HHS Public Access

Author manuscript

J Carbohydr Chem. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

J Carbohydr Chem. 2018 ; 47(5): 347–362. doi:10.1080/07328303.2017.1406095.

Pondering the Structural Factors that Affect 1,2-*trans*-Galactosylation: A Lesson Learnt from 3-*O*-*β*-Galactosylation of Galactosamine

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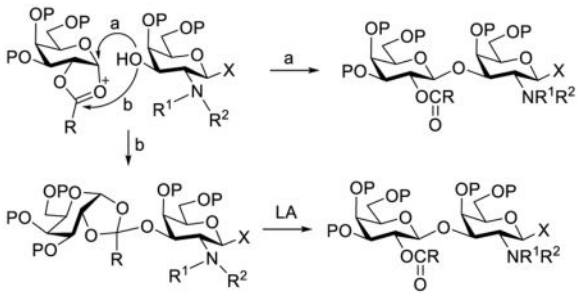
Abstract

Stereoselective formation of glycosidic bonds remains one of the most challenging topics in carbohydrate chemistry. The predominant method for stereoselective construction of 1,2-*trans*-glycosidic bonds is through the neighboring group participation effect (NGPE), which proved to be less successful in synthesizing Gal*β*(1→3)GalNAc disaccharide. The steric effect that overshadows NGPE and the impacts of substituents at the 3-*O*- and 2-*N*-positions of donors and acceptors, respectively, on this synthesis were systematically examined to lead to some practical guidelines for choosing protecting groups towards the successful synthesis of Gal*β*(1→3)GalNAc and similar disaccharides.

Graphical Abstract

The result is dependent on the properties of R, R^1 and R^2.



The result is dependent on the properties of R, R^1 and R^2.

Keywords

neighboring group participation effect; steric effect; galactose; galactosamine; *β*-glycosylation

Introduction

After over a century of research, stereoselective formation of glycosidic bonds still remains one of the most challenging topics in organic and carbohydrate chemistry. In this context,

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many new glycosylation strategies, such as *in situ* anomerization,[1] intramolecular aglycone delivery,[2] stereo-controlled or conformation-directed glycosylation,[3,4] etc., have been developed to achieve desired stereochemical outcomes. Among all existing strategies, stereoselective glycosylation based on the neighboring group participation effect (NGPE), involving mainly the protecting group at the 2-*O*-position of glycosyl donors (Scheme 1), is considered the most reliable strategy in constructing 1,2-*trans*-glycosidic bonds. Over the years, this strategy has been progressively improved. For example, reaction conditions have been extensively studied and perfected, whereas the pool of available participating groups has been expanded from ester to ether,[5] amine,[6] and heterocycle.[7] Consequently, NGPE-based glycosylation has become the ubiquitous method for constructing 1,2-*trans*-glycosidic bonds in complex carbohydrate synthesis.

However, occasionally the NGPE can be outweighed by other factors to give rise to unexpected glycosylation results. For instance, in the course of globo H synthesis, it was observed that constructing the β-linked disaccharide, Galβ(1→3)GalNAc, based on the NGPE was troublesome.[8,9] When a D-galactosamine derivative such as **2a** having the 2-*N*-position protected with a phthalyl (Phth) group (Scheme 2) was employed as the glycosyl acceptor, very low yields of the desired β-isomer were obtained along with substantial amounts of the α-isomer, even when a benzoyl group (Bz) with great potential of NGPE was utilized to protect the 2-*O*-position of the glycosyl donor **1a**. Therefore, in spite of some obvious advantages of the Phth group as a protecting group for amines, such as its excellent NGPE ability and the good stability of the resulting phthalimide, Phth-protected galactosamine derivatives have been rarely used as the glycosyl acceptor in the synthesis of Galβ(1→3)GalNAc. Even if the Phth group was used in the glycosyl acceptor synthesis, it was usually switched to a 2,2,2-trichloroethoxycarbonyl (Troc) or an azido group just before the glycosylation.[9,10] These findings call for a better mechanistic understanding of the NGPE to exploit its full potential in this synthesis.

In the meantime, Galβ(1→3)GalNAc is an important structural motif widely present in glycosphingolipids and many tumor-associated carbohydrate antigens (TACAs), such as GM1, TF, and globo series antigens. Because of its universal overexpression on cancer cells, a screening method, known as the Shams' test, to diagnose colorectal cancer has been developed based on the enzymatic detection of this structural motif.[11] Therefore, an efficient and reliable method to construct the Galβ(1→3)GalNAc motif should be very useful in the chemical synthesis of TACAs and related glycans. The current work aimed to address this frequently overlooked but nonetheless important problem by detailed analysis of the influence of various substituents in the glycosyl donor and glycosyl acceptor on the stereochemical outcome of 3-*O*-galactosylation of galactosamine.

Results and Discussion

As reported in the literature,[10,12–25] a number of TACAs and glycoconjugates containing the Galβ(1→3)GalNAc motif have been synthesized. Among these syntheses, the widely adopted strategy was based on the β-galactosylation of preassembled oligosaccharides or glycoconjugates that had a galactosamine residue at the non-reducing end (Table 1).[10,16–24] In this case, the protecting group at the galactosamine 2-*N*-position could be and indeed was

usually switched to the small and desired acetyl group before galactosylation (entries 1–9, Table 1), and most glycosylation reactions using 2-*O*-acyl protected galactosyl donors afforded good yields and stereoselectivity. However, if the galactosamine 2-*N*-position was protected with a Troc or a trichloroacetyl (TCA) group, it was not required to be switched to an acetyl group for the successful assembly of the Galβ(1→3)GalNAc motif (entries 10 and 11, Table 1).

