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DTBS EFFECT: THE UNIQUE STERICALLY DRIVEN DIRECTOR FOR α-GALACTOSYLATION

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Abstract – This review outlines the chemistry of 1,2-*cis* glycosylations and discusses the state-of-the-art methods, highlighting the DTBS(di-*tert*-butylsilylene)-directed α-galactosylation that was recently developed by our group. The key feature of our unique methodology includes extremely simple procedure, namely, the introduction of cyclic DTBS protecting group into 4- and 6-OH at *galacto*-type glycosyl donor. The power of the methodology was demonstrated by the syntheses of biologically relevant glycans including mucin-type glycosyl amino acids [α-*O*-GalNAc-Ser/Thr], α-galactosyl ceramides, globo-series glycolipids.

1. INTRODUCTION

In all organisms, a considerable number of glycoconjugates, which contain glycoproteins, glycolipids, and proteoglycans, play important roles in every biological event.[1] Typically, the glycans in glycoconjugates appear as *O*-glycoside linkages. There are two major types of *O*-glycosides–1,2-*cis* and 1,2-*trans* glycosides, as shown in Figure 1. Glycans are polyalcohol compounds that exhibit broad diversity with respect to the presence of a regioisomer and stereoisomer in the anomeric position. In the field of glycoscience, many glycochemists have made

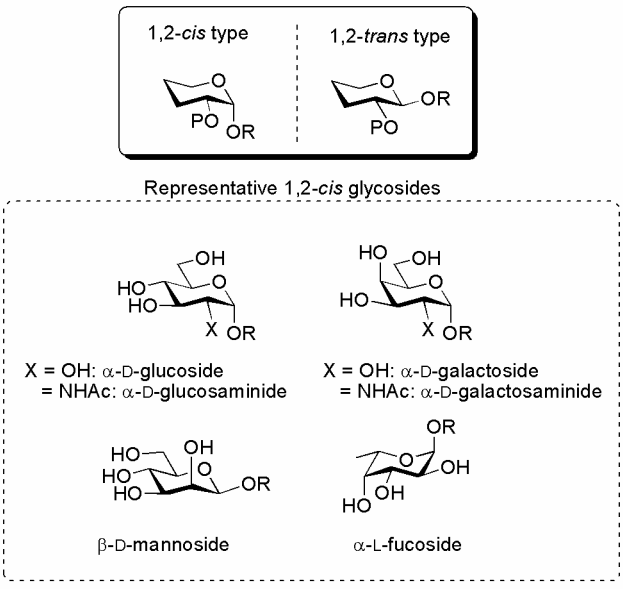


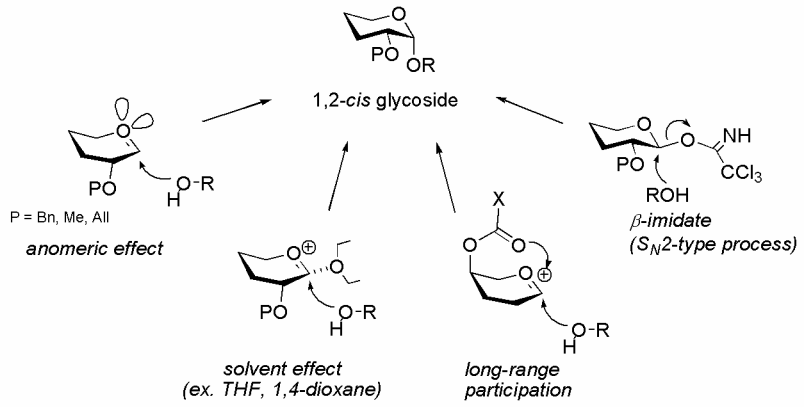
Figure 1. Structure of representative 1,2-*cis* glycosides

unfathomable efforts to efficiently construct an *O*-glycosidic linkage in a high regio-/stereo-selective manner.[2-4] For the syntheses of biologically attractive products, various elegant applications of the glycosylation reaction have been developed; some of these are listed below: In the case of *gluco*- and *galacto*-type glycoside formation, β(1,2-*trans*)-selectivity can be efficiently accomplished by exploiting the neighboring effect[5] of various acyl functionalities mounted on the C-2 hydroxyl group of a glycosyl donor or the nitrile solvent effect[6] under kinetically controlled conditions. For α(1,2-*cis*)-selective glycosylation, on the other hand, the glycosyl donors with non-neighboring functionality on C-2 have been commonly exploited in order to maximize the anomeric effect.[7] However, it is known that realizing efficient assemblies of α-sialoside,[8,9] β-mannoside,[10-13] and α-galactosaminide/glucosaminide[14] remains a considerably challenging task. Therefore, the development of innovative methodologies to accomplish such difficult glycosylations has been strongly desired in order to broaden the application scope of glycotechnology and glycobiology. This review focuses on the recent progress in α-glycosylation methods (Sections 2 and 3), and particularly discusses the DTBS(di-*tert*-butylsilylene)-directed α-selective galactosylation developed by our group (Section 4), which provides a solution to one of the long-standing problems in carbohydrate synthesis.

2. GENERAL METHODS FOR α-GLYCOSYLATION OF D-*gluco*-TYPE HEXOSES

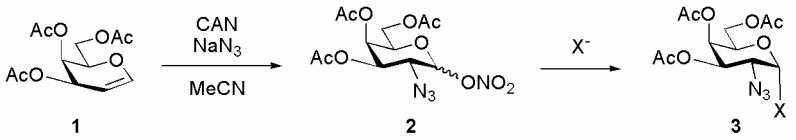
α-*Gluco*-type and β-*manno*-type glycosides represent 1,2-*cis*-glycosides of D-hexoses. The formation of these glycosidic linkages[15,16] are totally different synthetic issues with respect to anomeric stereocontrol–the anomeric effect is favored and utilized for α-glucosidation but is disfavored and suppressed for β-mannosidation. Herein, we outline the recent progress of the α-glycosidation of D-*gluco*-type sugars.

For α-*gluco*-type glycoside synthesis, as summarized in Scheme 1, glycosyl halides (chloride or bromide) have been proven to be versatile glycosylation agents by Koenigs and Knorr.[17] These glycosylations have been generally performed in the presence of mercury or silver salt, and Ag_2CO_3 has been occasionally used as an acid scavenger. The reactivity of the glycosyl halides is directly correlated to the nature of the protecting groups of sugar hydroxyl, especially at the C-2 position. The use of an ether-like substituent such as *O*-benzyl and *O*-methyl as a protecting group at the C-2 position is strongly recommended.



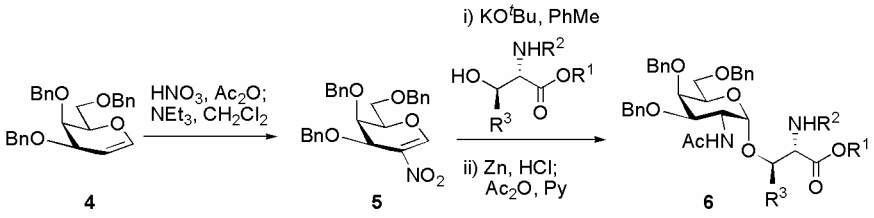
Scheme 1. Representative schemes for the formation of 1,2-*cis* glycosides

Moreover, it is important to choose appropriate solvents as the reaction media. Ether-type solvents[18] such as diethyl ether, THF and 1,4-dioxane coordinate with oxocarbenium ion to preferentially occupy the β-face due to the dipole-dipole interaction during the early stage of the glycosylation process. As a result, the attack of the acceptor is restricted to the α-face, thereby yielding the 1,2-*cis* glycoside. In addition, the long-range participation[19] of acyl-protecting groups at the C-4 or C-6 position of a glycosyl donor or the S_N2-type glycosylation accomplished using a reactive glycosyl β-trichloroacetimidate[20] donor has been exploited for the production of 1,2-*cis* glycoside.



Scheme 2. Preparation of 2-azido sugar by azidonitration method

However, it is well known that the synthesis of 1,2-*cis* glycoside on a 2-amino-2-deoxy sugar such as D-galactosamine (GalNAc) and D-glucosamine (GlcNAc) remains difficult due to the participating acetamide group, which is originally present at the C-2 position. Therefore, carbohydrate chemists have been required to circumvent this problem by using a nonparticipating azido-containing donor to fashion α(1,2-*cis*)-GalNAc/GlcNAc glycoside; this workaround was introduced by Paulsen in 1978.[21] However, the anomeric selectivity and yield of C-2 azide-based glycosylations varies greatly depending on the structures of the coupling partners; therefore, difficult separation procedures are occasionally required. In addition, the preparation of 2-azido sugar (**3**) in large scales tends to become problematic in terms of the overall yield or safety[22] (Scheme 2). In this context, Schmidt and co-workers have described a nitrogalactal-based approach, especially for the core sequence *O*-glycan, to skip the difficult task of azido introduction into the C-2 position.[23,24] In this method, 2-nitrogalactal (**5**) served as a good Michael acceptor during the reaction with the hydroxyl of a serine or threonine derivative, selectively undergoing conversion to an α-glycoside (Scheme 3). The nitro group of the resulting glycoside was hydrogenerated and acetylated to yield α-*N*-acetylgalactosaminyl Ser or Thr.



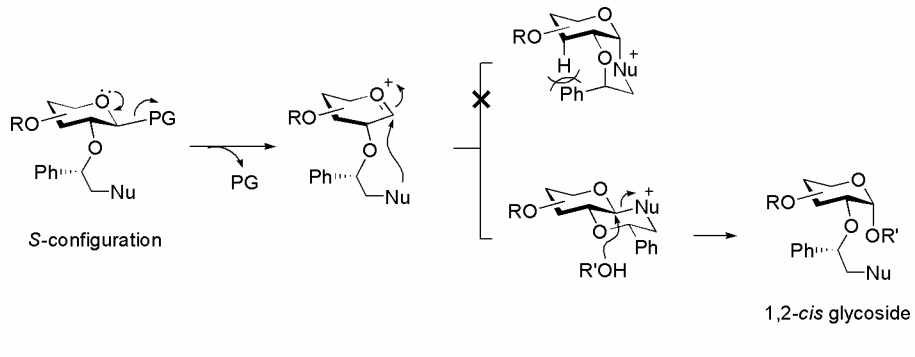
Scheme 3. Selective α-galactosidation with 2-nitroglycal donor

3. RECENT ADVANCES IN 1,2-*CIS*-GLYCOSYLATION

3-1. C-2-*S*-auxiliary Glycosyl Donor

Very recently, Boons and co-workers have developed a novel general method for the synthesis of 1,2-*cis* glycosides by utilizing the neighboring-group participation of the (1*S*)-phenyl-2-(phenylsulfanyl)ethyl group at the C2-hydroxyl position.[25] The participation of an (*S*)-ethoxycarbonylbenzyl auxiliary led to the formation of 1,2-*cis* glycosides, through a *trans*-fused dioxolenium ion intermediate. It was expected that the use of an auxiliary with (*S*)-stereochemistry would lead to the formation of a *trans*-fused dioxolenium ion exclusively, since the alternative *cis*-fused system would place the phenyl substituent in an axial position and induce unfavorable steric interactions. Displacement of the sulfonium ion by a hydroxyl group leads to the preferential and stereoselective formation of an α-glycoside (Scheme 4).

