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α-Glycosylation by D-Glucosamine-Derived Donors: Synthesis of Heparosan and Heparin Analogues That Interact with Mycobacterial Heparin-Binding Hemagglutinin

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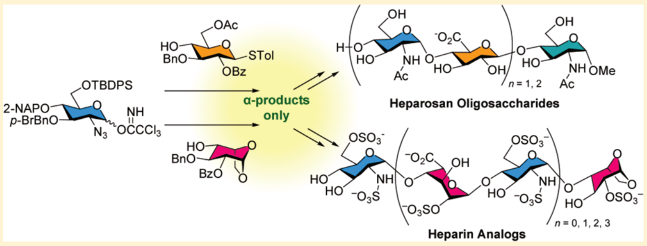
Supporting Information



ABSTRACT: Numerous biomolecules possess *α*-D-glucosamine as structural component. However, chemical glycosylations aimed at this backbone are usually not easily attained without generating the unwanted *β*-isomer. We report herein a versatile approach in affording full *α*-stereoselectivity built upon a carefully selected set of orthogonal protecting groups on a D-glucosaminyl donor. The excellent stereoselectivity provided by the protecting group combination was found independent of leaving groups and activators. With the trichloroacetimidate as the optimum donor leaving group, core skeletons of glycosylphosphatidyl inositol anchors, heparosan, heparan sulfate, and heparin were efficiently assembled. The orthogonal protecting groups were successfully manipulated to further carry out the total syntheses of heparosan tri- and pentasaccharides and heparin di-, tetra-, hexa-, and octasaccharide analogues. Using the heparin analogues, heparin-binding hemagglutinin, a virulence factor of *Mycobacterium tuberculosis*, was found to bind at least six sugar units with the interaction notably being entropically driven.

■ INTRODUCTION

The numerous important roles of carbohydrates in living systems are underscored by their high abundance and molecular diversity. Once regarded as merely structural support and energy storage molecules, sugars are now acknowledged as vital players in physiological and pathophysiological processes making them potential drug development targets.[1] They exist as glycoconjugates at the surface of every known cell, interacting and coordinating with various extracellular matrix elements.[2] Several natural products also carry sugar moieties that influence their biological potency.[3] With fewer monomer types than proteins, the exquisite complexity of carbohydrate polymers primarily arises from the multiple potential linkage points within the pyranosyl or furanosyl ring of each residue and the chirality of the anomeric center generated on assembly. Appreciation of the structure−function relationship of these biopolymers requires well-defined compounds, which are, unfortunately, difficult to isolate in sufficient quantity and purity from Nature. Thus, synthetic oligosaccharide preparations offer a dependable solution to vast demands.[4]

Several important biomolecules carry D-glucosamine in *α*-form as core component. For example, glycosylphosphatidylinositol (GPI)-anchors, which tether proteins of various functions to the cell surface, contain D-glucosamine *α*1→6-linked to *myo*-inositol (Figure 1).[5] *α*-D-Glucosamine is also an important feature, together with D-glucuronic and L-iduronic acid, in the repeating disaccharide of the structurally related heparan sulfate and heparin. Heparan sulfate is a linear polysulfated polysaccharide ubiquitously distributed on the

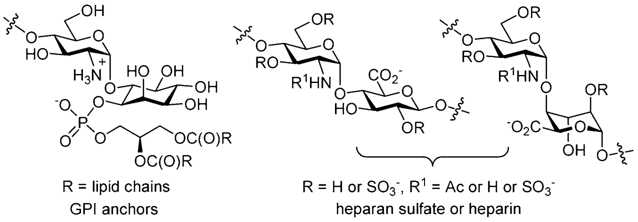


Figure 1. Structures of the *α*-D-glucosamine-containing disaccharide units found in GPI anchors, heparan sulfate, and heparin.

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cell surface as a component of proteoglycans. It is a known mediator of several biologically important events including viral and bacterial infection, cell growth, inflammation, wound healing, tumor metastasis, lipid metabolism, and diseases of the nervous system.[6] Found mainly in mast cells, heparin is a widely used clinical anticoagulant through its tight binding and consequent activation of antithrombin III.[7] On the basis of this interaction, the synthetic pentasaccharide fondaparinux is now effectively utilized for the prevention of venous thrombolitic events following surgery.[8] Alternating *N*-acetyl-*α*-D-glucosamine (GlcNAc*α*) and *β*-D-glucuronic acid all with 1→4 linkages are present in the capsular polysaccharide of *Escherichia coli* strain K5.[9] Termed heparosan, it is actively being employed in the preparation of the more complex heparan sulfate/heparin saccharides through chemoenzymatic transformations.[10] The highly immunogenic O-antigen of several serotypes of *Shigella* contains GlcNAc*α* in their repeating structure.[11] GlcNAc*α* is also present in the cell-wall teichoic acid of some *Staphylococcus* species.[12] Aside from these biopolymers, *α*-D-glucosamine is found in several natural products such as antibiotics (e.g., tunicamycins and neomycin)[13] and bacterial antioxidants (e.g., mycothiol and bacillithiol).[14]