Another strategy that has been adopted for the construction of glycans containing the Galβ(1→3)GalNAc motif was to assemble this disaccharide unit first (Table 2), which was then used as a glycosyl donor for the next glycosylation reaction.[13,25–27] In this case, the galactosamine 2-*N*-position was usually protected with a Troc or TCA group (entries 1–4, Table 2), which was demonstrated to possess the participating ability and facilitate subsequently NGPE-assisted glycosylation.[13,25,27,28] In addition, the 2-azido derivatives of galactosamine were also used as glycosyl acceptors for β-galactosylation to successfully fashion the Galβ(1→3)GalNAc motif (entry 5, Table 2), with the cost of further manipulation to convert the azido protection to the desired acetamino group.[9]

Clearly, galactosamine derivatives with the 2-*N*-position protected with Ac, Troc, and TCA group or as an azide could be successfully 3-*O*-β-galactosylated in good yields and stereoselectivity. In contrast, when the galactosamine 2-*N*-position was protected with a Phth group, the galactosylation reaction failed to give the desired β-anomer, even when an acyl group was present at the galactosyl donor 2-*O*-position (Scheme 2). These results may suggest the great influence of the protecting group at the galactosamine 2-*N*-position on its 3-*O*-galactosylation. The major and shared difference of the Phth group from Ac, Troc, TCA and azido groups is the steric bulk. Based on these discoveries, we suspected that during the galactosylation of galactosamine, the steric effect might surpass NGPE to affect the stereochemical outcome. To probe this hypothesis and find a solution to the problem, we designed and performed the following systematic studies.

First, we probed the reaction between glycosyl donor **1a** and glycosyl acceptor **2b**[29] with the Phth group in **2a** substituted for the Troc group, which should be less sterically demanding but as effective as Phth in directing stereoselective glycosylation.[26] Under the preactivation condition used in Scheme 2, the reaction between **1a** and **2b** went smoothly to give β-linked disaccharide **3a** ($J_{H1,2}$ = 7.9 Hz at δ = 4.86 ppm) as the only isomer in a very good yield (87%). This experiment clearly indicated that the bulky Phth group in **2a** was probably the major reason to cause failed glycosylation in Scheme 2.

Since steric interaction should involve two parties, next, we furthered our investigation to examine the influence of substituents in the glycosyl donor on this glycosylation reaction. Accordingly, we probed the reaction of **2a** with thioglycoside **1b** (Scheme 4), which had an acetyl group, instead of a Bz group, at the 2-*O*-position under the same condition and expected to obtain even better results than Scheme 3 due to reduced steric interactions. To our surprise, however, this reaction produced only a trace amount of desired disaccharide **3b** observed by mass spectrometry. TLC monitoring and analysis of the reaction proved that the activation of glycosyl donor **1b**, just like the activation of **1a**, was successful and complete, but the activated donor and acceptor did not react at −78 °C and no reaction was observed

even after the mixture was allowed to warm to room temperature. The reaction became very complex after overnight stirring at room temperature, whereas the acceptor was recovered.

To find out how the benzylidene ring in the glycosyl donor might have affected the reaction,[30] we synthesized tetraacetylated thioglycoside **1c** and studied its reaction with **2b** under the same preactivation condition. To our surprise again, this reaction was rather complex with orthoester **4** (Scheme 4), instead of the desired disaccharide, isolated as a major product in a low yield. The structure of **4** was characterized by NMR spectrometry. For example, the chemical shift of proton signal of the orthoester methyl group was 1.67 ppm opposed to that of the acetyl methyl groups (>2.0 ppm), and the chemical shift of the orthoester C-1 signal was 121.3 ppm as compared to 170 ppm for carbonyl signals of the three acetyl groups. Furthermore, the NOESY spectrum of **4** also showed strong NOE correlations between the orthoester methyl protons and H-3 and H-3′ protons.

It is well known that when 2-*O*-acylated sugars are used as donors, some glycosylation reactions, especially those of sterically demanding or relatively unreactive substrates, go through the mechanism involving orthoester intermediates that can rearrange to afford the desired glycosides under acidic conditions.[31–33] Alternatively, orthoester may be obtained as a common byproduct of glycosylation.[34] The isolation of orthoester **4** from the reaction mixture of **1c** and **2b** suggested that it was probably quite difficult for the acceptor **2b** to attack the anomeric carbon in the activated intermediate derived from **1c** but to attack the *C*-1 position of the participating 2-*O*-acetyl group instead. On the other hand, orthoester **4** could not rearrange to form the corresponding glycoside under the neutral or mildly basic condition, namely, in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) employed to neutralize the acid formed from the glycosylation reaction. This rationale can also be used to explain the low reactivity between **1b** and **2a** which in fact was even more sterically demanding than **2b**. We further hypothesized that the reaction of **1b** and **2a** might have formed the corresponding orthoester as well, but it was presumably unstable because of increased steric interactions and ring strains to give rise to complex results.

To gain more insights into this glycosylation reaction, we converted thioglycoside **2a** into methyl glycoside **2c** and investigated its coupling with **1b** and **1c** (Scheme 5), which allowed for glycosylation reactions under normal conditions, namely, in the presence of Lewis acids. Both reactions using *N*-iodosuccinimide (NIS) and silver triflate (AgOTf) as the promoters, which were also reported to promote orthoester rearrangement to generate glycosides,[35] proceeded smoothly to give the desired disaccharides **3c** ($J_{H1,2}$ = 8.3 Hz at δ = 4.57 ppm) and **3d** ($J_{H1,2}$ = 8.1 Hz at δ = 4.57 ppm) in good yields and stereoselectivity (β only). These results supported a hypothesis that in addition to steric factors, the failure of reactions in Scheme 4 to produce the desired disaccharides was probably because of the reaction condition used, which could not promote orthoester rearrangement.

To further confirm the above rationale, we studied the glycosylation reactions of **2c** with more sterically demanding donors **1a** and **1c** under the promotion of NIS and AgOTf (Scheme 6), which turned out to be very sluggish and gave only low yields of the desired disaccharides **3c** (25%, β major) and **3f** (20%, α:β = 1:1). A comparison of the results of Schemes 5 and 6 clearly indicated that steric interactions were indeed the major factor to

cause the failure of 3-*O*-galactosylation of galactosamine when larger protecting groups were present at the *C*-2-positions of both the donor and the acceptor.