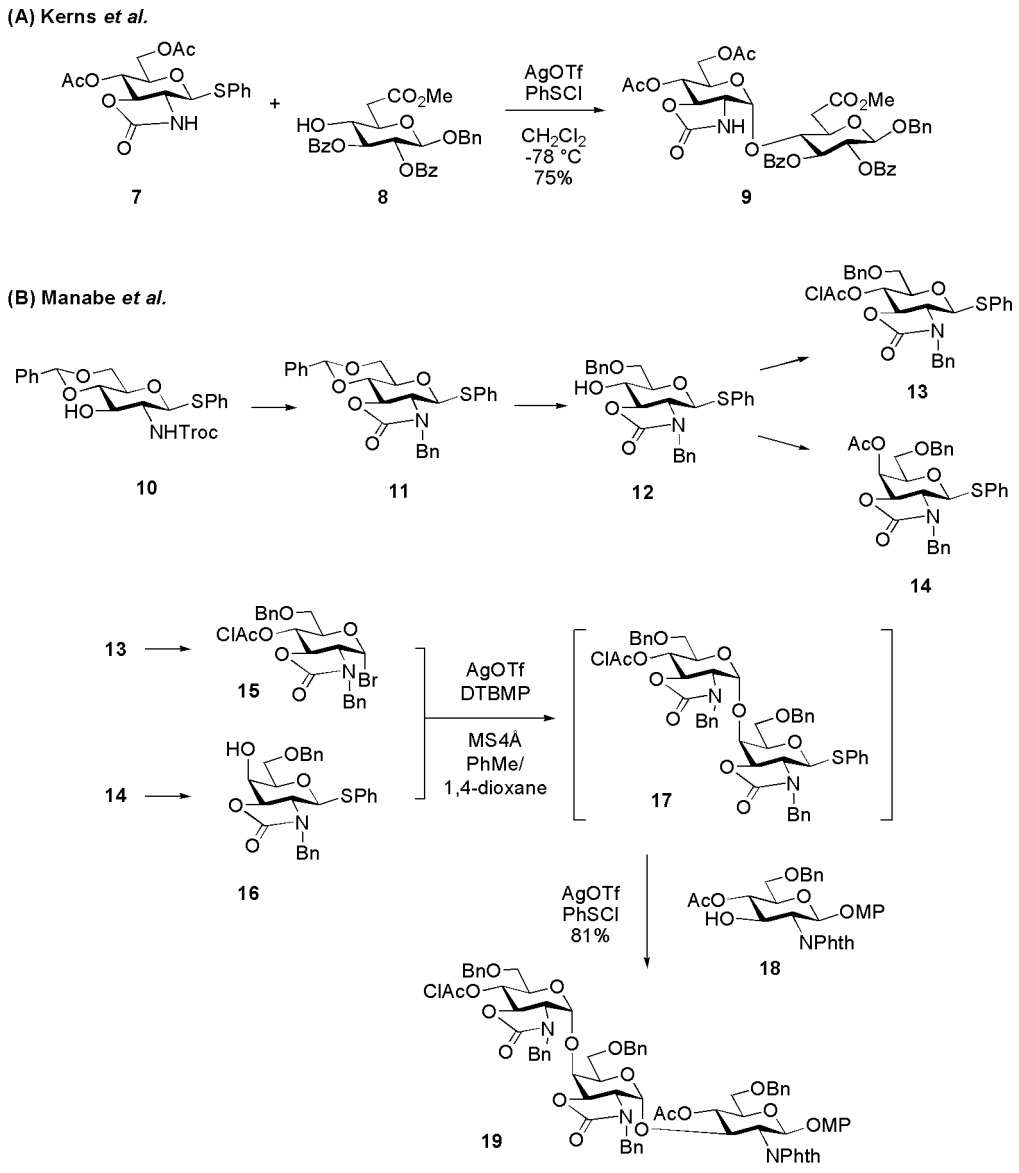
On the other hand, the use of an auxiliary with (*R*) configuration produced 1,2-*trans* glycosides; this glycosylation reaction proceeds through a *cis*-fused dioxolenium ion intermediate. They have also reported that the presence of an electron withdrawing substituent such as an ester at C-3 is of importance for the efficient formation of 1,2-*cis* glycoside. This is because the oxacarbenium ion is disfavored by the electron-withdrawing substituent at C-3, which may facilitate the participation.



Scheme 4. Concept of the glycosidation with C-2-*S*-auxiliary glycosyl donor

3-2. 2,3-Oxazolidinone Glycosyl Donor

In 2001, Kerns *et al*. reported[26] that a 2,3-oxazolidinone-protected D-glucosamine derivative, as a glycosyl donor, can be used for the formation of α(1,2-*cis*)-linked glycosides. They effectively synthesized heparin sulfate disaccharide by using 2,3-oxazolidinone donor (**7**), as shown in Scheme 5. Manabe *et al*. also demonstrated that *N*-benzyl-2,3-*trans*-oxazolidinone-fused glycosyl donors exhibit high α(1,2-*cis*) selectivities.[27] Due to their concise preparation as well as excellent nature as α-glycosyl donors as mentioned above, 2,3-oxazolidinone-fused derivatives are donors of choice for 1,2-*cis* glycosidic bond formation. It should be noted, however, that the anomeric stereoselectivities of glycosylation reactions occasionally depend on the activator and/or solvent system.



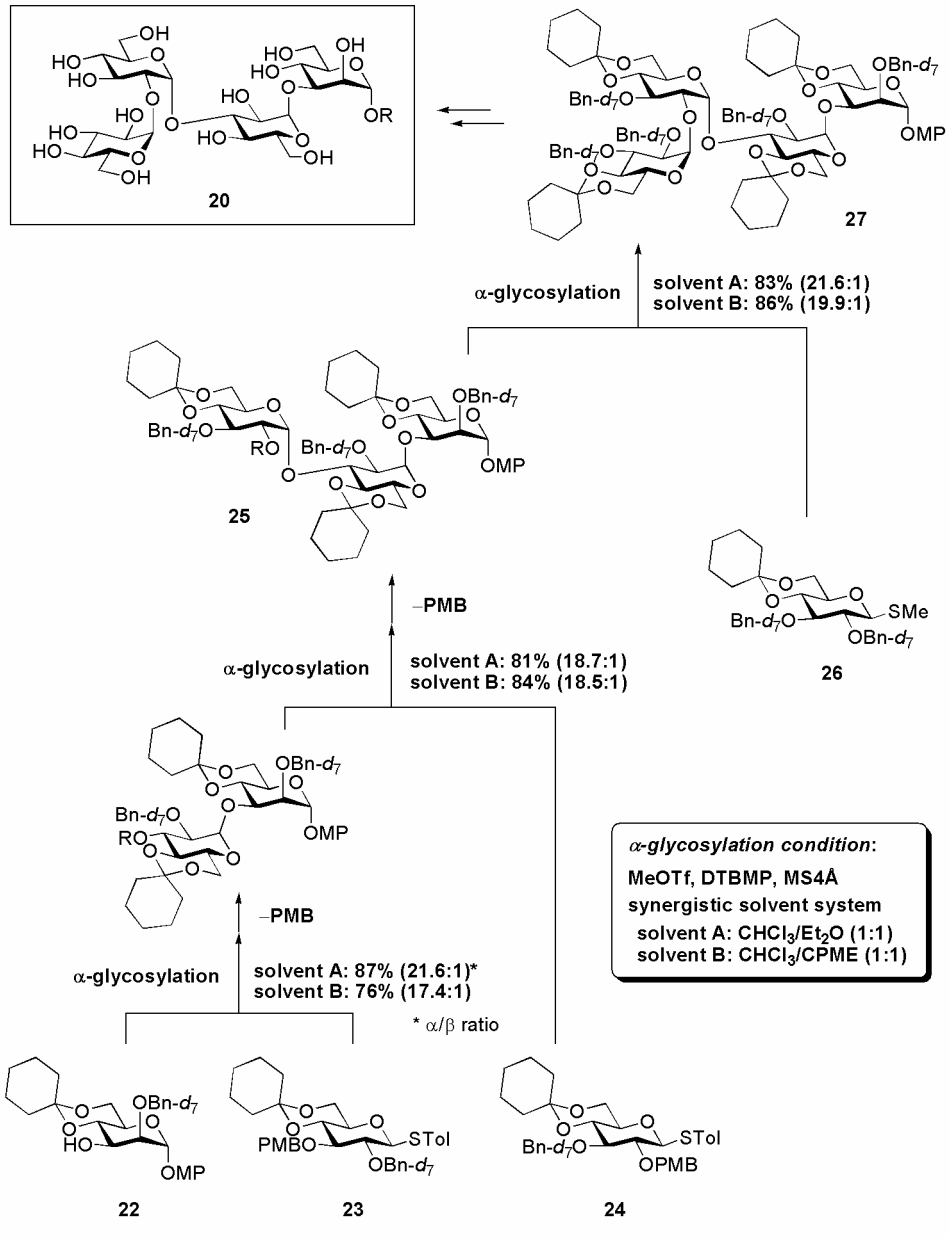
Scheme 5. 2,3-Oxazolidinone donors for the α-selective glycosylation

3-3. Synergistic solvent effect

As mentioned in Section 2, 1,2-*cis*-glycosides were preferentially obtained by virtue of the solvent effect of ethereal solvents such as diethyl ether, 1,4-dioxane,[28] CPME,[29] and THF. And the diastereoselectivity in these solvents can be understood considering the reverse anomeric effect. However, the extent of selectivity is difficult to predict precisely.

