A strategy for the one-pot synthesis of sialylated oligosaccharides

Zhiyuan Zhang, Kenichi Niikura, Xue-Fei Huang, and Chi-Huey Wong

Abstract: A new strategy has been developed for the synthesis of branched sialylated oligosaccharides using one-pot technology. Sialyl donors are in general too weak in reactivity to be used as the first glycosyl donors in the one-pot synthesis. When sialic acid is linked to a different sugar such as galactose, the reactivity is, however, significantly enhanced and can be tuned to enable the one-pot synthesis. A combination of NIS–TfOH–AgOTf was used for activation of the thioglycosides to improve the glycosylation yield when a hindered acceptor was used, as illustrated in the one-pot assembly of sialylated hexasaccharide.

Key words: one-pot synthesis, sialyl oligosaccharides, new activation.

Résumé : Faisant appel à une technologie monotope, on a développé une nouvelle stratégie pour réaliser la synthèse d'oligosaccharides sialylés ramifiés. La réactivité des donneurs sialyles est généralement trop faible pour les utiliser comme premiers donneurs de glycosyle dans une synthèse monotope. Lorsque l'acide sialique est lié à divers sucres, comme le galactose, la réactivité est toutefois considérablement augmentée et elle peut être ajustée pour utilisation dans une synthèse monotope. Lorsque des accepteurs stériquement empêchés ont été utilisés, on a fait appel à une combinaison de NIS–TfOH–AgOTf pour l'activation des thioglycosides afin d'augmenter le rendement de la glycosylation; cette technique est illustrée par la synthèse monotope d'un hexasaccharide sialylé.

Mots clés : synthèse monotope, saccharides sialylés, nouvelle activation.

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Introduction

Sialylated oligosaccharides are attractive targets for synthesis as most cancer cells carry sialylated oligosaccharides as unique markers on their surface (1). However, synthesis of sialylated oligosaccharides remains a major problem (2), mainly due to the low yield and low stereoselectivity in the sialylation step. Strategically, the difficult sialylation should be conducted at the early stage to maximize the overall yield and stereoselectivity. Sialic acid is, however, often found at the nonreducing end of oligosaccharides, and most methods developed for oligosaccharide synthesis start from the reducing end to the nonreducing end. The strategy based on Danishefsky et al.'s (3) glycal assembly, via epoxide intermediates, starts from the nonreducing end, but it is not applicable to sialic acid, as undesirable β-selectivity occurs.

Alternatively, fragment coupling using sialyl oligosaccharides as glycosylation reagents may minimize the problems. Another approach is based on the one-pot strategy (4), where an oligosaccharide is assembled rapidly from the nonreducing end to the reducing end by sequential addition of building blocks, with the most reactive one being added first. However, sialic acid thioglycosides (even a per-*O*-benzylated thioglycoside) are much less reactive and less influenced by the protecting group than the other thioglycosides (5). We have found that the major effect on the anomeric reactivity of sialic acid is from the carboxyl, not from the side-chain protecting groups. The reactivity difference between per-*O*-benzylated and per-*O*-acetylated sialosides is less than 100, compared to ~1×10^5 for the corresponding thioglycosides of the other sugars (5). Reduction of the carboxyl group does increase the reactivity by >1×10^4, but the α-selectivity is completely diminished and gives mainly the undesirable β-glycoside product (5). To tackle this problem, we decided to use sialylated disaccharides as building blocks in the one-pot synthesis, as the reactivity of a disaccharide or trisaccharide glycosyl donor is mainly determined by the reducing end unit (4*d*). Here we report the representative one-pot synthesis of a protected sialyl Lewis X hexasaccharide (1) to illustrate this strategy.

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Dedicated to the memory of Professor Raymond U. Lemieux.

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Experimental and results and discussion

As shown in Fig. 1, the target molecule could be assembled from the sialylated disaccharide **2**, the thiofucoside **4**, the 2-*N*-Phth glucoside **3**, and the lactoside **5**. This strategy is based on the following analysis: when a 2-*O*-acylated glycosyl donor couples with a 2-*N*-phthalimido protected 3,4-diol glucosyl acceptor (i.e., **2** + **3**), it is expected to give a 1,4-β linked product (5). This would allow the one-pot strategy to proceed through a fucosylation with **4** after the

