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Ligand-controlled Stereoselective Synthesis and Biological Activities of 2-Exomethylene Pseudo-glycoconjugates: Discovery of Mincle-Selective Ligands

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Dedication ((optional))

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Abstract: Glycoconjugate analogues in which the sp^3-hybridized C2 position of the carbohydrate structure (normally bearing a hydroxyl group) is converted to a compact sp^2-hybridized *exo*-methylene group are expected to have unique biological activities. We established ligand-controlled Tsuji-Trost-type glycosylation methodology to directly prepare a variety of these 2-*exo*-methylene pseudo-glycoconjugates, including glucosylceramide analogues, in an α- or β-selective manner. Glucocerebrosidase GBA1 cleaves these synthetic pseudo-β-glucosylceramides similarly to native glucosylceramides. The pseudo-glucosylceramides exhibit selective ligand activity towards macrophage-inducible C-type lectin (Mincle), but unlike native glucosylceramides, are inactive towards CD1d.

Introduction

Glycans (or glycoconjugates) exhibit diverse biological activities, depending on their constituent carbohydrates, stereoisomeric or anomeric form, substituents on the sugar ring, linkage pattern, and type of aglycone structure.[1] Thus, for the creation of biologically useful molecular tools and lead compounds for drug discovery, there is great interest in derivatives or analogues with more potent activity or different functions compared with native glycans. Many glycan derivatives, including derivatives of α-galactosyl ceramide (**α-GalCer**),[2,3] which activates NKT cells through binding to CD1d, and trehalose dimycolate (**TDM**) from *Mycobacterium tuberculosis*, which activates macrophage-inducible C-type lectin (Mincle) have been reported.[4–6] In this context, we are interested in glycan analogues (pseudo-glycans) having modifications of their carbohydrate skeletons while retaining the glycan structures as intact as possible.[7] For example, a ganglioside GM3 analogue with a *CHF*-glycoside linkage exhibits altered glycan conformation as a result of the gauche effect of the F-atom and shows more potent biological activity than native GM3 owing to its resistance to cellular hydrolytic enzymes.[8]

Modifications of the functionality at C2 on the sugar ring have been well studied (Figure 1A). In addition to the abundant 2-hydroxyl form, 2-amino- and 2-deoxy sugars also occur in nature, each with unique properties. Thus, changing the functionality at C2 position is a promising strategy for altering the function of native glycans, as exemplified by the fact that pseudo-glycans including 2-fluorosugars, act as mechanism-based inhibitors,[9,10] or exhibit superior biological activities,[11] or are useful for structural analysis.[12] Isolation or synthesis of other analogues with different functionality at C2[13–16] has also been reported. However, these examples all retain the sp^3-hybrized center at C2, and so can adopt a similar conformation to native glycans. In the present work, we designed glycan analogues with an *exo*-methylene functionality (2-exomethylene sugar, **1**) at C2. A 2-exomethylene sugar is expected to preferentially adopt the usual $^4C_1$ chair conformation, but with some distortion due to the sp^2-hybrized C2. Loss of the electronegative C2-substituent (**X**) might also increase the

electron density in the sugar, eliminate the possibility of hydrogen bonding, and enhance hydrophobic interactions. In addition, the newly introduced C-H bond is parallel to the sugar structure, while the π-bond is orthogonal. These features are expected to result in unprecedented electronic properties or intermolecular interactions.

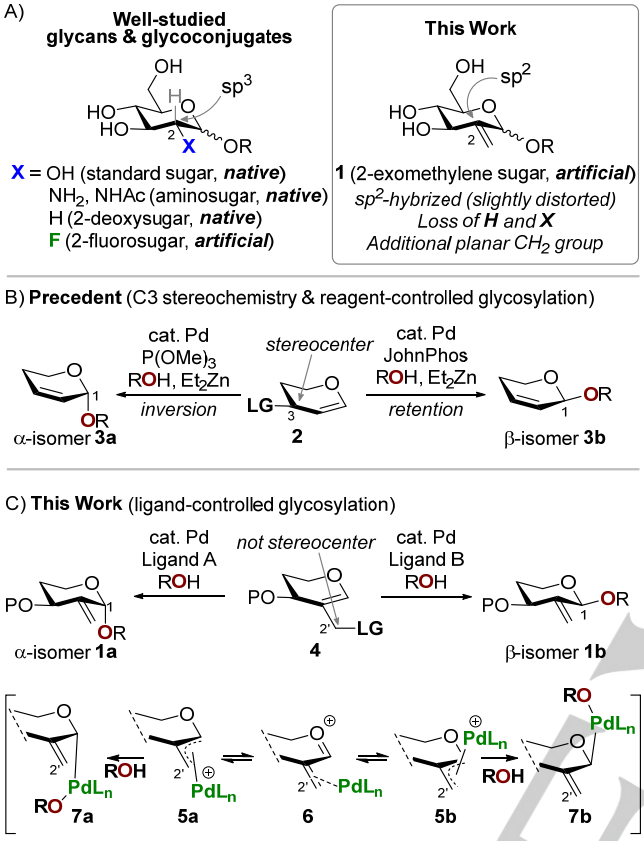


Figure 1. A) Conventional carbohydrate structures, an artificial 2-fluorosugar, and artificial glycan analogues with an exo-methylene functionality **1** (2-exomethylene sugars) synthesized in this work (**1** is depicted as standard $^4C_1$ conformation. The exomethylene group at C2 should point equatorially above); B) Representative precedent of reagent-controlled glycosylation of **2** to give 2,3-dehydro sugars **3a** or **3b**; C) Synthetic strategy for **1** in this work through ligand-controlled glycosylation of **4**, showing putative intermediates **5**-**7**.

