

S-Benzoxazolyl (SBox) Glycosides as Novel, Versatile Glycosyl Donors for Chemical O-Glycosylation

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Introduction:

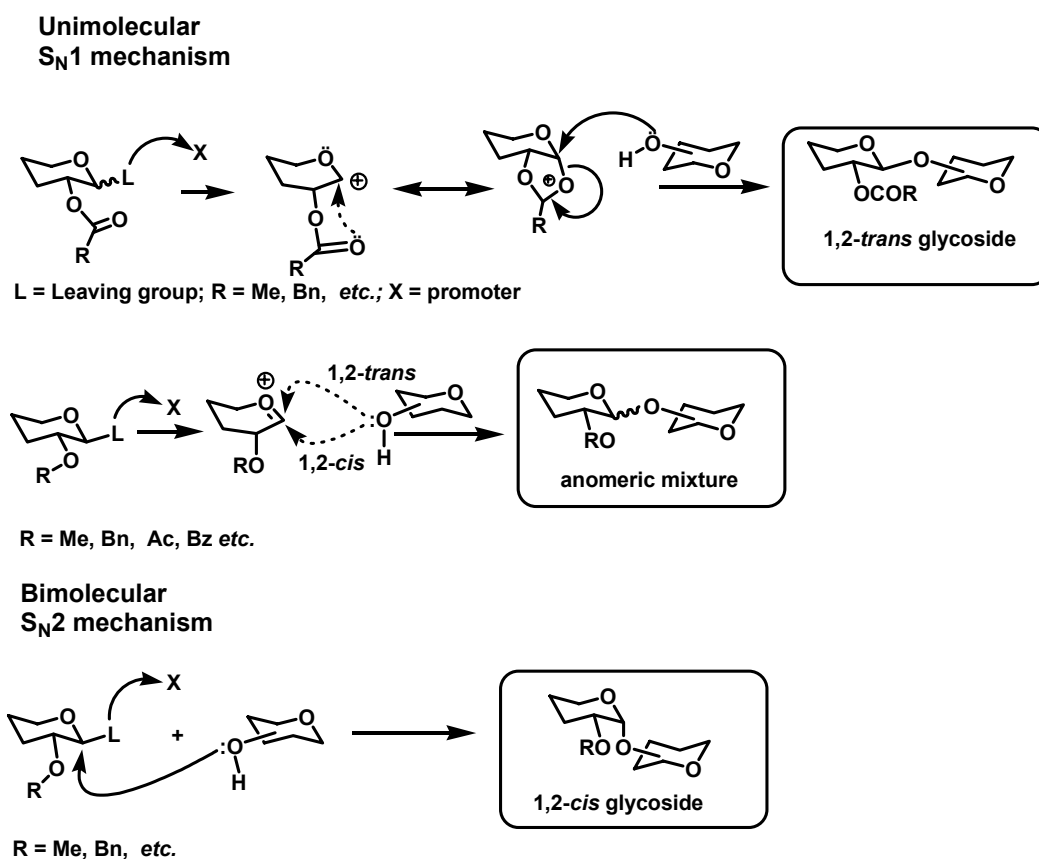
Carbohydrates have been known as a source of energy for a long time. Recent research has revealed that carbohydrates play an important role in a number of biological activities apart from storing energy.¹ It has been proven that carbohydrates play a key role in molecular recognition during antigen antibody interactions that help the host cell to identify a guest cell. The research has confirmed that these “sweet molecules of life” can be helpful in identification, analysis and treatment of many such incurable diseases as cancer, some severe bacterial infections and even HIV.²

The fact that complex sugar molecules exist in a living organism as oligosaccharides or glycoconjugates, creates a challenge for their chemical synthesis from simple monomers. This is the time when synthetic chemistry and methodology can give a major contribution to this extensively growing field. And keeping this goal in mind, this project is based on the following specific aims.

1. **Specific aim 1:** Method development: Invention of a new versatile glycosylation procedure.
2. **Specific aim 2:** Strategy design: Applications of the SBox glycosyl donors to convergent oligosaccharide synthesis; selective and/or orthogonal activation concept.
3. **Specific aim 3:** Application to target synthesis: Synthesis of the disialyl galactosyl globoside (DSGG), a biologically important tumor associated glycosphingolipid.