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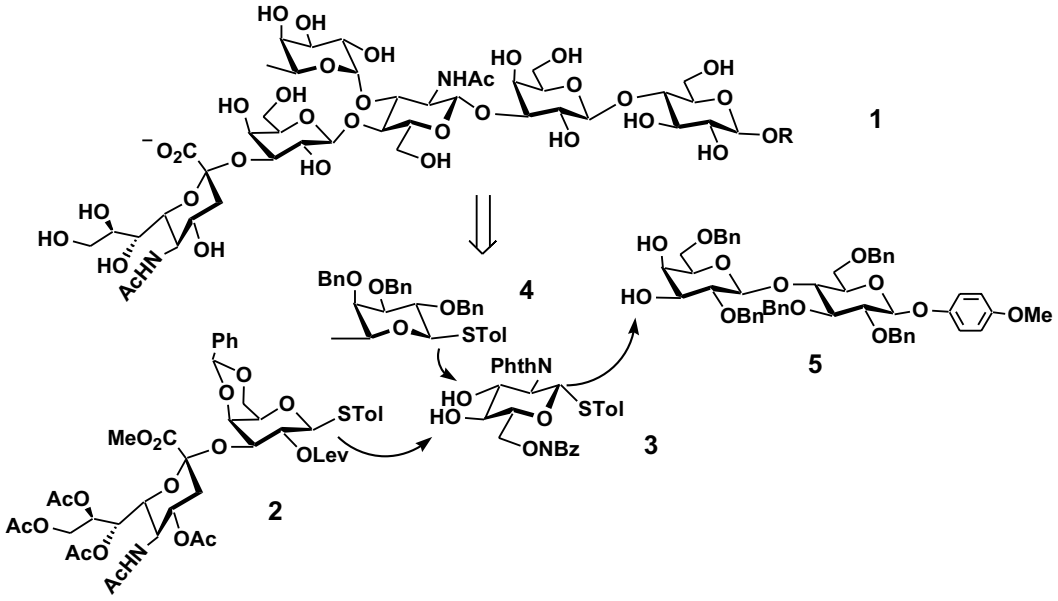
Fig. 1. Retro-synthetic analysis for the one-pot assembly of protected sialyl Lewis X hexasaccharide.



first step, followed by coupling with the final acceptor lactoside **5**.

We expect that the 2,3-linked disaccharide **2** would have a similar reactivity as the methylphenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (RRV = 285), and would be at least 10 times more reactive than 6-*O*-nitrobenzoyl-2-*N*-phthalimido β-glucoside **3**. Compound **2** was synthesized from phosphite **6** (7) and thiogalactoside **7** followed by acylation. The desired α-product **8** was isolated in 21% yield, and the lactonized product **9** was the major side product (8% yield). Protection of the 2-OH group using levulinic acid gave the desired product **2** in 86% yield.

To reduce the reactivity of the 2-*N*-Phth glucoside, a nitrobenzoyl group was selected as the 6-*O* protecting group. Monobenzoylation of the 2-*N*-phthalimido glucoside **3** was first tried with *p*-NO_2BzCl–DMAP–pyridine in THF. This reaction mainly gave two products, the 3,6-di-*p*-nitrobenzoylated compound **10** (35% yield), and 6-*O*-NBz **3** (53% yield), which were difficult to purify. However, pure compound **3** was easily obtained in 83% yield when we treated compound **10** with $(Bu_2Sn)_2O$ followed by *p*-nitrobenzoyl chloride.

The last component, lactoside acceptor **5**, was prepared (in 74% overall yield) from the free lactoside **11** (Fig. 2) through three steps and a single purification. An improved procedure, using DMF as the cosolvent together with α,α-dimethoxypropane under H_2SO_4 treatment, gave a good yield of isopropylidene lactoside. The crude product was subjected to further benzylation and TFA treatment to give the diol **5** after final purification.

Before we started the assembly, we measured the reactivities of compound **2** and compound **3**, using the HPLC method developed in this group. Compound **2** (RRV = 1308) was found to be 23 times more reactive than compound **3** (RRV = 57). To make sure that the glycosylation and the regio- and stereoselectivities would work as predicted during the one-pot synthesis, we tried a one-pot synthesis (Fig. 3) of the protected Lewis X trisaccharide **14**, and the product was isolated in more than 85% yield.