Very recently, Ishiwata *et al.* reported that combined halogenic and ethereal solvents had a synergistic effect to enhance the α-selectivity.[30] The α-glucosidation reactions in various mixed solvent system such as $CHCl_3/Et_2O$, $CHCl_3$/CPME, $(CH_2Cl)_2$/CPME, CH_2Cl_2/dioxane, and so on were investigated in detail. As a result, it was found that the subtle tuning of solvent composition, not only the mere presence of an

ethereal component, is of importance in maximizing the stereoselectivity in the glycosylation, while the α-selectivity was maximized when using a 1:1 mixture of $CHCl_3$ and ethereal solvents (Et_2O or CPME). The optimized solvent systems in $CHCl_3/Et_2O$ or $CHCl_3$/CPME were applied to the consecutive glycosylation reaction containing three α-glucosylations. Finally, they have accomplished the efficient synthesis of the tetrasaccharide Glc_3Man_1 [Glcα(1→2)Glcα(1→3)Glcα(1→3)Man] (**20**), which can be seen in *N*-linked glycans, using the combination of the synergistic solvent effect and their originally developed high-throughput screening (HTS) system[31] (Scheme 6).

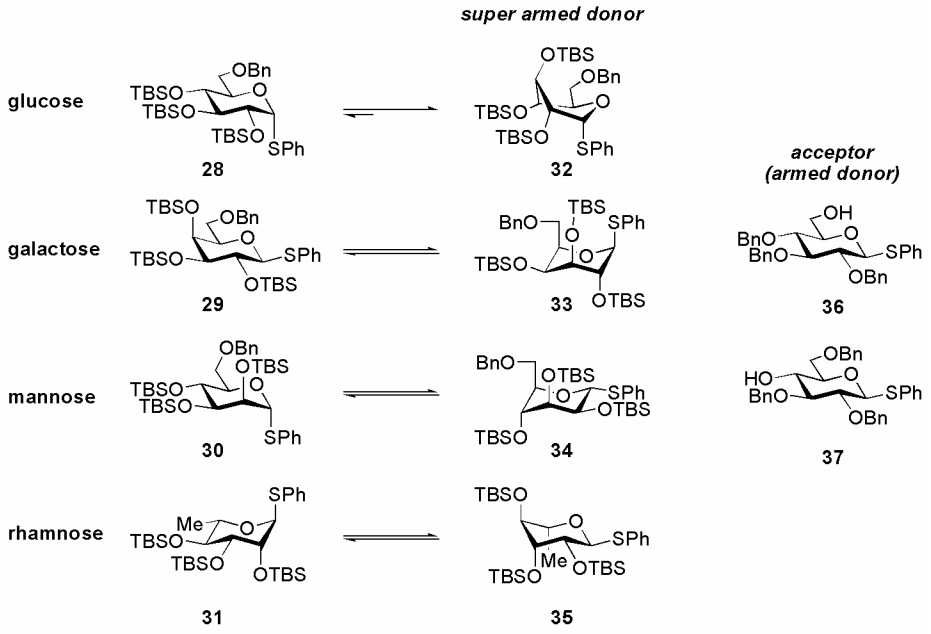


Scheme 6. Synthesis of the tetrasaccharide **20** through three consecutive α-glucosylation

3-4. Conformationally restricted glycosyl donor

Bols *et al.* have achieved the stereoselective glycosylation based on the conformational restriction of pyranose ring.[32] They addressed the reactivity of twisted-boat glycosyl donors, which was expected to enhance the reactivity in the case of an unusual glycosyl donor, considering the stabilizing effect of axial polar substituents on positive charges. Initially, they prepared the phenylthioglycoside of a 2,3,4-tri-*O*-*tert*-butyldimethylsilyl glucoside derivative (**28**) with a twisted-boat conformation. According to them, the glucosyl donor was activated much faster by the NIS-TfOH promoter system as compared to the phenylthioglycoside of tribenzylated glucoside (**36**), which is known as a highly reactive donor (armed donor), thereby producing the corresponding disaccharide with a high degree of β-selectivity. On the other hand, TBS-protected thiogalactoside (**29**), thiomannoside (**30**), thiorhamnoside (**31**) were also prepared analogously to thioglucoside (**28**). These donors (**33**~**35**) in which pyranose rings were flipped from the $^4C_1$–form into the $^1C_4$–form with bulky silyl protecting groups were subjected to the

Table 1. Glycosylation with axial-rich glycosyl donors (super-armed donor)



Entry	Donor	Acceptor	Condition	T [°C]	Product	% Yield[a]	α/β ratio
1	**32**	**36**	NIS TfOH MS4Å CH_2Cl_2	-85	**38**	86	1:6
2	**32**	**37**		-85	**39**	63	0:1
3	**33**	**36**		-85	**40**	73	1:0
4	**33**	**37**		-40	**41**	42	1:0
5	**34**	**36**		-85	**42**	79	10:1
6	**34**	**37**		-60	**43**	90	1:0
7	**35**	**36**		-85	**44**	68	7:1
8	**35**	**37**		-78	**45**	81	1:0

[a] Isolated yield.

glycosidation with **36** and **37** at very low temperature (-55 ~ -80 ˚C) to afford the desired disaccharides in good to excellent yields with very high α-selectivity. Importantly, when using the galactosyl donor (**33**), the reactions under various conditions were completely achieved with α(1,2-*cis*)-selectivity (Table 1). These findings demonstrated that the glycosyl donor which was forced ring-flipped or ring-twisted conformation was super-armed and provided exclusive stereoselectivity in glycosylation reaction (Figure 2).

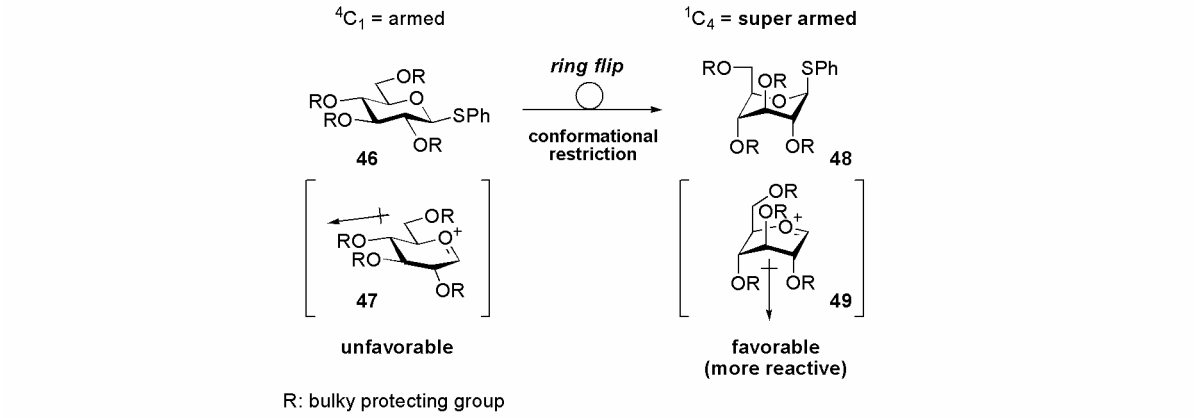
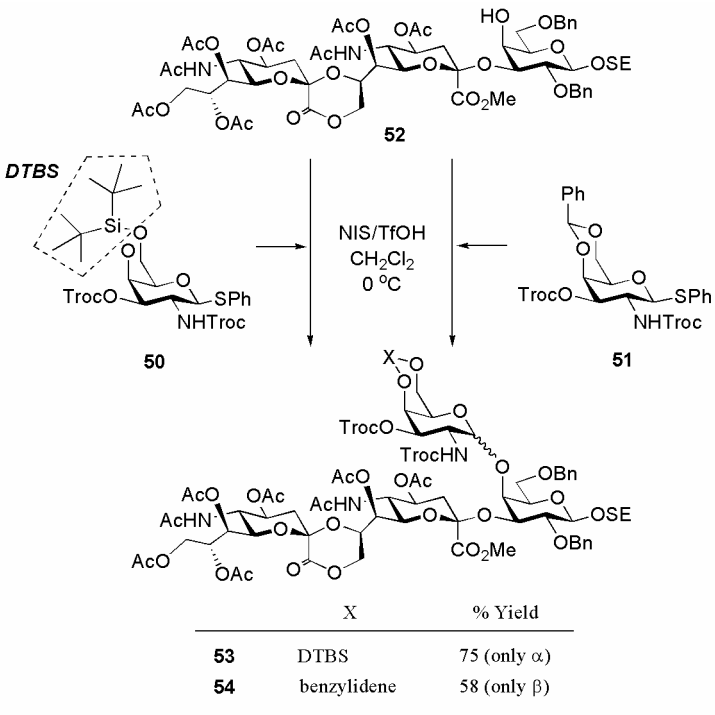


Figure 2. Ring flip caused by bulky hydroxy protection and dipoles in $^4C_1$ and $^1C_4$ conformation respectively

4. DTBS-DIRECTED α-GALACTOSYLATION

Up until now, as mentioned above, various types of methods for 1,2-*cis* glycosylation have been developed. However, there is no single method available for the production of all types of 1,2-*cis*-glycoside structures. Thus, even presently, chemists require a considerable amount of time to establish the best option providing a satisfactory yield of the desired 1,2-*cis*-glycoside. Recently, we discovered an unusual α(1,2-*cis*)-galactosylation using 4,6-*O*-di-*tert*-butylsilylene(DTBS)-protected galactose derivatives as a glycosyl donor.[33] The key feature of our glycosylation is the excellent α-selectivity that is compatible with the neighboring functionality on C-2 oxygen or nitrogen such as benzoyl, Troc, and Phth groups. In this section, we describe the new method in detail.