Results and Discussion

Strategy for Ligand-Controlled Glycosylation

In this work, we envisioned the stereo-selective synthesis of both α-glycosides **1a** and β-glycosides **1b** (Figure 1C). Previous reports describing the synthesis of **1** were based on olefination from 2-keto carbohydrates,[17–29] so that preparation of the corresponding ketones with α- or β-glycosides would be required. A more efficient strategy based on vinylogous glycosylation, simultaneous construction of the exomethylene functionality and stereoselective glycoside formation from the glucal-type precursor **4** promoted by acids or Pd catalyst has afforded **1a** with moderate α-selectivity.[30–40] The origin of the stereoselectivity is considered to be the stereo-electronic features of the substrate. In other words, highly stereoselective glycosylation to obtain **1a**, and especially **1b**, in the absence of the C2 functionality that controls the stereoselectivity in the conventional glycosylation method represents a significant synthetic challenge. Recent advances in reagent-controlled glycosylation without the aid of a functional group on the sugar molecule have enabled the stereoselective formation of α- or β-glycosides.[41–43] However, glycosylation to obtain the α- and β-isomers from the same substrate is rare. We considered that catalyst- or ligand-controlled glycosylation would be a promising approach.

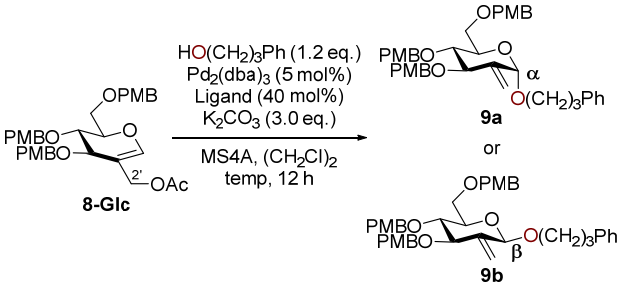
Specifically, we planned to employ Tsuji-Trost-type allylation with **4**, based on preliminary results reported by Vanker and co-workers.[33] Ferrier-type glycosylation of the precursor **2** to stereoselectively furnish α- or β-2,3-dehydro glycan analogues (**3a** or **3b**, Figure 1B) controlled by ligand[44] or catalyst[45] has already been reported. In contrast to their case, in which the stereochemistry of the π-allylpalladium complex should be regulated by the C3-chiral center, we considered that the precursor **4** without a stereocenter at the C2' position bearing the leaving group LG could in principle afford both intermediates (**5a** or **5b**). Subsequent reaction with harder acceptor molecules (ROH) through regio-selective inner-sphere reductive elimination[46-48] at the more electrophilic anomeric position would afford **1a** or **1b** depending on the stereochemistry of the Pd(II)-OR complex (like **7a** or **7b**).[49] Therefore, face-selective formation of intermediates **5** or **7** controlled by the ligand should enable the stereo-selective synthesis of compound **1**. Nevertheless, this superficially simple scenario involves inherent difficulties arising from the effects of substituent stereochemistry in the precursor **4** or the involvement the putative oxonium species **6**, which is a tautomeric structure of complex **5**.

Ligand Screening for Pd-catalyzed Glucal Glycosylation

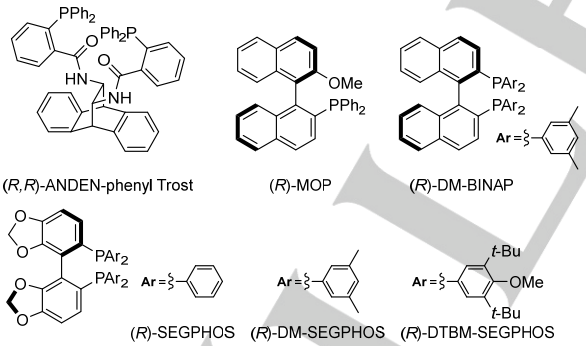
The glucal precursor was prepared in 5 steps from triacetyl D-glucal (Scheme S1). Based on initial findings, we employed acetate **8-Glc** for feasibility studies of ligand-controlled glycosylation (Table S1). When we used PPh_3 as a ligand, tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) as a source of palladium complex (Table S2) and the primary alcohol 3-phenylpropanol as a model acceptor molecule, the reaction scarcely occurred at 50 °C, but proceeded at elevated temperature to give **9a** in a highly α-selective manner (Table 1, entries 1-3). Next, we screened various phosphine ligands (see Table S3 for full data). As a result, 1,1'-bis(diphenylphosphino)ferrocene (dppf) enhanced both the reaction efficiency and α-selectivity, affording **9a** in good yield (Table 1, entry 4). Thus, we focused on dppf as a ligand for α-selective glycosylation. On the other hand, finding a ligand for β-selective glycosylation proved difficult since most achiral ligands produced **9a** as the major product. However, chiral ligands enabled selective formation of **9b**. Both enantiomers of the proven Trost ligands ANDEN-phenyl[49] and the monodentate ligand MOP selectively gave **9a** (entries 5-8). In the case of DM-BINAP ligands, the α-selectivity obtained with the (*R*)-isomer was moderate (2.3:1), whereas the (*S*)-isomer gave **9a** with high α-selectivity (16:1, entries 9 and 10), suggesting that (*R*)-DM-BINAP is a mis-matched ligand. A remarkable decrease of α-selectivity was observed with (*R*)-SEGPHOS (2.6:1, entry 12) and (*R*)-DM-SEGPHOS as ligands (1.9:1, entry 13). Finally, β-selective glycosylation proceeded with (*R*)-DTBM-SEGPHOS, and **9b** was formed in good yield with high selectivity at 120 °C

(76%, 1:10, entries 14 and 15). Low α-selectivity was observed with the corresponding (*S*)-isomer (60%, 1:1.7, entry 16), suggesting that reversal of the match/mismatch relationship occurs with the DTBM-SEGPHOS ligand.

