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Anomeric Reactivity-Based One-Pot Synthesis of Heparin-Like Oligosaccharides

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Abstract

A highly efficient one-pot methodology is described for the synthesis of heparin and heparan sulfate oligosaccharides utilizing thioglycosides with well defined reactivity as building blocks. L-idopyranosyl and D-glucopyranosyl thioglycosides **5** and **10** were used as donors due to low reactivity of uronic acids as the glycosyl donors in the one-pot synthesis. The formation of uronic acids by a selective oxidation at C-6 was performed after assembly of the oligosaccharides. The efficiency of this strategy with the flexibility for sulfate incorporation was demonstrated in the representative synthesis of disaccharides **17**, **18**, tetrasaccharide **23** and pentasaccharide **26**.

Keywords

Glycosylation; Heparin; Oligosaccharides; One-Pot Synthesis; Thioglycoside

Introduction

Glycosaminoglycans (GAGs) are a family of highly sulfated, linear polyanionic molecules that are found on most animal cell surfaces as well as in the basement membranes and other extracellular matrixes. Heparin and heparan sulfate are the most widely studied members of this family. Heparin is exclusively synthesized by tissue mast cells and is stored in cytoplasmic granules, whereas the closely related molecule heparan sulfate is expressed on cell surfaces and throughout tissue matrices.[1] They are composed of repeating disaccharide units of 1→4 linked uronic acid and D-glucosamine (Figure 1). The uronic acid residues typically consist of 90% L-iduronic acid and 10% of D-glucuronic acid. The interaction of these polyanionic molecules with proteins plays an important role in several biological recognition processes, including blood coagulation, virus infection, cell growth, inflammation, wound healing, tumor metastasis, lipid metabolism and diseases of the nervous system.[1,2]

The biosynthesis of heparin and heparan sulfate occurs by similar pathways.[2] Chain initiations occur in the Golgi apparatus. The first step in the pathway involves the attachment of a tetrasaccharide fragment to a serine residue in the core protein. This structure is then modified by a series of enzymatic transformations involving *N*-deacetylation followed by *N*-sulfation, substrate directed epimerization of glucuronic acid to iduronic acid moieties, and finally *O*-sulfation. Although these enzymatic modifications result in a mixture of very complex polysaccharides, structural studies have shown that heparin/heparan sulfates are composed of only 19 distinct disaccharide subunits, differing in their sulfation pattern and in the presence of either D-glucuronic or L-iduronic acid.

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To date, more than one hundred heparin-binding proteins have been identified. With the exception of the antithrombin III-heparin interaction[3], in which the minimal sequence of heparin pentasaccharide is required for binding, the structure and function of heparin interaction with proteins is poorly understood. This is mainly due to the complexity and heterogeneity of these polymers. With the discovery of increasing numbers of heparin-binding proteins, there is a need to characterize the molecular elements responsible for binding to a particular protein and modulating its biological activity. Since the first total synthesis of heparin pentasaccharide[4], numerous synthetic methodologies have been reported for the synthesis of heparin fragments.[5] Most of the strategies involve traditional stepwise oligosaccharide synthesis in which protecting group and anomeric leaving group manipulations, intermediate work-up and purification in each step are required. Access to differentially substituted derivatives is important for dissecting recognition and activity, as recently demonstrated in the synthesis of heparin[6] and chondroitin sulfates[7]. A rapid and truly practical strategy capable of creating diverse derivatives of heparin oligosaccharides with differential sulfation pattern would be useful for detailed functional studies of these important molecules. Recently, we reported a reactivity-based one-pot method for complex oligosaccharides synthesis. In this methodology, the oligosaccharide is assembled rapidly by sequential addition of thioglycoside building blocks, with the most reactive one being added first.[8] The generality of thioglycosides makes them convenient and attractive building blocks due to their stability, accessibility and compatibility.[9] Here we report an efficient one-pot strategy for the rapid assembly of representative heparin and heparan sulfate oligosaccharides.