Because of the 1,2-*cis* nature of the *α*-D-glucosamine glycosidic bond, control of its stereoselective formation without the *β*-isomer is challenging.[15] Typically, such couplings take advantage of the anomeric effect—the primary requirement of which is a nonparticipating group masking the 2-amine moiety. For this purpose, 2,3-*trans*-oxazolidinone, *p*-methoxybenzylideneamino group, and azide are the most prominent in recent literature. Initially applied by Kerns to D-glucosamine in 2001,[16] the ring-fused oxazolidinone provided good *α*-stereoselectivity; but its use was curtailed by high promoter loading and several side reactions. Later modifications by acetylation[17] or benzylation[18] at the nitrogen position, together with the proper selection of activators and solvent, gave better stereocontrol and yield. Nguyen and co-workers, on the other hand, found that nickel catalysts, through coordination with the approaching acceptor and the *p*-methoxybenzylideneamino group at the 2-C position, could deliver the stereoselective *α*-glycosidation of trichloroacetimidate donors.[19] The most commonly used 2-amino protecting group is the azide, first utilized for its nonparticipating property in glycosylation by Paulsen[20] more than 30 years ago. Nevertheless, it provided varying levels of *α*-stereoselectivity, often rendering anomeric mixtures that led to tedious purification. Drawing from the observation that the axially oriented 4-hydroxyl of L-iduronate acceptors provided full *α*-stereoselectivity on glycosylation with 2-azido-2-deoxy-D-glucosyl donors, Seeberger suggested that forcing the hydroxyl nucleophile of the acceptor to assume the axial position would enable excellent stereoselectivity.[21] Glycosylations of inositol-type acceptors following this concept, however, did not lead to the same outcome.[22] Alternatively, Boons and co-workers reported that an added thioether to the reaction mixture assists in the *α*-selective glycosidation of D-glucosaminyl trichloroacetimidate donors.[23]

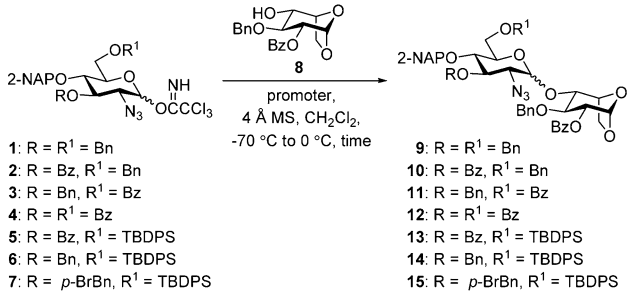
Our work on heparin and heparan sulfate synthesis[24] elevated the desire to find a highly useful strategy for stereoselective *α*-glucosaminylation. Accordingly, we present herein a versatile approach in generating the *α*-glycosidic bond built upon a carefully selected set of orthogonal protecting groups on a D-glucosaminyl donor. Using this donor, backbones of some naturally occurring sugars were efficiently generated including heparosan and heparin, which were both synthesized in various lengths. With analogues of heparin at hand, we further examined their interaction with heparin-binding hemagglutinin (HBHA), a virulence factor of *Mycobacterium tuberculosis*.[25]

■ RESULTS AND DISCUSSION

α-Stereoselective Glucosaminylation. Besides the familiar 2-C neighboring group assistance, other functional groups also influence the stereoselectivity of glycosylation. For example, remote acyl moieties, bulky substituents, and fused diol protecting groups all demonstrated potent stereodirecting effects in a variety of glycosyl donors.[26] Because the D-glucosamine residue of heparin/heparan sulfate—our motivation for this exploration—may be modified at the 2-N, 3-O, and 6-O positions and must be amenable to further coupling at 4-O, some degree of orthogonality among the protecting groups is needed. For the 2-C functionality, we chose the azido group for it imparts low steric hindrance and does not complicate NMR analysis of sugar derivatives.[27] It is also stable in many synthetic transformations, but is readily converted to the amine by reduction (e.g., hydrogenation or Staudinger reaction)[28] or directly to the acetamide by thioacetic acid treatment,[29] properties that nicely fit the D-glucosamine residues found in Nature. At 4-O, we required a blocking group that may both be a temporary and permanent protection. To this end, the 2-naphthylmethyl (2-NAP) group is suitable because it can be chemoselectively cleaved by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or simultaneously removed together with the other permanent arylmethyl groups by hydrogenolysis in later synthetic steps. With these primary requirements aside, we examined the effect of several protecting groups at the 3-O and 6-O positions on the stereoselectivity of glucosaminylation in CH_2Cl_2 using the trichloroacetimidate as leaving group and the known 1,6-anhydro-*β*-L-idopyranosyl 4-alcohol **8**[24f] as glycosyl acceptor (Table 1). The *α*-products of such couplings possess the backbone of the major repeating component of heparin. Employing catalytic trimethylsilyl triflate (TMSOTf) as promoter, the markedly higher yield and *α*/*β* ratio were notable when benzoyl (Bz) group was utilized instead of benzyl (Bn) (entries 1−4). This increase in stereoselectivity probably resulted from the remote assistance by the Bz group. The installation of *tert*-butyldiphenylsilyl (TBDPS) moiety at the 6-O position further gave better results (entries 5 and 6). Finally, when the *p*-bromobenzyl (*p*-BrBn) group was positioned at 3-O while TBDPS blocks 6-O, complete stereoselectivity was afforded generating the *α*-disaccharide **15** in 84% yield (entry 7). Both situated at the *β*-face of the donor, these large groups may have prevented the approach of the acceptor at the zone that would generate the unwanted stereoisomer. Notably, methods are available for the chemoselective removal of TBDPS and *p*-BrBn groups,[28b] rendering donor **7** with a protecting group pattern that holds a high level of orthogonality. Following the successful stereoselective glycosylation of acceptor **8** using donor **7** and TMSOTf, other typical trichloroacetimidate promoters were further examined. The imidate activation by silver(I) triflate (AgOTf) and triflic acid (TfOH) exclusively furnished the *α*-adduct **15** in 63% (entry 8) and 66% (entry 9) yields, respectively. As for $BF_3 \cdot Et_2O$, the yield was a meager 26%; 49% of **8** was recovered (entry 10).