The above discoveries together with literature results led us to conclude that when the Gal 2-*O*-position and galactosamine 2-*N*-position were both protected with large groups, such as the Bz and Phth groups respectively, severe steric interactions between the donor and the acceptor as shown in Figure 1A prohibited effective glycosylation, to result in poor reaction yields. The relatively poor stereoselectivity might result from a S_N2 type of glycosylation reaction, namely attack at the anomeric carbon of the oxonium intermediate by less sterically demanding nucleophiles such as triflate to form reactive glycosyl triflate followed by nucleophilic displacement of the triflate with galactosamine acceptor. Such reaction mechanism was reported previously.[36,37] This was applicable to glycosylation reactions carried out under both preactivation conditions (Scheme 2) and conventional conditions (Scheme 6). When the 2-*N*-Phth group in galactosamine acceptors was replaced with a smaller protecting group, such as Ac (Table 1) and Troc (Table 2 and Scheme 3), which would reduce steric hindrance, the reactions went smoothly under both conditions to afford the desired *β*-linked oligosaccharides in very good yields and stereoselectivity. The result of Scheme 3 further suggested that the glycosylation reaction might go through a mechanism of direct acceptor attack at the anomeric carbon of the oxonium cation intermediate of the activated donor with NGPE involvement (Figure 1B) to give high stereoselectivity, because the reaction was performed under mildly basic conditions that did not allow for orthoester rearrangement. This should be reasonable since a large group linked to the carbonyl carbon of the oxonium cation would make acceptor attack at the carbonyl carbon unfavorable. On the other hand, when the donor had an acetyl group at the 2-*O*-position, its reaction with acceptors having the large Phth group at the 2-*N*-position might go through the orthoester intermediate (Figure 1C), because nucleophilic attach at the less sterically hindered carbonyl carbon would become more favorable. This was supported by the isolation of a substantial amount of orthoester **4** and the complex results from the reactions in Scheme 4 under the preactivation condition that prohibited orthoester rearrangement to form glycosides. However, when glycosylation reactions were performed under acidic conditions that could promote orthoester rearrangement, they afforded the desired glycosides in good yields and excellent stereoselectivity (Scheme 5). Finally, when the protecting groups at both the Gal 2-*O*-position and the galactosamine 2-*N*-position were small, literature results (Tables 1 and 2) showed that the reactions gave the desired glycosides in relatively good yields and stereoselectivity, presumably through the mechanism outlined in Figure 1B.

In summary, we have systematically studied and analyzed the steric factors that might affect 3-*O*-*β*-glycosylation of galactosamine with galactose donors having acyl protecting groups at the 2-*O*-position, of which the NGPE failed in several cases to work or to assist stereoselective 1,2-*trans*-glycosylation. The failure of NGPE in directing stereoselective glycosylation was usually ascribed to solvent effects[9] or conformational influences.[8] Our results demonstrated that during 3-*O*-galactosylation of galactosamine steric interactions between the glycosyl donor and acceptor were also vital. Consequently, 2-*O*-benzylated galactosyl donors could not be used to react with galactosamine acceptors having 2-*N*-Phth protection effectively to give the desired 1,2-*trans*-glycosylation products, because the steric

factors might have outweighed the NGPE. Replacing the 2-*N*-Phth or 2-*O*-Bz group or both with less steric demanding protecting groups could alter the reaction pathways and put the NGPE back into action. These results may also be used to interpret the "matched and mismatched glycosylation" theory.[38]

It is also worth noting that 3-*O*-*β*-galactosylation of galactosamine derivatives could proceed smoothly for a variety of donors and acceptors under acidic conditions except for the 2-*O*-benzoylated donor and 2-*N,N*-phthalylated acceptor pair as shown in Scheme 6. Therefore, under these glycosylation conditions, a variety of combinations of protecting groups for the glycosyl donor and acceptor can be selected. However, under neutral and basic glycosylation conditions, relatively large acyl groups should be used to protect the donor 2-*O*-position in order to suppress orthoester formation. This has put limitations on our selection of the proper protections for the acceptor 2-*N*-position, that is, only small protecting groups should be used. Consequently, this work helped us develop some useful principles that can not only provide general guidelines for our future design of successful synthetic schemes for the Gal*β*(1→3)GalNAc motif but also help the synthesis of other similarly *β*-1,3-linked disaccharides.

Experimental Section

General Procedures

Chemicals and materials were purchased from commercial sources and were used as received without further purification unless otherwise noted. Molecular sieves 4Å (MS 4Å) were flame-dried under high vacuum and used immediately after cooling to rt under a N_2 atmosphere. Analytical TLC was carried out on silica gel 60Å F_{254} plates with detection by a UV detector and/or by charring with 10% (v/v) H_2SO_4 in EtOH. Flash column chromatography was performed on silica gel 60 (230–400 Mesh). NMR spectra were acquired on a 400, 500 or 600 MHz machine with chemical shifts reported in ppm (δ) and referenced with $CHCl_3$ (^{1}H NMR δ 7.26 ppm) or $CDCl_3$ (^{13}C NMR δ 77.0 ppm). Peak and coupling constant assignments are based on ^{1}H NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC experiments.

***p*-Tolyl (2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranosyl)-(1→3)- 4,6-*O*-benzylidene-2-(2,2,2-tri-chloroethoxycarbonylamido)-2-deoxy-1-thio-*β*-D-galactopyranoside (3a)—**Galactosyl donor **1a** (55 mg, 0.1mmol) and freshly activated MS 4Å (1.0 g) in CH_2Cl_2 (3 mL) was stirred at rt for 1 h. The mixture was cooled to −78 °C before extra dry silver triflate (77 mg, 0.3 mmol, 3.0 eq.) in acetonitrile (0.3 mL) was added to the reaction mixture, which was followed by the addition of *p*-TolSCl (16 μL, 0.1 mmol) 15 min later. After complete activation of galactosyl donor **1a** was confirmed by TLC, the CH_2Cl_2 solution (1 mL) of galactosamine acceptor **2b** (50 mg, 0.09 mmol) and TTBP (25 mg, 0.1 mmol) were added. The reaction was kept at −78 °C until its completion (in about 1 h), which was indicated by the disappearance of **2b**, and the reaction mixture was filtered through a Celite pad. The solid was thoroughly washed with DCM and the filtrates were combined and washed with saturated aq. solution of $NaHCO_3$. The organic layer was then dried over Na_2SO_4 and the solvent was removed by vacuum followed by purification of