Results and Discussion

For the synthesis of heparin and heparin sulfate, one has to overcome a range of synthetic difficulties imposed by the complex structure of heparin and heparin sulfate saccharides. Besides the careful design of protecting group strategy to enable the installation of sulfate groups on selected hydroxyl and amino functions, the stereoselective construction of the glucosamine-uronic acid backbone has to be developed. The use of uronic acid building blocks as glycosyl donor[10] is limited and is often avoided,[11] because uronic acids are prone to epimerization, have the inherent low reactivity imposed by the C-5 carboxyl group and complicate protecting group manipulations. Thus, in our synthetic approach, the formation of uronic acids by selective oxidation at the C-6 hydroxyl group was done after assembly of oligosaccharides. Control of the stereoselectivity of each glycosylation and generation of a significant reactivity difference between the thioglycosides are crucial for the successful synthesis of heparin oligosaccharides with the one-pot strategy. The reactivity of a sugar is highly dependent on its protecting groups and the anomeric activating group used. The reactivity differences between thioglycosides were mainly influenced by electron-donating and electron-withdrawing protecting groups. To this end, the hydroxyl groups to be sulfated were protected as acyl (acetyl and benzoyl) groups. Selective removal of acetate esters[12] in the presence of benzoates also enabled us to synthesize oligosaccharides with differential sulfation patterns. The primary hydroxyl groups to be selectively oxidized to uronic acids were protected as *tert*-butyldiphenylsilyl ethers. Finally, benzyl groups were installed on the remaining hydroxyls and the amino functionalities were masked as azides. Relative reactivity values (RRV) of monosaccharide building blocks were obtained by HPLC analysis with the established competitive assay method. [8a,13]

To test the above synthetic strategy, fully protected disaccharides **17**, **18**, tetrasaccharide **23** and pentasaccharide **26** (a binding epitope for antithrobin III) were selected as model saccharides. Thus, L-idopyranosyl, D-glucopyranosyl and azidoglucosyl thioglycosides were designed and prepared using above synthetic strategy (Scheme 1). The known 1,2,4,6-*O*-tetra-*O*-acetyl-3-*O*-benzyl-α/β-D-idopyranoside[14] was used as starting material for the construction of **5**. The reaction of **1** with *p*-toluenethiol in the presence of $BF_3{\cdot}Et_2O$ gave **2** in 93% yield.

Standard removal of acetate esters in **2** and formation of the 4,6-*O*-benzylidene acetal, followed by protection of the C-2 hydroxyl as a benzoate ester, which was chosen for its participating group assistance in the forthcoming glycosylation reaction. Subsequent acidolysis of the cyclic acetal afforded idopyranosyl thioglycoside **3** in 78% yield. Using standard methods, introduction of the *tert*-butyldiphenylsilyl group at C-6 (91%) and introduction of the levulinyl group at C-4 provided fully protected idopyranosyl thioglycoside **5** (RRV = 2656.4) in 89% yield. This route was also applied to the synthesis of glucopyranosyl thioglycoside **10** (RRV = 1443.5) starting from the known 1,2,4,6-*O*-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside[15] (Scheme 1).

Next azidoglucosyl thioglycoside building blocks **15** and **16** were synthesized following our previously reported methods (Scheme 2).[5f] Briefly, the C-3 hydroxyl was selectively protected as a benzyl ether after di-*n*-butyltin oxide activation.[16] Direct benzylidination of resulting **12** afforded **13** in 89% yield. Azidoglucosyl thioglycoside derivative **14** was formed through a two-step sequence: triflation of the free hydroxyl was followed by nucleophilic substitution with NaN_3 in DMF gave compound **14** in 83%. Removal of 4,6-*O*-benzylidene acetal and regioselective introduction of the acetyl group at C-6 afforded the fully protected azidoglucosyl acceptor **15** (RRV = 47.7). In a similar manner, selective opening of 4,6-*O*-benzylidene acetal in **14** using $PhBCl_2$ and Et_3SiH, followed by acetylation at C-6 afforded azidoglucosyl donor **16** (RRV = 53.7).

With all monosaccharide building blocks in hand, we turned our attention to the one-pot synthesis of heparin oligosaccharides. First, disaccharide formation by different thioglycoside activators was examined, using *N*-iodosuccinimide/trifluoromethanesulfonic (triflic) acid (NIS/TfOH),[8a] benzenesulfinyl piperidine (BSP)/triflic anhydride (Tf_2O)[8b] or *N*-(phenylythio)-ε-caprolactam/Tf_2O.[8c] These activators were previously used in several reactivity-based one-pot syntheses.[8] Since the reactivity of idopyranosyl thioglycoside **5** (RRV=2656.3) or glucopyranosyl thioglycoside **10** (RRV=1443.5) is much higher than that of **15** (RRV=47.7), glycosylation of donor **5** or **10** with azidoglucosyl thioglycoside acceptor **15** afforded the desired disaccharide **17** or **18** in excellent yield and stereoselectivity with no self coupling of **5** (Scheme 3). Slightly better yield was observed in both cases using NIS/TfOH as activator. In addition, disaccharide acceptor **20** was synthesized in a straightforward manner (Scheme 3). Glycosylation of **5** with methyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyronoside[10a] in the presence of NIS/TfOH afforded desired disaccharide **19** in 96% yield. Removal of the levulinyl group with NH_2NH_2/AcOH/Pyridine afforded disaccharide acceptor **20** in 95% yield.