We then focused on the effect of various leaving groups on the stereoselectivity of the 4-alcohol **8** glycosylation. The results

Table 1. Coupling of the 4-Alcohol 8 and Variously Protected D-Glucosamine-Derived Trichloroacetimidate Donors

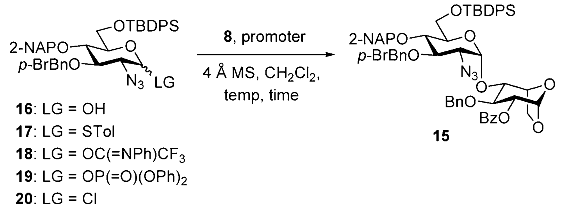


entry	donor	promoter[a]	time (h)	product (α/β[b])	yield (%)
1	**1**	TMSOTf	1.5	**9** (3.2/1)	63
2	**2**	TMSOTf	1.5	**10** (4.5/1)	71
3	**3**	TMSOTf	1.5	**11** (5.5/1)	72
4	**4**	TMSOTf	1.5	**12** (5.6/1)	73
5	**5**	TMSOTf	2	**13** (7.1/1)	89
6	**6**	TMSOTf	2.5	**14** (8.5/1)	76
7	**7**	TMSOTf	2	**15** (α only)	84
8[c]	**7**	AgOTf	2.5	**15** (α only)	63[d]
9	**7**	TfOH	2	**15** (α only)	66[d]
10	**7**	$BF_3 \cdot Et_2O$	1.5	**15** (α only)	26[d]

[a] 0.2 equiv TMSOTf, 5 equiv AgOTf, 0.2 equiv TfOH, and 1.2 equiv $BF_3 \cdot Et_2O$ were used. [b] Based on isolated yields; the α-isomer has $J_{1',2'}$ of 3.7−3.8 Hz, whereas the β-isomer has 7.6−8.0 Hz. [c] The reaction was carried out from −40 °C to rt. [d] Recovered acceptor **8**: 13% (entry 8), 17% (entry 9), and 49% (entry 10). MS: molecular sieves.

of the reaction leading to the disaccharide **15** are outlined in Table 2. Dehydrative glycosylation[30] (entry 1), glycosyl phosphate (entry 4), and glycosyl chloride (entry 5) gave modest results whereas thioglycoside (entry 2) and *N*-phenyl trifluoroacetimidate[31] (entry 3) provided good yields. In all these cases, precedence toward α-selectivity was observed. Thus, the importance of proper selection of protecting groups in achieving α-stereoselectivity is highlighted. Our protecting group combination exclusively afforded the α-isomer amidst the use of various leaving groups and promoters.

Table 2. Coupling of the D-Glucosaminyl Donors 16−20 and the 4-Alcohol 8

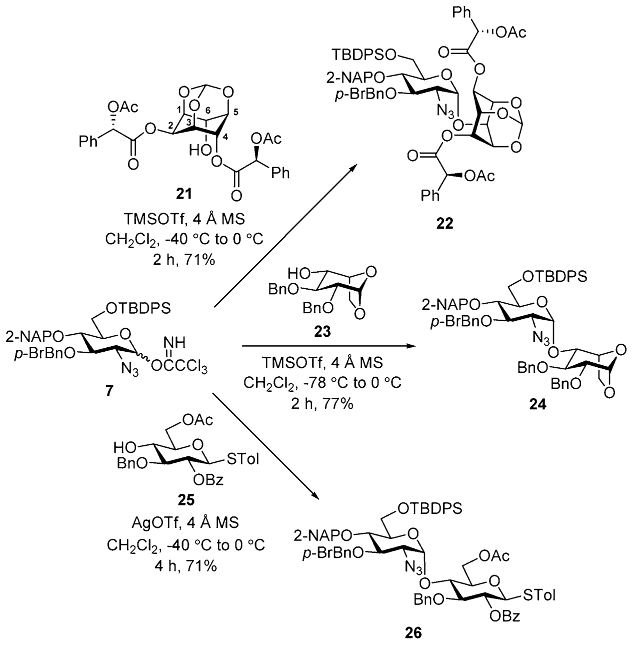


entry	donor	promoter	temp (°C)	time (h)	yield (%)
1	**16**	Me_2S/Tf_2O	rt	16	42[a]
2	**17**	NIS/TfOH	−78 to −20	4	79
3	**18**	TMSOTf	−40 to 0	2	73
4	**19**	TMSOTf	−40 to 0	3	48[a]
5	**20**	AgOTf	−20 to 0	2.5	30[a]