A: Method Development:

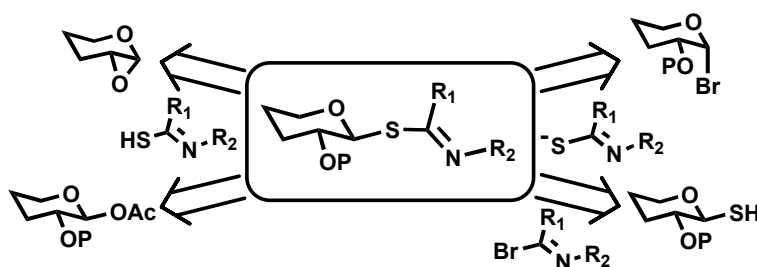
Background Information: One of the major hurdles in the synthesis of oligosaccharides is the stereoselectivity of glycosidic bond formation.³ It is well established that protecting groups overall, and those at the C-2 position of glycosyl donors in particular, significantly affect the stereoselectivity of glycosylation processes. Synthesis of *1,2-trans* derivatives can be achieved by using a participating substituent at C-2, while their *1,2-cis* counterparts are obtained by using a nonparticipating moiety (Scheme 1).³



Scheme 1: Synthesis of *1,2-trans* and *1,2-cis* glycosides

Our group has been interested in a class of glycosyl donors with a generic thioimidoyl leaving group ($\text{SCR}_1=\text{NR}_2$) taking into consideration the following advantageous

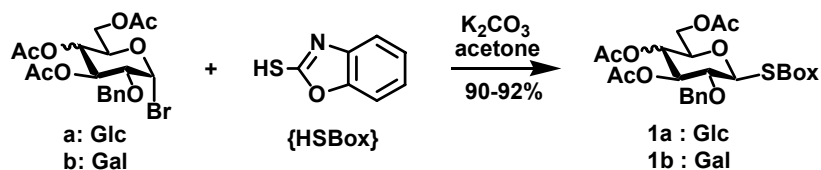
features of the substituted thioimidates. These compounds can be synthesized via a number of reaction pathways, e.g. from anomeric halides, acetates, 1,2-anhydro or 1-thiosugars (Scheme 2). Second, it is assumed that the reactivity of glycosyl donor could be adjusted by varying the R₁ and R₂ substituents. Third, due to the polyfunctional character of the leaving group, it is quite possible that these compounds could be activated via a number of conceptually different pathways.



Scheme 2: Synthesis of new class of glycosyl donors

The research project following this discussion is mainly focused on the synthesis and *O*-glycosidation of one specific class of glycosyl donors, so called *S*-benzoxazolyl (SBox) glycosides.

Previous Work: Our group has recently reported the synthesis of two new compounds, partially benzylated *S*-benzoxazolyl (SBox) glycosides, derivatives of D-glucose (**1a**), D-galactose (**1b**) and their assessment in 1,2-*cis* glycosylation.⁴ Synthesis of these donors was accomplished from the corresponding glycosyl bromides as precursors (Scheme 3).

