nature portfolio

Peer Review File

Carbene Catalysed Chirality-Controlled Site-Selective Acylation of Saccharides

Corresponding Author: Professor Yonggui Robin Chi

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is a very nice paper that describes the catalyst-controlled acylation of a number of minimally protected sugars. This is a very difficult problem, and one that has only recently been generally accepted as important by the mainstream synthetic community. Several different NHC-based catalysts have been brought to bear on the challenge, and excellent reports are described throughout the paper.

It is also very interesting that the acyl group ultimately comes in via the aldehyde as is required by the mechanism. This strategic choice also raises the possibility that acetals are possible intermediates in these reactions. Perhaps the authors can comment on this possibility.

The authors may wish to comment on any experiments that assure that the product ratios reflect the full kinetic selectivity. Is any acyl migration observed under the reaction conditions?

Table 1 reports ratios of products formed at 2- and 3- hydroxy sites. Is the 4-OH group fully intert under these reaction conditions?

The scope of the reaction is largely demonstrated on the aldehyde piece of these reactions, and it is quite impressive. The sugar side of the process is necessarily tougher to explpre, but the authors do a good job nonetheless, and show some results in the galctosyl series. Was mannosyl examined.

The DFT and NMT studies at the end are quite interesting and lend some rationality to the outcomes. The Si- and Re-face designations in this section are a bit misleading given the stereochemical situation presented by the pre-existing chirality in the nucleophile. It might be easier to read this section if these designations were dropped in favor of simple discussions of the differential energies of the transition states.

All in all, this is an excellent paper that adds to the field, and with the attention to the issues above, publication in Nature Communications will be warranted.

Minor point: The paper is a bit light on its acknowledgment of older precedent in the field, including direct work on the site-selective acylation of saccharides. The authors may wish to cite the older works of Miller, which some of the cited papers, for example: (a) “A Peptide-Based Catalyst Approach to the Regioselective Functionalization of Carbohydrates” Griswold, K. S.; Miller, S. J. Tetrahedron 2003, 59, 8869-8875. (b) “Site-Selective Derivatization and Remodeling of Erythromycin A Using Peptide-Based Chiral Catalysts” Lewis, C. A.; Miller, S. J. Angew. Chem. Int. Ed. 2006, 45, 5616-5619. (c) “Structure Diversification of Vancomycin through Peptide-Catalyzed, Site-Selective Lipidation: A Catalysis-Based Approach to Combat Glycopeptide-Resistant Pathogens” Yoganathan, S.; Miller, S. J. J. Med. Chem. 2015, 58, 2367-2377.
It also seems worth considering including some of this in Figure 1b.

Reviewer #2

(Remarks to the Author)

In this manuscript of Liu, Chi and co-workers, a thorough investigation into the selective functionalization and protection of the C2-OH and 3-OH groups in monosaccharides is presented. An extensive study on the substrate scope and late-stage modification of sacilin has been carried out, exhibiting remarkable regioselectivity and yields, thereby demonstrating the broad applicability of their methodology. Further investigation on the competitive reactions between a variety of sugars and different anomeric configurations, along with orthogonal acylation endowed this method with significant practical synthetic potential. The article is well-structured, and all new compounds are thoroughly characterized and described, including the absolute configuration of the new catalysts. This work represents an innovative strategy for the synthesis of carbohydrate-related products, offering an appealing approach for selectively acylation of monosaccharides. Despite the undeniable merits of the manuscript, there are some concerns that warrant attention before its publication:

1. The reactivity of the C4-OH group towards acylation is unclear. Have the authors observed any C4-OH acylation products? Clarification on the formation and rationale of potential C4-OH acylation would be beneficial.
2. The authors noted the significant influence of ether solvents on the efficiency of C2-OH acylation. Investigating the impact of ether solvents on the NHC G-catalyzed reactions could provide additional insights.
3. Examining the compatibility of aliphatic aldehydes within the reaction scope would help demonstrate the boundaries of the substrate applicability.
4. Exploring the use of reactive esters as acyl sources, as demonstrated in the previous work of the Chi group, may offer an alternative approach to avoid the use of DQ as an oxidant.
5. The density functional theory calculation contributes for the elucidation of the mechanism and origin of regioselectivity. However, the computational results were not well discussed in the manuscript. A more detailed discussion on the chirality recognition, match/mismatch stereochemical model, and the role of non-covalent interactions (if applicable) would enhance the manuscript. Moreover, a greater colour range of NCI analysis was suggested, as it was difficult to identify the non-covalent interaction in current range. And most importantly, the authors should emphasize how the calculations support the proposed key role of chirality match/mismatch in determining the regioselectivity, based on fundamental chemical principles, not the computed energies.
6. Some typos should be examined and carefully corrected: a) Page 2, line 108, entries 4-5; b) line 106, 1H NMR, eq. should be written formally as equiv.; c) Redraw the 4z in the Figure 2; d) Page 4, line 213, at a concentration of 0.1 mmol, the unit perhaps was wrong; e) azobenzene 8 should be in bold.

Reviewer #3

(Remarks to the Author)
The present manuscript describes a method to achieve site-selective mono-acylation of pyranosides using chiral N-heterocyclic carbine (NHC) as a catalyst via chirality match/mismatch between acyl and sugar substrates assisted by a hydrogen bonding network. The authors further applied their method on modification of salicin into chaenomeloidin, fluorescent labelling and the development of biomimetic receptors. DFT calculations supports their proposed catalytic mechanism. This manuscript can be published in this journal after modification as described below.
1. The authors did not give the background of acylation using aldehyde, NHC, base and DQ. The authors should first introduce such acylation in conjunction with the graphic and text, and further introduce whether this type of acylation reaction has an application in site-selective acylation of carbohydrates.
2. In the manuscript, the 3-OH of glucoside substrates and the 2-OH of α-pyranoside substrates can be site-selective mono-acylated via the authors' method. There are other methods that also can achieve the similar results. The authors should introduce these methods and compare them with their own. The authors' presentation of site-selective acylation of sugars is not systematic enough in introduction part.
3. The authors only gave two optimized transition state structures. They did not proposed a catalytic mechanism in detail. A detailed mechanism is necessary for understanding the manuscript.
4. The "Main Text:" should be removed in line 29. The footnote of Table 1 and Figure 2 should be revised (a, b,….should be revised a); b)…..). In the footnote of Figure 3, a) Selective…. Should be a) selective……. In Fig 2, "2-OH:3OH should be 2-OH:3-OH".
5. There are many sentences with repetitive content from line 119 to line 137. The authors should rewrite these two paragraphs.
6. If the method is not applicable to the 3-OH of other glycoside substrates and the 2-OH of α-pyranoside substrates, the authors should make this clear in the text.
7. In Figure 4a, the structure of glucoside was incorrectly drawn as galactoside.
8. In SI: p24, HRMS (ESI, m/z) calcd for C33H33O7 [M+H]+: 541.2221, found: 563.2219. Better using Figure SX instead of Supplementary Figure X.