[a] Recovered acceptor **8**: 16% (entry 1), 11% (entry 4), and 23% (entry 5). NIS: *N*-iodosuccinimide.

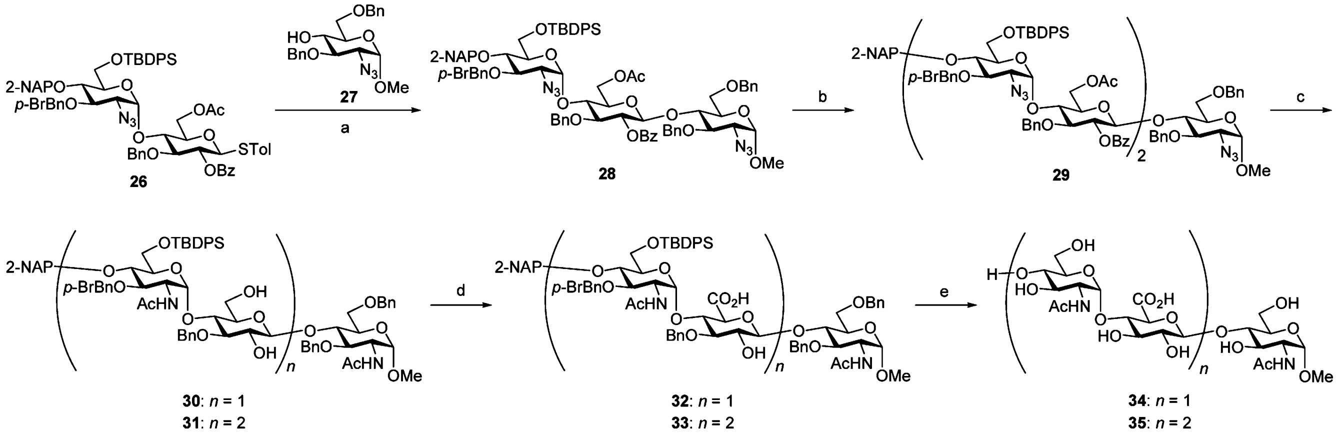
After establishing the effectiveness of our system with the 4-alcohol **8**, the trichloroacetimidate **7** was utilized in the assembly of some important sugar skeletons (Scheme 1). Glycosylation of the *myo*-inositol 6-alcohol **21**[32] afforded the α-pseudodisaccharide **22** in 71% yield as a single isomer. Compound **22** holds the core skeleton found in GPI anchors. The respective couplings of **7** with the 4-alcohols **23**[24d] and **25**[33] both led to the disaccharides **24** and **26**, which are also component backbones of heparin and heparan sulfate, in complete α-stereoselectivity and satisfactory yields. Additionally, **26** may be directly used for the synthesis of heparosan oligosaccharides. The preparation of compound **26** deserves more comment. Here, we utilized a trichloroacetimidate donor, a thioglycoside acceptor, and AgOTf as promoter. We found that promoting the reaction using TMSOTf, TfOH, or $BF_3 \cdot Et_2O$ furnished the desired product in poor yields (>13%), a likely consequence of the commonly observed sulfide aglycon transfer.[34]