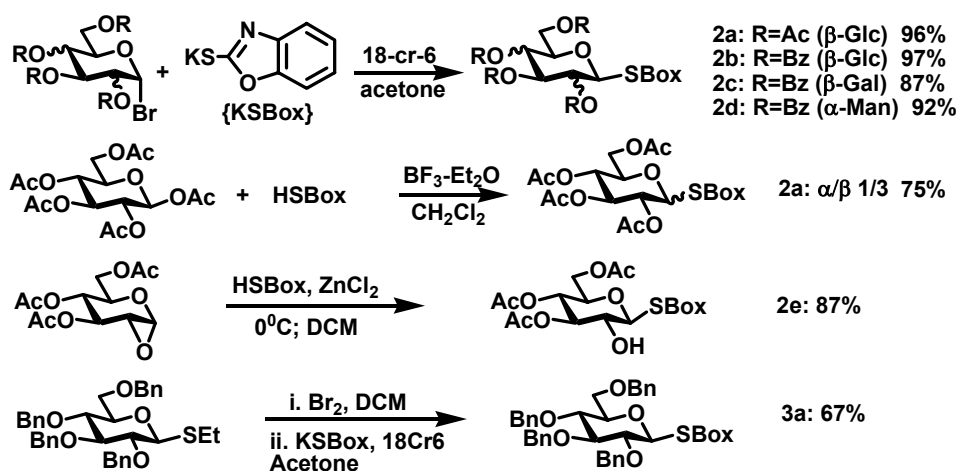


Scheme 3: Synthesis of 2-OBn SBox glycosides

The glycosyl donor properties of these compounds have been investigated in the presence of AgOTf and MeOTf. Overall the SBox glycosides have demonstrated improved stereoselectivity in 1,2-*cis* glycosylation of a range of glycosyl acceptors in comparison to that achieved with *S*-ethyl,⁵ *O*-pentenyl glycosyl donors.⁶

Ongoing Research:

Using the previous work as a starting point we decided to further develop this methodology. First of all, other pathways for synthesis of these glycosyl donors have been investigated. We demonstrated that the SBox glycosides could be obtained from a bromide and potassium salt of the HSBox (KSBox) in the presence of 18-Crown-6,⁷ glucose pentaacetate and HSBox in the presence of BF₃.Et₂O,⁷ and 1,2-anhydro sugars. Also, we demonstrated that *S*-ethyl glycosides could be converted into SBox glycosides via sequential bromination and the reaction with KSBox. As a result, a range of differently protected SBox glycosides of D-glucose (**2a**, **2b**, **2e**, **3a**), D-galactose (**2c**) and D-mannose (**2d**) has been prepared (Scheme 4).



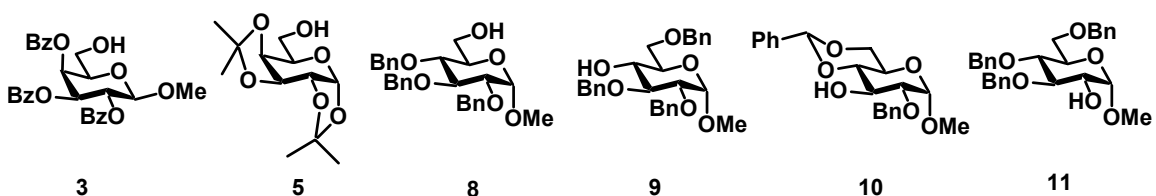
Scheme 4: Synthesis of new SBox glycosyl donors

A number of pilot experiments were performed to ensure stability of these compounds under various reaction conditions. Remarkably, the SBox moiety was found to be fairly stable under typical acetylation (Ac₂O/pyridine), deacetylation (MeONa/MeOH), benzylation (BnBr/NaH, DMF), triphenylmethylation (TrCl/pyridine), as well as benzylidene acetal formation {PhCH(OMe)₂, CSA} and cleavage (1% TFA/aq. CH₂Cl₂) conditions. The stability of the SBox moiety under various protecting group manipulation conditions makes these glycosides very useful glycosyl donors in convergent block oligosaccharide synthesis.

Having obtained a range of compounds suitable for the synthesis both 1,2-*trans* (Ac, Bz) and 1,2-*cis* (Bn) glycosides, we turned our attention to the optimization of the glycosylation reaction. A number of coupling experiments were performed to find the most suitable promoter for the SBox moiety activation. Many different promoters such as AgOTf, MeOTf, NIS/TfOH, CSA, PTSA, BF₃.Et₂O, AgCO₃/ TMSOTf, TfOH, Cu(OTf)₂, NBS were investigated. Amongst these, AgOTf, MeOTf and Cu(OTf)₂ proved to be the most powerful activators. Some of these reactions are summarized below in Table 1.

The per-*O*-benzylated donor (**3a**) showed somewhat lower stereoselectivity than its 2-*O*Bn-3,4,6-triacetyl analog,⁴ most likely due to the higher reactivity of **3a**. This somewhat lower stereoselectivity was then significantly improved by using a participating solvent system of toluene and dioxane (1:3).⁸ So far, we have demonstrated that per-benzoylated SBox glycosides serve as efficient glycosyl donors for stereoselective 1,2-*trans* glycosylations. This statement is substantiated by successful

synthesis of a range of disaccharide derivatives. Moreover, we found that Cu(OTf)₂ is able to activate one SBox donor over another. Protecting groups on these glycosyl donors seem to dictate this phenomenon. These results have raised the question of influence of protecting groups on the stereoselectivity of glycosylation reactions.