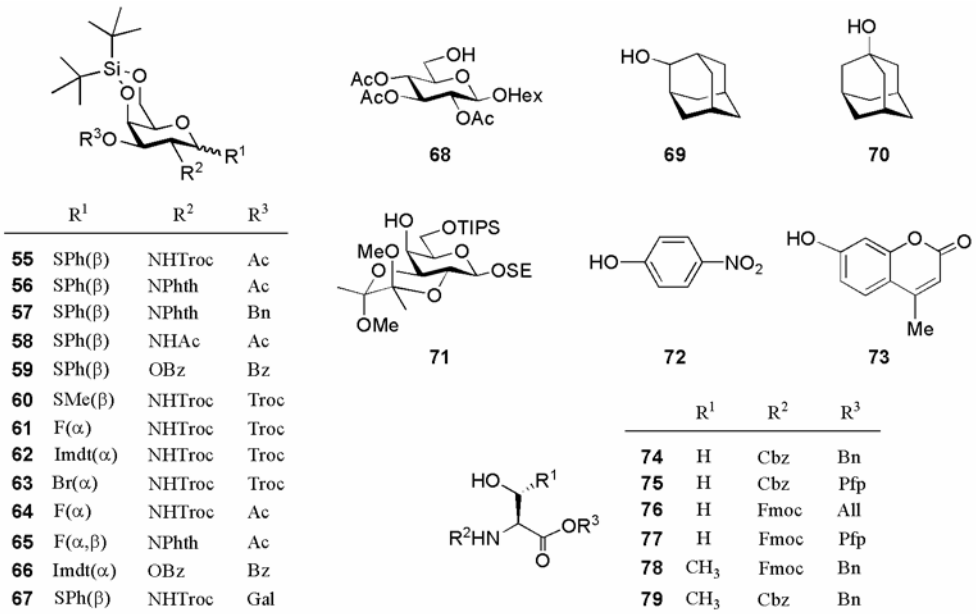
Scheme 7. First encounter with unusual α-galactosylation

4-1. Discovery of the DTBS-effect

The potential of the di-*tert*-butylsilylene (DTBS) group as an α-galactosylation enhancer was discovered by chance during a synthetic study on b-series gangliosides; in the study, phenyl 2-deoxy-4,6-*O*-di-*tert*-butylsilylene-3-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbam oyl)-1-thio-β-D-galactopyranoside (**50**), which was used as a surrogate of the 4,6-*O*-benzylidene-type donor (**51**), exhibited excellent α-selectivity in the coupling reaction[34] with trisaccharide acceptor (**52**) to afford tetrasaccharide (**53**). On the other hand, the corresponding 4,6-*O*-benzylidenated donor (**51**) afforded β-glycosyl product **54** exclusively (Scheme 7). The stereochemistries of each new glycoside in compounds **53** and **54** were confirmed to be α and β from the coupling constants between H-1 and H-2 in ^{1}H NMR spectra, δ 5.09 (d, $J_{1,2}$ = 3.4 Hz, H-1^{GalN}) in **53** and δ 5.05 (d, $J_{1,2}$ = 8.6 Hz, H-1^{GalN}) in **54**, respectively.

4-2. Scope of the DTBS-effect

Due to this unexpected and unusual result, we rechecked the anomeric configuration of the outcomes produced by the coupling reaction using tethered glycosyl donors (**50**) and (**51**). For this purpose, donors (**50**) and (**51**) were reacted with primary alcohol (**68**) to predominantly obtain α and β glycosyl products, respectively, with high yields (Table 2, entries 1 and 2). This, evidently, suggests that DTBS-tethering is responsible for α-predominant galactosylation. Furthermore, donor (**50**) also underwent coupling reactions with acceptors (**69**), (**70**), and MeOH in an α-selective and high-yielding manner (entries 3–5). Fortunately, α-anomeric products were preferentially afforded by the condensation of 3-*O*-Ac-GalNTroc



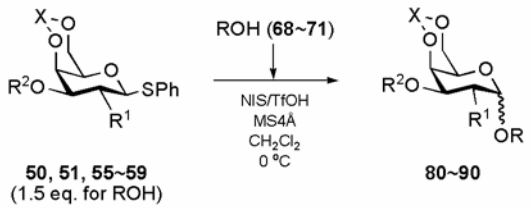
	R^1	R^2	R^3
55	SPh(β)	NHTroc	Ac
56	SPh(β)	NPhth	Ac
57	SPh(β)	NPhth	Bn
58	SPh(β)	NHAc	Ac
59	SPh(β)	OBz	Bz
60	SMe(β)	NHTroc	Troc
61	F(α)	NHTroc	Troc
62	Imdt(α)	NHTroc	Troc
63	Br(α)	NHTroc	Troc
64	F(α)	NHTroc	Ac
65	F(α,β)	NPhth	Ac
66	Imdt(α)	OBz	Bz
67	SPh(β)	NHTroc	Gal

	R^1	R^2	R^3
74	H	Cbz	Bn
75	H	Cbz	Pfp
76	H	Fmoc	All
77	H	Fmoc	Pfp
78	CH_3	Fmoc	Bn
79	CH_3	Cbz	Bn

Figure 3. Donors and acceptors used for α-galactosylation

(**55**), 3-*O*-Ac-GalNPhth (**56**), and 3-*O*-Bn-GalNPhth (**57**) donors with acceptor (**68**) in good yields as well (entries 6–8). In addition, it should be noted that even the C-2 acetamide group was accepted to exclusively obtain the α-product (entry 9). The phenylthioglycoside of a galactose derivative having benzoyl groups on both C-2 and C-3 hydroxyls–**59**–also attracted our attention; consequently, we successfully conducted α-selective and high-yielding glycosylations of **59**, which were unaffected by the reactivity of the acceptor hydroxyls (entries 10 and 11).

Table 2. α-Selective coupling of GalNand Gal donors with various acceptors



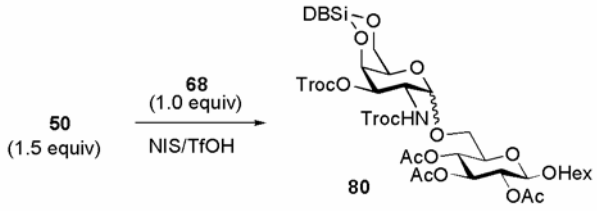
Entry	Donor	Acceptor	Time	Product	% Yield (α:β)
1	**50**	**68**	30 min	**80**	96:3
2	**51**	**68**	30 min	**81**	<7:93
3	**50**	**69**	30 min	**82**	91:7
4	**50**	**70**	30 min	**83**	90:5
5	**50**	**MeOH**	4 h	**84**	90:9
6	**55**	**68**	30 min	**85**	96:0
7	**56**	**68**	30 min	**86**	90:5
8	**57**	**68**	30 min	**87**	94:0
9	**58**	**68**	30 min	**88**	50:0
10	**59**	**68**	3 h	**89**	71:0
11	**59**	**71**	30 min	**90**	74:0

Second, the silylene-bridged glycosyl donor (**50**) reacted with glucosyl acceptor (**68**) in various solvents affected by NIS/TfOH[35,36] (Table 3, entries 1–5). As a result, the α-selectivity of this DTBS-directed coupling was found to be independent of solvent effects. Thus, in all cases, the α-anomer (**80**) was exclusively produced in 84% to 100% *de*. In particular, glycosylations, even in CH_3CN,[6] afforded α-glycoside as the sole product.

Third, DTBS-donor (**50**) was reacted with 2-adamantanol (**69**) in the presence of NIS and TfOH under various temperatures. It was found that the coupling reaction at 0 °C produced the best result, while lower temperatures reduced not only the yield of glycosides but also the stereoselectivity. (Table 4)

Next, in Table 5, various leaving group such as the methylsulfenyl,[35-37] fluoride,[38,39] and trichloroacetimidate[40] were examined. The glycosyl donors (**50**–**65**) were coupled with 2-adamantanol (**69**). All stereochemical outcomes in entries 1–7 were predominantly α with sufficiently high yields ranging from 68% to 91%. These results reveal the broad compatibility of DTBS-directed coupling with

Table 3. Glycosylation of galactosyl donor **50** with glucosyl acceptor **68** under various solvents

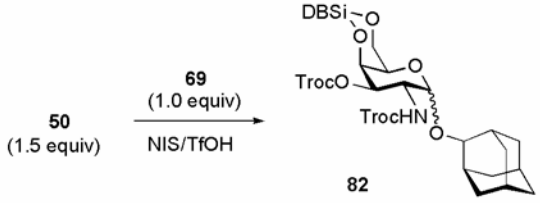


Entry	Solvent	T [°C]	t [h]	% Yield (α:β)
1	CH_2Cl_2	0	0.5	96:3
2	*n*-hexane	RT	8.0	58:3
3	PhMe	0	0.5	91:7
4	$MeNO_2$	0	0.5	93:6
5	MeCN	0→40	22	23:0

various leaving groups and promoters. Moreover, the results of entries 4–7, where α-fluoride or α-imidate donors were used, ruled out the possibility of the α-anomeric selectivity of the DTBS-directed reaction being attributable to the stereoconversion of the β-glycosyl donor *via* an S_N2-type mechanism. Interestingly, in contrast to these results, when insoluble silver silicate[41] was selected as a promoter for the glycosyl bromide (**63**), the glycosylation reaction predominantly produced the corresponding β-anomeric outcome (**83**) at 77% yield (entry 8). For entries 9 and 10, aryl alcohols[42] such as *p*-nitrophenol (**72**) and 4-methylumbelliferone (**73**) functioned as α-predominant coupling partners, thereby producing the corresponding galactosaminides (**91**) and (**92**) with high yields.

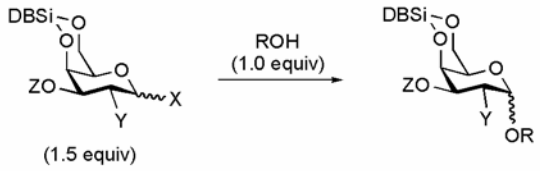
Furthermore, it is important to note that the relatively bigger difference between the R_f values of the α and β isomers having DTBS structures allowed us to separate them by silica gel chromatography within a short period. This was another unexpected advantage of the DTBS-tethering of glycosyl donor.

