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Design and Synthesis of Unnatural Heparosan and Chondroitin Building Blocks

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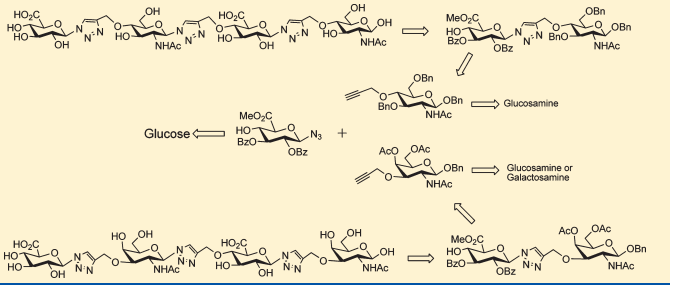
Supporting Information

ABSTRACT: Triazole linked heparosan and chondroitin disaccharide and tetrasaccharide building blocks were synthesized in a stereoselective manner by applying a very efficient copper catalyzed azide−alkyne cycloaddition (*CuAAC*) reaction of appropriately substituted azido-glucuronic acid and propargyluted *N*-acetyl glucosamine and *N*-acetyl galactosamine derivative, respectively. The resulting suitably substituted tetrasaccharide analogues can be easily converted into azide and alkyne unit for further synthesis of higher oligosaccharide analogues.



INTRODUCTION

The glycan chains of proteoglycans,[1] glycosaminoglycans (GAGs), correspond to the most abundant class of heteropolysaccharides in the body. These molecules control a wide range of physiological and pathological events[2−7] including cell−cell communication,[6] growth factor receptor and enzyme inhibition,[7] cell proliferation, angiogenesis and inflammatory processes, wound repair and healing,[8] and viral invasion.[6,9,10] GAGs are linear, highly sulfated anionic polysaccharides having repeating disaccharide motifs and are divided into families based on variations in stereochemistry and patterns of sulfation. It is through discrete structural features that they interact with GAG-binding proteins[11] to exhibit their biological effects. GAG structural diversity creates an enormous number of protein binding motifs and poses a daunting challenge to define the structural and functional properties of GAGs. Based on the difference the repeating disaccharide units comprising GAGs, they are classified into four main families: heparin/heparan sulfate, chondroitin sulfate/dermatan sulfate, hyaluronan, and keratan sulfate (Figure 1).[1] Sulfated GAGs are naturally found in all animals, and GAGs without sulfo group substitution, including heparosan, chondrotin, and hyaluronan, are also found in many bacteria.[12] GAGs are implicated in many diseases and are used therapeutically as drugs.[6−10]

Although GAGs are generally isolated by extraction from animal tissues, synthetic carbohydrate chemistry can offer a potentially more reliable route to homogeneous naturally occurring GAGs or for incorporating unnatural structures, such as enzymatically stable glycosidic linkages, into GAGs. One benefit of the latter approach is that an unnatural or "artificial" GAG may retain the same geometric and spatial characteristics of the native GAG while exhibiting stability toward hydrolase activity and might even potentially inhibit these enzymes, thus suggesting novel therapeutic applications. In addition, an unnatural glycosidic linkage might be finely tuned to be inert to subsequent synthetic transformations.

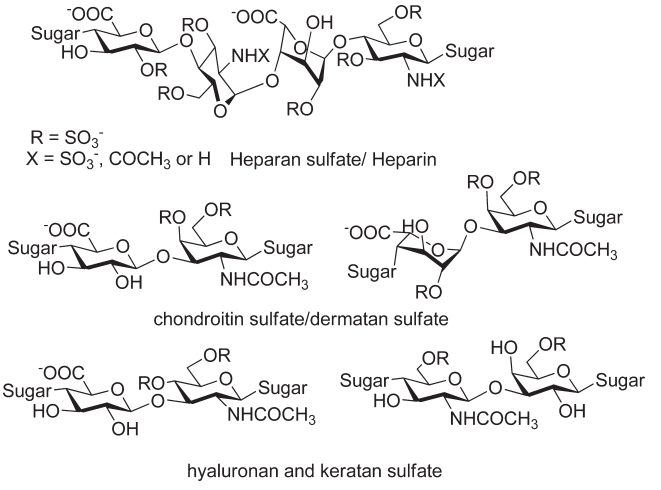


Figure 1. Structures of different glycosaminoglycans. Heparan sulfate contains primarily glucuronic acid, whereas heparin contains primarily iduronic acid and *N*-sulfoglucosamine. Heparosan has the same basic structure as heparan sulfate with R = H, X = $COCH_3$,and chondroitin has the same basic structure as chondroitin sulfate with R = H. For hyaluronan R = H.

