



Synthesis of Type-I and Type-II LacNAc-Repeating Oligosaccharides as the Backbones of Tumor-Associated Lewis Antigens

Riping Phang^{1,2} and Chun-Hung Lin^{1,2,3*}

¹ Department of Chemistry, National Taiwan University, Taipei, Taiwan, ² Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan, ³ Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan

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*Correspondence:

Chun-Hung Lin
chunhung@gate.sinica.edu.tw

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Type-I and Type-II LacNAc are Gal-GlcNAc disaccharides bearing a β 1,3- or β 1,4-linkage respectively. They exist as the backbones of Lewis antigens that are highly expressed in several cancers. Owing to the promise of developing carbohydrate-based anti-cancer vaccines, glycan synthesis at a large scale is indeed an important task. Synthesis of Type-I and Type-II tandem repeat oligomers has been hampered by the presence of GlcNAc residues. Particularly, *N*-protecting group plays a determining role in affecting glycosyl donor's reactivity and acceptor's nucleophilicity. This review discusses several representative studies that assembled desirable glycans in an efficient manner, such as chemoselective one-pot synthesis and chemoenzymatic methods. Additionally, we also highlight solutions that have been offered to tackle long-lasting problems, e.g., prevention of the oxazoline formation and change of donor/acceptor reactivity. In retrospect of scientific achievements, we present the current restrictions and remaining challenges in this less explored frontier.

Keywords: aglycon transfer, chemoenzymatic, chemoselective, glycosylation, one-pot synthesis, oxazoline, relative reactivity value, tumor-associated carbohydrate antigen

INTRODUCTION

Glycobiology has become a burgeoning field in cancer research in the past two decades (1–4). In comparison with traditional methods for cancer therapy (including chemotherapy, radiation and surgery) which still suffer from major adverse effects (5, 6), vaccine treatment to induce the self-immune system appears more beneficial because of its effective and safe approach (7–9). Intensive efforts have been made to develop carbohydrate-based anti-cancer vaccines. Several glycan vaccines are currently examined for clinical evaluations (10–12).

Cancer cells often display differential expression levels of unique carbohydrate epitopes that are not present in their normal counterparts. Noteworthily, altered glycosylation patterns in tumor cells have also been pinpointed as a hallmark of cancer (13–15). Aberrantly expressed glycans on cancer cells are known as tumor-associated carbohydrate antigens (TACAs), recognized as biomarkers to distinguish between malignant and normal cells (16–22). TACA can be classified into two classes: (i) glycoprotein antigens such as Tn, Thomsen-Friedenreich and sialyl-Tn; (ii) glycolipid antigens

including Globo series, gangliosides and blood group determinants (e.g., Lewis X (Le^x), Lewis Y (Le^y), and their sialylated derivatives) (23).

Lewis-type antigens contain a fucosylated backbone composed of alternating galactose (Gal) and *N*-acetylglucosamine (GlcNAc) residues. Based on the glycosidic linkage between Gal and GlcNAc, Gal β 1,4GlcNAc is defined as Type-II LacNAc, existing as the backbone of Le^x and Le^y . In contrast, Gal β 1,3GlcNAc is called Type-I LacNAc (or Lacto-*N*-biose), constituting Le^a and Le^b (24, 25). Significantly, these Lewis-type antigens also exist in glycosphingolipids, known to accumulate and overexpress in breast, prostate, lung, colon and ovary cancers (26).

TACA-based antitumor vaccines have shown great potential and high specificity in cancer immunotherapy. Initially, these carbohydrate antigens were isolated from tumor cells only, instead of normal cells (23, 27, 28). To explore the biological significance of tumor-associated antigens or any endogenous glycans, acquiring a sufficient quantity of desirable carbohydrates represents an important task. However, the immense complexity and heterogeneity of most natural glycans has not only made it impossible to isolate/extract desirable glycans from natural sources, but also impeded investigations to understand their binding modes and related mechanisms. To produce desirable glycans at a reasonable level, it is a pivotal demand to develop efficient, economic and scalable synthetic methods. As a consequence, this minireview places an emphasis on the synthetic methodology for Type-I and Type-II Lewis antigens. Meanwhile, we also discuss encountered problems and challenges, as well as future aspects about how scientists can provide satisfying solutions by standing upon the shoulders of giants.

SYNTHESIS OF TYPE-II LacNAc-REPEATING OLIGOSACCHARIDES

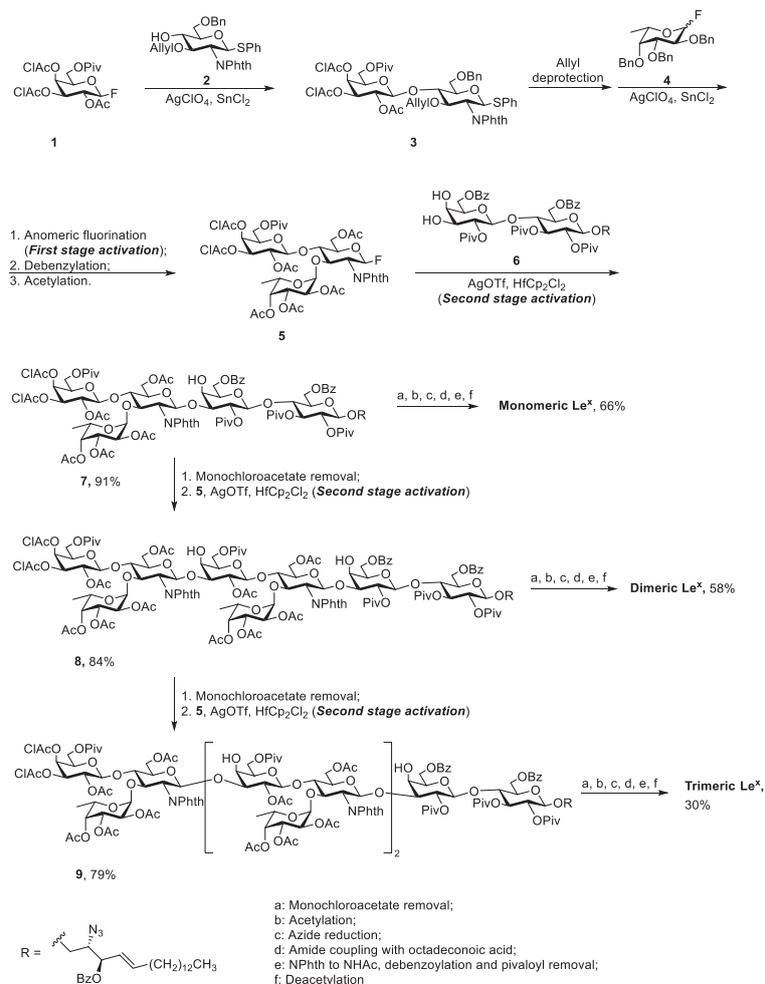
Glycosphingolipids (GSLs) play a crucial role in cell growth, infections, immune response and cancers (29, 30). Many GSLs containing the Le^x structure also play a role as TACAs (26). These antigens are highly expressed in tumors and identified as useful cancer markers (7, 24, 31, 32). To study potential applications for the prognostic and diagnostic usage, many GSLs of the Globo series that consist of the Type II backbone were synthesized, such as sialyl Le^x ceramide (33–35). The total synthesis of monomeric Le^x was first reported in 1987. To form the linkage of Gal β 1,4GlcNAc, the glycosylation employed an acceptor containing GlcNAc-3,4-diol (36). However, there were two existing problems, such as low regioselectivity (resulting from the similar reactivity of the diol) and the low coupling yield (caused by the disarmed nature of the glycosyl donor). Nicolaou and coworkers accomplished the total synthesis of the tumor-associated Le^x family of GSLs in 1990 (Scheme 1) (37). The monomeric, dimeric and trimeric Le^x (Figure 1) were synthesized by using a two-stage activation approach. The synthesis began with glycosyl fluoride **1** that reacted with