Table 1. Pd-catalyzed glycosylation of **8-Glc**: Effect of ligands



Entry	Lignad	Temps [°C][a]	Yield of **9** [%][b]	Ratio (**9a**:**9b**)[b]
1	PPh_3	50	6	20:1
2	PPh_3	70	30	15:1
3	PPh_3	95	49	20:1
4	**dppf**	95	**70**	**>20:1**
5	(*R,R*)-ANDEN-phenyl	95	30	2.9:1
6	(*S,S*)-ANDEN-phenyl	95	16	1.8:1
7	(*R*)-MOP	95	30	>20:1
8	(*S*)-MOP	95	57	18:1
9	(*S*)-DM-BINAP	95	34	16:1
10	(*R*)-DM-BINAP	95	48	2.3:1
11	(*S*)-SEGPHOS	95	41	>20:1
12	(*R*)-SEGPHOS	95	64	2.6:1
13	(*R*)-DM-SEGPHOS	95	50	1.9:1
14	(*R*)-DTBM-SEGPHOS	95	trace	Not determined
15[c]	**(*R*)-DTBM-SEGPHOS**	120	**76**	**1:10**
16[c]	**(*S*)-DTBM-SEGPHOS**	120	**60**	**1:1.7**



[a] Reaction was conducted in a sealed vial; [b] Determined by ^{1}H-NMR with dimethylsulfone as an internal standard. [c] Toluene was used as a solvent. dppf: 1,1'-bis(diphenylphosphino)ferrocene.

Scope of Donors and Acceptors

We further optimized the reaction conditions (see Tables S7-S13) for α- or β-selective glycosylation of **8-Glc** (conditions A or B). Under these conditions, glycosylation with a variety of acceptors was examined (Figure 2, isolated yields are shown). 3-Phenylpropanol gave the α-isomer **9a** (90%, 15:1) and β-isomer **9b** (73%, 1:13) under conditions A and B, respectively. Other primary alcohols, benzyl alcohol, furfuryl alcohol, and 3-thiophenemethanol, were similarly converted to **10-12** in good yield with high stereoselectivity. Furthermore, 2-pyridinemethanol afforded **13**, indicating that a basic heterocyclic moiety is tolerated under these conditions. On the other hand, glycosylation with secondary alcohol acceptors under conditions B was sluggish at 120 °C, but proceeded satisfactorily at 140 °C in xylene to give **14b** in a β-selective manner with sterically less-hindered cyclohexanol. The use of more hindered 2-adamantanol was also possible, affording **15b** with reasonable β-selectivity (66%, 1:3.5).

Glycosylation with the carbohydrate-based primary alcohols was successful, giving pseudo-1,6-disaccharides **16** and **17** in a highly α- or β-stereoselective manner. Both isomers of pseudo-1,3-disaccharides **18** were selectively obtained with 3-OH of galactose, though a high temperature (140 °C in xylene) was required for the formation of **18b**. Glycosylation with 4-OH glucose proceeded under conditions A to afford **19a**. However, conditions B also afforded **19a** at 170 °C in xylene. The reason for the undesired stereoselectivity is presumably steric hindrance of the acceptor molecule, resulting in ligand-independent glycosylation via oxonium-like intermediate **6**. However, the use of 4-OH of 1,6-anhydroglucose acceptor resulted in α- or β-selective formation of **20** in good yield.

This ligand-controlled glycosylation strategy was also applicable to the synthesis of pseudo-glycolipids. In this synthesis, we used **24-Glc** with acetyl protection of 6-OH for further transformations. Reaction with *N*-*t*-butoxycarbonyl (*N*-Boc) sphingosine acceptor under conditions A or B provided pseudo-α- or β-glycosyl sphingosine **21-Boc** in a highly stereoselective manner in good yield, suggesting that the allylic ether structure in the acceptor was well tolerated. The use of ceramide acceptors gave **21-C6**, **21-C12**, and **21-C18** with excellent (for α-isomers) or moderate (for β-isomers) yield and selectivity. Reactions with a phytoceramide acceptor also proceeded similarly to afford **22** in good yield. It should be mentioned that the Ac group on the donor was tolerant, but reaction with Ac-protected acceptors gave complex mixtures. Synthesis of pseudo-α- or β-glycosyl cholesterol **23** was also successful.

We then examined the reaction of the stereoisomer of the donor **8-Glc** (Scheme 1). Although the appropriate ligand for α-selective glycosylation should be changed from dppf to (*R*)-MOP (conditions C, Table S4), glycosylation of the C4 epimer **8-Gal** (Scheme S2) with 3-phenylpropanol provided **25** with complete stereoselectivity (Scheme 1A). For a secondary alcohol acceptor such as cyclohexanol or cholesterol, conditions C and B afforded both isomers of **26** and **27** in good yield with good selectivity. Interestingly, for the C3 epimer **8-All** (Scheme S3), stereoselectivity profiles by ligands were dramatically different (Scheme 1B). Thus, we re-examined the ligands (Table S5) and found that glycosylations of **8-All** gave the 2-exomethylene allosides **28a**-**30a** in a α-selective manner with (*S*)-DTBM-SEGPHOS (conditions D), an antipode of the β-selective ligand for **8-Glc**. On the other hand, selective formation of β-allosides **28b**-**30b** was realized with (*S*)-*t*Bu-PHOX (conditions E), and (*R*)-SEGPHOS (Table S5), an antipode of the α-selective ligand for **8-Glc**, also provided β-alloside **28b** as the major isomer. These results indicate that ligands are less affected by the C4 stereochemistry but are strongly influenced by the C3 stereochemistry, and the stereochemistry of the product can be controlled by using a ligand with matching chirality. Overall, the

established methodology can provide a wide range of 2-exomethylene-type pseudo-glycans.