Reviewer #4

(Remarks to the Author)
The work entitled "Carbene Catalyzed Chirally-Controlled Site-Selective Acylation of Saccharides" by Zhang, Chi and co-workers demonstrates the site-selective acylation of 6-O-trityl protected monosaccharides using chiral NHC catalyst, where aromatic aldehydes are acting as the acyl donor by the formation of reactive acyl azolium intermediate. The authors show the site selectivity of the acyl moiety incorporation between C3-OH and C2-OH by changing the chirality of the NHC catalyst. The reaction is studied with a variety of aromatic aldehydes.

The work is performed good to a large extent, particularly, developing the method of acyl transfer using chiral NHCs,

switching of site-selectivities by altered chiralities of the catalyst is unexceptional in monosaccharides, although protecting groups at C-4 and C-6 are different, examples are: acylation: Org. Lett. 2013, 15, 6178–6181; acetalization: J. Am. Chem. Soc. 2021, 143, 18592– 18604; Angew. Chem., Int. Ed. 2013, 52, 12932–12936. In the light of these and more reports, catalyst design is a concurrent theme and the development is not necessarily novel as a concept.
Further, a plenty of literature demonstrate the site-selective acylation of 6-O-protected pyranoside triols at C-3 carbon of a monosaccharide, using DMAP catalyst and acid anhydride reagent (e.g. Tetrahedron Lett. 2007, 48, 5031–5033; Carbohydr. Res. 2012, 359, 111–119 and J. Carbohydr. Chem. 2010, 29, 369– 378). In the light of this, acylations by the newly designed NHC catalyst are unexceptional, unless the compelling merits of NHC catalysis over DMAP catalysis for acylation reactions are described.

In glucopyranosides, 3-OH group of the derived triol is inherently reactive over other hydroxyl groups due to the intramolecular H-bonding network of the substrates. On the other hand, 2-OH acylation is challenging, and approaches are reported already by Dong and co-workers (Chem. – Eur. J. 2014, 20, 5013 – 5018) using the chiral Cu(II)-Ph-Box complexes. These developments put more queries on the novelty of the present work.

In the context of general carbohydrate chemistry, aliphatic acyl functionality, e.g. acetyl, pivaloyl, isobutyryl, are generally employed as protecting groups for their easy removal, as compared to the aromatic ester protecting groups. However, the present protocol is unsuitable for aliphatic aldehydes as acyl donors, although the authors incorporated compound 3w in Fig. 2 with an aliphatic substrate as the only example. Why is it so?. Due to this reason, the current method may not find practical usefulness for general carbohydrate chemistry acylations in the course of the synthesis of building blocks for oligosaccharide assembly. Thus merit of the work lies primarily for a broad spectrum of aromatic aldehydes along with aldehyde functionalized drug molecules, with excellent site-selectivity either at 3-OH or 2-OH.

For the reaction optimizations, in the case of 3-OH acylation K2CO3 is found to be the effective base, whereas for 2-OH acylation DABCO is the base of choice. What is the reason for this base dependency observation?. Effect of bases on rate and site-selectivity is studied in carbohydrate substrates (e.g. Org. Lett. 2004, 6, 945–948). Effect of solvents also merits an attention and discussion for the site selectivity.

Xylose is a case in point, where the alpha/beta anomers make either C-4 or C-2 more reactive, as an inherent property. However, the authors find this and all other substrates not possessing a reactivity difference when the anomers are studied. In effect, in none of the example, C-4 is ever affected. How do the authors establish the site of acylations so comprehensively. It is totally unclear neither from the text, nor from the supporting information section. How do the reactions are studied to estimate the product ratios, is it by NMR, HPLC or another method. Which method is being followed to estimate the selectivities.

Authors state that brominated NHC catalyst G exhibits the highest product selectivity and yield, whereas NHC H with strong electron-withdrawing group (-NO2) show reduced conversion rates and moderate yields (entry 8). This indicates that a change in electronic nature of the catalyst affects the reaction outcome (Nat. Chem. 2012, 4, 996-1003). How this electronic tuning on the catalyst affects the reaction rates and site-selectivity? This should be studied systematically.
The selective recognition of a particular carbohydrate substrate and / -anomer identification in the presence of a particular chiral catalyst is also very much reported in other site-selective acylation studies (using oligopeptide catalyst: Chem. – Eur. J. 2016, 22, 5914–5918, using chiral ligand and metal catalyst: Org. Lett. 2013, 15, 6178–6181). Reported methods were based on 4,6-O-benzylidene protected pyranoside diols. In the present study the authors extended the same concept for 6-O-protected triol system. Again the authors should mention the advantages of the current method over the reported ones.

In the fluorescent labelling experiment, the authors stated that in the presence of glucoside and galactoside mixture, under the standard condition of 3-OH acylation, glucoside is acylated selectively with azobenzene 8 over the galactoside. What is the reason for this substrate specific site selectivity?

The manuscript is largely focused on glucosides, with one example of galactoside 2-OBz (4ah). As monosaccharides come with that many epimers, in addition to anomers, the 1,2-cis and 1,2-trans dispositions and the selectivity offered by the NHC catalyst is important to note, for example, in the case of mannosides.

Lines 119-128 and next paragraph lines 129-137 state the same observations as a repeat. Make sure not to repeat the same text.

In SI all the compounds are properly characterized well, although not annotated, at least to differentiate the C-3 vs C-2 substitution. Check the yield percentages carefully.

Acylations are ever more important in chemical manipulations of the monosaccharides, although the presence in some natural product is also expected, ellagitannin is a grand example. Better references to highlight the importance of acylations covering the chemical manipulations should be included.

The merit of the present study is largely due to high site-selectivity either at 3-OH or 2-OH acylation reaction. A large variety of commercially available aromatic aldehydes were employed as acyl donors. This many aromatic acyl functionalized molecules may not be possible using acid anhydride derivatives, although one might pre-synthesize the acid anhydrides. The method finds its application for the efficient synthesis of the natural product chaenomeloidin as demonstrated by the authors. The orthogonal acylation strategy is an elegant application of the current method. The mechanism is studied by DFT calculations.

In the light of above points, the work is missing several important considerations to be of general value in carbohydrate chemistry and synthetic chemistry. The work at present might be suitable to a synthesis oriented journals, provided important points of characterizations, ratio calculations are addressed carefully.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I am satisfied withe revisions and recommend publication.

Reviewer #2

(Remarks to the Author)
The authors have well addressed previous comments. There is no more comments.

Reviewer #3

(Remarks to the Author)
I recommend the paper for publication in its current form.

Reviewer #4

(Remarks to the Author)
The authors addressed queries raised to the original submission. Important points are miserably missing due to the lack of highlighting the importance of inter- and intramolecular hydrogen bonding network in the monosaccharides. Hydrogen bonding schemes increase the nucleophilicity at a site. The absence of this interaction would make the hydroxyl group nucleophilicity lesser. The beta-anomers not undergoing acylations, for example at C-2 site is an example herein. Authors should highlight the importance of hydrogen bonding network for the nucleophilicities of hydroxyl groups at varied sites.