Table 4. Coupling of GalN-DTBS donor **50** and 2-adamantanol **69** under various temperature



Entry	T [°C]	t [h]	% Yield (α:β)	α/β ratio
1	30	0.5	87:12	7.2/1
2	0	0.5	91:7	13/1
3	-20	0.5	83:16	5/1
4	-40	4.0	65:24	2.7/1

Table 5. Examination of various leaving groups in the DTBS-directed galactosylation



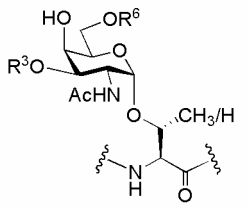
Entry	Donor	ROH	Promoter	T [°C]	t [h]	Product	% Yield[a] (α:β)
1	**50**	**69**	NIS/TfOH	0	0.5	**82**	91:7
2	**50**	**69**	DMTST[b]	0	21	**82**	83:15
3	**60**	**69**	NIS/TfOH	0	0.5	**82**	87:11
4	**61**	**69**	$SnCl_2/AgClO_4$[c]	0	1.5	**82**	82:0
5	**61**	**69**	$Cp_2HfCl_2/AgOTf$[d]	-20	0.5	**82**	71:0
6	**62**	**69**	TMSOTf[e]	0	0.5	**82**	91:8
7	**62**	**69**	$BF_3{\cdot}OEt_2$	0	0.5	**82**	68:9
8	**63**	**69**	Ag-silicate[f]	-20	2.0	**83**	6:77
9	**64**	**72**	$BF_3{\cdot}OEt_2/Et_3N$[g]	0	3.0	**91**	95:0
10	**65**	**73**	$BF_3{\cdot}OEt_2/Et_3N$	0	3.0	**92**	74:0

[a] Isolated yield. [b] See ref. [37]. [c] See ref. [38]. [d] See ref. [39]. [e] See ref. [40]. [f] See ref. [41]. [g] See ref. [42]. DMTST: dimethyl(methylthio)sulfonium trifluoromethanesulfonate

4-3. Synthesis of Glycosyl Amino Acids

Encouraged by the potential of the presented DTBS-directed α-galactosylation, we investigated the applicability of this method for the synthesis of biologically relevant glycans (Section 4-3, 4, 5)

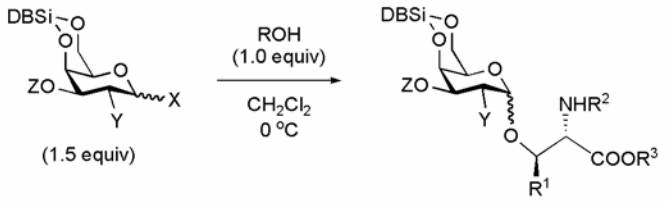
Mucins are cell-surface or secreted proteins that contain dense clusters of glycosylated serine and threonine residues.[43,44] GalNAc saccharide, which is linked to the hydroxyl group of serine or threonine through α-glycoside, represents the core unit, also known as the Tn antigen, in mucin-type glycoproteins. A wide variety of mucin-type core structures are generated from the GalNAc-α-serine/threonine motif by glycosylation at the C-3 and/or C-6 hydroxyl groups of GalNAc. As depicted in Figure 4, eight different types (cores 1–8) of core structures of mucin-type *O*-linked glycans are known. In many cases, these structures are modified by sulfation and the addition of NeuAc, Fuc, and/or repeating LacNAc units. Therefore, it is essential for the study of mucin-type glycans to establish the key protein-carbohydrate linkage (α-*O*-GalNAc-Ser/Thr) by a chemical method.[45] However, the synthesis of the α-glycosidic linkage between GalNAc (2-acetamido-2-deoxy-D-galactopyranose) and the hydroxyl



Core	R^3	R^6
1	Gal β(1→3)	
2	Gal β(1→3)	GlcNAc β(1→6)
3	GlcNAc β(1→3)	
4	GlcNAc β(1→3)	GlcNAc β(1→6)
5	GalNAc α(1→3)	
6		GlcNAc β(1→6)
7		GalNAc α(1→6)
8	Gal α(1→3)	

Figure 4. Core structure of mucin-type *O*-linked glycans

Table 6. α-Predominant formation of GalN-Ser/Thr linkages



Entry	Donor	ROH	Promoter	*t* [h]	Product	% Yield (α:β)
1	**50**	**74**	NIS/TfOH	0.5	**93**	95:2
2	**50**	**75**	NIS/TfOH	0.5	**94**	90:0
3	**62**	**76**	TMSOTf	0.5	**95**	97:0
4	**50**	**77**	NIS/TfOH	0.5	**96**	78:0
5	**50**	**78**	NIS/TfOH	0.5	**97**	93:0
6	**50**	**79**	NIS/TfOH	0.5	**98**	92:0
7	**58**	**74**	NIS/TfOH	0.5	**99**	65:0
8	**67**	**75**	NIS/TfOH	1.0	**100**	88:0

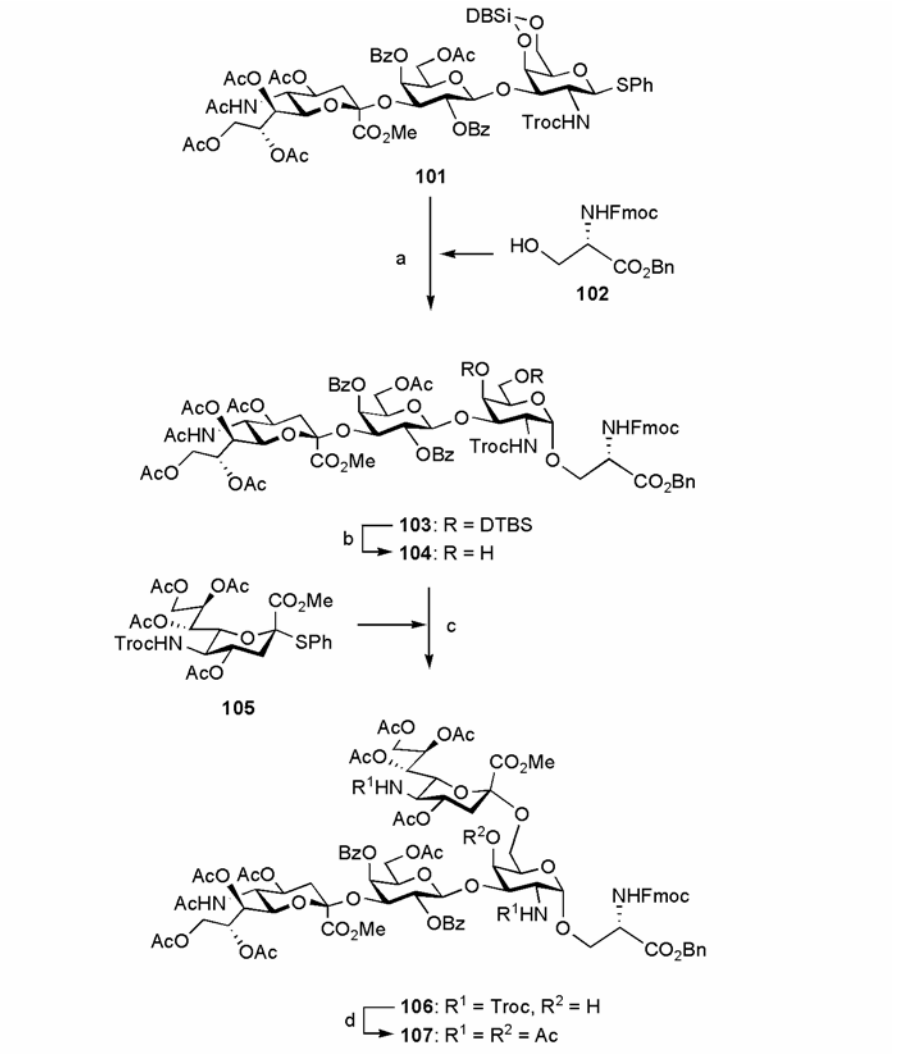
groups of serine and threonine has proven to be difficult due to the presence of the participating acetamide group at the C-2 position on the GalNAc residue. As mentioned above, the most common approach to realize the assembly of α-*O*-GalNAc linkage is to use a glycosyl donor that has a nonparticipating azido group at C-2. As a result, even simple *O*-linked glycosyl amino acids can be prepared by those with limited expertise in synthetic chemistry. Therefore, we believe that the presented DTBS-directed α-galactosylation process could become an attractive option for accomplishing the formation of core α-*O*-GalNAc-Ser/Thr linkage.

In accordance with our expectation, DTBS galactosyl donors have been successfully used in the efficient synthesis of α-galactosaminyl Ser/Thr sequences (Table 6). Thus, various types of Ser (**74**–**77**) as well as Thr (**78** and **79**) derivatives were α-selectively galactosylated by the GalNTroc donors (**50**) and (**62**) in high yields (entries 1–6). The acetamido-galactosyl donor (**58**) also served as an α-selective glycosylation unit to produce an α-*O*-GalNAc-Ser linkage in a moderate yield (entry 7). In entry 8, we found that the glycosidation of the Gal-GalN-Troc disaccharide donor (**67**) with Ser derivative (**75**) yielded the T-antigen structure (**100**) in 88% yield.

Moreover, the DTBS-containing trisaccharide (**101**) was applied to the coupling along with the Fmoc-Ser derivative (**102**) (Scheme 8). Fortunately, this coupling was also successful, producing the Neu5Acα(2→3)Galβ(1→3)GalNα(1→)Ser sequence (**103**) as a single α-isomer in high yield. These results extended the scope of the versatility of the DTBS effect. Further, to establish an α-sialyl branch on the C-6 of the GalN residue, the 4,6-DTBS group was cleaved by the action of the fluoride anion released from tri-*n*-butylammonium hydrogenfluoride (TBAHF)[46] without affecting the Fmoc moiety. The resulting 4,6-diol glycan (**104**) was then sialylated with the previously reported *N*-Troc sialyl donor (**105**)[47] to afford the tetrasaccharide (**106**) in 94% yield. Finally, the Troc groups within **106** were

chemoselectively replaced by acetyl groups to produce glycophorin A glycan frame (**107**).[48]

Up until now, even when monosaccharide donors were used, the anomeric selectivity and yield of α-galactosaminyl serine or threonine syntheses has remained elusive and unpredictable. This problem is even more pronounced when dealing with large oligosaccharides as glycosyl donors. Therefore, as the cassette approach[49] revealed, the α-*O*-GalNAc-Ser/Thr linkage is usually established prior to the elongation of the glycan chain. On the other hand, as exemplified above, DTBS-directed α-galactosylation allows us to incorporate Ser/Thr residue into the grown glycan chain at the endgame of the synthesis. It reveals that the presented DTBS-directed α-galactosylation has a wide range of practical applications with regard to the synthesis of *O*-glycans in mucin-type glycoproteins.[50]



Scheme 8. a) NIS/TfOH, MS4Å, CH_2Cl_2, 0 °C, 88% (only α); b) TBAHF, THF, H_2O, RT, 86%; c) NIS/TfOH, MS3Å, MeCN, CH_2Cl_2, -35 °C, 94% (α/β 73:21); d) i) Zn, AcOH, 40 °C, 68%; ii) Ac_2O, Py, RT, 68%. MS = molecular sieves, TBAHF = tri-*n*-butylammonium hydrogenfluoride.