The goal of this study was to develop novel synthetic approaches, with precise control over length, stereochemistry, linkage, and sulfation patterns, that lead to unnatural GAG chains as discovery tools to understand the biological roles of GAGs and explore their utility in treating diseases. Our initial targets are structural analogues of the disaccharide and tetrasaccharide

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backbones of heparosan and chondroitin. The synthetic approach should be useful in preparing larger and sulfated oligosaccharides for use in protein interaction studies.

In the current synthesis, the natural glycosidic linkage of these building blocks was replaced with a 1,2,3-triazole linkage. This synthesis relies on click chemistry,[13] a Cu(I)-catalyzed 1, 3-dipolar cycloaddition reaction (1,3-DCR) that affords superior regioselectivity, high tolerance of other functionalities, and almost quantitative transformation under mild conditions. The stereospecific (α or β) construction of glycosidic bonds is a critical challenge in carbohydrate chemistry and is often low-yielding, generating the undesired side products. The click reaction has been previously used in the synthesis of neoglycoconjugates[14] and the bioconjugation study of glycosides.[15] The 1,3-DCR utilizes relatively low-cost, nontoxic reagents and solvents alleviating the need for expensive and highly toxic glycosylation and coupling reagents. Finally, product purification is simple using precipitation or liquid−liquid extraction and typically not requiring chromatography to obtain pure products.

Herein, we report the synthesis of triazole linked tetrasacchaide analogue of heparosan and chondroitin. Heparosan is a repeating disaccharide unit of D-glucuronic (GlcA) acid and *N*-acetyl-D-glucosamine (GlcNAc) residues with a (1→4) linkage, [→4) β-GlcUA (1→4) α-GlcNAc 1→]$_n$ (Figure 1). It is a nonsulfated member of the heparin/heparan sulfate GAG family and is found in the capsule of certain pathogenic bacteria as well as the precursor in the biosynthesis of animal heparin and heparan sulfate. Heparin is a particularly important anticoagulant drug that has also been recently exploited in treating cancers and suppressing the inflammatory response.[1,2]

Chondroitin is composed of an alternating sequence of GlcA and *N*-acetyl-D-galactosamine (GalNAc) residues linked through alternating (β(1→3)) and (α (1→4)) linkages, [→4) β-GlcUA (1→3) α-GalNAc 1→]$_n$ (Figure 1). Chondroitin is a nonsulfated member of the chondroitin sulfate/dermatan sulfate family of GAGs and is found in the capsule of certain pathogenic bacteria as well as the precursor in the biosynthesis of animal chondroitin sulfate and dermatan sulfate. The chondroitin sulfate/dermatan sulfate family plays an important role in biological processes, such as neural development, viral invasion, cancer metastasis, arthritis, and spinal cord injury.[7−10]

Our synthetic strategy for preparing the triazole-linked oligosaccharide mimetics of heparosan and chondroitin (Figure 2) begins with the synthesis of suitably protected glucosamine and glucuronic acid building blocks having appropriately placed azide and alkyne functional groups.[10] This building block will also be required for further chain extension to prepare future polysaccharide targets.

Two monosaccharide units that can be easily be transformed to azido and the propargyluted saccharide building blocks are required for synthesis of the triazolyl linkages in the GAG oligosaccharide targets. Solution phase synthesis was selected and protecting groups and reaction conditions were carefully chosen to avoid side reactions and maximize the overall yield. The building blocks used for the synthesis of the heparosan and chondroitin tetrasaccharide analogues are shown in the retrosynthetic schemes (Figures 3 and 4). Two iterative 1,3-dipolar cycloaddition reactions will be used to form the required β-(1,4) or β-(1,3) linkages.

The chemical synthesis of GAGs is typically carried out using one of two general approaches. In the first, GlcA building blocks are directly utilized to react with a GlcN or GalN derivative.[16] The newly formed disaccharide analogue was transformed into either an acceptor by selective deprotection or a glycosyl donor through aglycone adjustment. Selective deprotection, aglycone adjustment, and additional glycosylation steps lead to synthesis of a GAG oligosaccharide. In the second, at first disaccharide analogue would be synthesized from two monosaccharide unit with an orthogonally protecting group, and then this disaccharide analogue would be used as an donor as well as acceptor for the next tetrasaccharide analogue and so on.