The above point also brings to the attention that site selective acylations are not limited to reactions using chiral catalysts only. Achiral catalysts that affect the hydrogen bonding are also competent for site selective acylations. Multivalent acylation catalysts are the case in point. The work on trivalent dialkylaminopyridine catalysts (J. Am. Chem. Soc., 2011, 133, 12220–12228 and Org. Biomol. Chem. 2024,22, 5134-5149) illustrate the site selective acylations that rely on the internal hydrogen bonding network for the site selectivities. A balanced consideration of the developments of site selective acylations in literature is important. A comprehensive presentation is critical in order to highlight the site selectivities.

Minor point: Pl check the 1H NMR splitting of monosaccharide ring protons, only doublet and doublet of a doublet are expected. Cases where the splitting is not clear, or appears to be a singlet or triplet, the same be denoted as apparent singlet or apparent triplet. There are no singlets and triplets to the ring protons.

Irrespective of the HMQC experiments enabling the regio-isomer identification as adopted in this work, it would be preferable to separately synthesise C-2 and C-3 acyl-functionalized derivatives by an altered synthetic route and validate the observations, at least in couple of instances. A weak cross peak denoting a particular regio-isomer may continue to be weak in interpretation also, particularly when the resonances are too close.

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October 16, 2024

(NCOMMS-24-33207-T)

Referee #1:

Main Scientific Questions

Q1. It is also very interesting that the acyl group ultimately comes in via the aldehyde as is required by the mechanism. This strategic choice also raises the possibility that acetals are possible intermediates in these reactions. Perhaps the authors can comment on this possibility.

Response: *Thanks very much for your valuable suggestions. Acetals and hemiacetals may be involved in the reaction process. However, under the present basic conditions, acetals from aldehyde and -OH groups are difficult to generate. Meanwhile, hemiacetals may occur but fail to be oxidized as acylated products due to the weak oxidizing environment (J. Org. Chem.* ***2017****, 82, 302–312; J. Am. Chem. Soc.* ***2022****, 144, 5441–5449, Chem. Commun.,* ***2013****,****49****, 6513-6515).*

Q2. The authors may wish to comment on any experiments that assure that the product ratios reflect the full kinetic selectivity. Is any acyl migration observed under the reaction conditions?

Response: *When the reactions time was prolonged, the regioselectivity and yields remained. No migration was observed.*

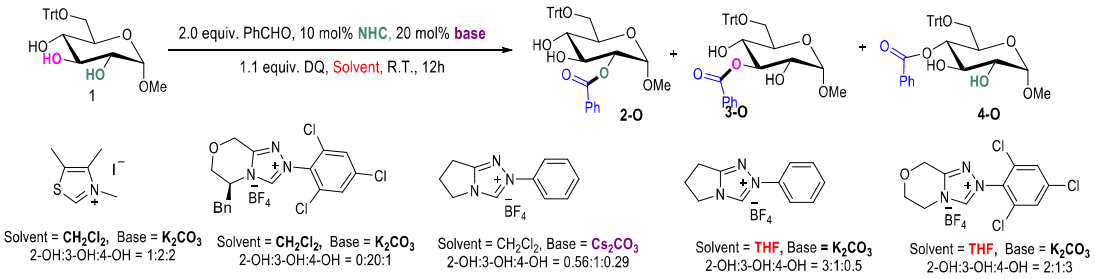
Following comments was added in the maintext:

"elongation of reaction time to 48 hours did not change the regioselectivity ratios and yields".

"During the screening. No acyl migration was observed under the reaction conditions".

Q3. Table 1 reports ratios of products formed at 2- and 3- hydroxy sites. Is the 4-OH group fully intert under these reaction conditions?

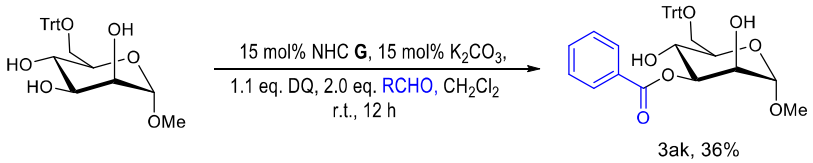
Response: *During the extensive screening, acylation on 4-OH could be identified in presence of some NHCs, for example:*



As shown above, the acylation on 4-OH could happen in these cases in a condition dependent manner. Under the optimized condition. 4-OH was successfully deactivated.

Q4. The scope of the reaction is largely demonstrated on the aldehyde piece of these reactions, and it is quite impressive. The sugar side of the process is necessarily tougher to explpre, but the authors do a good job nonetheless, and show some results in the galctosyl series. Was mannosyl examined.

Response: *Thanks for your valuable advice. We examined the mannosides. It can deliver good selectivity under optimized conditions albeit with poor yield. This example has been added into maintext as 3ak*



3ak, 36%

Q5. The DFT and NMR studies at the end are quite interesting and lend some rationality to the outcomes. The Si- and Re-face designations in this section are a bit misleading given the stereochemical situation presented by the pre-existing chirality in the nucleophile. It might be easier to read this section if these designations were dropped in favor of simple discussions of the differential energies of the transition states.

Response: *We thank the reviewer for her/his positive assessment of our work.*

We note that the Re and Si face designations are different for the aldehyde substrate and the thereafter formed acyl azolium intermediate. During the C–O bond formation step/transition state, the hydroxyl attacks the carbonyl carbon of the acyl azolium intermediate, as such the Re and Si face designations are with respect to the acyl azolium carbonyl group (C=O). This discussion provides the readers with better clarity on the possibility that the nucleophilic attack of carbonyl C=O of acyl azolium intermediate by the hydroxyl group of sugar can occur on either face (different energy barriers). Similar literature exists where this Re and Si face designations have been used (Org. Chem. Front. 2021, 8, 2413; Chem 2022, 8, 1518; Org. Chem. Front. 2021, 8, 3268; ACS Catal. 2021, 11, 3443; Chem. Sci., 2012,3, 2346; JPCA 2024 128, 6190).

We have clarified this in the manuscript to read:

"We examined the key transition state (TS) structures for the acylation step, allowing attacks from either the (Re)-face or the (Si)-face of the acyl azolium intermediate by the 2-OH and 3-OH groups. Thorough conformational samplings ensure that the most stable TS for each possibility is used for comparison (see Supplementary Information section 7.3 on conformational considerations)."

Q6. Minor point: The paper is a bit light on its acknowledgment of older precedent in the field, including direct work on the site-selective acylation of saccharides. The authors may wish to cite the older works of Miller, which some of the cited papers, for example: (a) "A Peptide-Based Catalyst Approach to the Regioselective Functionalization of Carbohydrates" Griswold, K. S.; Miller, S. J. Tetrahedron 2003, 59, 8869-8875. (b) "Site-Selective Derivatization and Remodeling of Erythromycin A Using Peptide-Based Chiral Catalysts" Lewis, C. A.; Miller, S. J. Angew. Chem. Int. Ed. 2006, 45, 5616-5619. (c) "SStructure Diversification of Vancomycin through Peptide-Catalyzed, Site-Selective Lipidation: A Catalysis-Based Approach to Combat Glycopeptide-Resistant Pathogens" Yoganathan, S.; Miller, S. J. J. Med. Chem. 2015, 58, 2367-2377.
It also seems worth considering including some of this in Figure 1b.