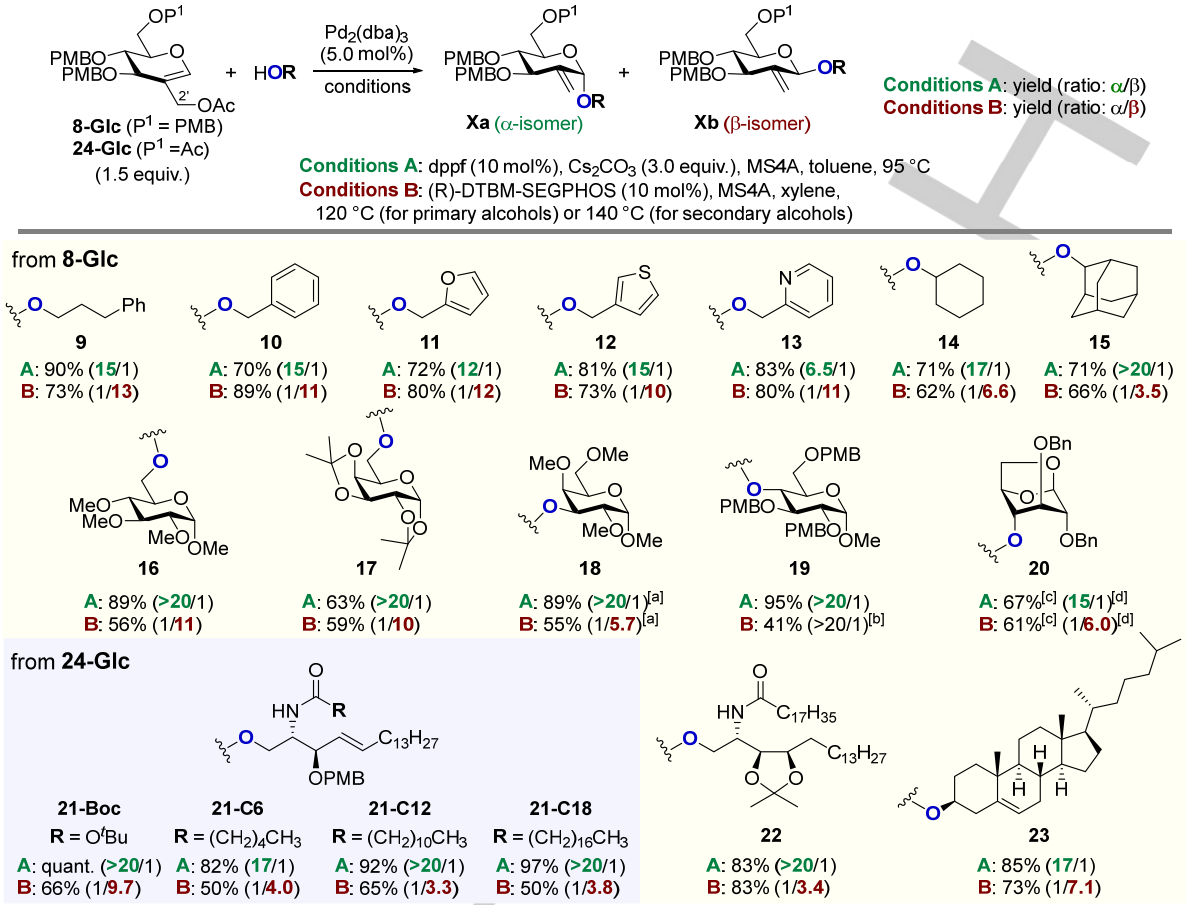
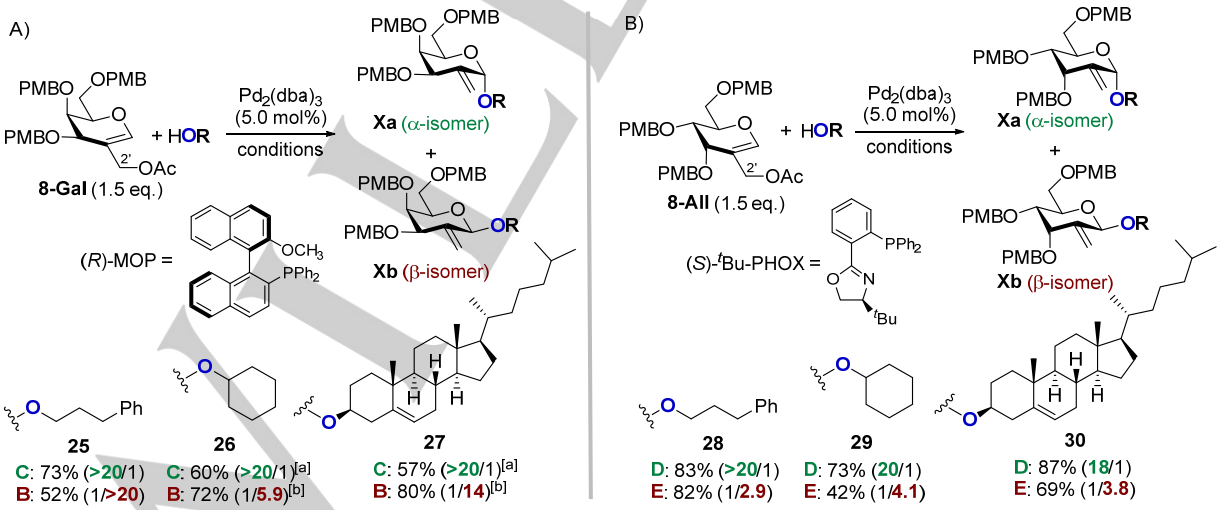