In an effort to extend the application of one-pot synthesis, syntheses of tetrasaccharide and pentasaccharide were next examined. For the one-pot tetrasaccharide synthesis (Scheme 4), fully protected idopyranosyl donor **5** was first coupled with azidoglucosyl acceptor **15** in the presence of NIS/TfOH at -45°C followed by slow warming to room temperature. After 3 h, α-methyl disaccharide acceptor **20** was added, followed by the addition NIS/TfOH at the same temperature. The fully protected tetrasaccharide **21** was obtained in 35% yield. With this methodology, protecting group and anomeric leaving group manipulations, intermediate work-up and purification can be avoided. Removal of the silyl ether protection group with HF·pyridine afforded **22** in 87% yield. The resulting primary hydroxyl groups were oxidized with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and NaOCl as a co-oxidant[5g] under basic conditions (pH = 10). The corresponding carboxyl groups were then esterified in the presence of MeI and $KHCO_3$ to give the desired fully protected tetrasaccharide **23** in 68% yield over two steps.

When the same approach was applied to the one-pot synthesis of the pentasaccharide, unfortunately, the yield in the first glycosylation was very low. This was partially attributed to

the bulky silyl group at C-6 on **18** which partially blocks the C-4 hydroxyl. Changing the silyl protection at C-6 to a smaller group was expected to increase the yield. Thus, the TBDPS groups of compounds **18** and **19** were deprotected using HF·pyridine in THF and oxidation of the resulting hydroxyl groups with TEMPO, NaOCl followed by methylation, and finally removal of levulinate afforded disaccharides **24** and **25** in 45% and 77% yield respectively. For the one-pot pentasaccharide synthesis (Scheme 5), azidoglucosyl donor **16** (RRV = 53.7) was first coupled with disaccharide acceptor **24** (RRV = 18.2), and then α-methyl disaccharide acceptor **25** was added to the reaction mixture. Under these conditions the fully protected pentasaccharide **26** was obtained in 20% yield. The corresponding *O*-sulfates, were obtained by consecutive saponification with LiOOH and *O*-sulfation with triethylamine-sulfur trioxide followed by palladium catalyzed hydrogenolysis and *N*-sulfation with pyridine-sulfur trioxide to provide the desired heparin pentasaccharide **27**. It is noted however that selective deprotection of the acetyl group, the benzoyl group, the methyl ester group and the benzyl group can be carried out with known procedures to create different sulfation patterns.