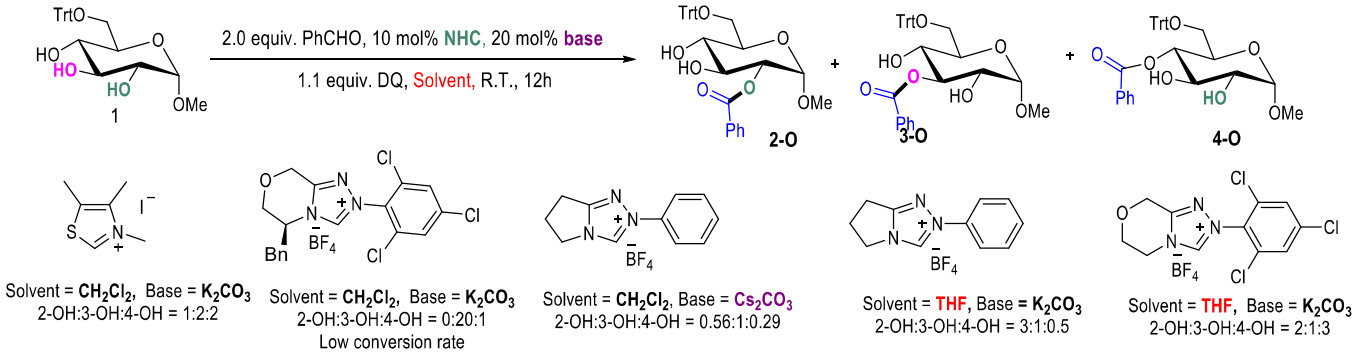
Response:*Thanks very much for you kind remind. These elegant works have been added into the introduction and Figure 1.*

Referee #2:

Main Scientific Questions

Q1. The reactivity of the C4-OH group towards acylation is unclear. Have the authors observed any C4-OH acylation products? Clarification on the formation and rationale of potential C4-OH acylation would be beneficial.

Response: *The C4-OH acylation products were generated in the condition dependent manner. The following Figure has been added into the Supplementary Information (Figure S4) and explained in the maintext.*



"Preliminary screening highlighted the challenges involved and revealed varying acylation ratios across the three secondary hydroxyl groups.(Figure S4). …4-OH was successfully deactivated under such circumstance."

Q2. The authors noted the significant influence of ether solvents on the efficiency of C2-OH acylation. Investigating the impact of ether solvents on the NHC G-catalyzed reactions could provide additional insights.

Response: NHC G-catalysed reactions have been investigated in the ether solvents such as THF, dioxane, Et_2O and iPr_2O (See Table S1, entry 20, 22, 23 and 24). They all deliver good regioselectivity albeit with lower yields. Ether solvents have less influence on 3-OH in presence of NHC G.

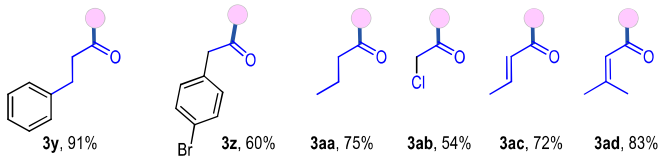
*We also examined by DFT calculation for NHC **G**. Computational studies using implicit solvation model show that the tested solvents (THF, Et_2O, and ipr_2O) also favor C3-OH acylation, Table. 1. It's worth noting that the discrepancy between calculated ratios and experimental ratios of C2/C3 may arise from the lack of additional interactions involving explicit solvent in the transition states and/or the resting states, as the implicit solvation model used treats the solvent as a continuum background reaction field. Additional solvent solubility of substrates and additives, which are not taken account into by the computational models, may also affect the exact yield and selectivity ratio.*

Table 1.Computational results for solvent effects.

Solvent	*NHC **G** (TS, kcal/mol)*		*Ratio(C2/C3)*
	TS_b1_G_O3_Re	***TS_b1_G_O2_Re***	***expt***
CH_2Cl_2	*0.0*	*3.7*	*C3 only*
THF	*0.0*	*4.5*	*C3 only*
Et_2O	*0.0*	*4.2*	*1:16*
iPr_2O	*0.0*	*4.1*	*1:20*

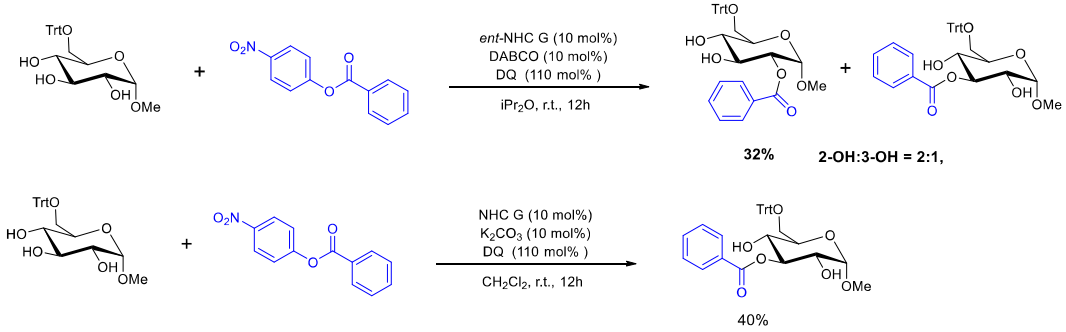
Q3. Examining the compatibility of aliphatic aldehydes within the reaction scope would help demonstrate the boundaries of the substrate applicability.

Response: *Six more aliphatic aldehydes were included in the reaction scope for 3-OH (Figure 2). The regioselectivity was still good enough, although the yields slightly decreased probably due to the low reactivity of aliphatic aldehydes.*



Q4. Exploring the use of reactive esters as acyl sources, as demonstrated in the previous work of the Chi group, may offer an alternative approach to avoid the use of DQ as an oxidant.

Response: *With 4-nitrobenzyl benzoate, 3-OH acylated product was obtained regio-selectively in presence of NHC G with 40% yield while 2-OH acylated product was obtained in presence of ent-NHC G with 32% yield and 2:1 regioselectivity (2-OH:3-OH).*



Q5. The density functional theory calculation contributes for the elucidation of the mechanism and origin of regioselectivity. However, the computational results were not well discussed in the manuscript. A more detailed discussion on the chirality recognition, match/mismatch stereochemical model, and the role of non-covalent interactions (if applicable) would enhance the manuscript. Moreover, a greater colour range of NCI analysis was suggested, as it was difficult to identify the non-covalent interaction in current range. And most importantly, the authors should emphasize how the calculations support the proposed key role of chirality match/mismatch in determining the regioselectivity, based on fundamental chemical principles, not the computed energies.