Entry	Donor	Acceptor	Promoter	Yield	Entry	Donor	Acceptor	Promoter	Yield	α/β Ratio
1	2b	3	MeOTf	92%	6	3a	5	Cu(OTf) ₂	89%	5/1*
2	2b	5	AgOTf	94%	7	3a	8	Cu(OTf) ₂	95%	6/1*
3	2b	8	AgOTf	91%	8	3a	9	Cu(OTf) ₂	67%	4/1*
4	2b	9	MeOTf	92%	9	3a	10	Cu(OTf) ₂	65%	4/1*
5	2b	10	MeOTf	86%	10	3a	11	Cu(OTf) ₂	68%	7/1*

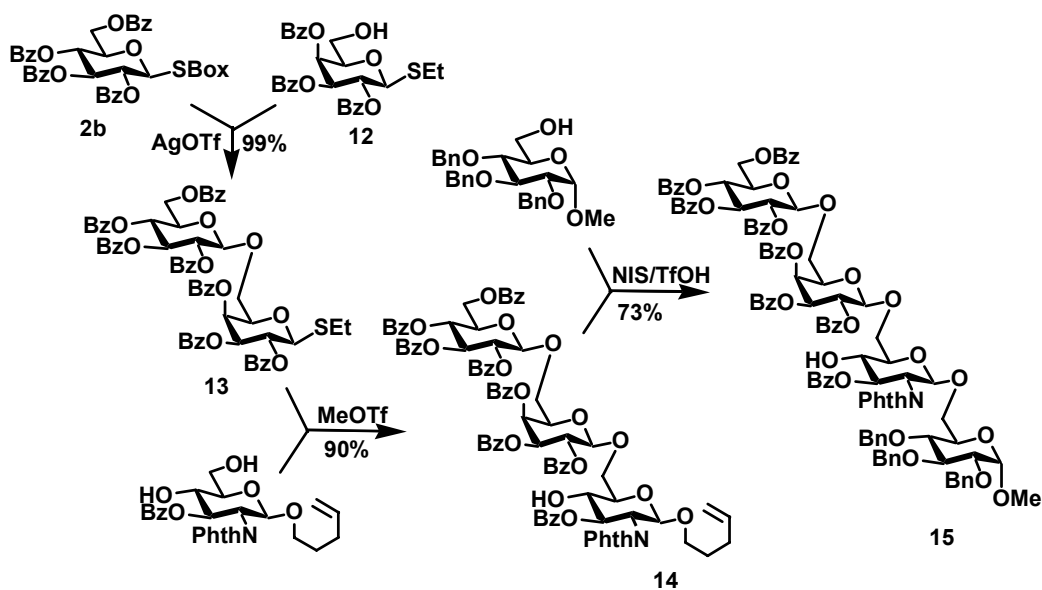
* These glycosylations were performed in 1:3 toluene: dioxane (v/v) as solvent

Table 1: Synthesis of 1,2-*cis* and 1,2-*trans* glycosides with new glycosyl donors

B: Strategy Design:

Previously, semi-orthogonality of the *O*-pentenyl and *S*-ethyl glycosides has been demonstrated.⁹ It has been proven that the *S*-ethyl glycosyl donors can be activated over the *O*-pentenyl glycosides in the presence of MeOTf. In turn, only activated (armed) *O*-pentenyl glycosyl donors can be activated over *S*-ethyl glycosides with iodonium dicollidine perchlorate (IDCP). Certain disadvantages of this strategy have stimulated our further search for convergent glycosylation strategies. Thus, we decided to incorporate the SBox glycosides in our studies.