Scheme 1. Glycosylation of Various Acceptors Using the D-Glucosaminyl Donor 7



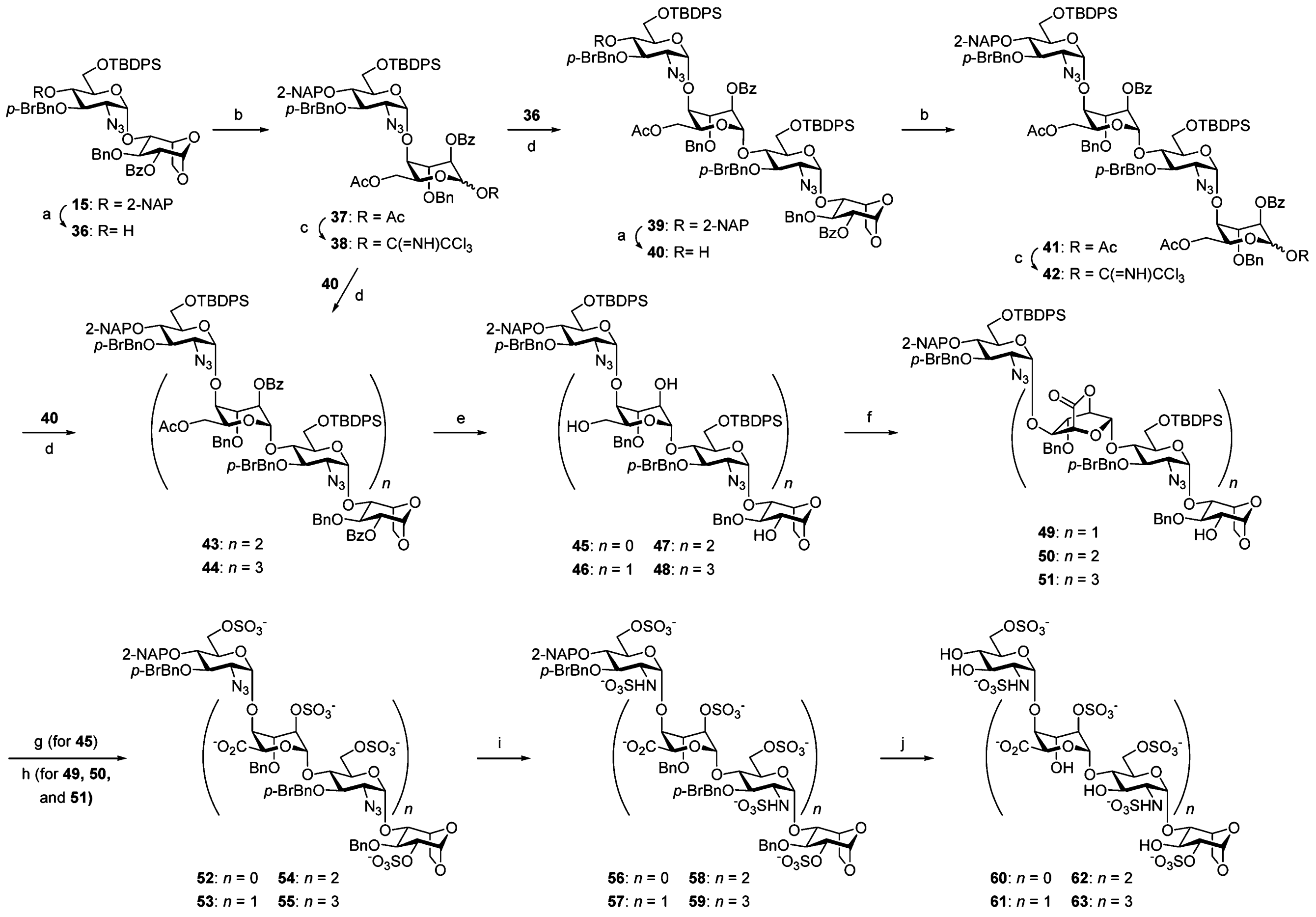
Synthesis of Heparosan Oligosaccharides. The enzymatic degradation of the *E. coli* strain K5 capsular polysaccharide supplies heparosan oligomers of various lengths.[10] However, the isolation of sufficiently pure compound of defined size from this mixture is very difficult. With compound **26** in hand, we could generate synthetic heparosan oligosaccharides with precise structure and molecular weight. Described in Scheme 2 is the preparation of heparosan tri- and pentasaccharides, which may both act as substrates in chemoenzymatic transformations toward heparin/heparan sulfate structures. Condensation of the thioglycoside **26** and the acceptor **27**[35] mediated by NIS and TMSOTf supplied the trisaccharide **28** in 86% yield. Here, the Bz group situated at the 2-O position of the donor assisted in generating the exclusive β-linkage ($J_{1',2'}$ = 8.6 Hz). Cleavage of 2-NAP in **28** by DDQ supplied the 4″-alcohol (78%), which upon further glycosylation by **26** may form the pentasaccharide skeleton **29**. Nonetheless, the glycosylation yield employing NIS and TMSOTf was only 12%, possibly because of the trisaccharide 4″-O-TMS formation, which may hinder effective coupling.

Scheme 2. Synthesis of the Heparosan Tri- and Pentasaccharide[a]



[a]Reagents and conditions: (a) NIS, TMSOTf, 4 Å MS, CH_2Cl_2, −78 °C to rt, 3 h; 86%. (b) (1) DDQ, CH_2Cl_2/H_2O = 18/1, 2 h; 78%; (2) **26**, NIS, TfOH, 4 Å MS, CH_2Cl_2, −78 °C to rt, 2 h; 64%. (c) (1) AcSH, Pyr, $CHCl_3$, 36 h; (2) NaOMe, MeOH, 16 h; **30**: 74%, **31**: 72%. (d) TEMPO, BAIB, CH_2Cl_2/H_2O = 2/1, 2 h; **32**: 90%, **33**: 77%. (e) (1) TBAF, AcOH, THF, 40 °C, 3 d; (2) H_2, $Pd(OH)_2/C$, phosphate buffer (pH 7), 2 d; **34**: 91%, **35**: 95%. Pyr: pyridine; TBAF: tetra-*n*-butylammonium fluoride.