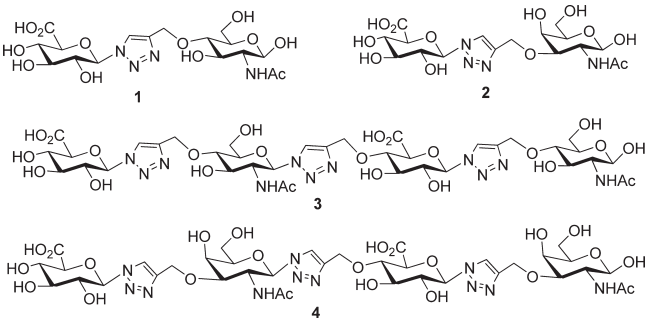


Figure 2. Triazole linked heparosan and chondroitin disaccharide and tetrasaccharide analogues.

Herein, we report the synthesis of heparosan and chondroitin disaccharide and tetrasaccharide analogues where GlcA is linked with GlcNAc/GalNAc through a triazole linkage instead of glycoside linkage. In this synthesis GlcA, having an azide functional group, will act as an acceptor and GlcNAc/GalNAc, having an alkyne functional group, will serve as a donor. This disaccharide unit will then be transformed into either an acceptor, having an azide functional group, or donor, having an alkyne functional group, through selective protection and deprotection.

Retrosynthetic analysis suggests that heparosan tetrasaccharide analogue **3** will be obtained from a disaccharide building block **1a** through the modification at the anomeric position of the GlcNAc residue to a glycosyl azide and the propargylation of the GlcA residue at the C4. Heparosan disaccharide analogue **1a** will be obtained from monosaccharide building blocks through the modification at the anomeric position of the GlcA residue to a glycosyl azide **5** and the propargylation of the GlcNAc residue **6** at the C4 (Figure 3).

Retrosynthetic analysis suggests that chondroitin tetrasaccharide analogue **4** will be obtained from a disaccharide building block **2a** through the modification at the anomeric position of the GlcNAc residue to a glycosyl azide and the propargylation of the GlcA residue at the C4. Chondroitin disaccharide analogue **2a** will be obtained from monosaccharide building blocks through the modification at the anomeric position of the GlcA residue to a glycosyl azide **5** and the propargylation of the GalNAc residue **7** at the C3 (Figure 4).

RESULTS AND DISCUSSION

Building Block Synthesis. Retrosynthetic analysis (Figures 3 and 4) shows that GlcA glycosyl azide **5** is common to both heparosan and chondroitin synthesis. To reach our goal, syntheses begin with properly protected GlcA glycosyl azide **5**. The C-1 and C-4 hydroxyl groups require orthogonal protection to allow the introduction of C-1 azido and a C-4 propargyl groups. The synthesis starts from commercially available phenyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside thioglycoside **8**. Thioglycoside **8** was protected using benzoyl chloride in pyridine

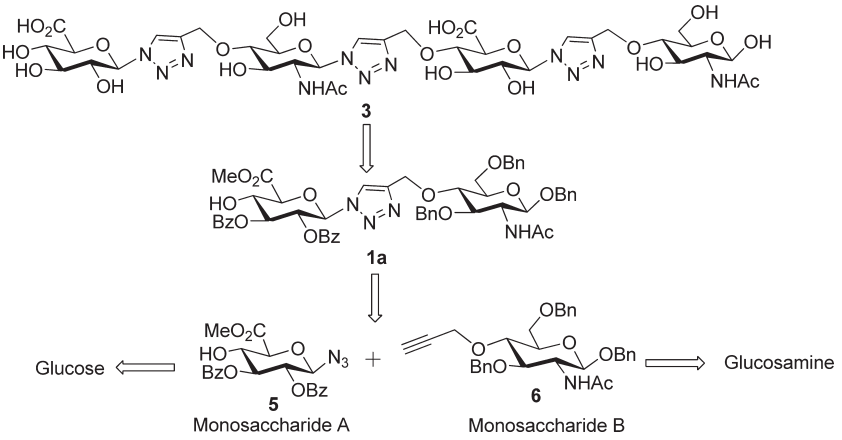


Figure 3. Retrosynthesis of heparosan disaccharide and tetrasaccharide analogues.