We investigated selective activation conditions of SBox glycosides in the presence of other common glycosyl donors, in this case acting as glycosyl acceptors. Thus, MeOTf or AgOTf allowed selective activation of SBox glycosides over the *O*-pentenyl moiety. Similarly, glycosylation of *S*-ethyl glycoside acceptor with **2b** donor in the presence of AgOTf provided **13** with complete stereoselectivity. Subsequently trisaccharide **14** was obtained via activation of the *S*-ethyl over *O*-pentenyl moiety in accordance with the semi-orthogonal technique. Finally, *O*-pentenyl moiety of **14** was activated with NIS/TfOH to allow **15** in 65% yield over three glycosylation steps. Clearly, no protective group manipulations were required during the synthesis of the tetrasaccharide **15**.⁷

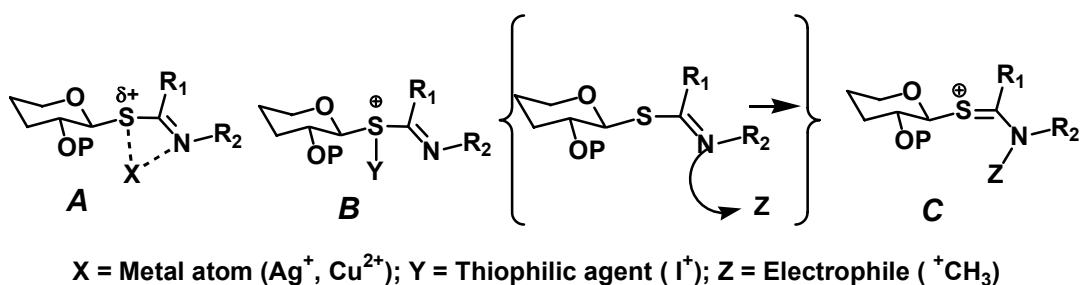


Scheme 5: SBox glycosides in convergent oligosaccharide synthesis

In brief, we demonstrated that SBox glycosides could be activated in the presence of other glycosyl donors and therefore may easily fit into convergent glycosylation strategies for rapid oligosaccharide assembly.

Proposed Research:

Mechanistic Studies: If compared to other well-known glycosyl donors with sulfur based leaving group, the SBox glycosides seem possess different properties. For example, S-ethyl glycosyl donors cannot be activated with AgOTf but SBox glycosyl donors show very good reactivity when AgOTf is used as a promoter. This fact has drawn our attention in order to understand the mechanism of glycosylations. The activation of these glycosyl donors can proceed in many different ways; some anticipated pathways are represented in Scheme 6.



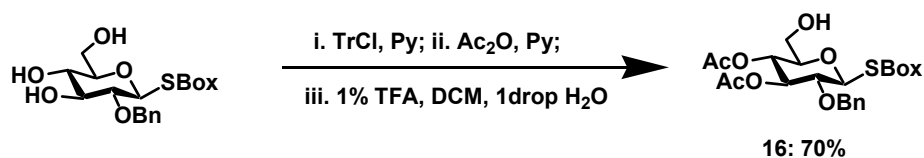
Scheme 6: Activation of SBox glycosides

If one could isolate the leaving group after the glycosylation is completed, it could be beneficial for understanding the activation mechanism. It might well be that all these processes may occur in the presence of other activators. For example, we demonstrated that NIS/ TfOH promoted SBox glycosidation proceeds according to the mechanism proposed for the S-aryl glycosides resulting in disulfide formation.⁵ We have been successful in isolation of the BoxS-SBox disulfide as the leaving group derived species under these conditions.

We have also attempted to isolate the departed aglycone as a metal complex, in the presence of $\text{Cu}(\text{OTf})_2$ or AgOTf. The leaving group seems to form a polymer when

activated in the presence of AgOTf. Having some prior concerns regarding the solubility of the formed complex, these studies were carried out in absence of molecular sieves. Our attempts to analyze this structure have failed so far, future investigations are thus proposed.

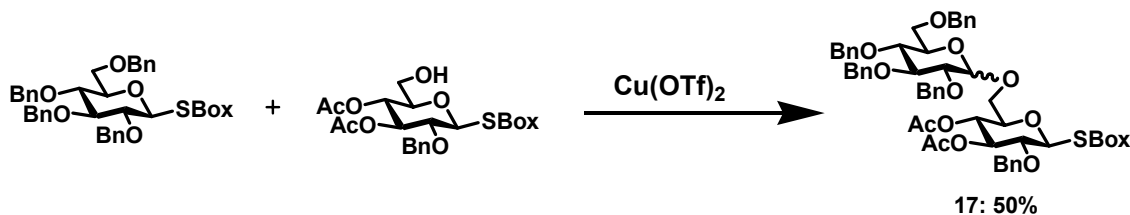
Method Development: The fact that the SBox glycosides have proven their applicability as glycosyl donors makes it important for us to investigate these glycosides as glycosyl acceptors. Making use of the stability of SBox glycosides under protecting group manipulation conditions, synthesis of a series of SBox glycosyl acceptors is one of our goals. For example, acceptor **16** can be easily obtained from a suitable synthetic precursor via routine protecting group sequence as shown in Scheme 7. Similarly, secondary glycosyl acceptors will be synthesized. Having a range of glycosyl acceptors in hands will allow us to test the ability of these compounds as glycosyl acceptors, in the presence of various other glycosyl donors, for example, *S*-ethyl, *O*-pentenyl, *S*-phenyl or halides.