Scheme 3. Synthesis of Heparin Analogues[a]



[a]Reagents and conditions: (a) DDQ, CH_2Cl_2/H_2O = 18/1, rt, 4 h; **36**: 92%, **40**: 93%. (b) Ac_2O, cat. $Cu(OTf)_2$, 0 °C; **37**: 99%, **41**: 98%. (c) (1) satd NH_3, MeOH/THF = 1/4, 0 °C, 6 h; (2) CCl_3CN, K_2CO_3; **38**: 90%, **42**: 85%. (d) cat. TMSOTf, 4 Å MS, CH_2Cl_2, −40 to 0 °C, 2 h; **39**: 98%, **43**: 82%, **44**: 84%. (e) NaOMe, CH_2Cl_2/MeOH = 1/1; **45**: 98%, **46**: 98%, **47**: 91%, **48**: 92%. (f) TEMPO, BAIB, CH_2Cl_2, 24 h; **49**: 93%, **50**: 78%, **51**: 86%. (g) (1) TBAF, AcOH, THF, rt, 5 h; 95%; (2) $SO_3 \cdot Et_3N$, DMF, 60 °C, 3 d; 92%. (h) (1) TBAF, THF, 50 °C, 24 h, then, LiOH, rt, 3 h; (2) $SO_3 \cdot Et_3N$, DMF, 60 °C, 3 d; **53**: 75%, **54**: 73%; **55**: 62%. (i) (1) PMe_3, NaOH, THF/H_2O = 9/1, rt, 5 h; (2) $SO_3 \cdot Pyr$, Et_3N, Pyr/DMF = 3/1, rt, 24 h; **56**: 86%, **57**: 75%, **58**: 79%; **59**: 69%. (j) H_2, $Pd(OH)_2/C$, phosphate buffer (pH 7), rt, 2 d; **60**: 94%, **61**: 90%, **62**: 94%, **63**: 87%.

Conversely, when TfOH was used in place of TMSOTf, the target compound **29** was isolated in 64% yield.

After constructing the oligosaccharide skeletons, functional group transformations were next carried out to achieve the desired materials. First, the azido groups of the trisaccharide **28** and pentasaccharide **29** underwent conversion to the acetamide using thioacetic acid. Subsequent treatment with NaOMe in MeOH resulted to deacylation delivering compounds **30** (74% from **28**) and **31** (72% from **29**). The primary hydroxyl groups in **30** and **31** were next oxidized to the carboxyls by employing a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxyl free radical (TEMPO) with excess [bis(acetoxy)iodo]benzene (BAIB) as co-oxidant to afford the acids **32** and **33** in 90% and 77% yields, respectively. Desilylation followed by global hydrogenolysis provided the target compounds **34** (91%) and **35** (95%), the structures of which were verified using NMR and mass spectroscopy (see the Supporting Information).

Synthesis of Heparin Analogues. Heparin, being more readily accessible, is often used as a model in probing the character of heparan sulfate-mediated interactions the cell surface. Indeed, over 100 heparin-binding proteins have been identified.[36] The major repeating component of heparin, among 48 disaccharide possibilities, is the N- and 6-O-sulfonated α-D-glucosamine 1→4-linked to 2-O-sulfonated α-L-iduronic acid. Acquisition of specific structurally defined heparin oligosaccharides from natural sources is a technical hurdle. Consequently, carbohydrate chemists developed several methodologies for their chemical and chemoenzymatic preparations.[24,37]

Scheme 3 illustrates our synthesis of the di-, tetra-, hexa-, and octasaccharide analogues of heparin starting from the disaccharide **15**. The 2-NAP group and the easily transformed anhydroidose moiety allow conversion into either a glycosyl acceptor or donor. Accordingly, DDQ treatment of the fully protected skeletons **15** and **39** gave the acceptors **36** (92%) and **40** (93%), respectively. On the other hand, copper(II) triflate [$Cu(OTf)_2$]-mediated acetolysis[38] of the same starting materials provided the diacetate **37** (99%) and the triacetate **41** (98%), which were both converted into the respective imidates **38** and **42** by successive exposures to a saturated NH_3 solution in MeOH/THF and CCl_3CN in the presence of K_2CO_3. With the use of the acceptors **36** and **40** and the donors **38** and **42**, the assembly of the heparin backbones **39** (98%), **43** (82%), and **44** (84%) under TMSOTf activation was achieved in a convergent and highly stereoselective manner. The W-coupling between the anomeric proton and 3-H, the small-to-zero coupling constant for 1-H, and the lack of NOE correlation between 1-H and 5-H of the involved L-idose residue are evidence that the newly formed glycosidic bond is α-configured and primarily exists in $^{1}C_{4}$ conformation. Here, we chose to keep the bicyclic skeleton of the reducing end sugar to eliminate the installation of a noncarbohydrate aglycone that often results in a mixture of isomers, causing purification problems. A possible advantage of a rigid ring system at the reducing end is the relative ease of co-crystallization with a binding protein due to the reduced motion of the terminal group.

