cs_2CO_3) was the optimal base for this β-selective anomeric *O*-alkylation.[20] Extensive NMR studies demonstrated the formation of predominant equatorial anomeric cesium alkoxides after deprotonation, due to the chelation of the cesium ion to the oxygen atoms. In addition, the chelating interactions of cesium ion with the oxygen atoms on the sugar are also supported by ^{133}Cs NMR experiments as well as 18-crown-6 titration studies.[20] Kinetic studies supported that the anomeric *O*-alkylation involves monomeric cesium alkoxides as the key reactive species. DFT calculations suggested that that the oxygen atoms at C2, C3, and C6 of the mannose enhances the acidity of the anomeric hydroxyl group to facilitate the deprotonation by Cs_2CO_3. In particular, the C2-oxygen atom is believed to play a major role in the chelation with the cesium ion. Such chelation preferentially favors the formation of equatorial anomeric alkoxides, leading to the highly stereoselective alkylation to form the β-anomer of the products. Based on experimental data and computational results, a revised mechanism for this β-mannosylation is proposed. We also isolated and characterized a side product, 3,4,6-tri-*O*-benzyl-D-fructose, which was presumably formed via an α-keto rearrangement of the open aldehyde intermediate.[20] This umpolung-type β-mannosylation has also been demonstrated in the efficient synthesis of the hexasaccharide core of complex fucosylated *N*-linked glycans. Application of this method to the stereoselective construction of other types of challenging glycosidic linkages, such as β-2-amino-2-deoxy-D-mannosides, and synthesis of complex biologically significant carbohydrate molecules are currently underway.

Experimental Section

General Procedure for Cs_2CO_3 Mediated *O*-Alkylation: To a mixture of sugar lactol donor (0.1 mmol, 1.0 equiv.), a sugar derived

triflate acceptor **6** (2.0 equiv.), and Cs_2CO_3 (2.5 equiv.) was added 1,2-dichloroethane (1.0 mL). The reaction mixture was stirred at 40 °C for 24 hours. The crude reaction mixture was purified by preparative TLC. The β-configuration of the newly formed mannosidic linkage was unambiguously assigned by measuring the $^1J_{C\text{-}H}$ for the anomeric carbon.

Synthesis of the Hexasaccharide Core of the Fucosylated *N*-Linked Glycans

Phenyl 3,6-Di-*O*-benzyl-4-*O*-trifluoromethylsulfonyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (57): To a solution of known compound **56**[40] (2.67 g, 4.6 mmol) in MeOH (25 mL) and THF (25 mL) was added 0.5 M NaOMe solution in MeOH (6.4 mL, 3.22 mmol). The mixture was stirred at room temperature for 7 hours. The reaction mixture was neutralized by the addition of Amberlyst IR-120 (H^+), filtered, and concentrated. The residue was purified by silica gel column chromatography (hexanes/EtOAc = 5:1 to 3:1) to give the corresponding alcohol (2.52 g, 94 %). To a solution of this alcohol (0.67 g, 1.2 mmol) and pyridine (1.0 mL, 12 mmol) in CH_2Cl_2 (3.0 mL) cooled to 0 °C was added Tf_2O (0.35 mL, 2.0 mmol) dropwise. The resulting mixture was stirred at 0 °C for 2 h before being quenched with ice water. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layer was washed sequentially with saturated $CuSO_4$ (50 mL × 3) and water (50 mL × 3), dried with anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with CH_2Cl_2 to afford the sugar derived triflate **57** (725 mg, 85 %). **^{1}H NMR (600 MHz, $CDCl_3$)** δ = 7.85 (m, 1H, H_{Ar}), 7.74 (m, 1H, H_{Ar}), 7.70 (m, 1H, H_{Ar}), 7.58 (dd, J = 7.3, 1.1 Hz, 1H, H_{Ar}), 7.42–7.32 (m, 7H, H_{Ar}), 7.25–7.18 (m, 3H, H_{Ar}), 6.98–6.93 (m, 3H, H_{Ar}), 6.90–6.83 (m, 2H, H_{Ar}), 5.54 (d, J = 2.9 Hz, 1H, *H*-4), 5.52 (d, J = 10.5 Hz, 1H, *H*-1), 4.70 (d, J = 12.6 Hz, 1H, -OC*H*$_2$Ar), 4.65 (d, J = 11.2 Hz, 1H, -OC*H*$_2$Ar), 4.52–4.45 (m, 2H, -OC*H*$_2$Ar, *H*-2), 4.38 (dd, J = 10.5, 2.9 Hz, 1H, *H*-3), 4.21 (d, J = 12.6 Hz, 1H, -OC*H*$_2$Ar), 4.01 (dd, J = 8.3, 5.6 Hz, 1H, *H*-5), 3.79 (dd, J = 9.2, 5.6 Hz, 1H, *H*-6a), 3.69 (dd, J = 9.2, 8.4 Hz, 1H, *H*-6b); **^{13}C NMR (150 MHz, $CDCl_3$)** δ = 168.05, 166.87, 137.36, 136.51, 134.26, 133.98, 132.81, 131.67, 131.62, 131.61, 129.01, 128.69, 128.44, 128.36, 128.34, 128.28, 128.24, 128.01, 123.80, 123.45, 118.68 (q, $^1J_{C\text{-}F}$ = 319.7 Hz), 84.21, 80.57, 75.14, 73.91, 72.97, 71.89, 67.20, 50.99.