the residue with silica gel column chromatography (hexane/EtOAc = 1/1) to afford **3a** as a white solid (86 mg, 87% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (d, 2H, J = 7.8 Hz, ArH), 7.59-7.54 (m, 3H), 7.45-7.35 (m, 7H, ArH), 7.22-7.16 (m, 10H, ArH), 6.97 (d, 2H, J = 7.8 Hz, ArH), 5.60 (dd, 1H, J = 9.9, 8.5 Hz, H-2), 5.53 (s, 1H, PhCH), 5.37 (s, 1H, PhCH), 5.27 (d, 1H, J = 7.7 Hz, NH), 4.88 (d, 1H, J = 10.7 Hz, H-1′), 4.86 (d, 1H, J = 7.9 Hz, H-1), 4.82 (d, 1H, J = 12.3 Hz, CCl_3CH_2), 4.67 (d, 1H, J = 13.5 Hz, $PhCH_2$), 4.57 (d, 1H, J = 13.5 Hz, $PhCH_2$), 4.45 (d, 1H, J = 3.2 Hz, H-4′), 4.40-4.27 (m, 4H. CCl_3CH_2, H-6a, H-3′, H-6a′), 4.23 (d, 1H, J = 2.9 Hz, H-4), 4.08 (d, 1H, J = 12.4Hz, H-6b), 3.98 (d, 1H, J = 13.0 Hz, H-6b′), 3.64 (dd, 2H, J = 10.4, 3.8 Hz, H-3, H-2′), 3.46 (s, 1H, H-5), 3.39 (m, 1H, H-5′), 2.29 (s, 3H, $PhCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.2, 154.0, 137.9, 137.8, 137.5, 133.6, 133.2, 129.9, 129.8, 129.6, 129.0, 128.5, 128.4, 128.1, 128.0, 127.7, 126.5, 101.0, 100.9, 100.0, 96.0, 85.4, 77.2, 77.19, 75.1, 74.1, 72.7, 71.1, 70.3, 70.0, 69.3, 69.2, 66.9, 51.5, 21.1; HR ESI-TOF-MS: $[M + Na]^+$ $C_{50}H_{48}Cl_3NO_{12}SNa^+$ m/z calcd 1014.1855, found 1014.1898.

_p_-Tolyl 3-_O_-[(3,4,6-tri-_O_-acetyl-β-D-galactopyranos-1,2-_O_-orthoacet)-yl]-4,6-_O_-benzylidene-2-(2,2,2-tri-chloroethoxycarbonylamido)-2-deoxy-1-thio-β-D-galactopyranoside (4)—Compound **4** was obtained as a white solid (yield 30%) from the reaction between **1c** and **2b** under the same conditions described above for the synthesis of **3a**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (d, 2H, J = 8.2 Hz, ArH), 7.47-7.45 (m, 2H, ArH), 7.38-7.36 (m, 3H, ArH), 7.03 (d, 2H, J = 8.0 Hz, ArH), 5.72 (d, 1H, J = 4.9 Hz, H-1), 5.56 (s, 1H, PhCH), 5.39 (m, 1H, J = 3.4, 2.0 Hz, H-4), 5.37 (d, 1H, J = 10.0 Hz, H-1′), 5.19 (d, 1H, J = 7.2 Hz, NH), 5.00 (dd, 1H, J = 7.0, 3.5 Hz, H-3), 4.78 (d, 1H, J = 12.2 Hz, CCl_3CH_2), 4.68 (d, 1H, J = 12.2 Hz, CCl_3CH_2), 4.46 (dd, 1H, J = 10.3, 3.1 Hz, H-3′), 4.39 (dd, 1H, J = 12.2, 2.0 Hz, H-6a′), 4.37 (d, 1H, J = 3.1 Hz, H-4′), 4.26-4.23 (m, 2H. H-2, H-5), 4.16 (dd, 1H, J = 11.4, 6.5 Hz, H-6a), 4.07 (dd, 1H, J = 11.7, 6.5 Hz, H-6b), 4.05 (dd, 1H, J = 12.5, 1.2 Hz, H-6b′), 3.58 (s, 1H, H-5′), 3.39 (m, 1H, H-2), 2.34 (s, 3H, $PhCH_3$), 2.06 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.67 (s, 3H, CH_3, orthoester); ^{13}C NMR (125 MHz, $CDCl_3$): δ170.4, 170.0, 169.8, 153.7, 138.4, 137.9, 134.2, 129.8, 129.1, 126.5, 121.3, 100.9,97.7, 95.3, 83.5, 75.1, 74.5, 71.1, 70.5, 69.9, 69.4, 69.2, 69.1, 65.7, 61.2, 51.3, 23.5, 21.2, 20.7, 20.68, 20.5; HR MALDI-TOF-MS: $[M + Na]^+$ $C_{37}H_{42}Cl_3NO_{15}SNa^+$ m/z calcd 900.1233, found 900.1273.

Methyl (2-_O_-acetyl-3-_O_-benzyl-4,6-_O_-benzylidene-β-D-galactopyranosyl)-(1→3)-4,6-_O_-benzylidene-2-deoxy-2-phthalimido)-β-D-galactopyranoside (3c)—A mixture of galactosyl donor **1b** (68 mg, 0.12 mmol), galactosamine acceptor **2c** (41 mg, 0.1 mmol) and freshly activated MS 4Å (1.0 g) in CH_2Cl_2 (3 mL) was stirred at rt for 1 h. The mixture was cooled to −78 °C before NIS (30 mg, 0.13 mmol) and dry AgOTf (8 mg, 0.03 mmol) were added to the reaction mixture. The reaction was allowed to warm to −30 °C and stirred for 1 h, when TLC indicated the completion of reaction. The reaction was quenched with Et_3N, followed by filtration through a pad of Celite. The solid was thoroughly washed with DCM and the filtrates were combined and washed with saturated aq. solution of Na_2SO_3, water, and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under vacuum followed by purification of the residue with silica gel column chromatography (hexane/EtOAc = 1/1) to afford the **3c** as a white solid (70 mg, 88%

yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.86-7.83 (m, 2H), 7.75-7.73 (m, 2H, ArH), 7.61-7.59 (m, 2H, ArH), 7.53-7.51 (m, 2H, ArH), 7.35-7.33 (m, 7H, ArH), 7.26-7.19 (m, 4H, ArH), 5.61 (s, 1H, PhCH), 5.40 (s, 1H, PhCH), 5.22 (dd, 1H, J = 10.0, 8.1 Hz, H-2), 5.14 (d, 1H, J = 8.5 Hz, H-1′), 4.82 (dd, 1H, J = 11.2, 3.6 Hz, H-3′), 4.73 (dd, 1H, J = 11.2, 8.5 Hz, H-2′), 4.57 (d, 1H, J = 8.3 Hz, H-1), 4.56 (d, 1H, J = 12.6 Hz, $PhCH_2$), 4.49 (d, 1H, J = 12.6 Hz, $PhCH_2$), 4.48 (d, 1H, J = 3.4 Hz, H-4′), 4.37 (dd, 1H, J = 12.2, 1.2, Hz, H-6a′), 4.11 (dd, 1H, J = 12.2, 1.2, Hz, H-6b′), 4.06 (d, 1H, J = 3.3 Hz, H-4), 3.99 (d, 1H, J = 11.4 Hz, H-6a), 3.91 (d, 1H, J = 11.4 Hz, H-6b), 3.59 (s, 1H, H-5′), 3.44 (s, 3H, OMe), 3.25 (s, 1H, H-5), 1.58 (s, 3H, OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 169.5, 128.3, 128.2, 128.1, 127.4, 126.6, 126.5, 101.3, 100.9, 100.5, 99.2, 77.5, 75.6, 73.0, 72.97, 70.9, 70.3, 69.3, 69.2, 69.1, 68.9, 66.9, 66.8, 56.1, 51.7, 29.7, 25.6, 20.3; HR ESI-TOF-MS: $[M + Na]^+$ $C_{44}H_{43}NO_{13}Na^+$ m/z calcd 816.2627, found 816.2655.

Methyl (2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-galactopyranoside (3d)—Compound **3d** was obtained as a white solid (65 mg, 85%) from the reaction between **1d** and **2c** under the same conditions described above for the synthesis of **3c**. ^{1}H NMR (500 MHz, CDCl3): δ 7.88-7.86 (m, 2H,, ArH), 7.76-7.74 (m, 2H, ArH), 7.62-7.60 (m, 2H, ArH), 7.42-7.36 (m, 3H,, ArH), 5.61 (s, 1H, PhCH), 5.30 (dd, 1H, J = 3.5, 1.2 Hz, H-4), 5.10 (dd, 1H, J = 10.5, 7.8 Hz, H-2), 5.06 (d, 1H, J = 8.0 Hz, H-1′), 4.83 (dd, 1H, J = 10.4, 3.4 Hz, H-3), 4.76-4.74 (m, 2H,, H-3′, H-2′), 4.57 (d, 1H, J = 8.1 Hz, H-1), 4.39 (ddd, 2H, J = 6.0, 4.9, 1.3 Hz, H-4′, H-6a′), 4.13 (dd, 1H, J = 12.3, 1.5 Hz, H-6b′), 4.10-4.05 (m, 2H, H-6a, H-6b,), 3.82 (dt, 1H, J = 6.5, 1.1 Hz, H-5), 3.60 (s, 1H, H-5′), 3.44 (s, 3H, OCH_3), 2.13 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.88 (s, 3H, $COCH_3$), 1.42 (s, 3H, $COCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.3, 168.9, 167.4, 137.9, 134.4, 134.3, 131.9, 131.8, 129.1, 128.3, 126.6, 123.9, 123.5, 101.6, 101.1, 99.3, 75.8, 75.0, 71.1, 70.9, 69.4, 68.7, 67.0, 66.9, 61.4, 56.3, 51.6, 32.1, 29.8, 29.5, 22.8, 20.88, 20.86, 20.7, 20.6, 19.9; HR HR ESI-TOF-MS: $[M + Na]^+$ $C_{36}H_{39}NO_{16}Na^+$ m/z calcd 764.2161, found 764.2194.

Methyl (2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*α,β*-D-galactopyranoside (3e)—A mixture of galactosyl donor **1a** (68 mg, 0.12 mmol), galactosamine acceptor **2c** (41 mg, 0.1 mmol) and freshly activated MS 4Å (1.0 g) in CH_2Cl_2 (3 mL) was stirred at rt for 1 h. The mixture was cooled to −78 °C before NIS (30 mg, 0.13 mmol) and dry AgOTf (8 mg, 0.03 mmol) were added. The mixture was allowed to warm to −30 °C and stirred for 1 h, whereas TLC showed incomplete reaction. Thus, the mixture was warmed to rt and stirred at rt overnight. TLC showed that the acceptor disappeared but no further progress of the reaction was observed. The reaction was quenched with Et_3N followed by filtration through a pad of Celite. The solid was thoroughly washed with DCM and the filtrates were combined and washed with saturated aq. solution of Na_2SO_3, water, and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under vacuum, which was followed by purification of the residue with silica gel column chromatography (hexanes/EtOAc = 1/1) to **3e** as a white solid (22 mg, 25% yield) along with recovered acceptor **2c** (about 50%). Purified major isomer **3e-*β***: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.93-7.86 (m, 4H), 7.78-7.76 (m, 2H, ArH), 7.50-7.39 (m, 5H, ArH), 7.33-7.16 (m, 13H, ArH), 5.50 (d, 1H, J = 4.0 Hz,