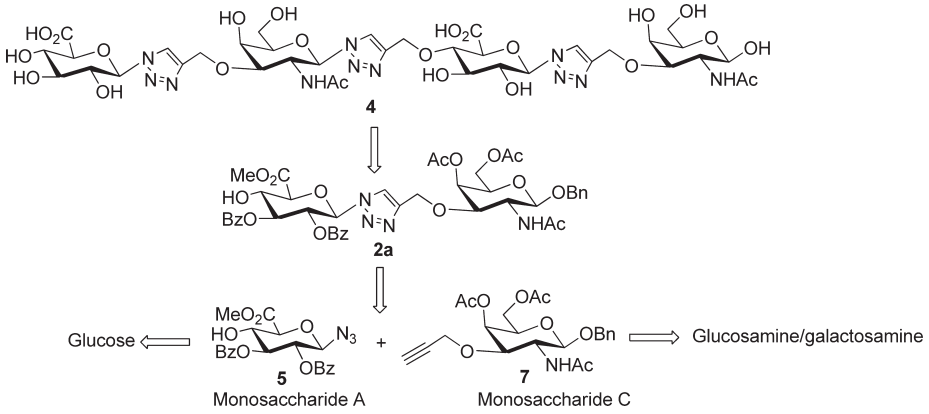
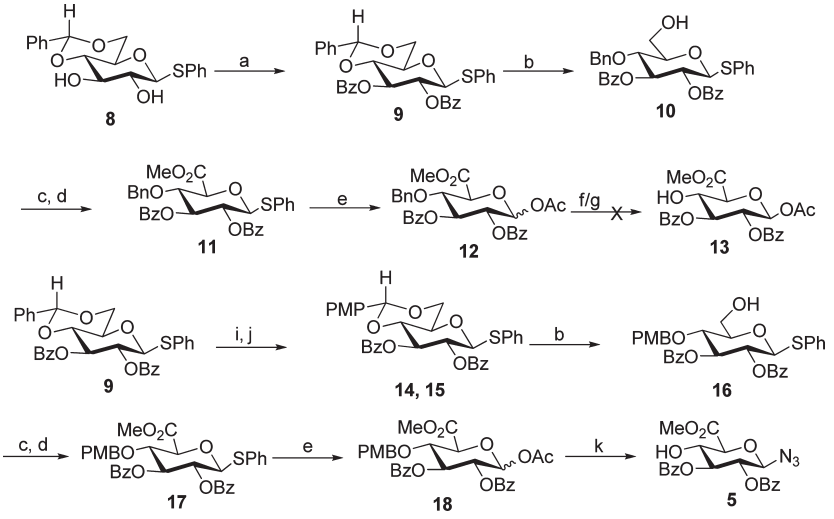


Figure 4. Retrosynthesis of chondroitin disaccharide and tetrasaccharide analogues.

to obtain the 2,3-di benzoate ester **9**. The neighboring group participation of benzoyl at C-2 should allow β-directed azidation of the thiophenyl moiety. The 4,6-*O*-benzylidene acetal of compound **9** was selectively reduced by treatment with $PhBCl_2$ and triethylsilane[16f] at −78 °C for 1 h to afford the corresponding 4-*O*-benzyl derivative (**10**). The remaining primary hydroxyl group at C-6 was oxidized using a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and [bis(acetoxy)iodo]-benzene (BAIB),[17] followed by esterification with Cs_2CO_3 and MeI affording the completely protected thioglycoside **11**. Direct conversion of **11** to azido glycoside[18] with NIS, $TMSN_3$ and TMSOTf failed to give the desired product. Conversion of the thioglycoside to acetate **12** (α:β/3:7) was accomplished in quantitative yield with NIS in the presence of acetic acid.[19] The β-selectivity is due to the directing effect of benzoate group at C2 position. Prior to introducing an azido group at C-1, the reductive removal of C4 benzyl ether has been attempted under hydrogenation condition using H_2/$Pd(OH)_2$ and H_2/Pd−C in MeOH and catalytic amount of HCl, but the reaction failed to proceed. We ascribe the failure of this reaction to steric hindrance at the C-4 position. At this point we decided to revise Scheme 1 modifying a benzylidine 2,3-dibenzoate derivative (**9**). Acid hydrolysis of **9** using *p*-TSA in MeOH and CH_2Cl_2 mixture for 8 h afforded diol **14**, which was reprotected with *p*-methoxybenzylidene dimethyl acetal at 50 °C in the presence of catalytic 10-camphorsulfonic acid as *p*-methoxybenzylidene thioglycoside **15**.[20] The *p*-methoxy-benzylidene acetal **15** was selectively reduced to the corresponding 4-*O*-*p*-methoxybenzyl derivative (**16**) by treatment with $PhBCl_2$ and triethylsilane[16] at −78 °C for 30 min under anhydrous condition, keeping the C-6 hydroxyl group free. Oxidation of the C-6 hydroxyl of **16** followed by esterification with Cs_2CO_3 and MeI to afford **17** in 75% yield. The thioglycoside was converted to acetate **18** (α:β/2:8) in 61% yield with NIS in the presence of acetic acid at 20 °C. The anomeric acetate **18** was converted to the β-azide derivative **5** in good yield, by treatment with $TMSN_3$ and $SnCl_4$. During this reaction the *in situ* removal of *p*-methoxybenzyl group at the C-4 position was observed. After chromatographic separation, the compound was fully characterized with spectroscopic analysis and HRMS. Disappearance of the acetate peak at 2.04 ppm in the NMR spectrum confirmed the complete conversion to the C-1 azide, and the coupling constant at the anomeric proton at 4.95 ppm as a doublet (J = 8.7 Hz) confirmed the stereospecific formation of the β-conformation. The *in situ* removal of *p*-methoxybenzyl group eliminates the need to modify the disaccharide building block in assembling the tetrasaccharide analogue.