NPhth thioglucoside **2** to obtain disaccharide **3**. After selective deprotection of the allyl group, the resulting acceptor was subjected to the orthogonal glycosylation with fucosyl fluoride **4**, leading to the formation of α -fucosyl trisaccharide. Further transformation steps generated the key building block (**5**), including the first stage activation–anomeric fluorination, removal of the benzyl groups by hydrogenation and the subsequent acetylation.

To avoid the aforementioned low glycosylation yield arising from a ceramide acceptor, Schmidt's procedure using 2-azidosphingosine was applied for the preparation of acceptor **6**. The azide was found effective to prevent the formation of intramolecular hydrogen bonding (38). Further glycan elongation from the reducing end to nonreducing end was accomplished by glycosylation of **6** with **5** to produce pentasaccharide **7**. Product **7** was elongated to give octasaccharide **8** after the removal of monochloroacetate and the subsequent glycosylation with **5**. Likewise, undecasaccharide **9** was generated in the same manner. Interestingly, although 3- and 4-hydroxyl groups at the nonreducing Gal of **7** and **8** were unmasked at the same time, the next step of glycosylation occurred at 3-OH with exclusive regioselectivity, which was attributed to the steric hindrance of the axial 4-OH. Products **7–9** were subjected to the same procedure to give monomeric, dimeric and trimeric Le^x , respectively, in excellent overall yields. The procedure was to remove all the protecting groups installed in hydroxyl groups, to convert the azide (in the ceramide moiety) to the desirable acyl amide, and transform the NPhth (at C2 of Gal) to NHAc.

Koeller reported a chemoenzymatic approach to prepare sialyl-trimeric- Le^x (Scheme 2) (39). Glycosylation of acceptor **10** with glycosyl donor **11** was promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give β 1,4-linked disaccharide **12** (50%) and the β 1,3-linked regioisomeric product (8%). Notably, no α -linked product was isolated because of the neighboring group participation of 2-OAc of donor **11**. Moreover, several steps were carried out to produce disaccharide donor **13-D**, including deacetylation of **12**, silylation of the Gal 6-OH, deprotection of the anomeric TBS ether, and the subsequent conversion to the trichloroacetimidate (**13-D**). The two following steps, attachment of **13-D** with allyl alcohol and incomplete Zemplén deacetylation (NaOMe in MeOH), led to the formation of acceptor **13-A**. The coupling of **13-D** and **13-A** afforded tetrasaccharide product that was subjected to acetylation for the purpose of product purification and characterization. The tetrasaccharide product (**14**) was obtained after incomplete Zemplén deacetylation. A similar approach was conducted to yield tri-LacNAc hexasaccharide **15**, including [2 + 4] glycosylation between donor **13-D** and acceptor **14**, peracetylation, and global deprotection.

To prepare sialyl-trimeric- Le^x , the remaining glycosylation steps utilized an enzymatic method to incorporate the necessary sialic acid and L-fucose residues. Enzymatic sialylation was performed by using recombinant human α 2,3-sialyltransferase in the presence of CMP-NeuAc to introduce sialic acid to 3-OH of the nonreducing Gal. Human α 1,3-fucosyltransferase VI was



SCHEME 1 | Nicolaou's two-stage activation strategy for total synthesis of the tumor-associated Lex family of glycosphingolipids.

applied to attach L-fucose to 3-OH of three GlcNAc units where 3 equivalents of GDP-fucose were present. This combination of chemical and enzymatic synthesis greatly simplified the preparation of this complex deca-saccharide.

Furthermore, Wong and coworkers established relative reactivity values (RRVs) to index the reactivity of glycosyl donors (40). The measurement relied on an HPLC-based competition assay. The resulting database collects the RRVs of hundreds of various thioglycosides. The computer software "OPTIMER" was thus developed to dissect glycan synthesis, which appeared useful to design an efficient one-pot synthesis. That is to say, a desirable glycan is analyzed by OPTIMER to come up with a plausible, high-yielding one-pot synthesis where the glycan can be assembled by consecutive coupling of suitable thioglycosides (their RRVs listed in the database). However, the capability of the OPTIMER is confined by the limited number of verified RRV's building blocks. In 2018, Wong and coworkers further developed an algorithm for hierarchical one-pot synthesis called "Auto-CHO" (41). Based on the machine

learning, Auto-CHO can predict more than 50,000 virtual building blocks (with predicted RRVs) from 50 to 154 real building blocks (with verified RRVs). Both OPTIMER and Auto-CHO have made glycan synthesis to be performed in a designed and analytical manner. In the synthesis, the most reactive thioglycoside reacts with the second most reactive thioglycoside and this process can be repeated by coupling of the resulting product (that then serves as the donor) with another thioglycoside (acceptor) in the order of decreasing anomeric reactivity (reducing RRV), elongating glycans from the nonreducing to the reducing end (**Scheme 3**). This development has several advantages. One is to simplify typically tedious procedures in carbohydrate synthesis without purification of intermediate products. The operation of one-pot synthesis greatly speeds up the synthesis and increases the overall yield.