Phenyl *O*-2,4-Di-*O*-benzyl-3,6-di-*O*-(*para*-methoxybenzyl)-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-gluopyranoside (60): To a mixture of mannopyranosyl donor 58[13] (1.02 g, 2.0 mmol), sugar-derived triflate acceptor **57** (3.7 g, 5.0 mmol), and Cs_2CO_3 (2.0 g, 6.0 mmol) was added 1,2-dichloroethane (20 mL). The reaction mixture was stirred at 40 °C for 24 hours. The crude reaction mixture was purified by silica gel column chromatography (hexanes/EtOAc = 5:1 to 1:1) to give disaccharide **59** (1.73 g, 80 %). To a solution of 59 (1.0 g, 0.93 mmol) in DMF (4.0 mL) cooled to 0 °C was added NaH (75 mg, 1.86 mmol, 60 % in mineral oil) portion wise. The resulting mixture was stirred at 0 °C for 1 h before BnBr (0.17 mL, 1.4 mmol) was added. The reaction mixture was warmed up and stirred at ambient temperature for 3 h before being quenched with water. The resulting mixture was extracted with EtOAc three times, and combined organic extracts were washed with water and brine, dried with anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexanes/EtOAc = 5:1 to 3:1) to give the title compound **60** (1.01 g, 94 %). The $^1J_{C\text{-}H}$ of mannosidic anomeric carbon for 60 was determined to be 159.0 Hz. $[\alpha]_D^{23}$ = +5.9 (*c* 1.0, $CHCl_3$); FT-IR (thin film): 3064, 3032, 2938, 2863, 1777, 1714, 1678, 1512, 1455, 1388, 1250, 1174, 1102, 1070, 917, 807, 701 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.80 (d, J = 7.3 Hz, 1H, H_{Ar}), 7.70 (m, 1H, H_{Ar}), 7.65 (m, 1H, H_{Ar}), 7.58 (d, J = 7.3 Hz, 1H, H_{Ar}), 7.50–7.44 (m, 2H, H_{Ar}), 7.42–7.38 (m, 2H, H_{Ar}), 7.37–7.27 (m, 11H, H_{Ar}), 7.24–7.13 (m, 9H, H_{Ar}), 6.89–6.81 (m, 4H, H_{Ar}), 6.78–6.69 (m, 5H, H_{Ar}), 5.52 (d, J = 9.9 Hz, 1H, *H*-1), 4.96 (d, J = 13.0 Hz, 1H, -OC*H*$_2$Ar), 4.91 (s, 2H, -OC*H*$_2$Ar), 4.86 (d, J = 10.9 Hz, 1H, -OC*H*$_2$Ar), 4.60 (d, J = 12.0 Hz, 1H, -OC*H*$_2$Ar), 4.56 (s, 1H, H-1′), 4.55–4.49 (m, 2H, -OC*H*$_2$Ar), 4.49–4.41 (m, 4H, -OC*H*$_2$Ar), 4.38 (d, J = 11.6 Hz, 1H, -OC*H*$_2$Ar), 4.32–4.23 (m, 2H, *H*-3, *H*-2), 4.02 (dd, J = 9.8, 8.0 Hz, 1H, *H*-4), 3.90 (t, J = 9.6 Hz, 1H, *H*-4′), 3.81–3.78 (m, 4H, *H*-2′, -OC*H*$_3$), 3.77–3.70 (m, 5H, -OC*H*$_3$, *H*-6a, *H*-6′a), 3.66–3.59 (m, 3H, *H*-6b, *H*-6′b, *H*-5), 3.42–3.34 (m, 2H, *H*-3′, *H*-5′); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 168.08, 167.31, 159.25, 158.98, 139.03, 138.91, 138.63, 138.06, 133.86, 133.69, 132.82, 132.06, 131.80, 131.69, 130.74, 130.48, 129.33, 129.28, 128.87, 128.58, 128.38, 128.25, 128.06, 128.00, 127.94, 127.85, 127.78, 127.72, 127.66, 127.45, 126.82, 123.44, 123.33, 113.86, 113.73, 101.79, 83.37, 82.50, 79.26, 78.57, 76.04, 75.21, 75.11, 74.99, 74.81, 74.18, 73.59, 73.07, 71.61, 69.22, 68.91, 55.36, 55.31, 54.86; LRMS (ESI) calculated for $C_{70}H_{69}NNaO_{13}S$ $[M + Na]^+$ 1187.44, found 1187.30.