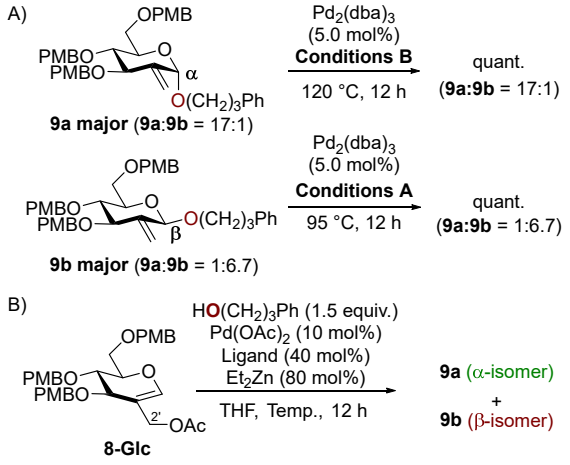
Figure 2. Ligand-controlled glycosylation of **8-Glc** or **24-Glc** (isolated yields are shown). [a] **8-Glc** (3.0 equiv.); [b] Reaction was conducted at 170 °C; [c] Isolated yields of the major isomer; [d] Determined by ^{1}H-NMR of the crude material



Scheme 1. Ligand-controlled glycosylation of (A) **8-Gal** and (B) **8-All**. Condition B: (*R*)-DTBM-SEGPHOS (10 mol%), MS4A, xylene, 120 °C (for primary alcohols) or 140 °C (for secondary alcohols); conditions C: (*R*)-MOP (10 mol%), Cs_2CO_3 (3.0 equiv.), MS4A, toluene, 95 °C; conditions D: (*S*)-DTBM-SEGPHOS (10 mol%), MS4A, xylene, 120 °C (for primary alcohols) or 140 °C (for secondary alcohols); conditions E: (*S*)-^{t}Bu-PHOX (10 mol%), Cs_2CO_3 (3.0 equiv.), MS4A, xylene, 140 °C. [a] *R*-MOP (40 mol%) was used.; [b] Donor (1.0 equiv.) and acceptor (HOR) (1.5 equiv.) were used.

Control Experiments and Mechanistic Considerations

Several additional experiments provided mechanistic insights into the stereoselectivity in the ligand-controlled glycosylation. When the α-glycoside **9a** was subjected to conditions B (β-selective glycosylation condition), no conversion of α-glycoside **9a** to β-glycoside **9b** was observed at all (Scheme 2A, Scheme S4). Transformation from **9b** to **9a** under conditions A was also not detected, indicating that glycoside formation is kinetically controlled.

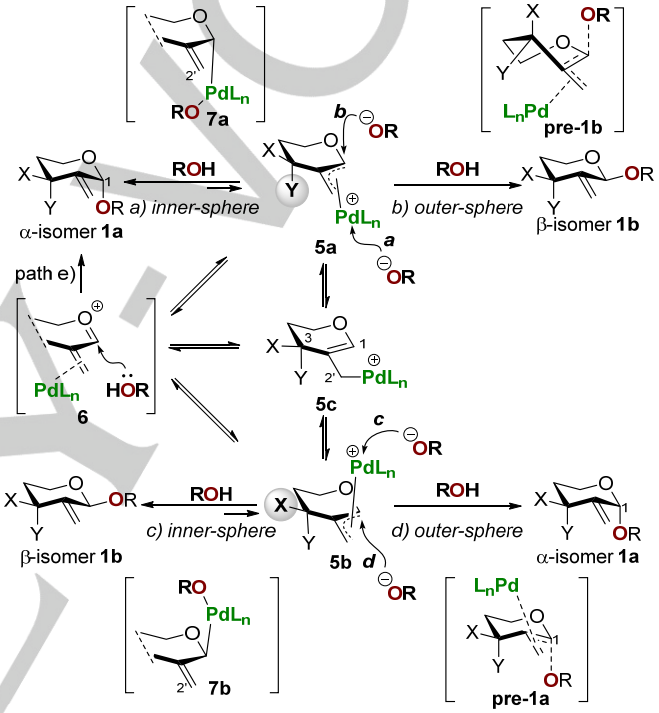


Entry	Ligand, Temp.	Yield (**9a**:**9b**)[a]
1	dppf, 95 °C	54% (>20:1)
2	dppf, rt	72% (>20:1)
3	(*R*)-DTBM-SEGPHOS, 95 °C	59% (>20:1)
4	(*R*)-DTBM-SEGPHOS, rt	12% (11/1)

Scheme 2. A) Reversibility of Pd-catalyzed glycosylation; B) Investigation of the effect of Et_2Zn. [a] Diastereomeric ratios were determined by ^{1}H-NMR with dimethylsulfone as an internal standard.