Scheme 7: Synthesis of the SBox acceptors

Strategy Design (Chemoselective Activation): As aforementioned we have discovered that one SBox glycoside can be activated over another, in the presence of Cu(OTf)₂ (Scheme 8). Remarkably, our observations do not correlate with classic armed-disarmed glycosylation concept.¹⁰ Further studies could be beneficial in order to understand the

nature of these glycosylation reactions. These studies will reveal if this phenomenon is a property of SBox glycosides or a novel aspect of a general character.



Scheme 8: Chemoselective activation strategy for SBox glycosides

Application to Synthesis: As aforementioned, carbohydrates of the cell surface play important role in various biological processes. Renal cell carcinoma (RCC) is a highly metastatic type of cancer that preferentially metastasizes to lung nodes. Preliminary clinical investigations have proven the high degree of expression of DSGG (Fig. 1) on the surface of RCC.¹¹ It is also anticipated that a specific type of receptor might be present in lung tissue that recognizes DSGG and mediates the metastasis of renal cell carcinoma to the lung. Further evaluation of these interesting features has not yet emerged, as the isolation of large quantities of pure biological material, sufficient for quantitative adhesion determination is cumbersome. These findings make the synthesis of DSGG and its biological evaluation a very challenging and important target.

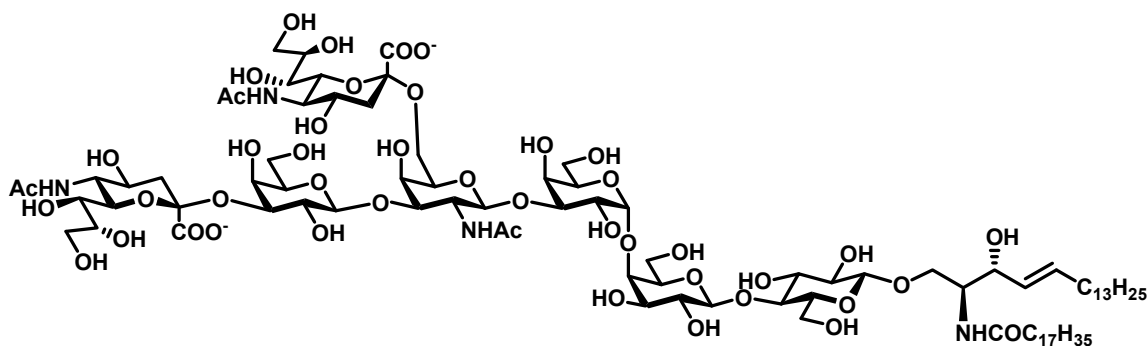
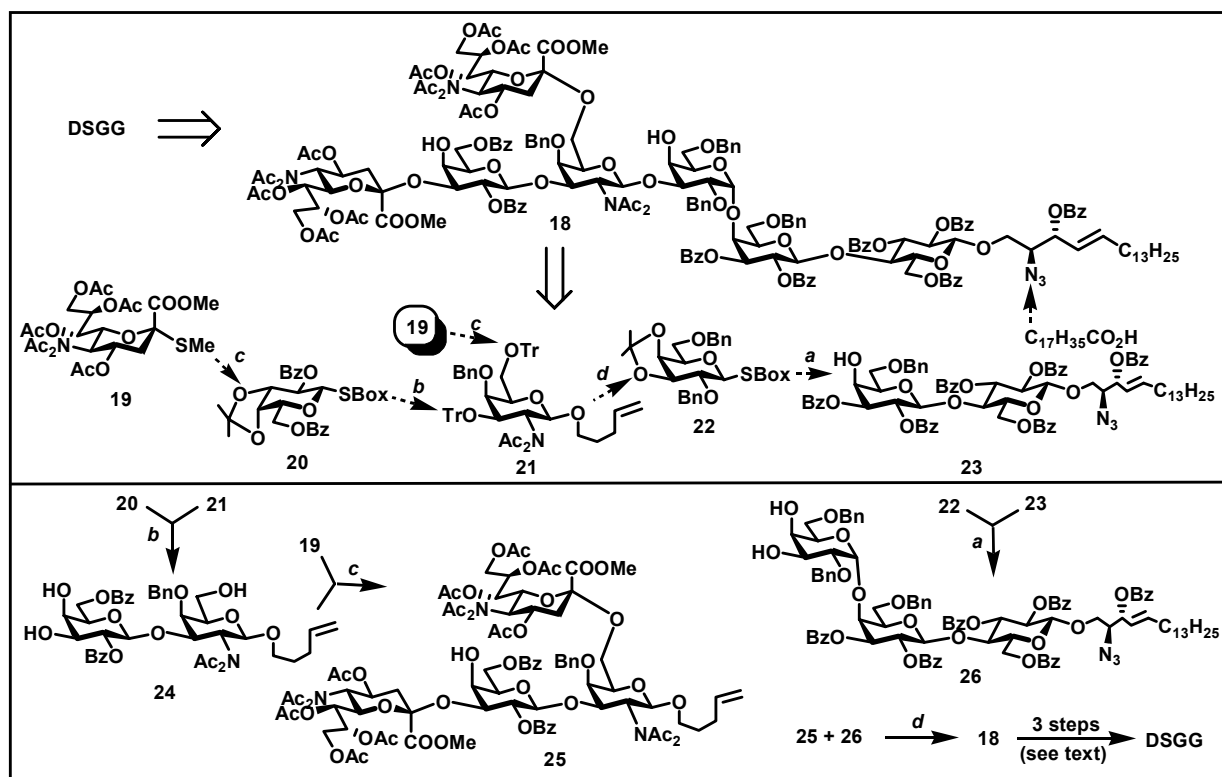