Response: *We thank the reviewer for her/his positive assessment of our work.*

*We appreciate the reviewer's insightful comments and have addressed their concerns by providing a more detailed discussion of the key computational results, including an in-depth geometrical analysis. During our careful re-evaluation of the TS conformations for both the NHC **G** and **ent-G** systems, we discovered that the calculated barrier differences between the most stable, selectivity-determining TS structures differ slightly from the values originally reported. These discrepancies have been corrected in the revision. Importantly, this correction does not change the overall conclusions of our study; in fact, the revised values yield O2:O3 selectivity ratios that are even more consistent with experimental observations. We have updated the text*

accordingly and revised Figure 4 in the main text, along with Supplementary Figures 5-8, to reflect these changes.

Additionally, we have refined the NCI analysis by simplifying the colour range to three distinct colours (blue, white, red) to enhance the clarity and understanding of the non-covalent interactions presented.

*We have revised the values (highlighted) for NHC **G** (entry 9) to read:*

*However, the calculated TS barriers suggest that 3-OH acylation via **TS_b1_G_O3_Re** has a barrier that is 3.7 kcal/mol lower than 2-OH acylation via **TS_b1_G_O2_Re**. This predicts that O3-acylation is kinetically most favorable, resulting in an estimated regioselectivity ratio of > 516:1 over O2-acylation, consistent with the experimentally observed exclusive 3-OH acylated product.*

*We have added the following discussion on the model system using NHC **G** (entry 9) in the manuscript to read:*

*"The lower energy barrier of O3-acylation TS, **TS_b1_G_O3_Re**, over O2-acylation TS, **TS_b1_G_O2_Re**, may be attributed to the late transition state and more favorable non-covalent interactions (NCIs) observed in the former. In **TS_b1_G_O3_Re**, the O–C bond formed between hydroxyl group of sugar and carbonyl carbon of acyl azolium intermediate is shorter (2.18 Å) than in **TS_b1_G_O2_Re** (2.48 Å, Figure S6); in addition, the π-π interaction is presumably stronger in the former (3.64Å) than the latter (3.68Å, Supplementary Figure 5). Additionally, the NCI plots suggests that **TS_b1_G_O3_Re** may benefit from more attractive interactions than in **TS_b1_G_O2_Re** (Figure S7), thereby greatly stabilizing the transition state structure."*

*We have revised the value (highlighted) for NHC **ent-G** (entry 17) to read:*

*Overall, the calculated TS barriers for the NHC **ent-G** catalysed model reaction suggest that 2-OH acylation via the most stable **TS_b2_ent-G_O2_Si** is lower by 1.8 kcal/mol compared to 3-OH acylation via **TS_b2_ent-G_O3_Re**.*

*and the discussion on the model system using NHC **ent-G** (entry 17) to read:*

*"This predicts that O2-acylation is kinetically most favorable, translating to a O2 : O3 acylation ratio of 28:1, agrees qualitatively well with our experimentally observed 2-OH acylated product selectivity (20:1). This lower barrier for O2-acylation TS, **TS_b2_ent-G_O2_Si**, can be attributed to the π-π interaction between the trityl group of sugar and the bromo-phenyl group of the acyl azolium intermediate (Figures S8 and S9). In contrast, **TS_b2_ent-G_O3_Re** experiences some steric hindrance between the trityl group and the acyl azolium intermediate (Figure S9)."*

Q6. Some typos should be examined and carefully corrected: a) Page 2, line 108, entries 4-5; b) line 106, 1H NMR, eq. should be written formally as equiv.; c) Redraw the 4z in the Figure 2; d) Page 4, line 213, at a concentration of 0.1 mmol, the unit perhaps was wrong; e) azobenzene 8 should be in bold.

Response: *Fixed with thanks*

Referee #3:

Main Scientific Questions

Q1. The authors did not give the background of acylation using aldehyde, NHC, base and DQ. The authors should first introduce such acylation in conjunction with the graphic and text, and further introduce whether this type of acylation reaction has an application in site-selective acylation of carbohydrates.

Response: *Following introduction on NHC was added*

"N-heterocyclic carbenes (NHCs) are important organocatalysis in reactions like acyl transfer, conjugate additions, and umpolung reactivity. Their tunable electronic and steric properties of NHCs allow chemists to modify their structure for specific catalytic applications, making them highly adaptable for a wide range of reactions. Their effectiveness in controlling reaction selectivity, both regio- and stereoselectivity, has led to widespread use in modern organic synthesis. Our groups have developed programmable selective acylation of saccharides using the combination of carbene and boronic acid[35]."

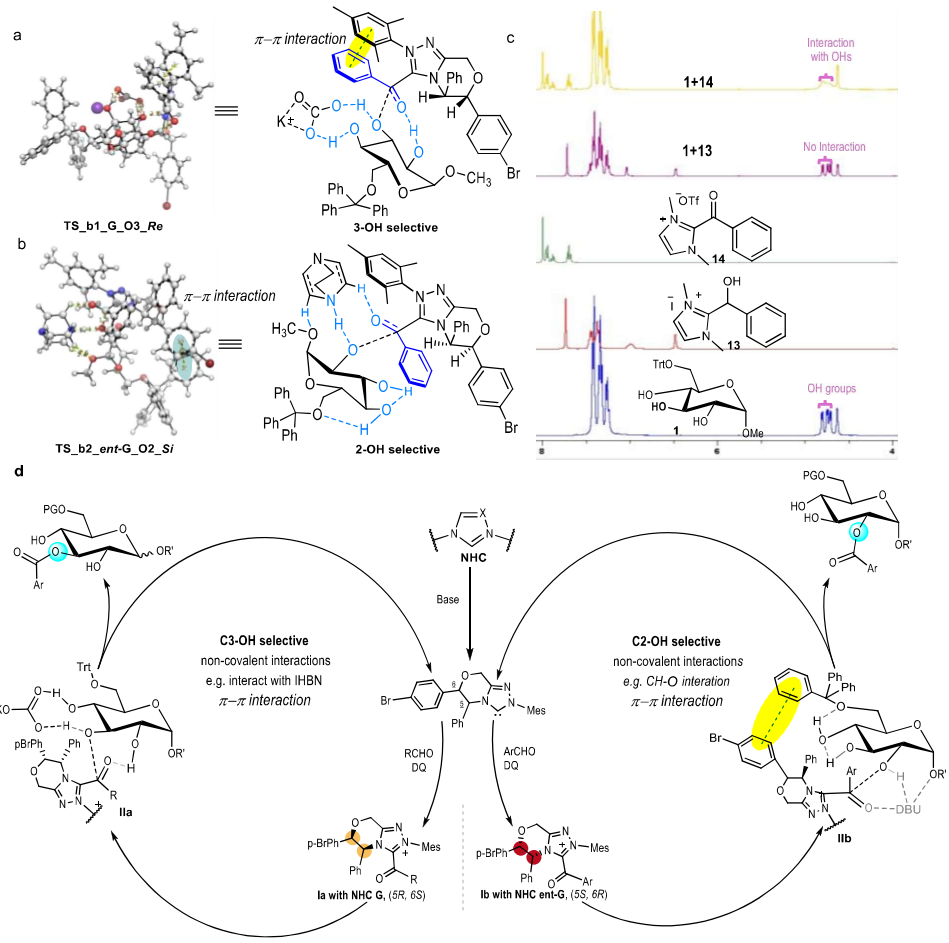
2. In the manuscript, the 3-OH of glucoside substrates and the 2-OH of α-pyranoside substrates can be site-selective mono-acylated via the authors' method. There are other methods that also can achieve the similar results. The authors should introduce these methods and compare them with their own. The authors' presentation of site-selective acylation of sugars is not systematic enough in introduction part.