Compounds **15**, **39**, **43**, and **44** were next subjected to multifunctional group transformations leading to the target heparin analogues. Deacylation was conveniently carried out under Zemplén conditions to give the alcohols **45**−**48** in excellent yields. TEMPO oxidation of **46**−**48** resulted to the lactones **49** (93%), **50** (78%), and **51** (86%). The presence of the cyclic ester was identified through the correlation between the 2-H and 6-C of the internal L-iduronyl residue in the HMBC spectra (Figure 2) and corroborated by the relatively high IR C═O stretching band observed around 1790 cm^{-1}. Desilylation of **45** was accomplished using TBAF/AcOH reagent system to furnish the 2,6′-diol (95%), which was then treated with $SO_3{\cdot}Et_3N$ to afford the disulfate **52** in 92% yield. For **49**−**51**, the basic TBAF affected not only the removal of TBDPS groups, but also the lactone ring opening as observed using TLC. LiOH was subsequently added to the solution to ascertain the complete cleavage of the lactone. O-Sulfonation of the partially purified materials gave the polysulfated compounds **53** (75% from **49**), **54** (73% from **50**), and **55** (62% from **51**). Here, O-sulfonations were verified by the downfield shifts (>0.5 ppm) in the resonances of the respective geminal protons. The smooth azido-to-amino conversion was successfully implemented using PMe_3 in THF/H_2O with added NaOH to avoid the interference of the free carboxylic acid during the course of the reaction.[39] Treatment with $SO_3{\cdot}Pyr$ resulted in the amine sulfonation delivering the products **56** (86% from **52**), **57** (75% from **53**), **58** (79% from **54**), and **59** (69% from **55**). While O-sulfonation could be detected from ^{1}H NMR downfield shifts, no such trend occurs during N-sulfonation. Thankfully, we found that this functionalization can be identified using the highly indicative downfield shifts (4−5 ppm) in the ^{13}C resonance of the nitrogen-bonded carbon (Figure 3). We also figured, upon comparison with the spectra of **32** and **33**, that N-acetylated and N-sulfonated glucosamine residues can be differentiated by inspection of their ^{13}C chemical shifts. Finally, the compounds were subjected to hydrogenolysis to remove all arylmethyl groups and supply the heparin analogues **60**, **61**, **62**, and **63** in 94%, 90%, 94%, and 87% yields, respectively. The structures of these final compounds were confirmed by NMR and mass spectroscopic analysis (see the Supporting Information).

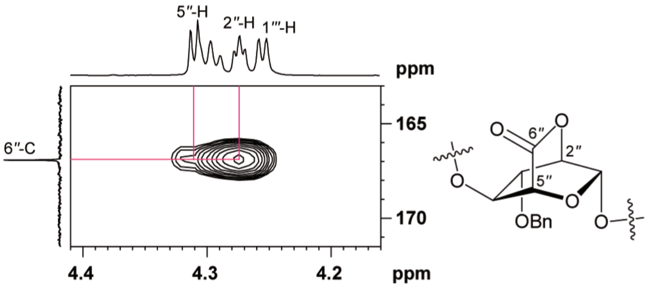


Figure 2. The HMBC spectrum of compound **49** identifying the lactone structure.

Interaction of the Heparin Analogues and HBHA$_{60-199}$. The invasion of alveolar macrophages by *M. tuberculosis* has an outstanding role in the pathogenesis of tuberculosis, a devastating disease that leaves millions dead each year.[40] Besides this interaction, the bacteria also infect respiratory epithelial cells initiated upon adherence to heparan sulfate proteoglycans. The responsible bacterial adhesin is HBHA, a 199-amino acid protein, the absence of which severely impairs the extrapulmonary dissemination of the pathogen.[41] HBHA has a coiled coil N-terminal domain associated with the agglutination property of the protein and a lysine-rich C-terminal region predisposed for binding with the polyanionic sugars of epithelial cells.[42]

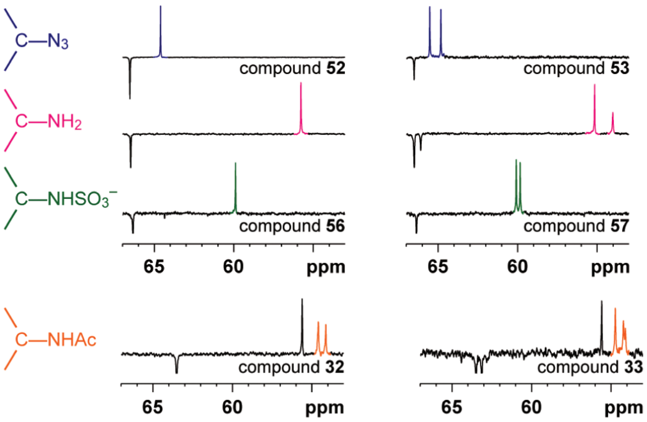


Figure 3. Comparisons of carbons attached to azido, amino, sulfonatamido, and acetamido groups in DEPT-135 spectra (solvent: CD_3OD).