Wong and Mong then applied the reactivity-based chemoselective glycosylation strategy to synthesize Le^y hexasaccharide 21 (**Scheme 4**) (42). Through the retrosynthetic

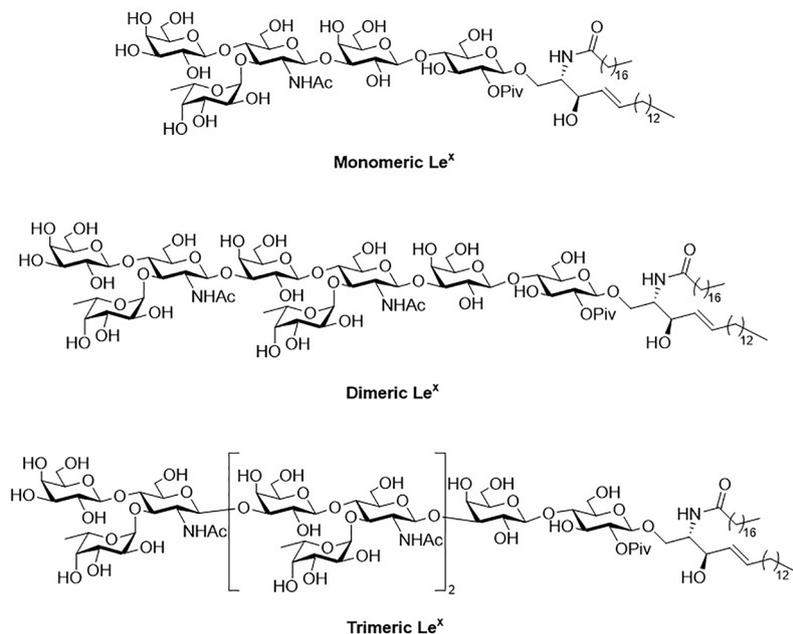
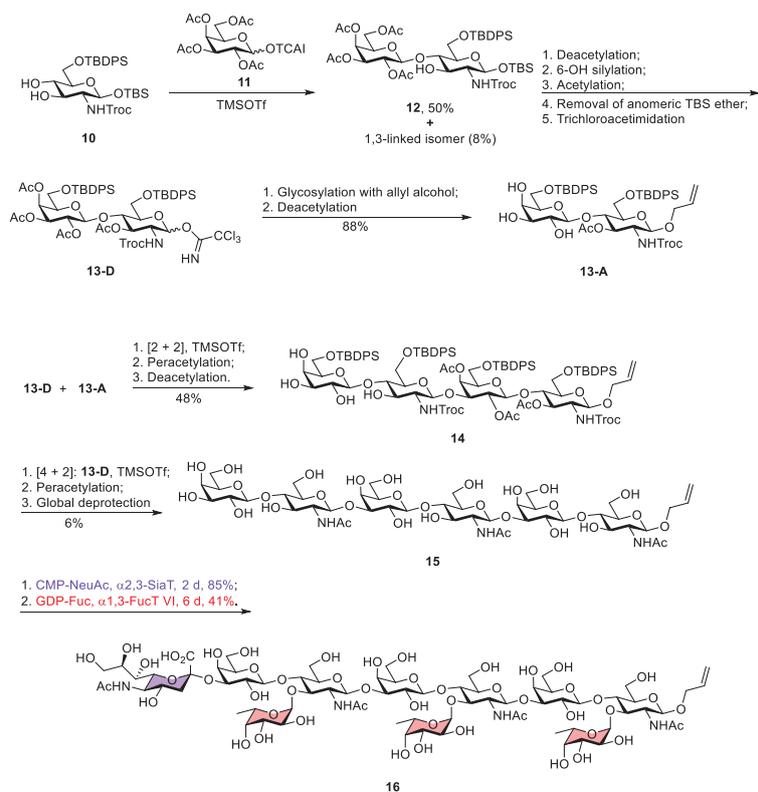
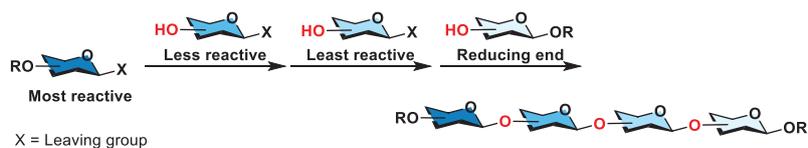


FIGURE 1 | Structure of monomeric, dimeric and trimeric Le^x.



SCHEME 2 | Koeller's chemoenzymatic synthesis of Sialyl-Trimeric-Lex **16** (TCAl, trichloroacetimidate; TBS, tert-butyl(dimethyl)silyl; TBDPS, tert-butyl(diphenyl)silyl; Troc, 2,2,2-trichloroethoxycarbonyl).



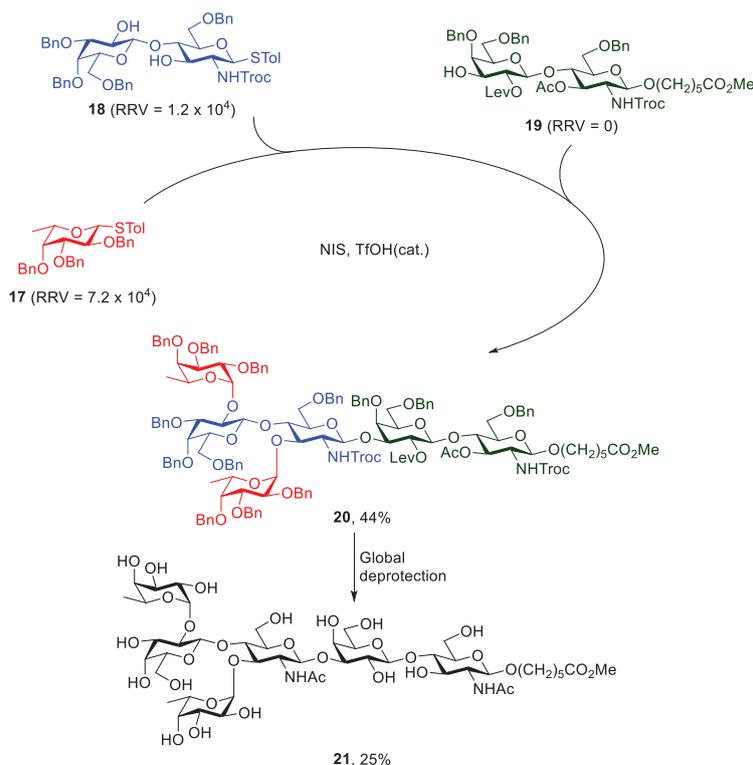
SCHEME 3 | General scheme for reactivity-based one-pot glycosylation.