H-1), 5.37 (dd, 1H, *J* = 10.6, 4.0 Hz, H-2), 5.26 (s, 1H, PhCH), 5.09 (d, 1H, *J* = 8.2 Hz, H-1′), 5.01 (s, 1H, PhCH), 4.72-4.63 (m, 3H, H-2′, H-3′, $PhCH_2$), 4.58 (d, 1H, *J* = 12.2 Hz, $PhCH_2$), 4.25 (dd, 1H, *J* = 12.3, 0.7, Hz, H-6a′), 4.03 (dd, 1H, *J* = 10.5, 3.4, Hz, H-3), 3.98 (m, 2H, H-4, H-4′), 3.82 (dd, 1H, *J* = 12.2, 1.6 Hz, H-6b′), 3.47 (s, 1H, H-5′), 3.41 (s, 3H, OMe), 3.36 (dd, 1H, *J* = 12.5, 1.3 Hz, H-6a), 3.28 (s, 1H, H-5), 2.98 (dd, 1H, *J* = 12.2, 1.7 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.5, 167.9, 166.0, 137.6, 137.4, 134.5, 134.3, 133.2, 131.8, 131.6, 129.7, 129.6, 128.9, 128.6, 128.4, 128.3, 128.1, 128.0, 127.6, 127.5, 126.2, 126.1, 123.5, 123.4, 100.8, 100.3, 99.1, 98.7, 76.4, 74.0, 73.6, 73.4, 72.1, 70.8, 69.0, 68.4, 66.4, 63.4, 56.4, 52.3, 36.6, 29.7; HR ESI-TOF-MS: $[M + Na]^+$ $C_{49}H_{45}NO_{13}Na^+$ *m*/*z* calcd 878.2783, found 878.2804.

Methyl (2,3,4,6-tetra-*O*-benzoyl-*α*-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*α*,*β*-D-galactopyranoside (3f)—Compound **3f** was obtained as a white solid (85% yield for both isomers) from the reaction between **1c** and **2c** under the same conditions described above for the synthesis of **3e**, and the two anomers were separated by silica gel column chromatography (hexane/EtOAc = 1/1). **3f-*α***: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95-7.92 (m, 4H, ArH), 7.88-7.87 (m, 1H, ArH), 7.82-7.80 (m, 3H, ArH), 7.44-7.37 (m, 7H, ArH), 7.31-7.26 (m, 2H, ArH), 7.20-7.11 (m, 7H, ArH), 5.79 (d, 1H, *J* = 2.2 Hz, H-4), 5.74 (dd, 1H, *J* = 10.6, 3.3 Hz, H-3), 5.72 (d, 1H, *J* = 3.9 Hz, H-1), 5.50 (dd, 1H, *J* = 11.0, 3.9 Hz, H-2), 5.17 (s, 1H, PhCH), 5.14 (d, 1H, *J* = 7.6 Hz, H-2′), 4.85-4.82 (m, 2H, H-1′, H-3′), 4.28 (d, 1H, *J* = 12.3 Hz, H-6a′), 4.20-4.18 (m, 2H, H-5, H-4′), 4.02 (dd, 1H, *J* = 10.5, 8.1 Hz, H-6a), 3.93-3.90 (m, 2H,, H-6b, H-6b′), 3.43 (s, 3H, OCH_3), 3.40 (s, 1H, H-5′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.0, 167.5, 166.3, 165.4, 165.3, 165.0, 137.1, 134.4, 134.3, 133.5, 133.4, 133.2, 133.0, 131.5, 131.4, 129.8, 129.77, 129.74, 129.6, 129.4, 129.1, 129.0, 128.6, 128.5, 128.46, 128.4, 128.35, 128.3, 128.2, 127.9, 125.9, 124.0, 123.1, 100.2, 99.1, 95.3, 73.8, 72.1, 69.2, 69.0, 68.3, 67.8, 67.4, 66.4, 61.3, 60.4, 56.3, 51.8, 29.7; HR ESI-TOF-MS: $[M + Na]^+$ $C_{56}H_{47}NO_{16}Na^+$ *m*/*z* calcd 1012.2787, found 1012.2790. **3f-*β***: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95-7.93 (m, 2H, ArH), 7.89-7.87 (m, 1H, ArH), 7.82-7.78 (m, 4H, ArH), 7.66-7.65 (m, 1H, ArH), 7.62-7.53 (m, 7H, ArH), 7.40-7.30 (m, 12H, ArH), 7.13-7.10 (m, 2H, ArH), 6.00 (d, 1H, *J* = 5.2 Hz, H-1), 5.63-5.62 (m, 1H), 5.51 (s, 1H, PhCH), 5.31 (dd, 1H, *J* = 5.9, 4.2 Hz), 5.07 (d, 1H, *J* = 8.5 Hz, H-1′), 4.58 (dd, 1H, *J* = 11.0, 8.8 Hz), 4.52 (dd, 1H, *J* = 114, 6.8 Hz), 4.39-4.36 (m, 2H), 4.34-4.32 (m, 2H), 4.26 (dd, 1H, *J* = 10.5, 5.6 Hz), 4.16 (d, 1H, *J* = 3.8 Hz), 4.04 (dd, 1H, *J* = 12.2, 1.5 Hz), 3.44 (s, 1H), 3.39 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.2, 167.4, 165.9, 165.0, 164.9, 137.7, 135.5, 134.0, 133.8, 133.5, 133.3, 133.2, 131.9, 131.8, 129.8, 129.75, 129.72, 129.4, 129.0, 128.8, 128.4, 128.35, 128.3, 128.2, 126.4, 126.38, 125.8, 123.6, 122.9, 119.9, 101.3, 98.9, 98.3, 74.5, 72.7, 69.4, 69.2, 69.17, 68.6, 66.6, 66.0, 62.2, 56.2, 51.7, 29.7; HR ESI-TOF-MS: $[M + Na]^+$ $C_{56}H_{47}NO_{16}Na^+$ *m*/*z* calcd 1012.2787, found 1012.2811.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank NIH/NCI for the support (grant R01CA095142) of this research work.