Encouraged by this result, we then conducted the assembly of the four components **2**–**5** in a one-pot procedure. Our experience suggests that exclusively dry conditions are the key to a successful glycosylation step, and these conditions are even more important in the one-pot procedure as more reagents and substrates are added to the reaction flask. The relative amounts of building blocks used is also one of the important factors. Normally, the use of less equivalents of the acceptor at each step can have many advantages. First, it increases the selectivity; for example, if the concentration ratio of the donor and the acceptor in the beginning is 1.5, the ratio would roughly be ~2.0 in the middle of the reaction, and much higher near the end. Second, the excess amount of donor can still be activated, even though all of the acceptor is consumed and trapped by the released succinimide to form the glycosyl succinimide. When the last acceptor is an *O*-linked glycoside, using an excess of the acceptor will increase the yield. Through several attempts at the synthesis of the SLe X hexasaccharide, we found that the optimal ratio of building blocks (**2**:**3**:**4**:**5**) is 1.3:1:1.5:2.

We first tried the one-pot assembly using NIS–TfOH as the promoter. TLC analysis showed a good result in the first step (coupling **2** with **3**); however, almost no reaction was observed in the second step, probably due to the steric hindrance at 3-OH after the 4-OH position was glycosylated to the disaccharide **2**. In addition, most of the thiofucoside **4** was converted to its succinimide derivative.

To reduce the amount of the succinimide, we have developed a new procedure (Fig. 4) using NIS–TfOH–AgOTf. In this reaction the side product TolS-I is generated, as shown in Scheme 1. In the presence of AgOTf, it reacts with TolS-I to generate another more powerful promoter, TolS-OTf (8), which can further activate the donor. In this manner, 0.5 equiv of NIS, together with 0.5 equiv of AgOTf, activate 1 equiv of the donor. As an extended application of this reaction, we used 1.5 equiv of compound **2** and 1.5 equiv of NIS in the first step (Fig. 4). The resultant mixture ideally is composed of about 1 equiv of trisaccharide product, 0.5 equiv of succinimide derivative of compound **2**, and

Fig. 2. Preparation of building blocks. (*a*) TMSOTf, MS AW-300, CH_2CI_2–CH_3CN; (*b*) Levilunic acid, EDC, DMAP, CH_2CI_2; (*c*) $(Bu_3Sn)_2O$, *o*-NBzCl, 82%; (*d*) CMA, α,α-dimethoxy isopropane; (*e*) NaH, BnCl, DMF; (*f*) TFA–CH_2Cl_2, overall 82% yield.

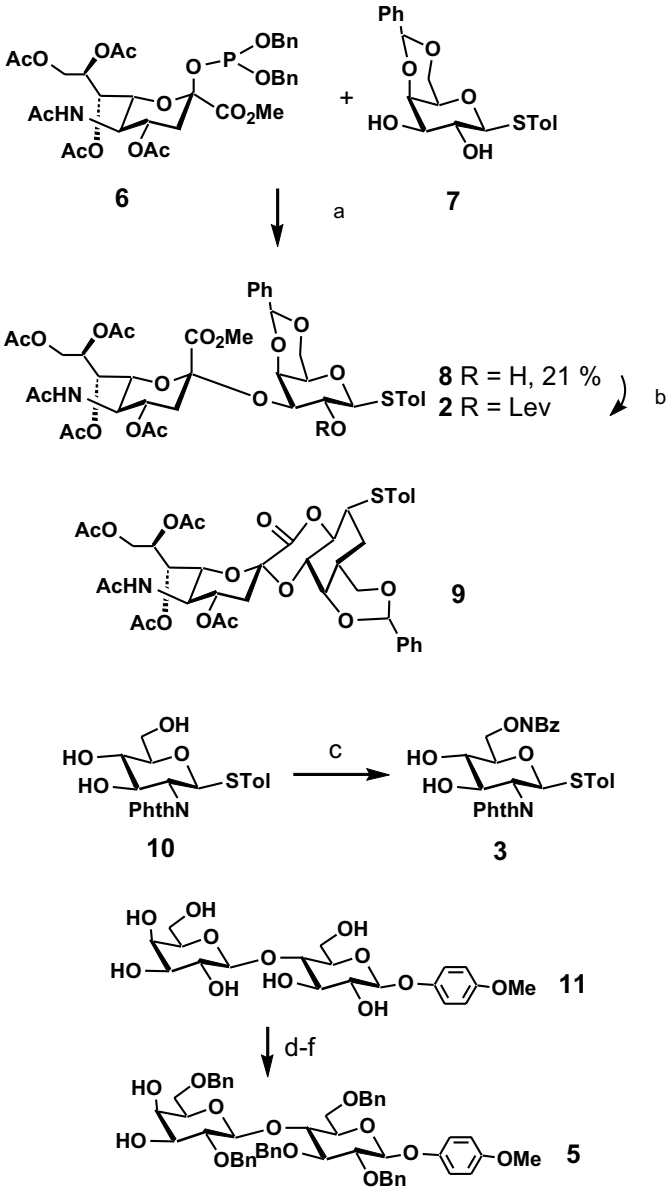


Fig. 3. One-pot synthesis of a protected Lewis X trisaccharide.