Response: *The introduction has been revised. More reference on site-selective acylation of sugars have been presented.*

Our focus is on the selective functionalization of sugars, including acylation, as we believe that selectivity remains one of the grand challenges in glycochemistry. To address this complex issue, the continuous development of innovative tools and methodologies is crucial. We are confident that, with the growing understanding in this field, these challenges will eventually be overcome by scientists.

Q3. The authors only gave two optimized transition state structures. They did not proposed a catalytic mechanism in detail. A detailed mechanism is necessary for understanding the manuscript.

Response: *The reaction pathway has been proposed in Figure 4 and below*



Q4. The "Main Text:" should be removed in line 29. The footnote of Table 1 and Figure 2 should be revised (a, b,….should be revised a); b)…..). In the footnote of Figure 3, a) Selective…. Should be a) selective……. In Fig 2, "2-OH:3OH should be 2-OH:3-OH".

Response*: Fixed and thanks*

Q5. There are many sentences with repetitive content from line 119 to line 137. The authors should rewrite these two paragraphs.

Response*: Fixed and thanks*

Q6. If the method is not applicable to the 3-OH of other glycoside substrates and the 2-OH of α-pyranoside substrates, the authors should make this clear in the text.

Response: *Some glycoside substrates with acylation at 3-OH have been demonstrated in Figure 2 (3ae -3ak). α-pyranosides acylated at 2-OH have been demonstrated in Figure 2 (4a-4ai). However, our method of acylation at 2-OH is not suitable for β-pyranoside substrates.*

We have added following statement in our maintext:

"Further exploration of glucoside alterations revealed the indispensable role of the α-anomeric configuration in selectivity (4ab-4ai). This method is not applicable to acylation on 2-OH of β-pyranoside substrates"

Q7. In Figure 4a, the structure of glucoside was incorrectly drawn as galactoside.

Response*: Fixed with thanks*

Q8. In SI: p24, HRMS (ESI, m/z) calcd for C33H33O7 [M+H]+: 541.2221, found: 563.2219. Better using Figure SX instead of Supplementary Figure X.

Response*: Fixed with thanks*

Referee #4:

Main Scientific Questions

Q1. The work is performed good to a large extent, particularly, developing the method of acyl transfer using chiral NHCs, switching of site-selectivities by altered chiralities of the catalyst is unexceptional in monosaccharides, although protecting groups at C-4 and C-6 are different, examples are: acylation: Org. Lett. 2013, 15, 6178–6181; acetalization: J. Am. Chem. Soc. 2021, 143, 18592– 18604; Angew. Chem., Int. Ed. 2013, 52, 12932–12936. In the light of these and more reports, catalyst design is a concurrent theme and the development is not necessarily novel as a concept.

Further, a plenty of literature demonstrate the site-selective acylation of 6-O-protected pyranoside triols at C-3 carbon of a monosaccharide, using DMAP catalyst and acid anhydride reagent (e.g. Tetrahedron Lett. 2007, 48, 5031–5033; Carbohydr. Res. 2012, 359, 111–119 and J. Carbohydr. Chem. 2010, 29, 369– 378). In the light of this, acylations by the newly designed NHC catalyst are unexceptional, unless the compelling merits of NHC catalysis over DMAP catalysis for acylation reactions are described.

Response: *Thank you for insightful comments. I appreciate your recognition of the effort in developing the acyl transfer methodology using chiral NHCs. Regarding the point about site-selectivity in monosaccharides, our work aims to expand on the understanding of how subtle changes in chirality influence reactivity in systems with minimally protected glycosides.*

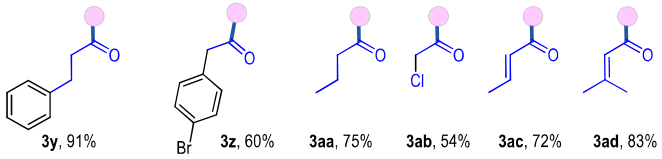
We believe that by building on previous work (e.g., Org. Lett. 2013, J. Am. Chem. Soc. 2021, and Angew. Chem. 2013, Tetrahedron Lett. 2007, 48, 5031–5033; Carbohydr. Res. 2012, 359, 111–119 and J. Carbohydr. Chem. 2010, 29, 369– 378), our contributions lie in refining catalyst design for more precise control of selectivity. Our findings push forward the practical applications of NHC catalysis in carbohydrate chemistry, offering new insights and methods for selective functionalization.

Q2. In glucopyranosides, 3-OH group of the derived triol is inherently reactive over other hydroxyl groups due to the intramolecular H-bonding network of the substrates. On the other hand, 2-OH acylation is challenging, and approaches are reported already by Dong and co-workers (Chem. – Eur. J. 2014, 20, 5013 – 5018) using the chiral Cu(II)-Ph-Box complexes. These developments put more queries on the novelty of the present work.

Response: *Thank you for insightful comments. The regioselective functionalization on sugars was far from resolved even though many elegant works have been reported. Our approach using NHC catalysis offers a distinct advantage in terms of controlling regioselectivity and stereoselectivity without relying on metal catalysts, as seen in the Cu(II)-Ph-Box complex method.*

Q3. In the context of general carbohydrate chemistry, aliphatic acyl functionality, e.g. acetyl, pivaloyl, isobutyryl, are generally employed as protecting groups for their easy removal, as compared to the aromatic ester protecting groups. However, the present protocol is unsuitable for aliphatic aldehydes as acyl donors, although the authors incorporated compound 3w in Fig. 2 with an aliphatic substrate as the only example. Why is it so?. Due to this reason, the current method may not find practical usefulness for general carbohydrate chemistry acylations in the course of the synthesis of building blocks for oligosaccharide Thus merit of the work lies primarily for a broad spectrum of aromatic aldehydes along with aldehyde functionalized drug molecules, with excellent site-selectivity either at 3-OH or 2-OH.

Response: *Six more aliphatic aldehydes were included in the reaction scope for 3-OH (Figure 2). The regioselectivity was still good enough, although the yields slightly decreased probably due to the low reactivity of aliphatic aldehydes.*



3y, 91% **3z**, 60% **3aa**, 75% **3ab**, 54% **3ac**, 72% **3ad**, 83%

Q4. For the reaction optimizations, in the case of 3-OH acylation K2CO3 is found to be the effective base, whereas for 2-OH acylation DABCO is the base of choice. What is the reason for this base dependency observation?. Effect of bases on rate and site-selectivity is studied in carbohydrate substrates (e.g. Org. Lett. 2004, 6, 945–948). Effect of solvents also merits an attention and discussion for the site selectivity.