The addition of Et_2Zn was found to be critical for Ferrier-type glycosylation of the precursor **2**, shown in Figure 1B.[44] Lee and co-workers reported that outer-sphere reductive elimination could be accelerated by zinc alkoxides formed *in situ* in the reaction of bidentate phosphine-coordinated allylpalladium species with aliphatic alcohol as a nucleophile.[50,51] According to Lee's report, we used $Pd(OAc)_2$ as a palladium source instead of $Pd_2(dba)_3$, and glycosylations using the α-selective ligand dppf or β-selective ligand (*R*)-DTBM-SEGPHOS were investigated in the presence of Et_2Zn. Reaction with dppf proceeded even at room temperature but provided α-glycoside **9a**, not β-glycoside **9b**, in a highly selective manner. This was the same stereochemical outcome as in the case of the reaction in the absence of Et_2Zn (Scheme 2B, entries 1-2). On the other hand, addition of Et_2Zn in the reaction with (*R*)-DTBM-SEGPHOS ligand provided α-glycoside **9a** in a highly selective manner even at room temperature. The reaction did not occur at room temperature in the absence of either Et_2Zn or $Pd(OAc)_2$ (Table S14). These results indicate that oxidative addition is enhanced by the addition of Et_2Zn.

As mentioned above, conditions B in the absence of Et_2Zn produced β-glycoside **1b**, presumably via an inner-sphere process from **5b** via **7b** (Scheme 3). If this is the case, it would be reasonable to consider that formation of **1a** by addition of Et_2Zn occurs through the outer-sphere process via the chair-type **pre-1a**. Similarly, if the generation of α-glycoside **1a** follows the inner-sphere process via **7a** from **5a**, the results of entries 1 and 2 (Scheme 2B) can be interpreted as indicating that the production of **1b** from **5a** in the outer-sphere process is significantly disfavored, so that **1a** is formed via the inner-sphere process even in the presence of Et_2Zn. Formation of **1b** may be kinetically unfavorable due to the instability of the skew-boat-type transition state **pre-1b** in the outer-sphere pathway. Overall, our results suggest that the reactions under our optimum conditions should occur via an inner-sphere process.



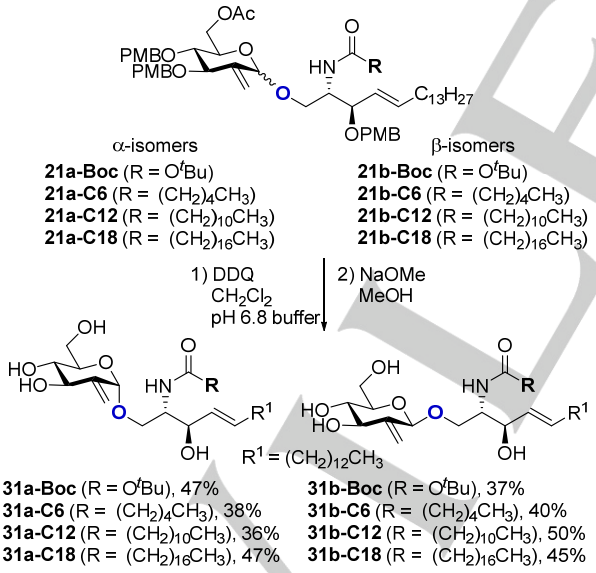
Scheme 3. Plausible reaction pathways of allylpalladium complexes **5a**, **5b**, and **5c**.

The key π-allylpalladium intermediates **5a** and **5b** could be interconvertible through π-σ-π equilibration via σ-allylpalladium **5c** (Scheme 3). In the case of **8-Glc** and **8-Gal** (X = OPMB, Y = H), given the proximity to the olefin of glycals and steric repulsion with the substituent X at C3, the formation of **5a** (or the following intermediate **7a**) seems likely to be kinetically and thermodynamically favorable. These speculations are supported by the fact that **1a** was preferentially formed with almost all ligands, including chiral ligands. The fact that preferential formation of **1b** was observed only with the bulky (*R*)-DTBM-SEGPHOS can be reasonably explained by assuming that (*R*)-DTBM-SEGPHOS reversed the relative stability of the π-allylpalladium intermediates. Namely, formation of **5b** (or **7b**) might be thermodynamically preferred over **5a** (or **7a**) with (*R*)-DTBM-SEGPHOS. On the other hand, in the case of **8-All** (X = H, Y = OPMB), where the C3 substituent was swapped, most ligands gave a mixture of **1a** and **1b** with low selectivity (Table S5). These facts indicate that the relative stabilities of **5a** (or **7a**)

and **5b** (or **7b**) may be similar. However, **1a** and **1b** could be selectively generated by using the appropriate chiral ligands, suggesting that these ligands alter the thermodynamic stability of one of the intermediates. In addition, in the reaction to produce **28** from **8-AII**, we observed increased β-selectivity with increasing reaction temperature (Table S6). These results support our hypothesis that the thermodynamic stability of the intermediates is a significant determinant of the stereoselectivity.

Synthesis of pseudo-GlcCer

With the above synthetic methodologies in hand, we next investigated the biological activities of 2-exomethylene sugar-containing pseudo-glycans. β-Glucosyl ceramides (**β-GC**, Figure 3A) are important glycosphingolipids found in various organisms and act as common biosynthetic precursors of a variety of glycolipids including gangliosides.[52,53] Dysfunction of glucocerebrosidase GBA1, one of the enzymes responsible for degradation of **β-GC**, is known to be associated with Gaucher disease.[54] In order to evaluate whether the 2-exomethylene sugar-containing **pseudo-β-GCs** act as analogues of **β-GCs**, we synthesized **pseudo-β-GCs** (**31b-C6**, **31b-C12**, and **31b-C18**) and the corresponding α-isomers, **pseudo-α-GC** (**31a-C6**, **31a-C12**, and **31a-C18**), with different chain lengths of the fatty acid part from the each isomer of the Tsuji-Trost products **21-C6**, **21-C12**, and **21-C18**, using conventional de-protection methods (Scheme 4). It should be noted that coupling constants (^3J) of both **31a** and **31b** at H3-H4 and H4-H5 were very similar to those of glucose derivatives, suggesting that the 2-exomethylene glycosides adopt chair-like conformation as we speculated.