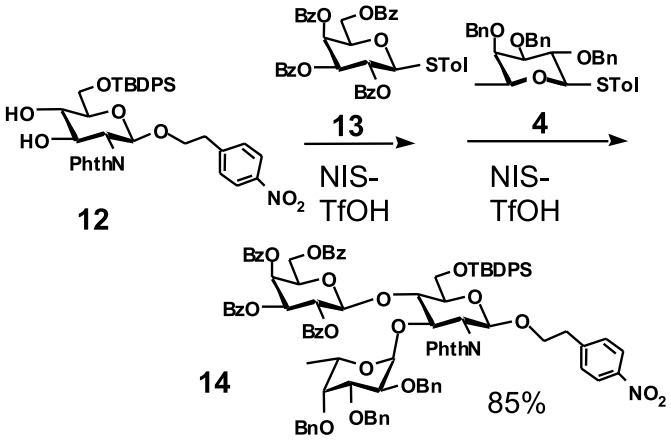
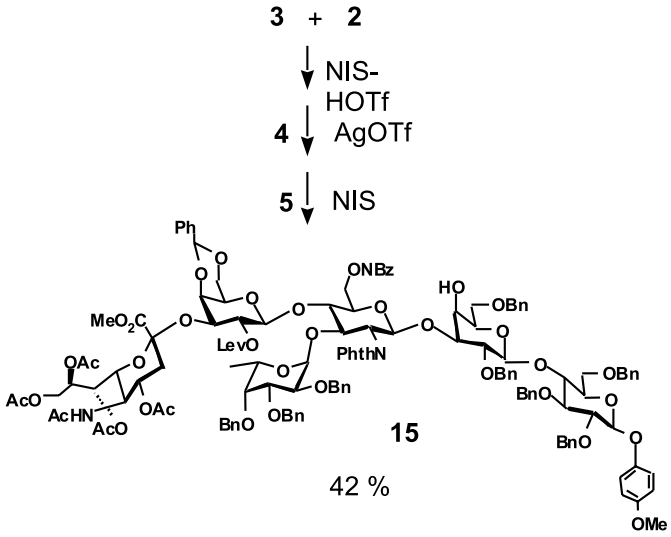
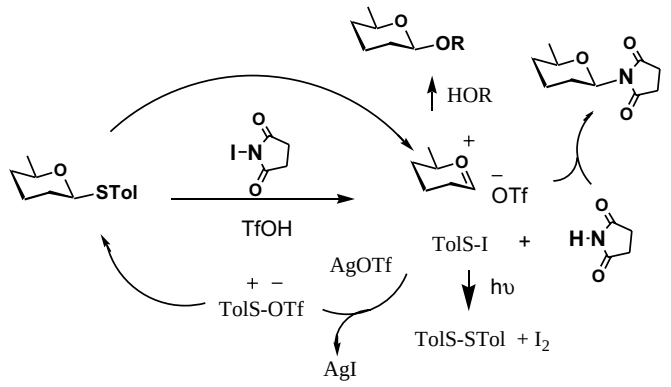


Fig. 4. One-pot synthesis of sialyl Lewis X hexasaccharide.



Scheme 1. Proposed mechanisms of NIS–TfOH and AgOTf activation of thioglycosides.



1.5 equiv amount of ToS-I. Therefore, addition of AgOTf after adding thiofucoside **4** in the second step would generate an adequate amount of TolS-OTf (8) to activate the trisaccharide and to give the tetrasaccharide. In the last step we used NIS for the coupling, as the lactoside diol **5** (2.5 equiv) is much more reactive than succinimide, and less hindered. By using this one-pot procedure, we were able to isolate the final hexasaccharide **15** in 42% yield after flash chromatography.[2]

In summary, we have successfully developed a one-pot synthesis of sialyl oligosaccharide using a sialylated disaccharide and other building blocks in combination with regioselective glycosylation. We have also developed a new method using NIS–TfOH–AgOTf for activation of thioglycosides. Further application of the one-pot strategy in

[2] Supplementary material may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc.ca/cisti/irm/unpub_e.shtml).

the syntheses of more complicated and highly branched natural oligosaccharides is in progress.

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