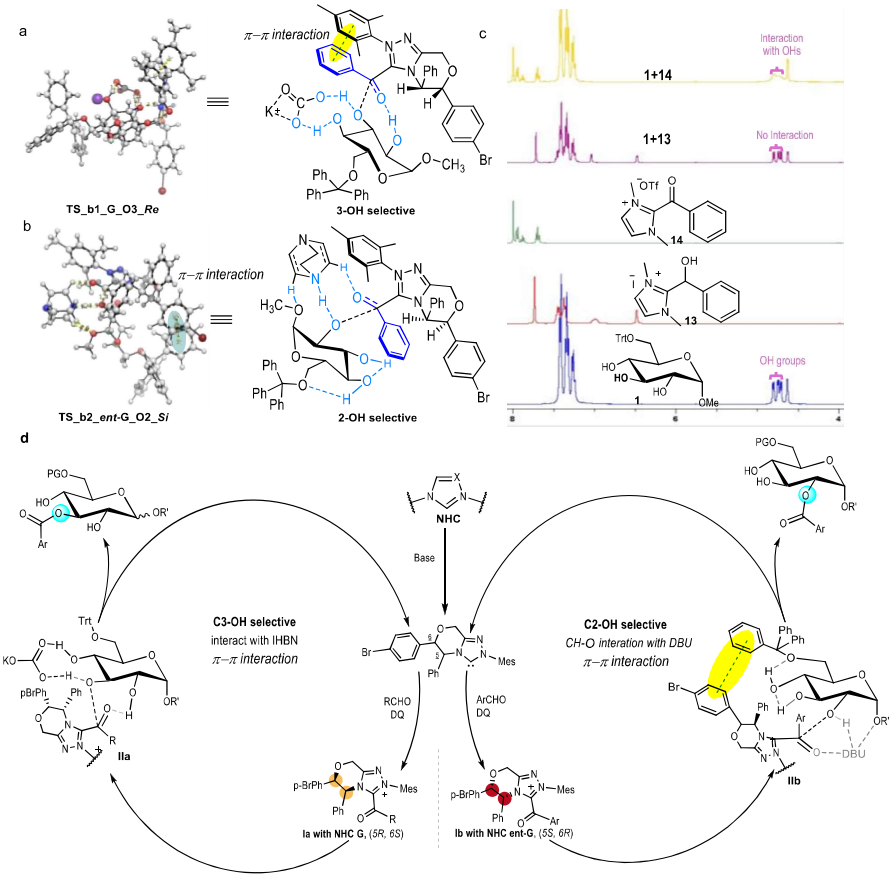
Response: *We screened the bases for acylation on 2-OH and 3-OH (see Table S1 and S2). Unfortunately, bases did not have a clear influence on the rate and site selectivity in presence of NHC G and ent-G. It's probably because their participation patterns into the transition state of bases were different in carbene-catalysed oxidative reactions (see Figure 4). Solvents have obvious effect on site selective acylation of 2-OH while they have very little effect on acylation of 3-OH.*

"This suggests that solvents affect the acylation of the 2-OH group, and ether solvents are favourable for the site selectivity"

Q5. Xylose is a case in point, where the alpha/beta anomers make either C-4 or C-2 more reactive, as an inherent property. However, the authors find this and all other substrates not possessing a reactivity difference when the anomers are studied. In effect, in none of the example, C-4 is ever affected. How do the authors establish the site of acylations so comprehensively. It is totally unclear neither from the text, nor from the supporting information section. How do the reactions are studied to estimate the product ratios, is it by NMR, HPLC or another method. Which method is being followed to estimate the selectivities.

Response: *4-OH was acylated in our extensive screening (see Figure S4 or following). Using optimized NHCs (NHC G and ent G), 4-OH was successfully deactivated due to steric hindrance, and non-covalent interaction including π–π interaction (chirality match /mismatch, See Figure 4). It's also worthy to note that our methodology of acylation onto 2-OH was only applicable to α-anomers. The maintext has been revised accordingly.*

The determination of regioselectivity ratios has been denoted under Tables/Figures. The regioselectivity ratios were determined by 1H NMR



Q6. *Authors state that brominated NHC catalyst G exhibits the highest product selectivity and yield, whereas NHC H with strong electron-withdrawing group (-NO2) show reduced conversion rates and moderate yields (entry 8). This indicates that a change in electronic nature of the catalyst affects the reaction outcome (Nat. Chem. 2012, 4, 996-1003). How this electronic tuning on the catalyst affects the reaction rates and site-selectivity? This should be studied systematically.*

Respones: *In presence of NHC H, the reactions finished more quickly within 4 hours (To make it clear, the reaction times was typically indicated in the footnote d and e under Table 1). The conversion rate become lower (79%, substrate's recover ratio:21%) than NHC G (quant.) without eroding the selectivity. These findings indicate that electronic tuning on NHC catalysts can affect the reaction rate and equilibrium. Stronger electron withdrawing groups could facilitate the reaction rate, albeit with reaching an unfavourable equilibrium. This could be complementary to Burke's outstanding research on electronic tuning of acyl donors and their counterions (Nat. Chem. 2012, 4, 996-1003).*

Following discussion was added in the manuscript to read.

"Notably, NHC F(entry 6), brominated NHC G (entry 7)and nitrated NHC H (entry 8, reaction time 4hours) all exhibited the excellent selectivity with 85%, 90% yield and 79% yield. This finding suggests that electronic tuning of N-heterocyclic carbene (NHC) catalysts can significantly impact both reaction rate and equilibrium. Introducing stronger electron-withdrawing groups may accelerate the reaction rate, although this could push the equilibrium toward less favorable conditions. This aligns with Burke's notable research on electronic tuning

of acyl donors and their counterions, where similar strategies of electron manipulation are employed to control reactivity and selectivity in catalytic processes[51].”

Q7. The selective recognition of a particular carbohydrate substrate and □/□-anomer identification in the presence of a particular chiral catalyst is also very much reported in other site-selective acylation studies (using oligopeptide catalyst: Chem. – Eur. J. 2016, 22, 5914–5918, using chiral ligand and metal catalyst: Org. Lett. 2013, 15, 6178–6181). Reported methods were based on 4,6-O-benzylidene protected pyranoside diols. In the present study the authors extended the same concept for 6-O-protected triol system. Again the authors should mention the advantages of the current method over the reported ones.