analysis and consultation of the RRV database, Le^y **21** can be divided into three basic units: thiotoluenyl fucoside **17** (RRV = 7.2×10^4), thiotoluenyl disaccharide **18** (RRV = 1.2×10^4) and disaccharide **19** containing a linker in the reducing end (RRV = 0). Sequential one-pot synthesis was carried out by coupling **17** of the highest RRV with the less reactive acceptor **18**, followed by reaction with the least reactive reducing-end acceptor **19**. Fully protected Le^y hexasaccharide **20** was obtained in 44% yield.

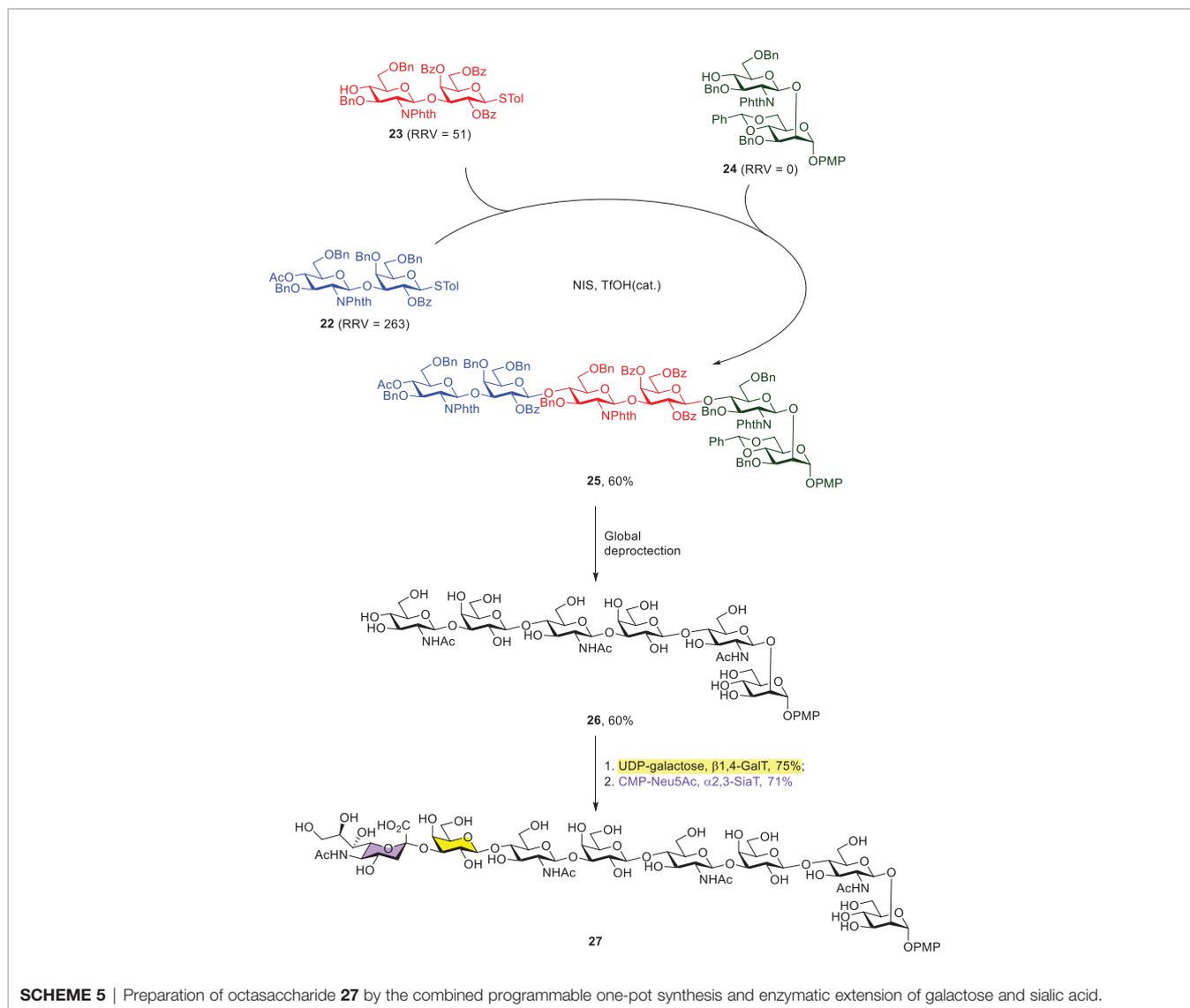
Another representative example of Wong's programmable one-pot synthesis was to integrate chemical and enzymatic glycosylations. The target glycan contained three Type-II LacNAc-repeating units that were attached with an additional mannose at the reducing end and sialic acid at the nonreducing end (Scheme 5) (43). Three thioglycoside building blocks (**22–24**) with RRVs of 263, 51 and 0, respectively, were prepared for the subsequent [2 + 2 + 2] one-pot synthesis, leading to the

formation of fully protected hexasaccharide **25** in 60% yield. Further global deprotection steps afforded compound **26**, including sequential removals of all of the *N*-phthaloyl groups, acetyl groups and benzyl ethers, and acetylation of the resulting amines. Lastly, the enzymatic incorporations of galactose and sialic acid residues were successfully achieved by using β 1,4-galactosyltransferase and α 2,3-sialyl transferase to give **27** and both reactions were carried out in good yields.