Benzyl *O*-2,4-Di-*O*-benzyl-3,6-di-*O*-(*para*-methoxybenzyl)-β-D-mannopyranosyl-(1→4)-*O*-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-gluopyranosyl-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)]-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-gluopyranoside (62): To a mixture of donor 60 (87 mg, 0.075 mmol), acceptor **61**[36c] (45 mg, 0.05 mmol), activated 4 Å molecular sieves (300 mg), and NIS (84 mg) was added CH_2Cl_2 (2.5 mL). The solution was cooled to –40 °C and TfOH (2.0 μL) was added. The resulting mixture was stirred at this temperature overnight and then filtered through celite. The filtrate was quenched and washed with saturated $Na_2S_2O_3$ aqueous solution. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude reaction mixture was purified by preparative TLC (EtOAc/CH_2Cl_2/toluene = 1:5:5) to furnish the title tetra-saccharide **62** (57 mg, 58 %). $[\alpha]_D^{23}$ = –3.3 (*c* 1.0, $CHCl_3$); FT-IR (thin film): 3065, 3033, 2934, 2867, 1777, 1716, 1614, 1514, 1454, 1388, 1249, 1091, 1038, 749, 724, 700 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.88 (d, J = 7.3 Hz, 1H, H_{Ar}), 7.82 (d, J = 7.2 Hz, 1H, H_{Ar}), 7.76–7.56 (m, 7H, H_{Ar}), 7.50 (d, J = 7.3 Hz, 1H, H_{Ar}), 7.46–7.11 (m, 32H, H_{Ar}), 7.07 (m, 1H, H_{Ar}), 7.04–6.91 (m, 6H, H_{Ar}), 6.87–6.69 (m, 12H, H_{Ar}), 5.59 (d, J = 8.4 Hz, 1H, H-1), 5.01–4.91 (m, 5H), 4.91–4.81 (m, 4H), 4.80–4.72 (m, 2H), 4.66–4.47 (m, 9H), 4.45–4.26 (m, 8H), 4.21–4.16 (m, 2H), 4.12 (dd, J = 10.7, 8.5 Hz, 1H), 4.07–4.01 (m, 2H), 3.99 (dd, J = 10.2, 2.8 Hz, 1H), 3.90–3.85 (m, 2H), 3.83–3.77 (m, 4H), 3.77–3.72 (m, 3H), 3.68 (s, 3H), 3.65–3.57 (m, 3H), 3.41 (dd, J = 10.8, 3.0 Hz, 1H), 3.38 (dd, J = 9.4, 3.0 Hz, 1H), 3.35 (ddd, J = 9.8, 5.1, 1.7 Hz, 1H), 3.28 (ddd, J = 9.9, 3.0, 1.6 Hz, 1H), 1.01 (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 168.27, 167.89, 167.78, 167.67, 159.19, 158.91, 139.21, 139.17, 139.05, 138.96, 138.86, 138.73, 138.07, 137.16, 133.91, 133.73, 133.67, 133.58, 132.01, 131.79, 131.66, 130.87, 130.59, 129.28, 129.19, 128.62, 128.60, 128.51, 128.42, 128.34, 128.23, 128.19, 128.10, 128.07, 128.05, 127.91, 127.89, 127.74, 127.72, 127.62, 127.60, 127.57, 127.55, 127.47, 127.44, 127.37, 127.08, 126.95, 126.72, 123.55, 123.31, 123.20, 113.81, 113.68, 101.45, 96.90, 96.86, 96.70, 82.46, 79.57, 79.24, 77.71, 77.33, 76.09, 76.05, 75.82, 75.30, 75.09, 75.06, 74.84, 74.82, 74.69, 74.63, 74.43, 74.36, 73.79, 73.39, 73.20, 73.09, 72.52, 71.30, 69.96, 69.27, 68.51, 66.07, 63.94, 56.68, 55.95, 55.35, 55.25, 16.51; LRMS (ESI) calculated for $C_{119}H_{118}N_2Na_2O_{24}$ $[M + 2Na]^{2+}$ 1002.89, found 1002.80.