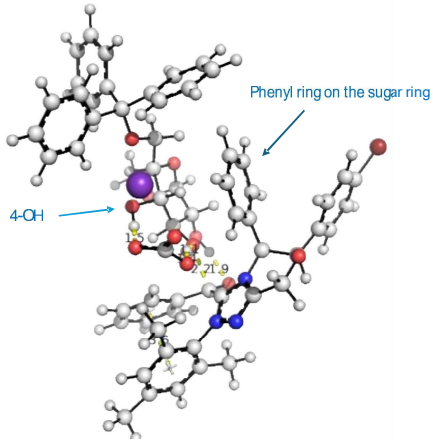
Response: *Thank you for bringing attention to the previously reported work on selective recognition of carbohydrate substrates and α/β-anomer identification in site-selective acylation studies. We appreciate your reference to methods using oligopeptide catalysts (Chem. – Eur. J. 2016) and chiral ligand-metal systems (Org. Lett. 2013) with 4,6-O-benzylidene protected pyranoside diols.*

In our study, we sought to extend these concepts to a 6-O-protected triol system, a substrate that presents additional challenges in terms of regioselectivity and functionalization due to the absence of 4,6-O-benzylidene protection. Our NHC catalyst system enables a selective acylation at C-3 and C-2, achieving a high degree of control in a more complex situation, where competing reactivities of multiple hydroxyl groups could be more problematic.

One of the key advantages of our method over the reported ones is its broader substrate scope and the ability to manipulate acylation site-selectivity with minimal protecting group interference, which can be difficult to achieve with metal or oligopeptide catalysts in more complex systems.

Q8 In the fluorescent labelling experiment, the authors stated that in the presence of glucoside and galactoside mixture, under the standard condition of 3-OH acylation, glucoside is acylated selectively with azobenzene 8 over the galactoside. What is the reason for this substrate specific site selectivity?

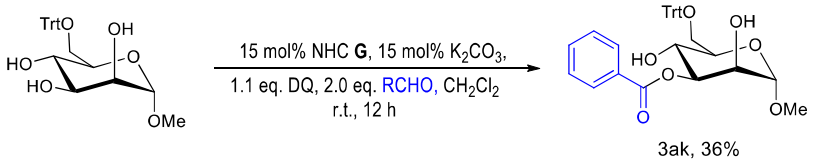
Response: *According to the DFT results (See below), equatorial 4-OH (glucosides) participates in the interaction with KCO_3^-. Axial 4-OH (galactosides) has obvious steric hindrance with the pheny ring of the NHC **G**. These factors make the acylation onto 3-OH of galactosides unfavorable. Consequently, acylation onto glycosides over galactosides happens.*



TS_b1_G_O3_*Re*

Q9 The manuscript is largely focused on glucosides, with one example of galactoside 2-OBz (4ah). As monosaccharides come with that many epimers, in addition to anomers, the 1,2-cis and 1,2-trans dispositions and the selectivity offered by the NHC catalyst is important to note, for example, in the case of mannosides.***

Response: *Thanks for your valuable advice. We examined the mannosides. It can deliver good selectivity at* 3-OH *under optimized conditions albeit with poor yield (see below). However, in presence of NHC ent -G, only trace of 2-OH acylated product was obtained. The epimerization of reaction center itself led to poor yield and/or regioselectivity.*



3ak, 36%

Q10. Lines 119-128 and next paragraph lines 129-137 state the same observations as a repeat. Make sure not to repeat the same text.

Response *: Fixed and thanks.*

Q11. In SI all the compounds are properly characterized well, although not annotated, at least to differentiate the C-3 vs C-2 substitution. Check the yield percentages carefully.

Response: *Thanks. Yields have been checked again.*

Q12. Acylations are ever more important in chemical manipulations of the monosaccharides, although the presence in some natural product is also expected, ellagitannin is a grand example. Better references to highlight the importance of acylations covering the chemical manipulations should be included.

Response: *Importance for synthesis of naturally occurring compounds has been included with ellagitannin as example. Corresponding literature has been cited.*

"Furthermore, Synthesis of natural products with multiply acyl groups is challenging such as Ellagitannin[22-24]"

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November 8, 2024

(NCOMMS-24-33207A)

Three referees were satisfied with the revisions and recommend publication.

Referee #4:

Main Scientific Questions

Q1：The authors addressed queries raised to the original submission. Important points are miserably missing due to the lack of highlighting the importance of inter- and intramolecular hydrogen bonding network in the monosaccharides. Hydrogen bonding schemes increase the nucleophilicity at a site. The absence of this interaction would make the hydroxyl group nucleophilicity lesser. The beta-anomers not undergoing acylations, for example at C-2 site is an example herein. Authors should highlight the importance of hydrogen bonding network for the nucleophilicities of hydroxyl groups at varied sites.

Response: *Thank you for your valuable feedback. We acknowledge that hydrogen bonding network can significantly impact nucleophilicity at different sites, particularly by enhancing or diminishing the electron density of hydroxyl groups based on their environment. We have revised the manuscript to highlight this point accordingly:*

"Our approach relied on the chirality match/mismatch to achieve specific transformations of hydroxyl groups, facilitated by inter- and intramolecular hydrogen bonding network in the monosaccharides to increase the nucleophilicity at the corresponding site".

"The distinct interactions between acyl azoliums (IIa and IIb) and the substrate indicate that the inter- and intramolecular hydrogen bonding network plays a significant role in modulating the reactivity and selectivity of hydroxyl group functionalization. The OH groups of the saccharides then engage with the acyl azoliums in a chirality match/mismatch manner, yielding regioselective outcomes."

Q2:The above point also brings to the attention that site selective acylations are not limited to reactions using chiral catalysts only. Achiral catalysts that affect the hydrogen bonding are also competent for site selective acylations. Multivalent acylation catalysts are the case in point. The work on trivalent dialkylaminopyridine catalysts (J. Am. Chem. Soc., 2011, 133, 12220–12228 and Org. Biomol. Chem. 2024,22, 5134-5149) illustrate the site selective acylations that rely on the internal hydrogen bonding network for the site selectivities. A balanced consideration of the developments of site selective acylations in literature is important. A comprehensive presentation is critical in order to highlight the site selectivities.

Response: *Thank you for your valuable advice. We have added it in Figure 1 and the introduction.*

"Hamachi's tri-DMAP systems illustrated that achiral organocatalysts are also competent for site selective acylations[47,48]"

Q3: Pl check the 1H NMR splitting of monosaccharide ring protons, only doublet and doublet of a doublet are expected. Cases where the splitting is not clear, or appears to be a singlet or triplet, the same be denoted as apparent singlet or apparent triplet. There are no singlets and triplets to the ring protons.

Response: *Thank you for your valuable advice. We have fixed them following your suggestions.*

Q4: Irrespective of the HMQC experiments enabling the regio-isomer identification as adopted in this work, it would be preferable to separately synthesise C-2 and C-3 acyl-functionalized derivatives by an altered synthetic route and validate the observations, at least in couple of instances. A weak cross peak denoting a particular regio-isomer may continue to be weak in interpretation also, particularly when the resonances are too close.

Response: *Thank you for your valuable advice.*

We approached the assignment of ^{1}H NMR peaks with caution. To ensure reliability, at the outset of this project, we synthesized 2-O, 3-O, and even 4-O acylated products via different synthetic routes to validate our results. The NMR spectra of these reference compounds align consistently with our methodological findings. These were clarified by adding to the supplementary information section 4.2