The RRV database covers a wide range of thioglycosides with various protecting groups, thus paving the way for simplifying complex carbohydrate synthesis in a one-pot manner. When using Gal β 1,3/4GlcNAc-derived disaccharides to assemble Type-I or Type-II LacNAc-repeating glycans, the effect of neighboring group participation plays a role in forming β -glycosidic linkage exclusively. Therefore, *N*-protecting group installed at glucosamine residues appears to be crucial to determine the



SCHEME 4 | Wong's and Mong's reactivity-based one-pot synthesis of Ley hexasaccharide **21**.



reactivity and stereoselectivity at the same time. In addition to the factor of donor reactivity, the acceptor's nucleophilicity also plays an important role in impacting the stereoselectivity and glycosylation yield. Generally, an electron-rich glycosyl acceptor (protected by electron-donating groups) favors β -glycosylation, whereas an electron-poor acceptor (protected by electron-withdrawing groups) tends to display α -selectivity (44). Moreover, to match between donor's reactivity and acceptor's nucleophilicity is another challenging issue. Once the donor's glycosidic bond is cleaved in a typical glycosylation reaction, the acceptor needs to react with the resulting intermediate and form a new glycosidic linkage. If the donor is highly active or the acceptor is a weak nucleophile, the reaction usually gives a low yield or no formation of the desired product. Romano reported an interesting investigation towards the glycosylation reactivity of lactosamine disaccharides by using various *N*-protecting groups in the donors and protecting groups in the acceptors (**Scheme 6**) (45).

The NHTroc-protected lactosamine showed the lowest yield (20% by 1st generation acceptors and 78% by 2nd generation acceptors), assuming acceptors **31** and **32** had the same reactivity (i.e., the remote *N*-protecting group in the reducing glucosamine residue did not affect the reactivity). Likewise, acceptors **33** and **34** were considered to be equally reactive. In contrast, the NHTFA-protected lactosamine displayed the highest glycosylation reactivity (40% by 1st generation acceptors and 94% by 2nd generation acceptors) with no oxazoline formation. Moreover, the benzylated acceptors (the 2nd generation) significantly produced higher yields, as compared to the peracetylated acceptors (the 1st generation). Gratifyingly, the result was well explained by the fact that benzylated acceptor has higher reactivity than the acetylated ones, which was in agreement with that obtained by Wang and coworkers (46). Owing to the electron-withdrawing feature of ester-type protecting groups, the reduced nucleophilicity indeed decreased the glycosylation yields (see the reactions using acceptors **31** and **32**, vs. **33** and **34** in **Scheme 6**). As a consequence, to optimize glycosylation conditions,

selection of different protecting groups appears to be critical to tune donors' reactivity and acceptors' nucleophilicity.

SYNTHESIS OF TYPE-I LacNAc REPEATING OLIGOSACCHARIDES

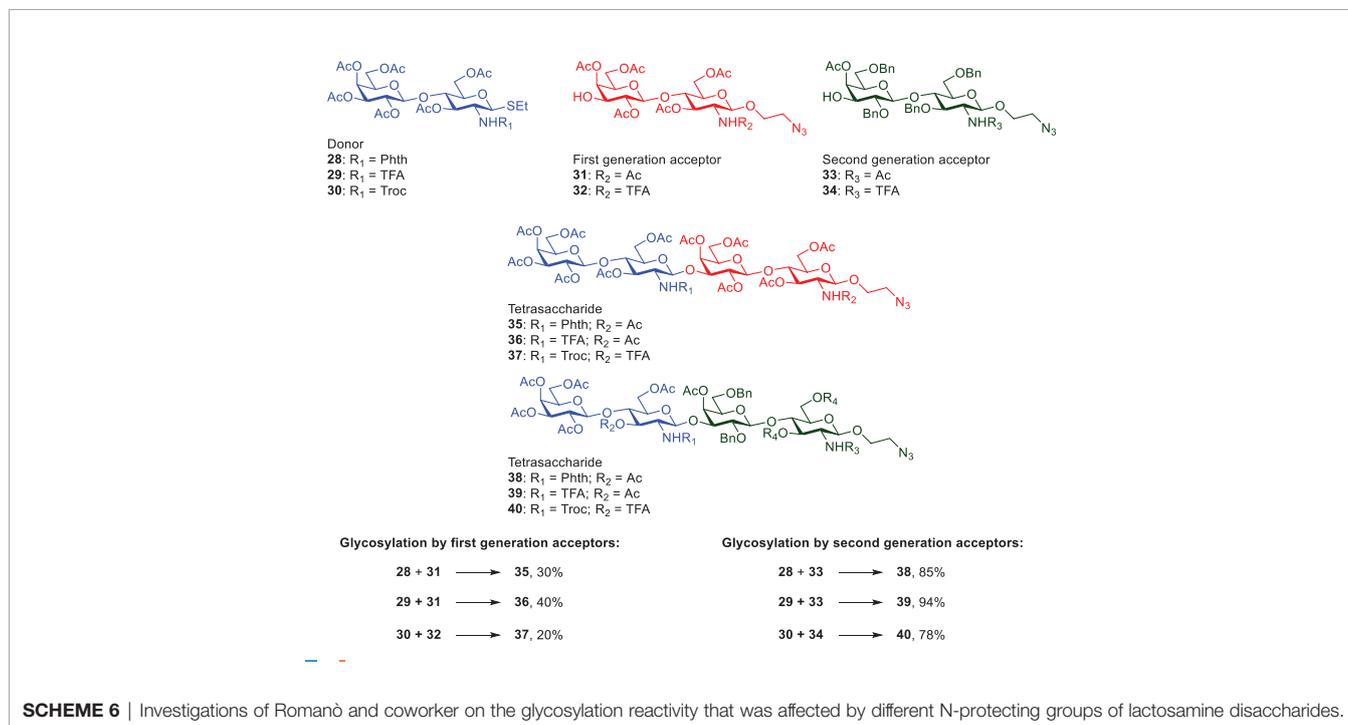
In comparison with naturally prevalent Type-II LacNAc, Type-I LacNAc also exists in several antigens of Lewis blood groups, such as Le^a, Le^b and sialyl Lewis A (sLe^a). These glycans are involved in many biological processes including tumor metastasis (47). Recent studies indicated that Type-I LacNAc-tandem repeats were isolated from SW1116 human colorectal carcinoma cell line (28, 48, 49) and shown as the specific ligands of the tumor-associated human galectin 3 (22, 50). Type-I LacNAc-containing glycans can be utilized to gain novel insights about their functional roles, such as interactions with carbohydrate-binding proteins (e.g., galectin 3), fertilization, pathogen adhesion, and inhibitory activity against tumor cells. Therefore, there is a rising urge for chemists to develop efficient synthesis for these glycans or related glycoconjugates.