Conclusion

In conclusion, we have developed a one-pot strategy for the synthesis of heparin-like oligosaccharides. Carefully designed monosaccharide building blocks (e.g., **5**, **10**, **15** and **16**) with well defined reactivity were successfully used in the representative one-pot synthesis of disaccharides **17**, **18**, tetrasaccharide **23** and pentasaccharide **26**. No self coupling of the building blocks was observed in each case, illustrating the importance of quantitative reactivity determination for the implementation of programmable one-pot synthesis. Heparin pentasaccharide derivative **27** was obtained after global deprotection and sulfation. We believe that this new strategy has potential for rapid synthesis of various heparin analogs for the study of their biological properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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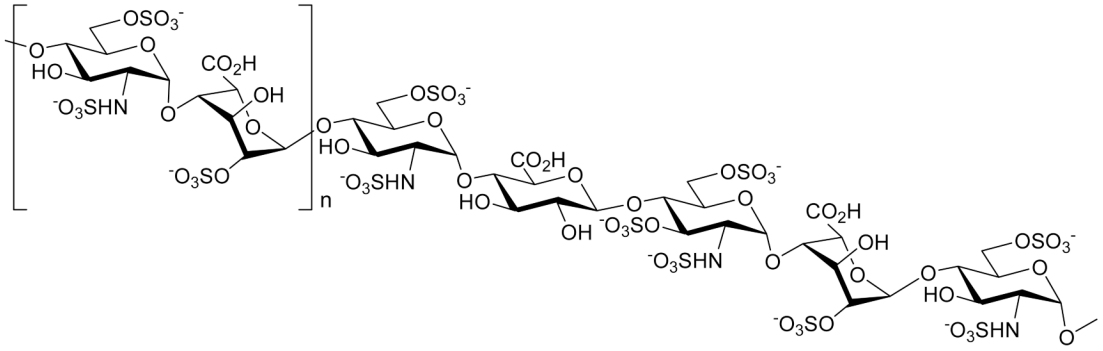
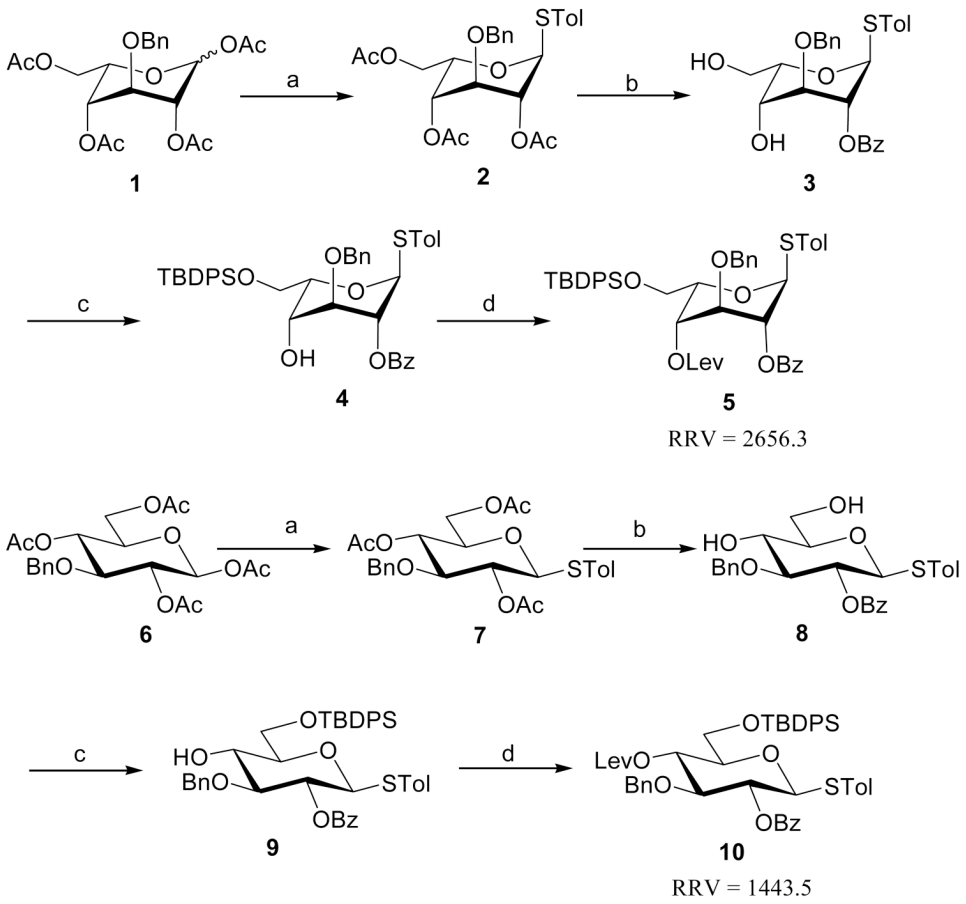