Benzyl-*O*-2-*O* Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-*O*-[2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-*O*-2,4-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-*O*-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-gluopyranosyl-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)]-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-gluopyranoside (66): To a solution of tetra-saccharide **62** (57 mg, 0.029 mmol) in CH_2Cl_2 (3.0 mL) was added a solution of pH 7 (1.0 mL) buffer and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (18 mg, 0.079 mmol). The resulting

mixture was stirred at room temperature for 3 h before additional amount of DDQ (13 mg, 0.057 mmol) was added. The mixture was stirred for another 2 hours and another portion of DDQ (13 mg, 0.057 mmol) was added. After 2 hours, the mixture was quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate (10 mL × 3). The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude reaction mixture was purified by preparative TLC (EtOAc/CH_2Cl_2 = 1:10) to furnish the corresponding diol **63** (37 mg, 74 %). The spectroscopic data was consistent with the data reported in the literature.[36c] To a mixture of diol acceptor **63** (74 mg, 0.043 mmol), donor 64 (95 mg, 0.13 mmol), activated 4 Å molecular sieves (100 mg) was added CH_2Cl_2 (1.0 mL). The solution was cooled to –20 °C and TMSOTf (1.0 μL) was added. The resulting mixture was slowly warmed up to room temperature over 2 hours. The reaction was quenched with Et_3N (200 μL) and filtered through celite. The filtrate was washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The residue was purified by preparative TLC (EtOAc/toluene = 1:10) to afford hexasaccharide **66** (115 mg, 95 %). $[\alpha]_D^{23}$ = –7.0 (*c* 1.0, $CHCl_3$); FT-IR (thin film): 3091, 3061, 3030, 2933, 2872, 1777, 1717, 1496, 1455, 1390, 1266, 1099, 1077, 737, 696 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 8.07 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.83–7.78 (m, 2H), 7.76 (d, *J* = 7.4 Hz, 1H), 7.67–7.63 (m, 2H), 7.61–7.55 (m, 2H), 7.49–7.43 (m, 2H), 7.43 (m, 1H), 7.40–7.34 (m, 6H), 7.33–7.08 (m, 60H), 7.06–7.00 (m, 2H), 6.97 (t, *J* = 7.6 Hz, 2H), 6.95–6.92 (m, 2H), 6.90–6.87 (m, 2H), 6.76–6.68 (m, 4H), 6.63–6.55 (m, 1H), 6.50 (t, *J* = 7.5 Hz, 2H), 5.72 (t, *J* = 2.5 Hz, 1H), 5.54 (t, *J* = 2.3 Hz, 1H), 5.46 (d, *J* = 8.3 Hz, 1H), 5.20 (d, *J* = 2.0 Hz, 1H), 4.95–4.78 (m, 12H), 4.74–4.56 (m, 8H), 4.55–4.37 (m, 11H), 4.34 (d, *J* = 11.3 Hz, 1H), 4.29 (d, *J* = 12.3 Hz, 1H), 4.27–4.18 (m, 4H), 4.17–4.10 (m, 3H), 4.09–4.04 (m, 2H), 4.00–3.91 (m, 7H), 3.90–3.83 (m, 2H), 3.80 (m, 1H), 3.76–3.69 (m, 5H), 3.64 (dd, *J* = 10.8, 1.5 Hz, 1H), 3.61–3.53 (m, 5H), 3.29 (dd, *J* = 10.8, 3.4 Hz, 1H), 3.24–3.19 (m, 2H), 0.95 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 168.05, 167.87, 167.64, 165.56, 165.07, 139.01, 138.91, 138.89, 138.78, 138.76, 138.70, 138.41, 138.20, 138.04, 138.01, 137.94, 137.18, 133.75, 133.55, 133.24, 132.76, 131.95, 131.80, 131.63, 130.10, 130.00, 129.97, 129.95, 128.74, 128.58, 128.57, 128.54, 128.52, 128.49, 128.46, 128.37, 128.33, 128.30, 128.24, 128.21, 128.18, 128.02, 128.01, 127.98, 127.89, 127.87, 127.85, 127.77, 127.67, 127.63, 127.61, 127.59, 127.57, 127.53, 127.46, 127.43, 127.38, 127.15, 126.91, 126.84, 126.78, 123.52, 123.25, 102.06, 99.60, 98.34, 96.91, 96.69, 82.21, 79.77, 79.46, 78.35, 78.23, 77.80, 76.51, 76.31, 75.54, 75.24, 75.20, 75.17, 75.06, 74.81, 74.78, 74.58, 74.54, 74.47, 74.43, 74.16, 74.13, 73.85, 73.55, 73.41, 73.36, 72.66, 72.48, 72.10, 71.60, 71.03, 69.94, 69.08, 69.04, 69.00, 68.48, 67.99, 66.55, 66.04, 63.93, 56.64, 55.92, 16.51. LRMS (ESI) calculated for $C_{171}H_{166}N_2Na_2O_{34}$ $[M + 2Na]^{2+}$ 1419.56, found 1419.80.