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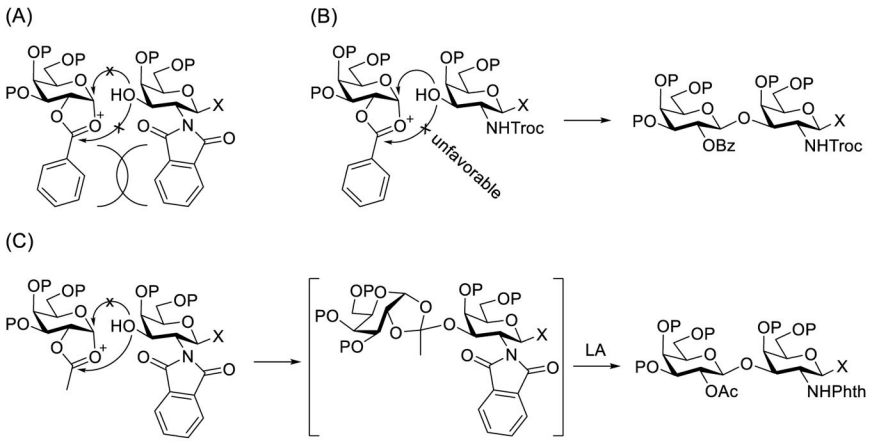
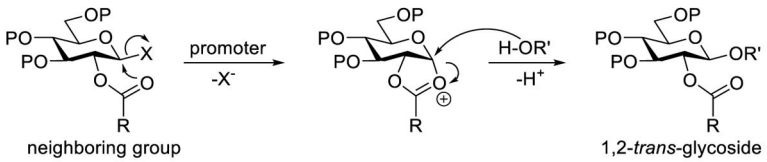
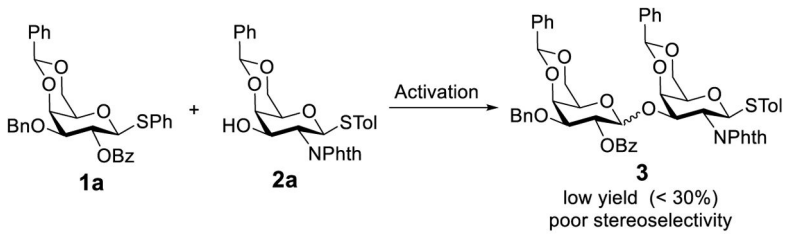


Figure 1.
Steric interactions during the synthesis of Galβ(1→3)GalN motif

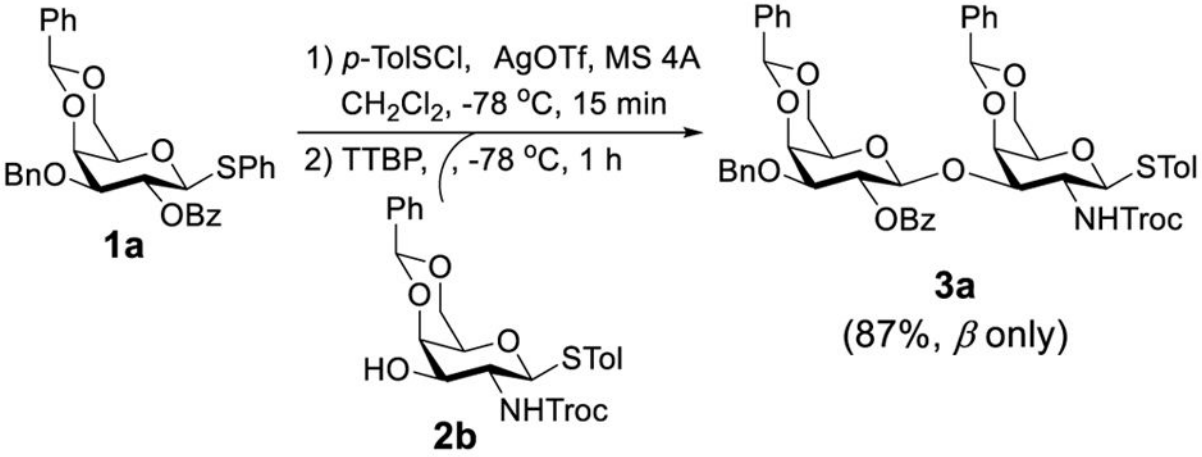


Scheme 1.
NGPE-directed 1,2-*trans*-glycosylation



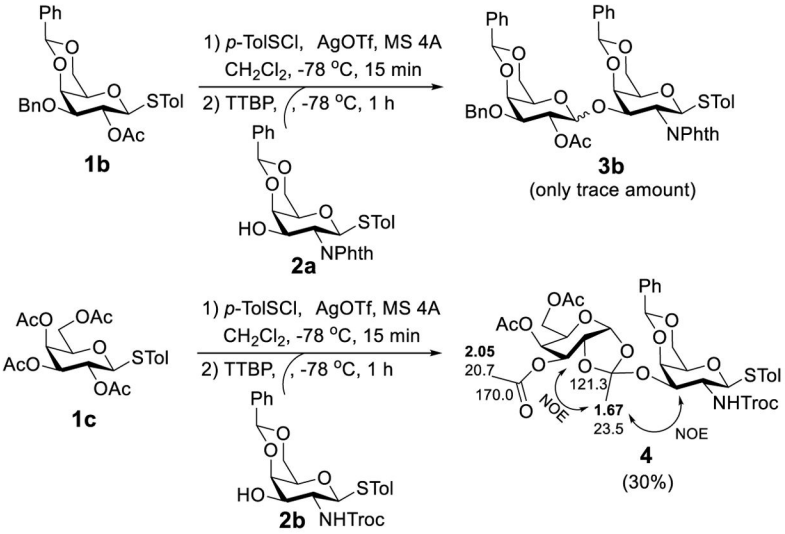
Scheme 2.