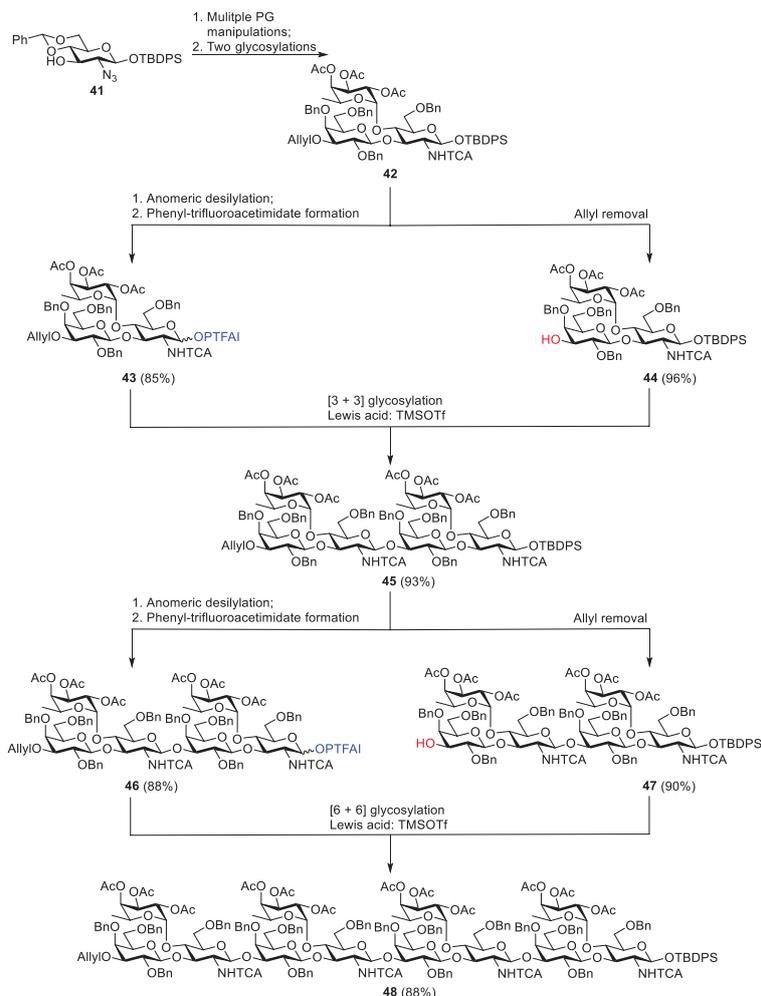
The first synthesis of Le^a-tandem repeat was developed and reported by Ishida et al. in 2016 (Scheme 7) (51). The fucose-containing trisaccharide **42** was derived from monosaccharide **41** through multiple protecting group manipulations, followed by two glycosylation steps. Next, **42** was converted to either the corresponding *N*-phenyl-trifluoroacetimidate donor (**43**) via anomeric desilylation and acetimidation, or the trisaccharide acceptor (**44**) via selective removal of the allyl group at C-4 of Gal residue. The Lewis acid (TMSOTf)-catalyzed [3 + 3] glycosylation resulted in the formation of hexasaccharide **45** in

93% yield. The same strategy was also applied to provide hexasaccharide donor **46** and hexasaccharide acceptor **47** that were subjected to [6 + 6] glycosylation with the same Lewis acid to produce dodecasaccharide **48**. A linker was introduced at the reducing end of **48** to produce this Le^a-tandem repeat oligosaccharide. Although there was a high or excellent yield in every step, this synthetic procedure was tedious and laborious, considering the number of reaction steps and multiple protecting groups employed. As a consequence, to develop a more convenient approach has drawn increasing attention.

Elling and coworkers developed a one-pot and sequential approach to prepare Type-I LacNAc oligosaccharides by using two recombinant glycosyltransferases. The procedure started from chemically derivatized GlcNAc-linker-*t*Boc **49** (Scheme 8). Two recombinant bacterial enzymes, were added to the reaction mixture alternatively, including *E. coli* O55:H7 β 1,3-galactosyltransferase (β 1,3GalT) and *H. pylori* β 1,3-*N*-acetylglucosaminyltransferase (β 3GlcNAcT). Di-, tri-, tetra- and pentasaccharides were synthesized in high overall yields. However, β Gal3T showed low activity towards longer Type-I LacNAc glycan acceptors, resulting in unsatisfying yields for hexa-, hepta- and octasaccharides. The problem was not solved albeit with extra additions of enzymes (β 3GalT, alkaline phosphatase) and donor (UDP-Gal).

In comparison with the alternative enzyme usage, the one-pot, simultaneous usage of β 3GalT and β 3GlcNAcT was examined with different activity-based enzyme ratios (Scheme 8) (52, 53). Varying ratios led to different product distributions. Interestingly, when the ratio of β 3GalT/ β 3GlcNAcT was 5/1, acceptor **49** was consumed completely, generating disaccharide **50** (~80%) as the main product and tetrasaccharide **52** (14%) after 72 h. Nevertheless, this one-pot procedure was restricted





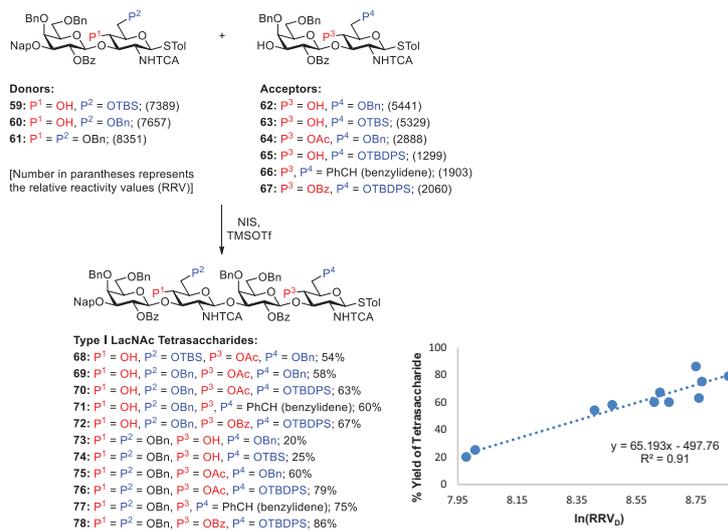
SCHEME 7 | Synthesis of Lea-tandem repeat dodecasaccharide **48** by Ishida and coworkers (TBDPS, *t*-butyldiphenylsilyl; PTFAI, *N*-phenyltrifluoroacetimidate).

towards the synthesis of trisaccharide and longer glycans. The method usually produced a mixture of products, representing another problem in purification of desirable glycans. Additionally, several challenges are associated with enzymatic methods. For instance, most glycosyltransferases and related enzymes are not commercially available. To prepare any of them for the synthetic purpose is time-consuming and may not be cost-effective. At this stage, it is not feasible to synthesize long Type-I oligomers by using β 3GalT and β 3GlcNAcT unless superior enzymes or/and reaction conditions are identified.