Fig. 1: Structure of DSGG

DSGG is a complex heptasaccharide that consists of two terminal neuraminic acid units, which are linked via (2→3) and (2→6)-bonds to the galactose and galactosamine units respectively. The remaining part of the molecule consists of D-galactose unit, which is linked via 1,2-*cis* glycosydic bond to a lactose unit (Galβ1→4Glc) at the reducing end, which is glycosylated with the ceramide unit, a biantennary saturated lipid.

According to the retrosynthetic analysis, the final assembly of **18**, a synthetic precursor of DSGG, will be accomplished from simple building blocks **19-22** in just four sequential glycosylation steps (a-d, Scheme 9). Glycosylation of **23** with **22** in the presence of AgOTf, followed by mild acidic hydrolysis with 10% TFA/DCM will afford the trisaccharide intermediate **26**.



Scheme 9: Retrosynthetic scheme for synthesis of DSGG

Synthesis of the intermediate **24** (step b) will be performed with the use of elegant 'ditrityl' glycosylation protocol, according to which it should be possible to invert the reactivity of the primary and secondary hydroxyls protected as trityl ethers.¹² Thus, building block **21** will be regioselectively glycosylated at C-3 in the presence of TrClO₄. Subsequently, the acid labile groups will be deprotected as described for synthesis of **26**. The 6,3',4'-triol **24** will be regioselectively disialylated (step c) with access of sialyl donor **19**¹³ in the presence of MeOTf to afford the tetrasaccharide **25**. The latter will be subsequently reacted with **26** in the presence of NIS/ TMSOTf (step d) to complete the assembly. The protecting groups in **18** are designed to minimize the number of deprotection steps and improve the overall yield.

Conclusions:

In conclusion, we have proposed the elaboration of a novel glycosylation methodology. The SBox glycosyl donors are easy to synthesize, stable under functional group manipulation conditions, and provide excellent stereoselectivity and yields. These compounds are highly chemoselective toward other glycosyl donors and hence can be applied in synthesis of complex structures of biological interest. Total synthesis of tumor associated DSGG is also proposed.

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