Attempted assembly of the Galβ(1→3)GalNAc motif in globo H synthesis



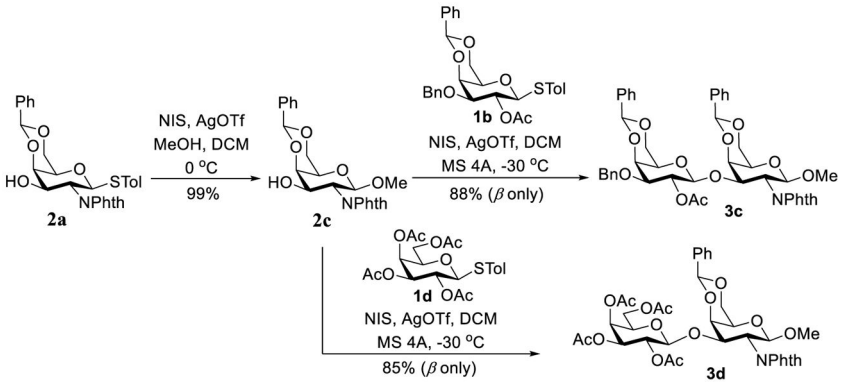
Scheme 3.

Galactosylation of less sterically demanding **2b** under preactivation condition



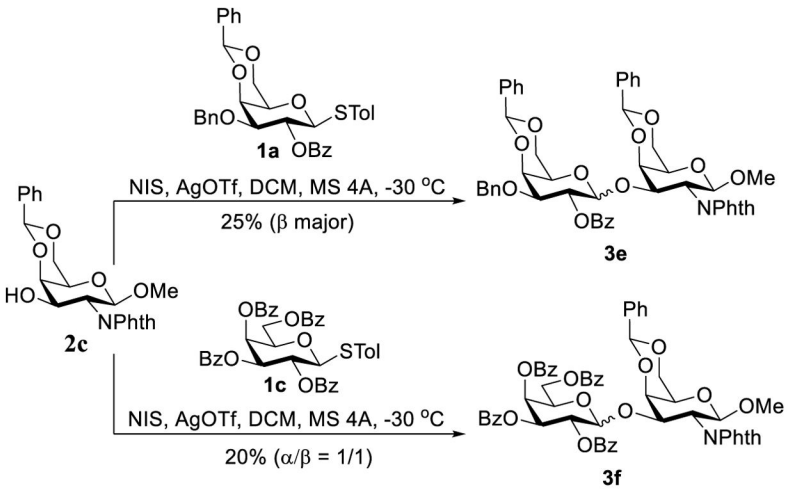
Scheme 4.

Galactosylation using 2-*O*-Ac-protected donors under preactivation condition



Scheme 5.

2-*O*-Ac-directed galactosylation under acidic condition



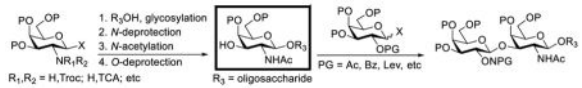
Scheme 6.

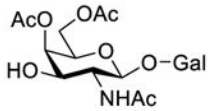
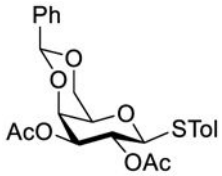
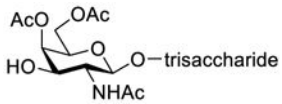
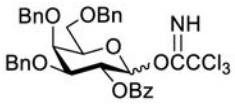
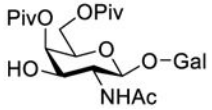
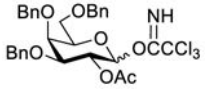
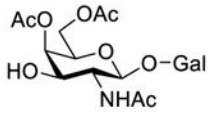
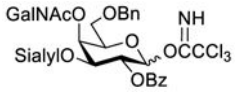
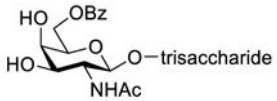
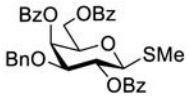
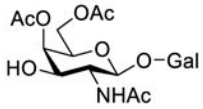
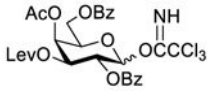
2-*O*-Bz-directed galactosylation under acidic conditions

J Carbohydr Chem. Author manuscript; available in PMC 2019 January 01.

Table 1

Construction of the Galβ(1→3)GalNAc motif using GalNAc in oligosaccharides or glycoconjugates as the galactosyl acceptor

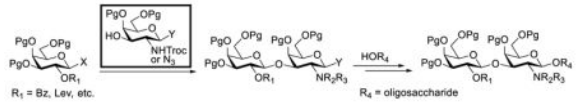


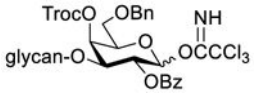
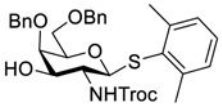
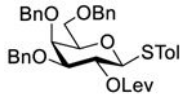
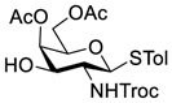
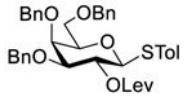
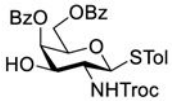
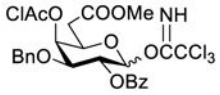
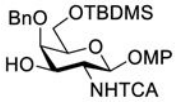
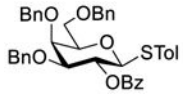
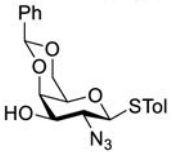
entry	glycosyl acceptor	galactosyl donor	promoter	β-isomer yield (%)	ref.
1			NIS/TfOH	63%	23
2			TMSOTf	52%	24
3			TfOH	84%	22
4			TMSOTf	75%	21
5			NIS/TfOH	66%	20
6			TMSOTf	77%	19

entry	glycosyl acceptor	galactosyl donor	promoter	β-isomer yield (%)	ref.
7			$Hg(CN)_2$	67%	18
8			TMSOTf	70%	17
9			$BF_3{\cdot}OEt_2$	40–60%	12,15
10			NIS TBDMSOTf	32% (2 steps)	17
11			TMSOTf	90%	16

Table 2

Construction of the Galβ(1→3)GalNAc motif using GalNTroc, GalNTCA, and 2-azido derivatives of galactosamine as glycosyl acceptors



entry	galactosyl donor	glycosyl acceptor	promoter	β-isomer yield (%)	ref.
1			TMSOTf	95%	28
2			NIS/TfOH	45%	27
3			NIS/TfOH	67% (2 steps)	26
4			TMSOTf	71%	13
5			TolSCl/AgOTf	72%	9