We examined the interaction of the heparin analogues **60**−**63** and a truncated form of HBHA (amino acid 60−199) using isothermal titration calorimetry (ITC) (Table 3). A commercially acquired 3 kDa heparin was also tested for comparison. Aggregation problems were incurred during the purification of the full-length HBHA expressed in *E. coli*; hence, we settled with its slightly shortened form. Moreover, the hexahistidine tag was cleaved from our recombinant protein prior to ITC measurement because it caused measurable decrease in binding affinity. Here, the protein solution in Tris buffer (pH 7.6) was titrated with the sugar dissolved in the same solvent at 25 °C. To account for the contribution of heat of dilution, the buffer without the protein was also titrated with the sugar solution. No $HBHA_{60-199}$ binding was detected for the disaccharide **60** and tetrasaccharide **61**. On the other hand, the associations of $HBHA_{60-199}$ with the hexasaccharide **62**, the octasaccharide **63**, and the commercial heparin were all found to be endothermic with the sugar−protein binding stabilized by entropic factors. A representative titration isotherm is shown in Figure 4. The release of the ordered water molecules from the interface between $HBHA_{60-199}$ and the sugar molecules are quite possibly the major driving force for the adhesion process. Compound **63** has a slightly better binding than the 3 kDa heparin with both sugars having nearly 1:1 binding stoichiometry with $HBHA_{60-199}$. The binding affinity is lower for hexasaccharide **62**. These data suggests a length of at least 6 sugar units for effective HBHA binding to occur.

Table 3. Thermodynamic Parameters of Sugar-Binding with $HBHA_{60-199}$ Obtained Using ITC[a]

sugar	n[b]	K_D[c] (μM)	ΔH (cal·mol^{-1})	ΔS (cal·mol^{-1}·K^{-1})	ΔG (cal·mol^{-1})
63[d]	1.0	19	3500	33.3	−6430
63	1.0 (±0.1)	4.2 (±0.4)	4370 (±150)	39.3 (±0.3)	−7330
62	1.1 (±0.2)	19 (±7)	2400 (±90)	29.7 (±1.0)	−6450
61	–[e]	–	–	–	–
60	–[e]	–	–	–	–
3 kDa heparin	1.2 (±0.1)	5.3 (±1.1)	4220 (±1140)	38.3 (±4.2)	−7200

[a] The values reported are averages of results from three experiments with standard deviations indicated in parentheses. [b] Sugar-to-protein binding ratio. [c] The actual parameter measured is association constant, the inverse of dissociation constant (K_D). [d] The protein utilized has an N-terminal hexahistidine tag; the values reported came from a single experiment. [e] No binding was detected.

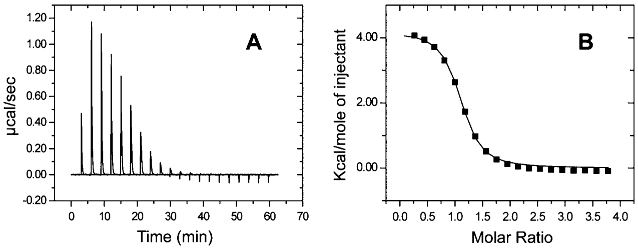


Figure 4. Compound **63**−$HBHA_{60-199}$ ITC titration profile showing an endothermic interaction. Panel A shows the raw titration data and panel B shows the integrated heats of binding generated from the raw data after subtracting the heats of dilution.

■ CONCLUSIONS

We have successfully developed a powerful donor for the formation of 2-deoxy-2-amino-α-D-glucosides in high stereoselectivity and good yields. The α-isomer was exclusively generated regardless of promoter and leaving group. Moreover, excellent α-stereoselectivities were afforded upon coupling with selected glycosyl acceptors to form the core structures of important sugars including GPI anchors, heparosan, heparan sulfate, and heparin. The orthogonal protecting groups built within our donor were effectively transformed and manipulated to furnish heparosan oligosaccharides and heparin analogues. Examination of the interaction of the prepared heparin analogues and $HBHA_{60-199}$ revealed an entropically driven binding for the octasaccharide and hexasaccharide. Our disaccharide and tetrasaccharide heparin analogues did not bind to HBHA. From these examples, we have shown the flexibility of our donor in oligosaccharide synthesis. Further pursuit of other relevant bioactive compounds is currently in progress.

■ EXPERIMENTAL SECTION

General Procedure for the Synthesis of Compounds 9−15. A solution of the trichloroacetimidate donor (**1**−**7**, 1.2 equiv) and the 4-alcohol **8** (1.0 equiv) in CH_2Cl_2 (10 mL per total gram of donor, acceptor, and molecular sieves) with freshly dried 4 Å molecular sieves (1.5 g per total gram of the donor and the acceptor) was stirred at room temperature for 1 h under N_2 atmosphere. The mixture was cooled to −78 °C, TMSOTf (0.2 equiv) was added to the solution, and the mixture was gradually warmed up to 0 °C. After stirring for 1.5−2.5 h, Et_3N (0.3 equiv) was added to quench the reaction and the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure to furnish a residue, which was purified by flash column chromatography to give the expected disaccharide. The yields of compounds **9−15** are listed in Table 1.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures and ^{1}H and ^{13}C NMR spectra for relevant compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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