Scheme 4. Synthesis of pseudo-α-glucosylceramides **31a** or pseudo-β-glucosylceramides **31b** containing a 2-exomethylene sugar moiety.

pseudo-β-GlcCer as a substrate of GBA1

Upon treatment of these pseudo-glycolipids with commercially available GBA1 (Cerezyme®, imiglucerase), new bands corresponding to each ceramide was detected only from **pseudo-β-GC** but not from **pseudo-α-GC**, suggesting that the glycosidic linkages of **31b-C6**, **31b-C12**, and **31b-C18** are cleaved by GBA1 (Figure 3B). However, GBA1 degraded **31b-C18** less effectively than native **β-GC**, which has a C18 fatty acid chain, but the degradation efficiency gradually increased as the fatty acid chain in **pseudo-β-GC** was shortened. Thus, **pseudo-β-GC** is recognized by GBA1, but the affinity or enzymatic reaction rate is affected to some extent by the 2-exomethylene functional group.

pseudo-GlcCer as a Ligand of CD1d or Mincle

β-GC is an endogenous ligand for Mincle, one of the C-type lectin receptors that sense damage and trigger inflammatory responses.[55] Mincle was originally identified as a receptor of trehalose dimycolate (**TDM**),[4] which constitutes the cell walls of *Mycobacterium tuberculosis* and related species and triggers potent immunological responses. These facts and other findings suggest that Mincle recognizes glucose-based or mannose-based glycolipids with both α- or β-glucosides linkages, although to different degrees.[56] On the other hand, the existence of small amounts of **α-GC** (Figure 3A) as endogenous glycolipids in mammalians has been suggested,[57] and related molecules have been isolated from other organisms or plants.[58–60] However, the possibility that **α-GC** is a ligand for Mincle has never previously been discussed. On the other hand, α-galactosylceramides (**α-GalCer**, KRN7000), the C4 epimers of **α-GC**, are well-known ligands of CD1d, which serves to present glycolipids as antigens and activate NKT cells, showing immunostimulatory and antitumor activity.[3] It has been reported that **α-GC** also act as CD1d ligands, although they are generally less potent than **α-GalCer**.[61,62] In the present study, we investigated how 2-exomethylene glucose-containing pseudo-glycolipids function as ligands of Mincle and CD1d.

Binding of these pseudo-GCs to CD1d or Mincle was evaluated on NFAT-GFP reporter cells expressing these molecules. For CD1d, co-expression of T-cell receptor (TCR) of iNKT cells (clone: DN32.D3)[63] together with **α-GalCer** treatment strongly induced GFP expression (Figure 3C). In contrast, neither **pseudo-α-GC** nor **pseudo-β-GC** activated GFP expression at all (Figure 3C and S3). This indicates that the 2-OH group in **α-GC** or **α-GalCer** plays a critical role in binding to CD1d and/or recognition by iNKT TCR. This finding is consistent with the results of a crystallographic analysis of **α-GalCer** complex with CD1d (Figure S4).[64]

For both mouse and human Mincle, co-expression of FcRγ along with treatment of **TDM** induced GFP expression even at 1 ng/well, as reported previously (Figure 3D).[4] In contrast, analogues of **β-GC** (**pseudo-β-GC**) showed very weak GFP-inducing ability (Figure 3D and Figure S5). Mincle activation could be induced by native **β-GC** with a long-chain fatty acid (C24:1),[54] so it is possible that **pseudo-β-GC** with C_{12} or C_{18} fatty acid could not fully activate Mincle. On the other hand, we found that **pseudo-α-GCs**, **31a-C12** and **31a-C18** exhibited remarkable GFP-inducing ability even at 10 ng/well, whereas the corresponding native **α-GC**(C_{12}) and **α-GC**(C_{16}) were found to be less effective. These results indicate that introduction of the 2-exomethylene into **α-GC** enhanced the Mincle ligand ability of native **α-GC**. Interestingly, in the case of **pseudo-α-GC**, **31a-C12** is more active than **31a-C18**. This may be a unique feature of glycolipids with the α-glucoside structure, since a similar

tendency was observed in α-glucosyl diacylglycerol derivatives,[65] despite the fact that glycolipids that are Mincle ligands often have longer lipid lengths, as in TDM and **β-GC**. Effective Mincle activation was not observed at much shorter lipid lengths (Figure S5), suggesting the lipid length of around C12 would be appropriate. Finally, we confirmed that cytokine MIP-2 production from bone marrow-derived dendritic cells (BMDCs), which is a known effect of Mincle activation, was induced by **31a-C12**, while the corresponding native **α-GC**(C_{12}) was less effective (Figure 3E). These results indicate that the introduction of the 2-exomethylene group can modulate the biological activity of native glycolipids.