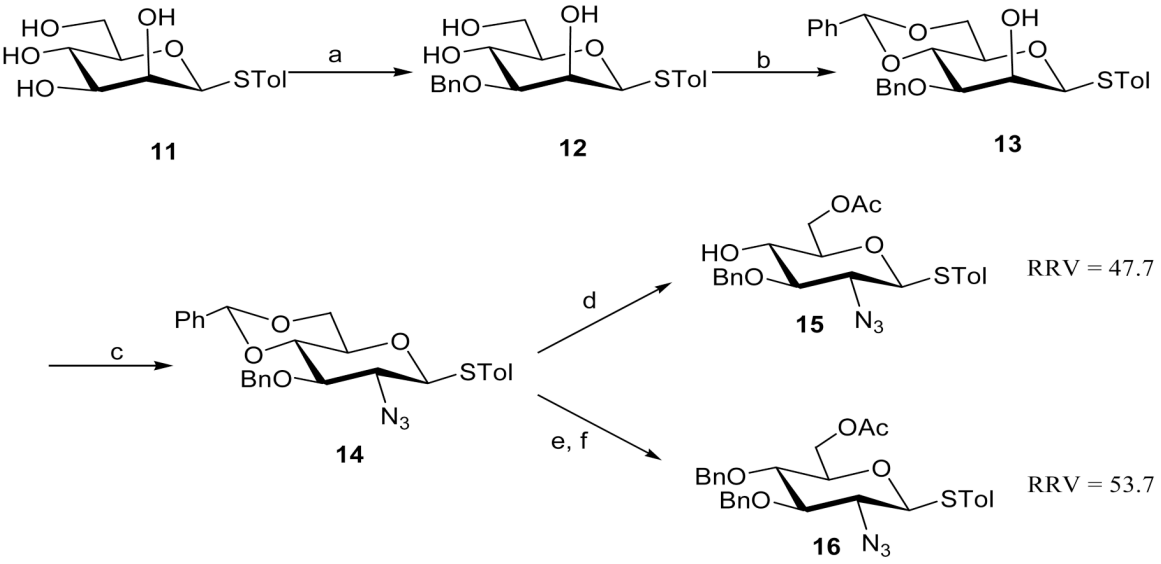
Figure 1.
Schematic view of heparin



Scheme 1.

Preparation of the 1-thio uronic acid building blocks **5** and **10**[a]

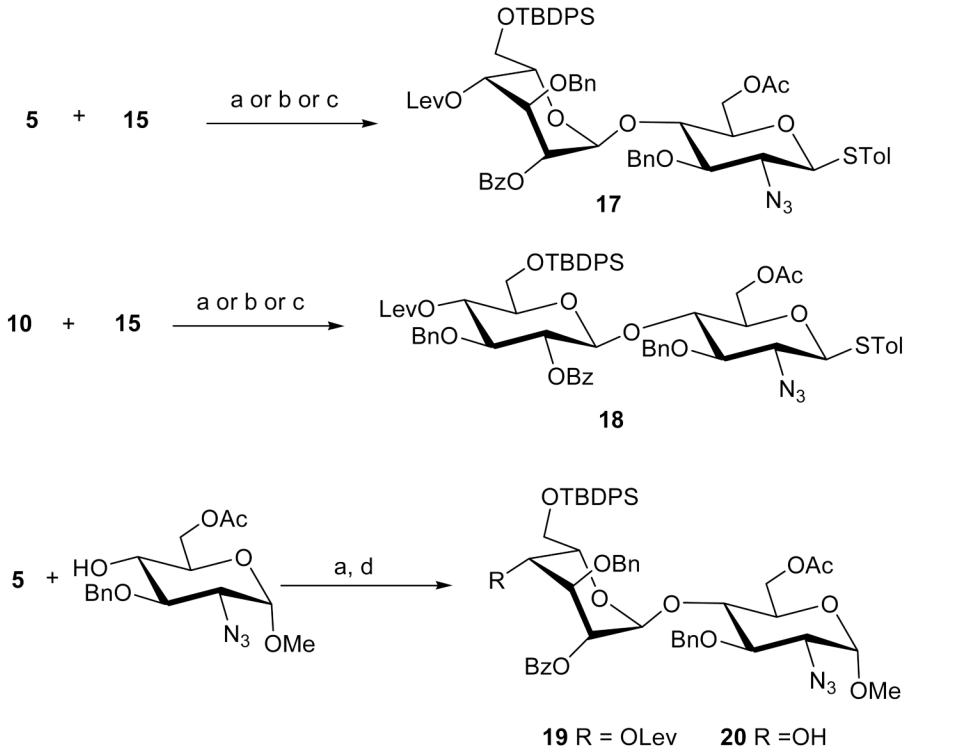
[a]Reagents and conditions: (a) TolSH, $BF_3{\cdot}Et_2O$, **2**: 93% (9:1 α/β), **7**: 87%; (b) i. NaOMe, MeOH; ii. $PhCH(OMe)_2$, *p*-TsOH, CH_3CN/DMF; iii. BzCl, Pyridine; iv. 60% TFA in H_2O, CH_2Cl_2, **3**: 78%, **8**: 81%; (c) TBDPSCl, Pyridine, **4**: 91%, **9**: 95%; d) Lev_2O, Pyridine, **5**: 89%, **10**: 91%.