Moreover, inspired by Wong's success of the reactivity-based one-pot synthesis in Type-II LacNAc oligosaccharides, Lin and coworkers demonstrated a one-pot [1 + 1 + 2] glycosylation. The procedure started with a high-RRV galactoside **54** as the donor (RRV = 16500) to couple with a low-RRV, trichloroacetamide (NHTCA)-protected glucosamine acceptor **55** (RRV = 126), as shown in **Scheme 9**. In the presence of *N*-iodosuccinimide (NIS) and TMSOTf as the promoter, the glycosylation produced

disaccharide **56** as a mixture of α/β isomers (ratio of α/β = 1/7) (**54**). The loss of the exclusive stereoselectivity was mainly due to the bulky *O*3-*t*-butyldimethylsilyl group of **54** that caused a distorted chair conformation. The next glycosylation utilized NIS/AgOTf as the milder promoter after donor **54** and acceptor **55** were completely consumed. Although **56** existed as a mixture of α/β isomers, the coupling with disaccharide **57** produced tetrasaccharide **58** of the desired β,β,β -stereochemistry in a total 45% yield, without detection of the isomeric α,β,β -product. To explain the stereochemistry outcome, **57** was glycosylated with either pure α -isomer (Gal- α 1,3-GlcNHTCA, **56 α**) or β -isomer (Gal- β 1,3-GlcNHTCA, **56 β**). Interestingly, **56 α** was activated faster than **56 β** and thus rapidly hydrolyzed without forming the expected tetrasaccharide of α,β,β -stereochemistry.

The idea of performing the one-pot synthesis of tetrasaccharide **58** can be further extended to synthesize Type-I LacNAc oligosaccharides in an efficient and convenient way, but there were problems to be resolved. First, the methoxy group



SCHEME 10 | The importance of exceeding the RRV threshold for efficient synthesis of Type-I oligomers demonstrated by Lin and coworkers.

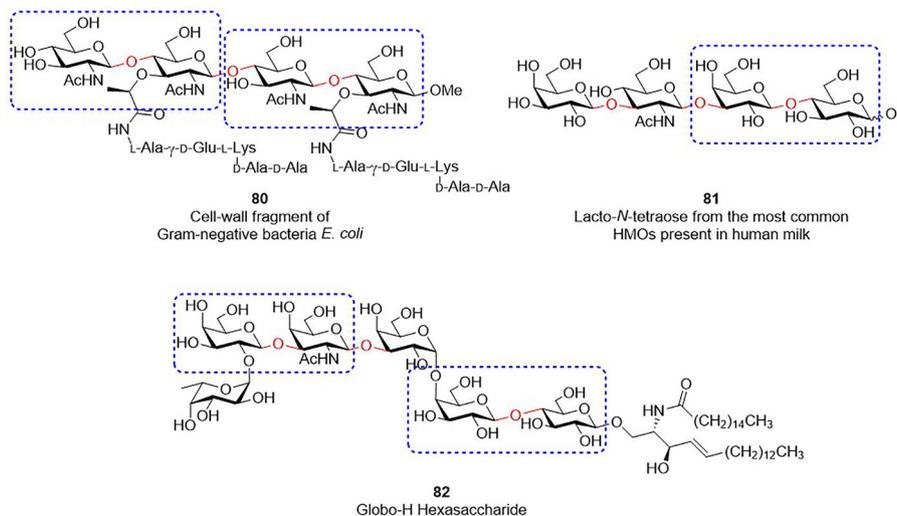


FIGURE 2 | Glycans containing β -1,3/4 glycosidic bonds (indicated as red color). Disaccharides marked by rectangles are analogous to Type-I or Type-II LacNAc.

peptidoglycans contain the repeating unit of GlcNAc- β 1,4-MurNAc(pentapeptide) (see the structure of **80** in **Figure 2**). Since MurNAc is a GlcNAc derivative, GlcNAc- β 1,4-MurNAc can be considered as the modified version of Type-II LacNAc. Human milk oligosaccharides are known to contain β -1,3/4-linkage sugar residues, including Gal- β 1,4-Glc, Gal- β 1,3-GlcNAc and Gal- β 1,4-GlcNAc (e.g., Lacto-*N*-tetraose **81**). Likewise, Globo-H hexasaccharide (**82**), one of TACAs, has the moieties of Gal- β 1,3-GalNAc and Gal- β 1,4-Glc. Preparation of these glycans or glycoconjugates unavoidably encounter various

problems, like what we previously discussed. As a consequence, the aforementioned ideas and approaches are presumably useful, such as the concept of RRV threshold and the ways to prevent the formation of stable oxazoline side-products, making it possible to produce these oligosaccharides at a suitable scale with high efficiency. With continuing advances in the field of carbohydrate synthesis, the expansion of these developed methods and related applications will serve as an important stepping stone toward a foreseeable promise for efficient glycan synthesis operated with a rational design and analysis.

CHALLENGES AND FUTURE PERSPECTIVES

In the past two decades, tremendous efforts have been made to develop an efficient methodology for preparing complex oligosaccharides. To assemble complicated glycan structures in a more rapid, efficient and massive way, many strategies have been studied, including protecting group manipulation, one-pot glycosylation, chemoenzymatic synthesis, or even the combination of these strategies (e.g., protecting-group controlled enzymatic glycosylation) (56). Additionally, a solid-phase oligosaccharide synthesizer, enabling automated glycan assembly, was developed by Seeberger to prepare desired glycans in a fast and reproducible fashion. Through the resin equipped with a photolabile linker as a solid support, a set of Lewis, Type-I/II oligosaccharides (Le^a, Le^b, Le^x, Le^y, lactotetraosyl, neolactetraosyl) were synthesized successfully by using a large quantity of glycan building blocks (5–8 equiv) (57). However, the assembly of Type-I and Type-II LacNAc-tandem repeat glycans still represents a long-lasting challenge due to several limiting factors shown as follows.