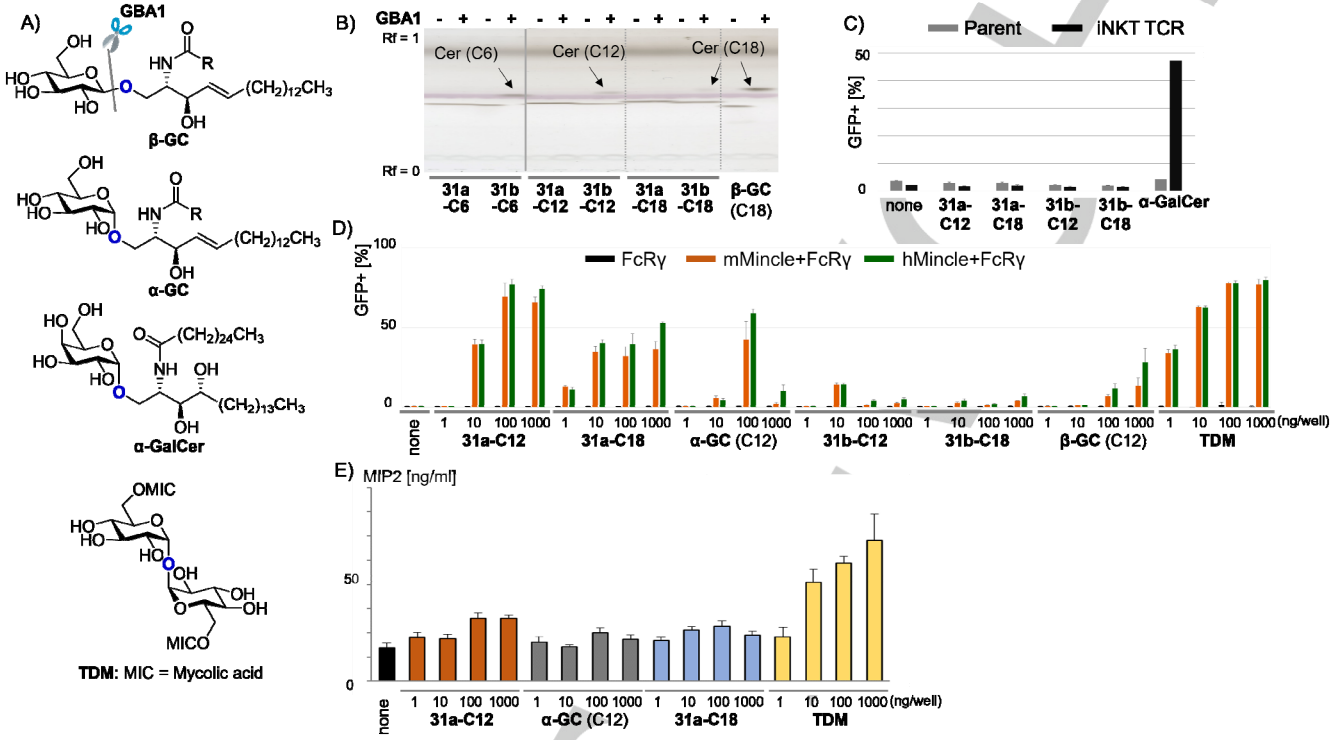


Figure 3. A) Structures of native β-glucosyl ceramides (**β-GC**), α-glucosyl ceramides (**α-GC**), α-glucosyl ceramides (**α-GalCer**, KRN7000) and trehalose dimycolate (**TDM**). B) TLC analysis of cleavage reactions of 500 μM **pseudo-β-GC**, **pseudo-α-GC**, and **β-GC** by GBA1 (20 μg) in the presence of 35% DMSO, 0.25% Triton X-100, and 0.6% sodium taurocholate. C) NFAT-GFP reporter cells expressing CD1d alone and CD1d + T-cell receptor of mouse iNKT cells were stimulated with 5 μg/well of **pseudo-β-GC**, **pseudo-α-GC**, and **α-GalCer** (C26:0). GFP expression was analyzed by flow cytometry. D) NFAT-GFP reporter cells expressing FcRγ alone, FcRγ + mouse Mincle, and FcRγ + human Mincle were stimulated with **pseudo-β-GC**, **pseudo-α-GC**, **β-GC**, **α-GC**, and **TDM**. GFP expression was analyzed by flow cytometry. E) WT BMDCs were stimulated with **31a-C12**, **α-GC**(C_{12}), **31a-C18**, or **TDM** for 1 d. Concentrations of MIP-2 in the supernatants were measured by ELISA. Data are presented as mean ± SD of triplicate assays (C-E).

Conclusion

In conclusion, we have achieved ligand-controlled, stereoselective syntheses of both α- and β-glycosides containing a 2-exomethylene group. The pseudo-α-glucosylceramides exhibit selective ligand activity toward the C-type lectin Mincle, but in contrast to native α-glucosylceramides, are inactive towards CD1d. This suggests that the 2-hydroxyl group of native glucosylceramides is important for the activation of CD1d, but not for the activation of Mincle. As exemplified in this study, 2-exomethylene pseudo-glycans would be new class of useful analogues, which alter and tune the biological activity compared to the native glycans due to lack of the 2-OH and 2-amino groups. Furthermore, 2-exomethylene pseudo-glycans could be useful precursors of derivatives with various functionalities at C2. Further investigations to expand the ability of 2-exomethylene pseudo-glycans are underway.

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Keywords: glucosylceramides • glycan analogues • reagent-controlled glycosylation • Tsuji-Trost allylation • immune receptor

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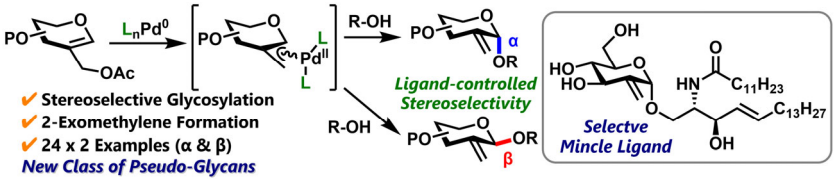

Phosphine ligand-controlled Tsuji-Trost-type glycosylation enabled stereoselective synthesis of α- and β-pseudo-glycoconjugates with an exomethylene group at the C2 position. These compounds are Mincle-selective ligands that do not bind to Cd1d, suggesting that the 2-OH of natural glycoconjugates is critical for CD1d binding.

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