Scheme 2.

Preparation of azidoglucosyl acceptors **15** and **16**[a]

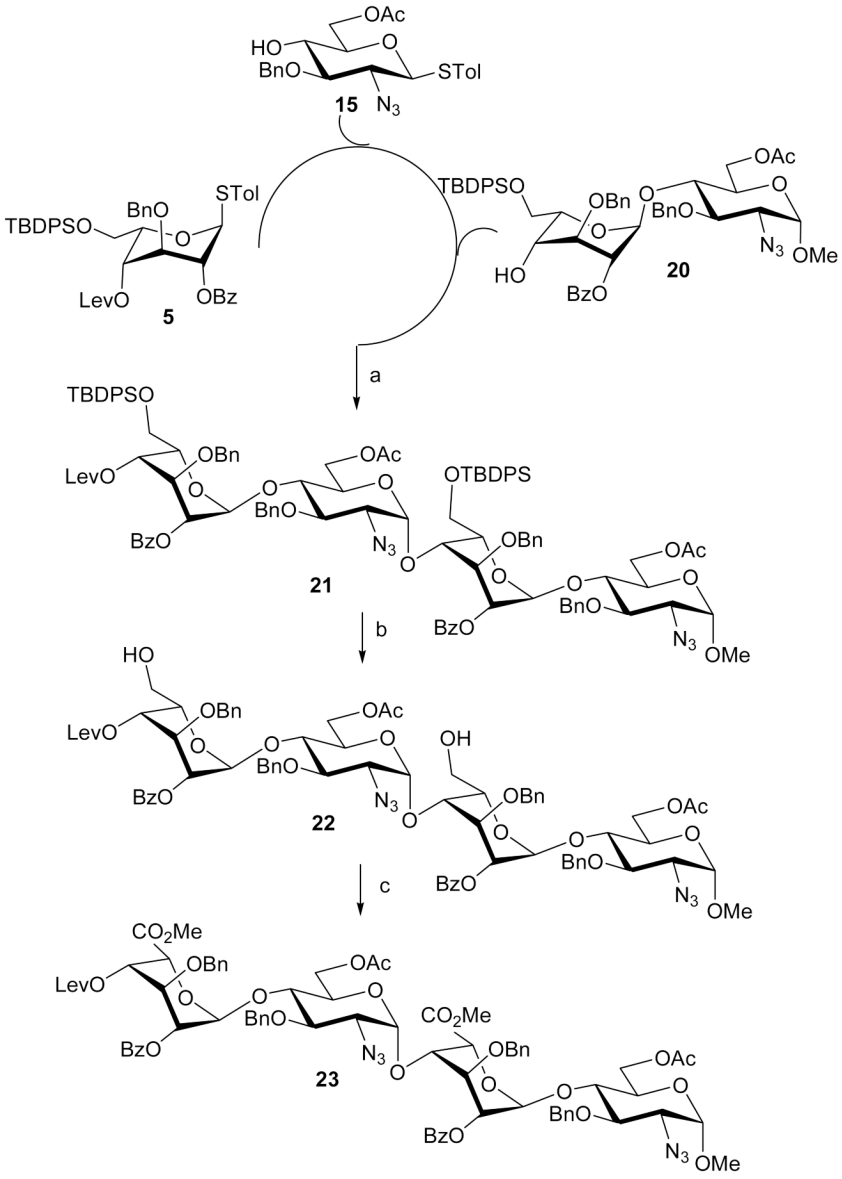
[a]Reagents and conditions: (a) i. Bu_2SnO, Toluene ii. Bu_4NBr, BnBr, 65% in two steps; (b) $PhCH(OMe)_2$, CSA, 89%; (c) i. Tf_2O, Pyr-CH_2Cl_2, ii. NaN_3, DMF, 83% in two steps; (d) i. 80% AcOH, ii. AcCl, Pyridine, **15**: 89%; (e) $PhBCl_2$, Et_3SiH, CH_2Cl_2, 92%; (f) Ac_2O, Pyridine, **16**: 95%;



Scheme 3.