Acknowledgments

We are grateful to National Science Foundation (CHE-1464787 to X. L. and J. Z.), National Institutes of Health Common Fund Glycosciences Program (1U01GM125290 to P. L. and 1U01GM125289 to J. Z.), The University of Toledo, and University of Michigan–Dearborn for supporting this research. Calculations were performed at the Center for Research Computing at the University of Pittsburgh and the Extreme Science and Engineering Discovery Environment (XSEDE) supported by the NSF. We thank Dr. Yong Wah Kim for assistance in the ^{133}Cs NMR studies, Mr. James K. Dunaway and Rodney A. Park from University of Toledo, and Mr. Luke Harding and Justin Woodward from University of University of Michigan–Dearborn for experimental assistance.

Keywords: Carbohydrates · Glycans · Glycosylation · Mannosylation · Synthetic methods

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[25] A small amount of side product, 3,4,6-tri-*O*-benzyl-D-fructose **37** (Table 1), was always detected in all of the previous anomeric *O*-alkylation reactions involving 3,4,6-tri-*O*-benzyl-D-mannose **7** as the lactol donor. Presumably, **37** was obtained via an α-keto rearrangement of the open aldehyde intermediate derived from lactol **7**.
[26] This cesium carbonate-mediated β-mannosylation via anomeric *O*-alkylation gives comparable results in 1,2-dichloroethane (DCE) and chloroform ($CHCl_3$).
[27] Extensive NMR studies of anomeric cesium alkoxide (**45**) at room temperature or 40 °C did not show much difference.
[28] Attempted crystallization of anomeric alkoxides **42**, **45**, and **48** using various solvents or mixed solvents, e.g. dichloromethane, 1,2-dichloroethane, chloroform, and dichloromethane/*n*-pentane, was unsuccessful.
[29] Although the data prove that monomeric cesium alkoxide **42**β should be the real reactive species for this anomeric *O*-alkylation at 0.1–0.2 M of initial concentration of mannose **7**, other forms of cesium alkoxide **42**β, e.g., dimeric form, can not be ruled out at higher concentration.
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[34] Deprotonation of C2-OH is not required for this anomeric *O*-alkylation to occur, as anomeric O-alkylation of 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose also afford the desired β-mannoside in good yield and anomeric selectivity. However, we can not rule out the possibility that the C2-OH of mannose is deprotonated in a thermodynamically unfavorable, rate-limiting step to generate a bis-alkoxide which is rapidly alkylated at the anomeric position. We sincerely thank one of the reviewers for pointing this out.
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Received: March 10, 2020