4-4. Synthesis of α-Galactosyl Ceramides and iGb3 Glycolipid

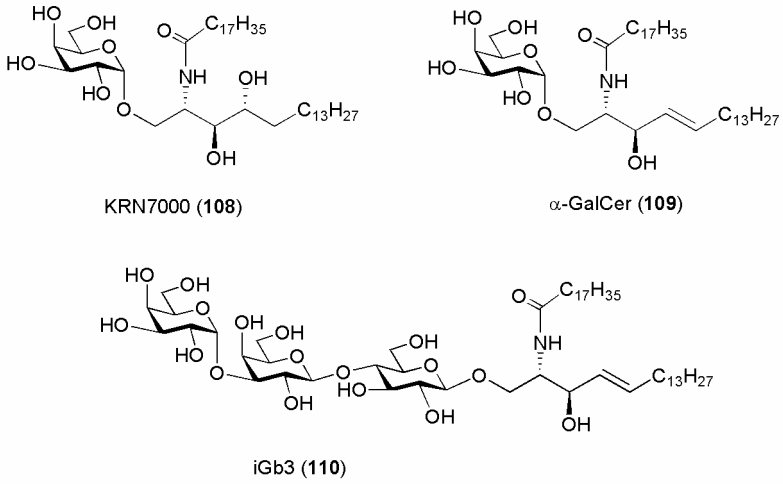
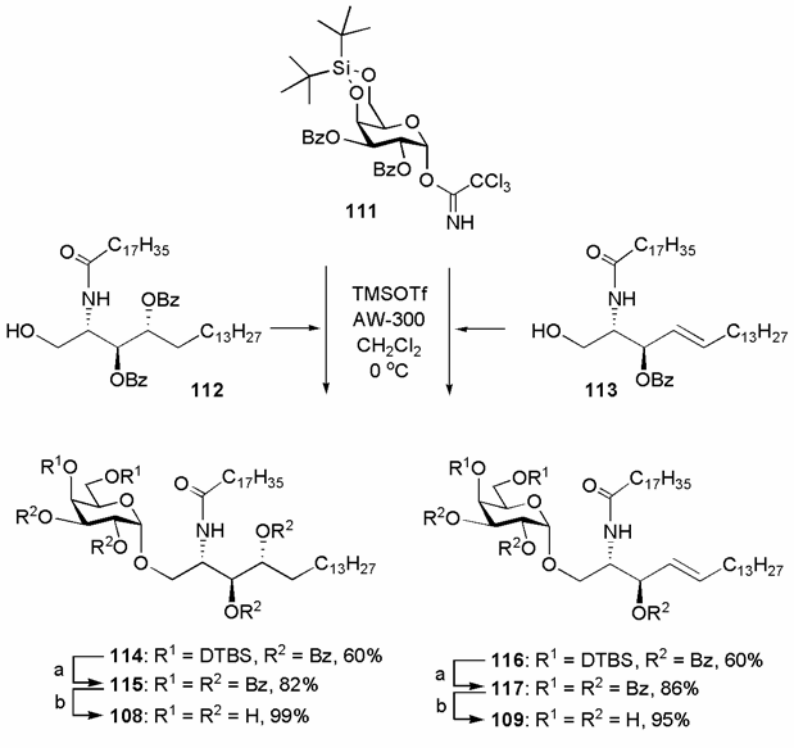


Figure 5. Structure of α-GalCers (**108**, **109**) and iGb3 **110**

To further assess the utility of the presented DTBS-directed α-galactosylation, the synthesis of α-galactosyl ceramides and iGb3 glycolipid was investigated.[51]

α-Galactosyl ceramides (α-GalCers), which are obtained from CD1d, are recognized by a T cell antigen receptor on an NKT cell, thereby resulting in the stimulation of NKT cells. NKT cells are essential not only for defense against pathogens but also for the initiation of adaptive immune responses and regulating autoimmune responses.[52-54] Recently Zhou *et al*. suggested that isoglobotrihexosylceramide (iGb3) acts as an endogenous ligand for NKT cell responses to infections, malignancy and in autoimmunity.[55] Therefore α-GalCers and iGb3 have become an attractive new candidate for the treatment of microbial infections, cancer, and autoimmune diseases.

So far, several methods for the syntheses of α-GalCer[56-61] and iGb3[62,63] have been reported. Per-*O*-benzylated galactosyl donors with leaving groups such as fluoride, chloride, aryl sulfenyl, and trichloroacetimidate have been used for the formation of α-galactosyl linkages. However, the α-selectivity and yield of glycosylation strictly depend on the structure of the glycosyl acceptor. In particular, the galactosylation of the ceramide part is not characterized by high stereoselectivity or yield. Therefore, we believe that the proposed DTBS-directed galactosylation process can be utilized for the preparation of α-linkage in compounds **108**, **109**, and **110** (Figure 5).

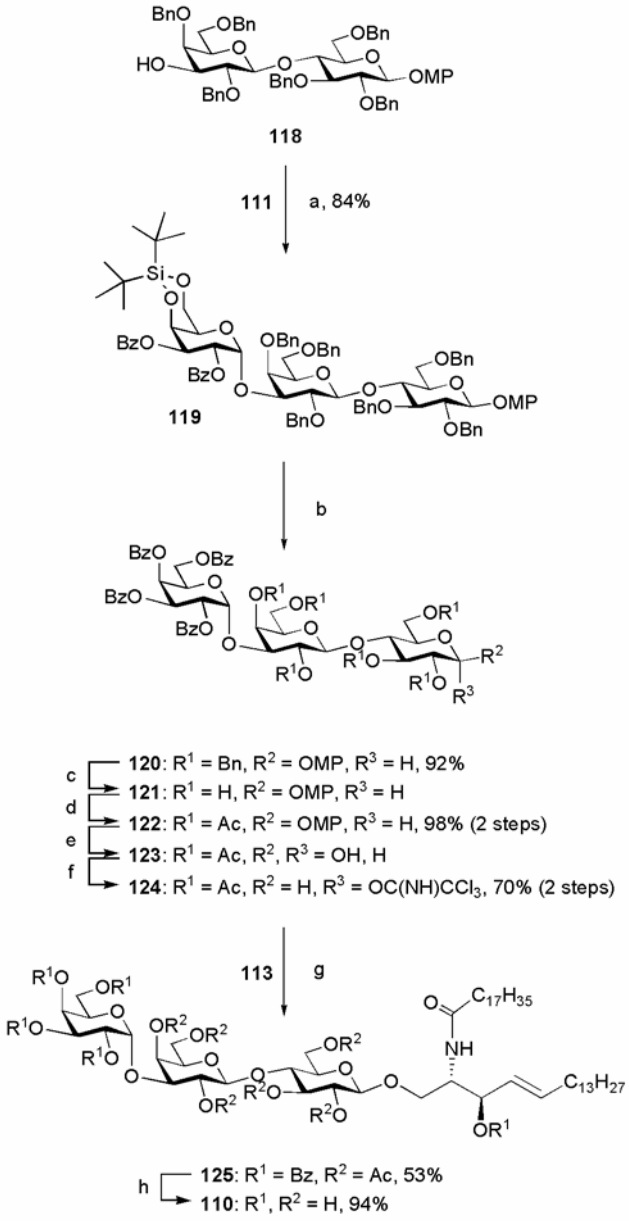


Scheme 9. a) TBAHF, then Bz_2O, DMAP, Py; b) NaOMe, MeOH

Having prepared the DTBS-galactosyl

donor (**111**), we conducted the DTBS-directed α-galactosylation of ceramide acceptors (**112**)[51] and (**113**).[64,65] In both cases, the glycosylation was performed in the presence of TMSOTf at 0 °C to exclusively afford α-GalCer sequences (**114**) and (**116**) as single isomers in 60% yield. The removal of the 4,6-*O*-DTBS group from **114** and **116** by TBAHF and sequential benzoylation yielded per-*O*-benzoylated α-GalCers (**115**) and (**117**) in 82% and 86% yields, respectively. Finally, debenzoylation of **115** and **117** with NaOMe in MeOH provided KRN7000 (**108**) and the analogue (**109**) in 99% and 95% yields, respectively (Scheme 9).

As depicted in Scheme 10, the synthesis of iGb3 (**110**) was also investigated, again using DTBS-directed



Scheme 10. a) TMSOTf, AW-300, CH_2Cl_2, 0 °C; b) TBAHF then Bz_2O, DMAP, Py; c) H_2, $Pd(OH)_2$/C, 1,4-dioxane, 40 °C; d) Ac_2O, Py; e) CAN, MeCN-H_2O-toluene; f) CCl_3CN, DBU, CH_2Cl_2; g) TMSOTf, AW-300, CH_2Cl_2, 0 °C; h) NaOMe, MeOH-THF, 40 °C.

α-galactosylation. The known lactoside acceptor (**118**)[66] was glycosylated with the common DTBS-protected galactosyl donor (**111**). As expected, the desired α-glycoside (**119**) was obtained as a single isomer in 84% yield. Subsequently, the trisaccharide (**119**) was transformed into the trichloroacetimidate form (**124**) in five steps. The glycosylation of the lipid acceptor (**113**) with trichloroacetimidate donor (**124**) was achieved in the presence of TMSOTf to produce a 53% yield of completely protected isoglobotrihexosylceramide (**125**). Finally, the deacylation of compound **125** with NaOMe in MeOH-THF afforded the target iGb3 (**110**).