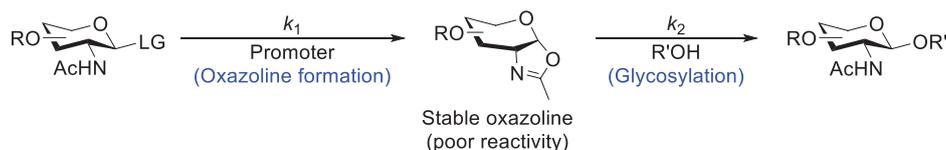
Firstly, the intrinsic low nucleophilicity of 3/4-OH of glucosamine acceptor has impeded the synthesis of Galβ1,3GlcNAc and Galβ1,4GlcNAc disaccharide precursors at the very beginning (44, 58, 59). Both hydroxyl groups are easily affected by adjacent protecting groups. The latest discovery by Wang and coworkers also pinpointed the deactivating effect of electron-withdrawing group (e.g. benzoate) to reduce the hydroxyl nucleophilicity that is indexed in terms of acceptor nucleophilic constant (Aka) (46). Although it seems feasible to compensate the poor nucleophilicity of acceptor by increasing the donor reactivity, the effort usually leads to rapid hydrolysis before formation of a desired glycosidic linkage. The problem of preparing Type-I LacNAc is more serious than preparing Type-II LacNAc because 3-OH is less reactive than 4-OH (58). This is in agreement with the analysis of Wang's Aka values (perbenzylated glucoside's Aka value of 4-OH is 2.68, whereas that of 3-OH is 1.62), as well as the results of Lin's (50, 54, 55) and Madsen's work (58). The nucleophilicity is known to be influenced by steric effect (e.g., primary vs. secondary alcohol) and electronic effect (electron-withdrawing vs. electron-donating group) as mentioned previously. Additionally, other effects were reported to influence acceptor's reactivity, such as hydrogen bonding and conformational change. To enhance the acceptor reactivity is thus a complex issue.

The reactivity-based chemoselective glycosylation represents an effective strategy by tuning and matching the RRVs of donors

and acceptors. However, this strategy is usually accompanied by aglycon transfer of the acceptor, especially when the nucleophilicity of the hydroxyl group is much lower than the anomeric thio-moiety (60–64). To circumvent this problem, Glidersleeve et al. surveyed a series of thio-aglycon groups and discovered that thio-2,6-dimethylphenyl (SDMP) can block the aglycon transfer completely because of the corresponding steric hindrance (65). Therefore, a SDMP-based RRV database may possibly provide a satisfying solution for chemoselective one-pot glycosylation.

Another concern is how to select *N*-protecting group of glucosamine acceptors. Various amide- or carbamate-type *N*-protecting groups have been utilized for the purpose of the neighboring group participation to produce β-stereoselectivity (66–71). However, most of them still display limitations during glycosylation. For example, the synthesis of *O*-sulfated sLe^x antigens by Chen et al. started from *N*-phthalimido-3,4-diol glucosamine derivatives as glycosyl acceptors. The glycosylation exhibited good regioselectivity towards 4-OH, owing to the differential steric hindrance of the two hydroxyl groups. The C3-hydroxyl group is near the bulky *N*-phthalimide and thus hindered for reaction (72). In comparison with *N*-phthalimide, 2,2,2-trichloroethoxycarbamate (NHTroc) is less bulky, but it has to be removed either under harsh conditions (e.g., Zn and acidic condition under a high temperature) (73), or by a radical method which often results in a significant amount of a dichloroethoxycarbonylated byproduct (74). Although trichloroacetamide (NHTCA) or trifluoroacetamide (NHTFA) have been widely used and can be easily deprotected (75–78), the formation of TCA-oxazoline as a stable side product is known to be problematic (55, 79). For example, the chemical synthesis of a hyaluronic acid decasaccharide by Huang et al. was hampered by the substantial formation of TCA-derived oxazoline side products (79). Despite the addition of TMSOTf to suppress their formation by shifting the equilibrium from oxazoline to oxazolinium ion (80), a significant amount of the oxazoline species was still detected during the elongation of longer saccharides (55, 79).

Moreover, when a relatively acidic oxazolinium accumulates to a high level in glycosylation reactions, it would possibly activate NIS and then thioglycosides, with concomitant formation of a deprotonated yet stable oxazoline (81). Therefore, to resolve this issue, Henrik and coworkers demonstrated the importance of balancing the rate between the oxazoline formation (rate constant k_1 in **Scheme 11**) and the subsequent glycosylation (k_2), which can be achieved by tuning the amount and Lewis acidity of the catalyst (e.g., Bi(OTf)₃, Fe



SCHEME 11 | General scheme for the glycosylation with a GlcNAc-derived donor where an oxazoline is formed as a stable intermediate.

(OTf)₃-DMSO). The glycosylation yield was thus improved significantly by maintaining a low concentration of the oxazoline under the reaction condition. This approach was found useful to obtain good yields for the acceptors containing a primary hydroxyl group, but the reactions for those containing a secondary hydroxyl still gave moderate yields. Meanwhile, Hashimoto and coworkers used triflimide to convert glycosyl diethylphosphite to α -glycosyl triflimide for the subsequent glycosylation, which successfully avoided the oxazoline formation (82, 83).

Even though these aforementioned methods were established to overcome the oxazoline formation, their efficacy is usually dependent on promoters, donors or acceptors. There is still lack of systematic investigations that are expected to offer a universal solution to the majority of glycosylation reactions. The examination of *N*-protecting groups likely shows a great promise when taking account of the fact that *N*-protecting groups are essential for controlling the glycosylation stereoselectivity and tuning the saccharide reactivity at the same time. If *N*-protecting groups are

able to form corresponding oxazolines, they can also alter the reactivity of oxazoline and thus shift the oxazoline/oxazolinium equilibrium. If the past is any indication of the future, undoubtedly, the development of an advanced methodology will be mostly emphasized on chemoselective one-pot synthesis with prior analysis on the reactivity of donors and acceptors.

AUTHOR CONTRIBUTIONS

Writing, figures, and original draft preparation, RP. Writing, reviewing, and editing, C-HL. Both authors contributed to the article and approved the submitted version.

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