One-pot synthesis of disaccharide derivatives **17** – **20**

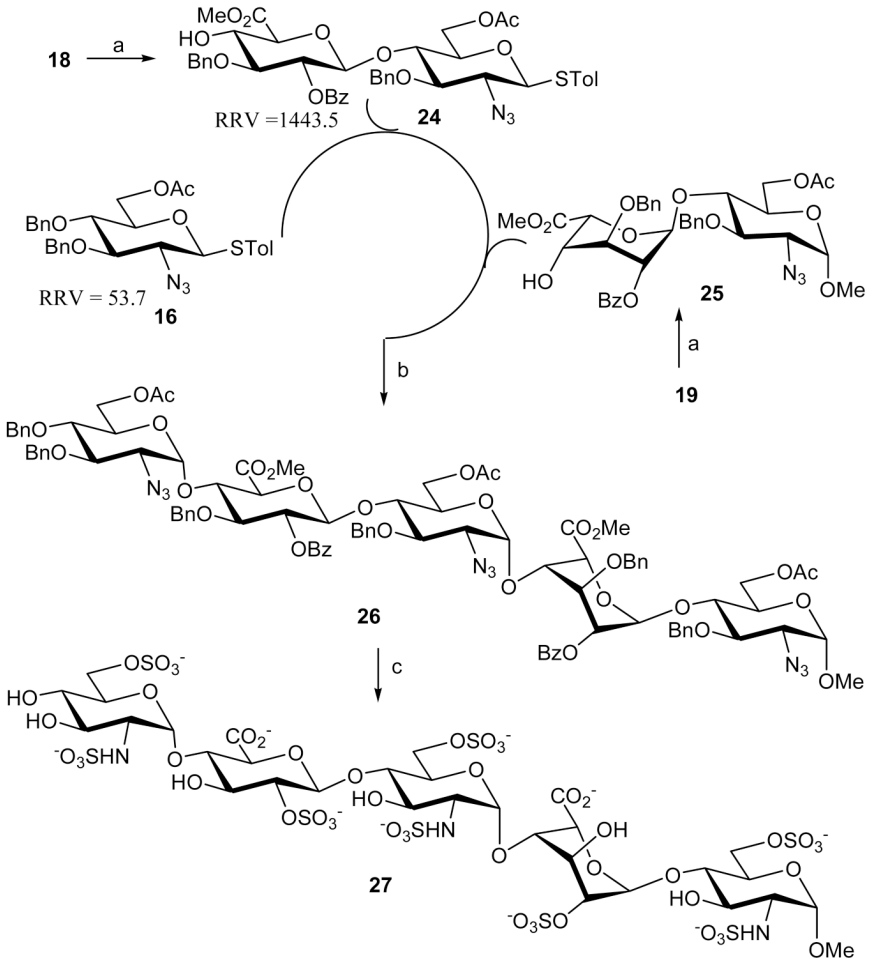
[a]Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, -45°C to room temperature, **17**: 92%, **18**: 89%, **19**: 96%; (b) BSP, Tf_2O, CH_2Cl_2, -45°C to room temperature, **17**: 72%, **18**: 75%; (c) *N*-(phenylythio)-ε-caprolactam, Tf_2O, CH_2Cl_2, -45°C to room temperature, **17**: 85%, **18**: 88%; (d) NH_2NH_2/AcOH/Pyridine, **20**: 95%.



Scheme 4.

One-pot synthesis of tetrasaccharide derivative **23**[a]

[a]Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, -45°C to room temperature, 35%; (b) HF·Pyr, THF, 87%; (c) i. TEMPO, KBr, NaOCl, CH_2Cl_2, H_2O, ii. MeI, $KHCO_3$, DMF, 68% in two steps.



Scheme 5.

One-pot synthesis of pentasaccharide derivative **27**[a]

[a]Reagents and conditions: (a) i. HF·Pyr, THF; ii. TEMPO, KBr, NaOCl, CH_2Cl_2, H_2O, then MeI, $KHCO_3$, DMF; iii. NH_2NH_2/AcOH/Pyridine, **24**: 45%, **25**: 77%; (b) i. NIS, TfOH, CH_2Cl_2, -45°C to room temperature; ii. NIS, TfOH, CH_2Cl_2, -45°C to room temperature, 20%; c) i. LiOOH, THF; ii. $Et_3N{\cdot}SO_3$, DMF; ii. H_2, Pd/C; *iv*- $Pyr{\cdot}SO_3$, H_2O, 33%.