Moreover, Lee *et al*., very recently, exploited DTBS-directed α-galactosylation for the efficient synthesis of α-GalCer.[67]

4-5. Synthesis of 4-Methylumbelliferyl T-antigen

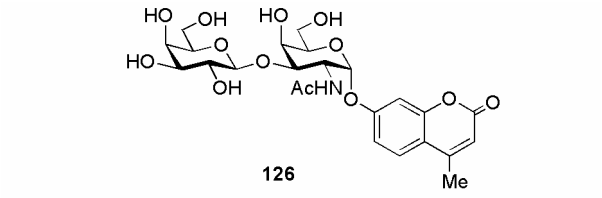
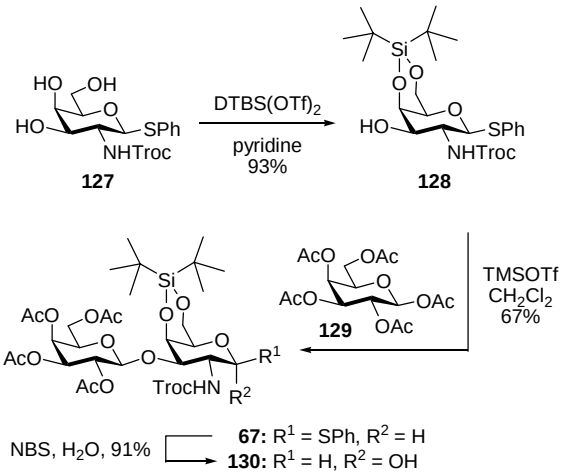


Figure 6. Structure of 4-methylumbelliferyl T-antigen

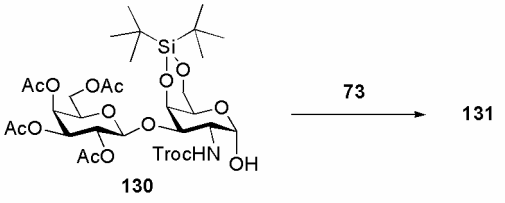
In order to extend the scope of the application of DTBS-directed α-galactosylation, the facile synthesis of 4-methylumbelliferyl (4-MU) T-antigen (**126**) was also investigated (Figure 6). 4-MU glycosides have been a popular type of fluorogenic probe for hydrolase action because of the potent fluoromeric property of their phenolic counterpart liberated by enzymatic hydrolysis.[68,69] However, it is typically difficult to synthesize 4-MU glycosides.[70] In particular, as in the case of other types of glycosides, the synthesis of the corresponding α-glycosaminides is extremely arduous with regard to circumventing the participatory effects of the *N*-acetyl group.

To the best of our knowledge, only Lemieux *et al.* succeeded in synthesizing 4-MU-α-GalNAc using 2-azidogalactosyl chloride as a glycosyl donor. Unfortunately, their synthetic protocol involved many laborious manipulations. Moreover, the glycosyl donor could only be coupled with 4-methylumbelliferone (4-MU-OH) in poor yield (33%).[71] To solve this synthetic issue, we envisaged using the presented DTBS-directed α-galactosylation process for 4-MU T-antigen synthesis.[72]



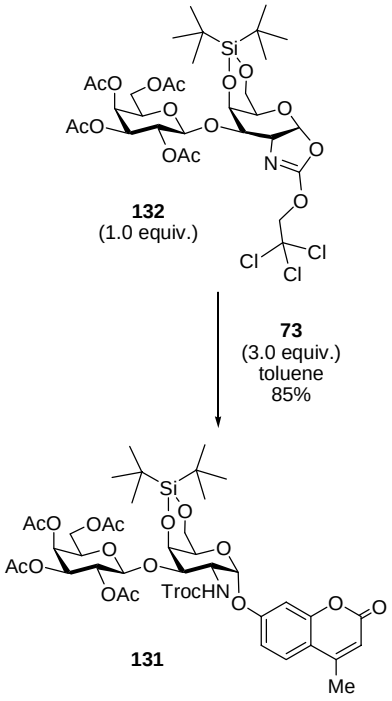
Scheme 11. Preparation of Galβ(1→3)GalN disaccharide

Table 7. 4-Methylumbelliferylation with Mitsunobu reaction[a]



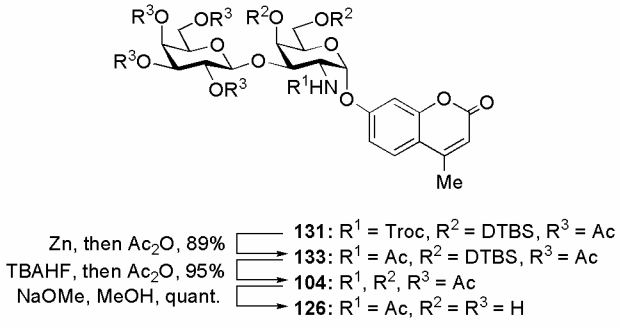
Entry	Phosphine / Azo cmpd[b]	4-MU-OH [eq.]	Solvent	% Yield[c] (α:β)
1	TPP / DEAD[d]	3.0	THF	47:5
2	TBP / ADDP[e]	3.0	THF	20:—
3	TBP / TMAD[f]	3.0	THF	8:—
4	DPPE / DIAD[g]	3.0	THF	no reaction
5	TPP / DEAD	3.0	toluene	74:8
6	TBP / ADDP	3.0	toluene	62:15
7	TPP / DEAD	8.0	toluene	80:9

[a] Every reaction was conducted under reflux condition. [b] TPP: Triphenylphosphine, TBP: Tributylphosphine, DPPE: 1,2-Bis(diphenylphosphino)ethane, DEAD: Diethyl azodicarboxylate, ADDP: 1,1'-(Azodicarbonyl)dipiperidine, TMAD: 1.1'-Azobis(*N,N*'-dimethylformamide), DIAD: Diisopropyl azodicarboxylate. [c] Isolated yield. [d] See ref. [74]. [e] See ref. [75]. [f] See ref. [76,77]. [g] See ref. [78].



Scheme 12. Coupling of **132** and **73** in the absence of activators

By taking advantage of the compatibility of the proposed α-selective galactosylation process with the acyl functionality on C-2 amino groups, the *N*-Troc-protected disaccharide (**130**) was designed. As depicted in Scheme 11, the hemiacetal derivative (**130**) was efficiently prepared via a three-step manipulation by using a known starting material–**127**.[73] Next, the hemiacetal was subjected to the Mitsunobu reaction with 4-MU-OH in the presence of various combinations of trialkyl phosphines (TPP,[74] TBP,[75-77] DPPE[78]) and azocompounds (DEAD,[74] ADDP,[75] TMAD,[76,77] DIAD[74,78]), as summarized in Table 7. Surprisingly, the anomeric configuration of **130** was almost retained, and α-glycoside (**131**) was the predominant output in all reactions. The yield of **131** increased with the reaction temperature (entries 5 and 6), and the use of excess 4-MU-OH (8.0 equiv) resulted in optimum product yield (80%) (entry 7). In contrast, at a lower temperature, the trichloroethoxyoxazole (**132**) was produced as a major byproduct, which was observed as a reaction intermediate of **130** during the Mitsunobu reaction. Accordingly, the oxazole (**132**) could



Scheme 13. Final deprotections

also be reacted with 4-MU-OH in toluene under reflux to afford α-glycoside (**131**) in 85% yield (Scheme 12). Finally, as depicted in Scheme 13, 4-MU glycoside (**131**) was transformed into **126** through global deprotection steps.

4-6. Proposed Reaction Mechanism

The X-ray crystallographic analysis of compound **56** showed that its six-membered ring comprised of a 4,6-*O*-DTBS and that a C4-C5-C-6 bond assumed a nearly half-chair (sofa) conformation, which resulted in the *tert*-butyl group being positioned closer to the anomeric carbon (Figure 7). Although it is static, it implies the steric hampering of the DTBS group during glycosylation. On the other hand, it is known that the six-membered ring comprising a corresponding benzylidene acetal typically assumes a chair conformation.

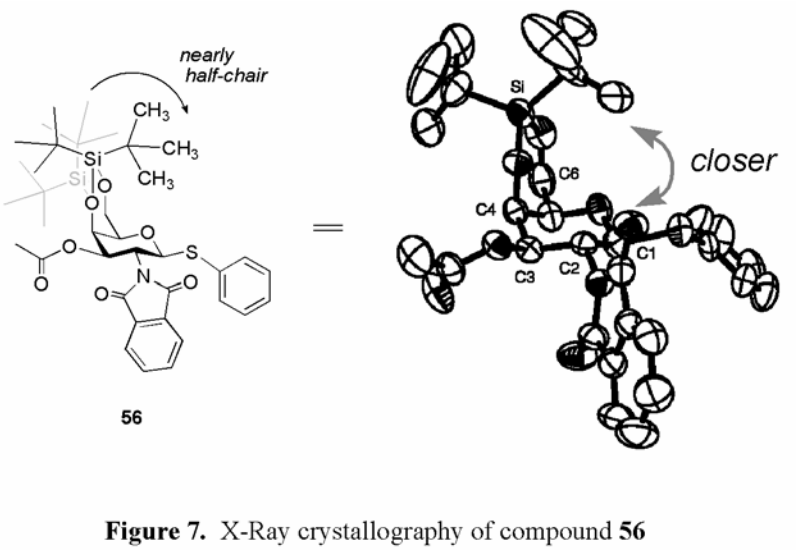


Figure 7. X-Ray crystallography of compound **56**

Miljković *et al.* demonstrated[79] that the electron donation from the axially oriented substituent at the C-4 carbon atom of the galactopyranoside ring contributes to the stabilization of the oxocarbenium ion generated during the glycosylation process. As summarized in Figure 8, in the D-*galacto*-type series, the rate of acetolysis of methyl glycoside depends strongly on the electronegativity of the C-4 substituent because of the 1,3-*syn*-diaxial electronic interaction between an axially oriented electronegative group at C-4 and the axially oriented sp^3 hybridized nonbonding electron pair of the ring oxygen. In the D-*gluco* series, the dependence of acetolysis rates on the electronegativity of the C-4 substituent can only be explained as a "through-bond" interaction (inductive effect) with the ring oxygen, which is apparently rather small. On the other hand, in the D-*galacto* series, the very large influence of the electronegativity of the axially oriented C-4 substituent on the acetolysis rate cannot be ascribed to this small through-bond interaction. The only possible explanation for the unusually large kinetic effect observed in the D-*galacto* series is a strong "through-space electron donation" of the axially oriented electronegative substituent at the C-4 to the oxocarbenium ion under formation. This effect, which destabilizes the neutral

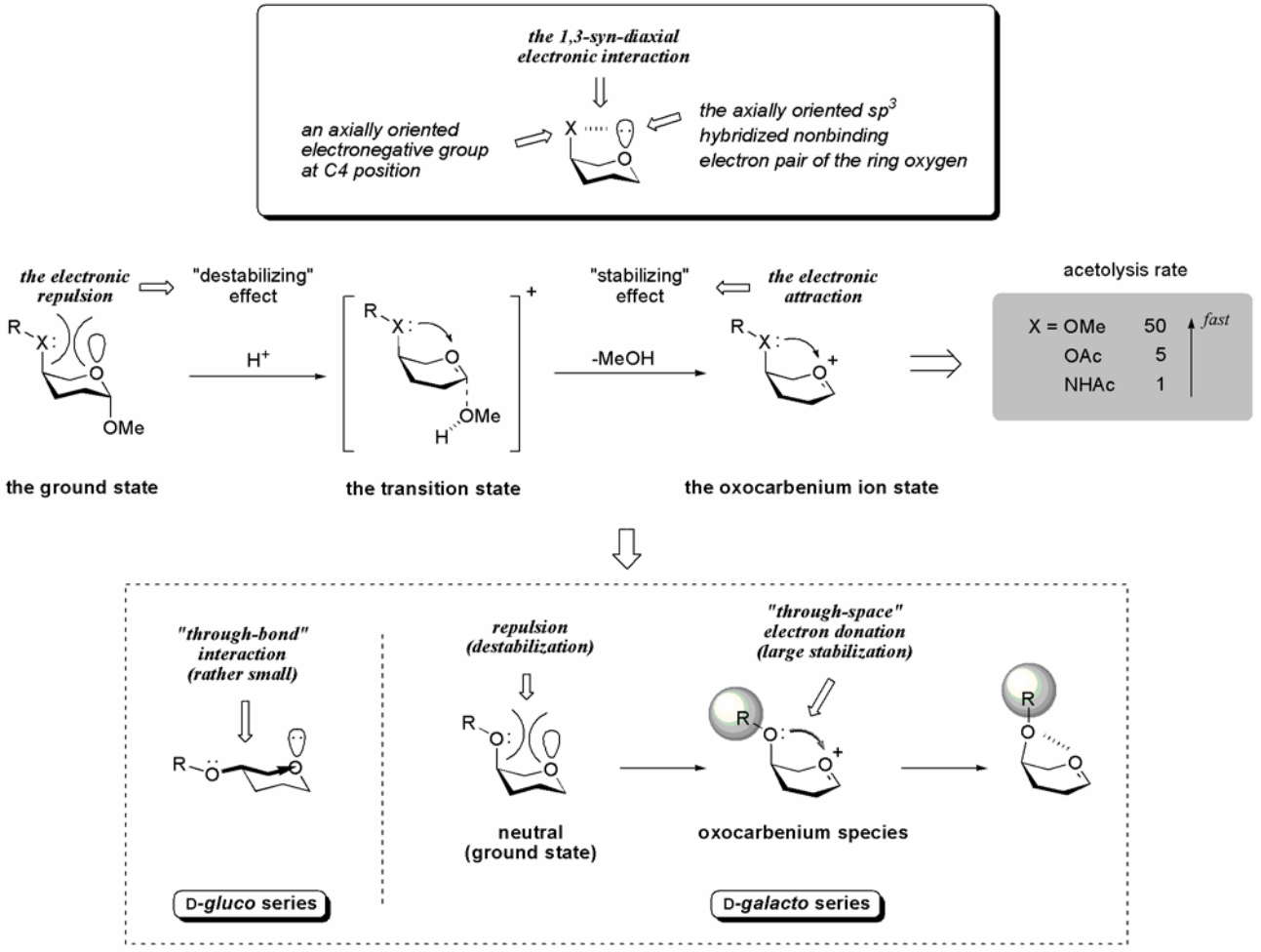


Figure 8. Through-space electron donation theory

galactopyranoside due to electrostatic repulsion, becomes stronger as the oxocarbenium species appears. In addition, the results of *Ab initio* calculation showed that the interatomic distance between the electronegative C4 oxygen and the oxygen of the oxocarbenium ion decreases. These findings form the theoretical basis of the strong steric hampering exerted by the ^{t}Bu group on the anomeric center during glycosylation. In other words, on the generation of the oxocarbenium ion, the electron-rich C4 oxygen, owing to $^{t}Bu_2Si$ group, donates an electron toward the electron-poor oxygen of oxocarbenium ion (**136**), which entails a closer positioning of ^{t}Bu groups to anomeric center (Figure 9, route A). This prevents a nucleophile from attaching to the β-side on **138**, which could be a plausible explanation for the enormous α-selectivity resulting from the DTBS effect. Further, the fact that most of the DTBS-directed α-glycosylations have very short reaction times (almost less than 30 min.) would strongly support that the oxocarbenium ion is stabilized by electron donation.

Conversely, Shuto *et al.* have reported[80,81] that conformational features and the reactivity of the anomeric position of sugars are affected by the anomeric effect as a stereoelectronic effect. In other words, the stereoselectivity of S_N1-type glycosylations of pyranoses may be controlled effectively by the kinetic anomeric effect when conformationally restricted glycosyl donors are employed. Therefore, the following

process as another route in the DTBS-directed α-galactosylation was assumed (Figure 9, route B).

After activation of a glycosyl donor (**135**) confomationally restricted by cyclic DTBS group, the transition state (**140**) would assume the $^4C_1$-chairlike form as a result of conformational restriction of pyranose backbone, in which conformation the anomeric center would be pyramidal. Subsequently, the orbital interaction known as the kinetic anomeric effect, which is a stereoelectronic effect resulting from n→σ* hyper conjugation between the nonbonding orbital of the oxygen atom in the ring and the antibonding orbital of the anomeric carbon-heteroatom bond in their planar arrangement, takes place and stabilizes the transition state during bond-forming (or bond-cleavage) processes at the anomeric center, where nucleophilic attack occurs through an axial trajectory on **142**.

At the present, we speculate that the DTBS-directed α-galactosylation takes place through either route A or B (or their hybrid route as shown in Figure 9). Further effort toward elucidating of the mechanism is in progress.

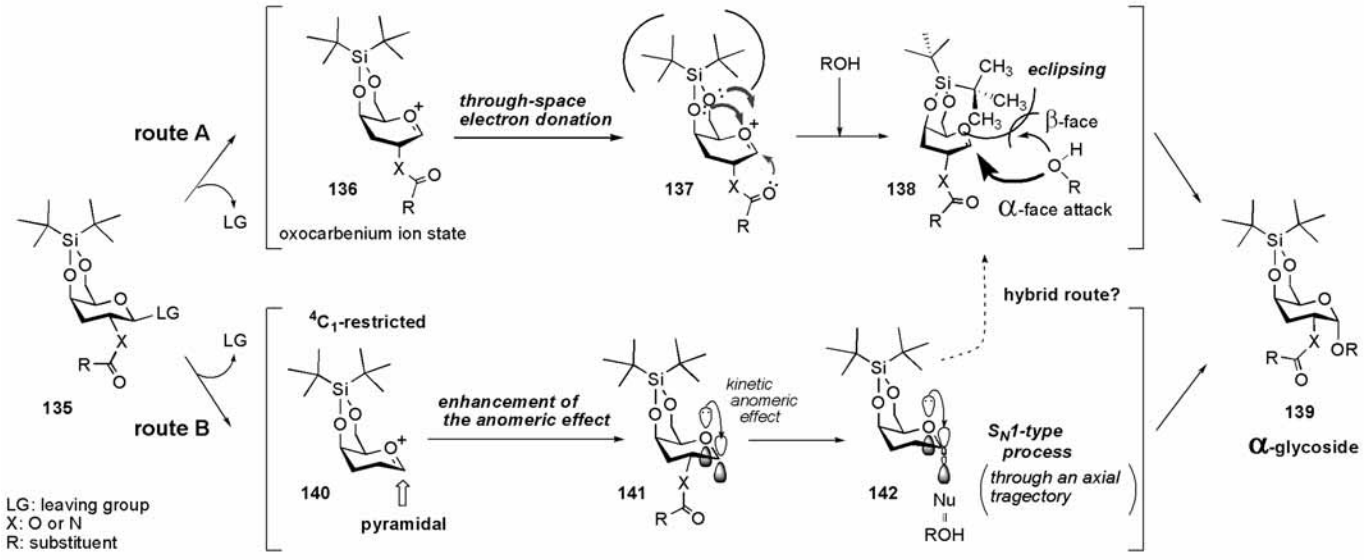


Figure 9. Proposed reaction mechanism in the DTBS-directed α-galactosylation

5. CONCLUSION

Considerable efforts have been made for studying the synthesis of 1,2-*cis* glycosides. Although the assembly of simple 1,2-*cis* glycosides in D-glucose/galactose is a simple task that does not require any particular experience in carbohydrate chemistry, the construction of complex *O*-linked glycans containing D-GlcNAc/GalNAc had remained difficult to achieve.

In this context, we present in this review the 4,6-*O*-DTBS-directed α-galactosylation methodology in which the neighboring participations of the NHTroc, NPhth, NHAc, and OBz groups on the selectively produced β-glycoside was ineffectual. We observed that the DTBS-directed glycosylation process is characterized by extremely high yield and α-selectivity, regardless of the various reaction conditions, which is in clear contrast to known methodologies. Furthermore, it was found that the DTBS-directed

α-galactosylation process has a wide range of applications as exemplified by the high α-selectivity and high-yielding synthesis of biologically significant α-*O*-GalNAc-Ser/Thr in mucin-type glycoprotein, glycophorin A glycan frame, and α-GalCers. Very recently, other groups have reported the synthesis of natural compounds containing α-Gal moiety by using the DTBS-directed α-galactosylation process presented in this study.[67,82] These reports suggest that our protocol greatly expands the current scope of this important synthesis of 1,2-*cis* galactosides and will undoubtedly form the basis for further development.

ACKNOWLEDGEMENTS

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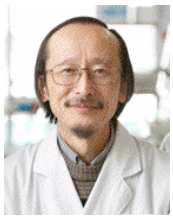

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