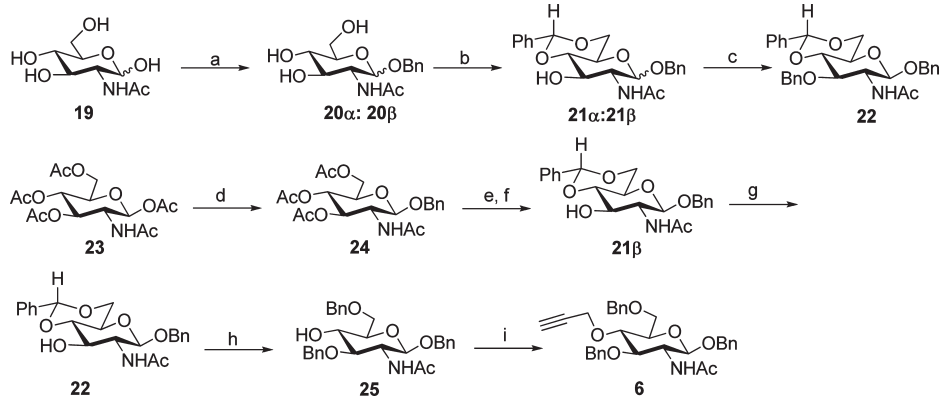
Next, we undertook the synthesis of the GlcNAc β-benzyl glycoside derivative. Although several methods are reported for the synthesis of selective β-*O*-benzyl glucosamine derivatives,[21] we selected an approach that treated GlcNAc (**19**) with sodium hydride and benzyl bromide in DMF at room temperature for 3 h affording benzyl glycoside **20** as a mixture of α:β anomers (1:4).[22] To facilitate separation, the C-4 and C-6 hydroxyl group

Scheme 1[a]



[a] Reagents and conditions: (a) benzoyl chloride, pyridine, rt; (b) $PhBCl_2$, mol sieves powder 4 Å, Et_3SiH, CH_2Cl_2, −78 °C−rt; (c) BAIB, CH_2Cl_2, H_2O, TEMPO, rt; (d) MeI, Cs_2CO_3, DMF; (e) NIS, AcOH, CH_2Cl_2, 20 °C; (f) H_2, 20% $Pd(OH)_2/C$, MeOH, 1 N HCl or (g) H_2, 10% Pd/C, MeOH, 1 N HCl; (i) *p*-TSA, MeOH, CH_2Cl_2; (j) *p*-OMe-$PhCH(OMe)_2$, 10-camphorsulfonic acid, 50 °C; (k) $SnCl_4$, $TMSN_3$, CH_2Cl_2, 0 °C.

Scheme 2[a]



[a] Reagents and conditions: (a) NaH, BnBr, DMF, 0 °C−rt; (b) $PhCH(OMe)_2$, *p*-TSA, DMF, rt; (c) $Ba(OH)_2$, BaO, BnBr, DMF, 0 °C−rt; (d) $Sc(OTf)_3$, BnOH, DCE, 90 °C; (e) 0.5 M NaOMe, MeOH, 0 °C; (f) $PhCH(OMe)_2$, *p*-TSA, DMF, rt; (g) $Ba(OH)_2$, BaO, BnBr, DMF, 0 °C−rt; (h) Et_3SiH, CH_2Cl_2, $BF_3 \cdot Et_2O$, 0 °C; (i) $Ba(OH)_2$, BaO, propargyl bromide, DMF, 0 °C.

was protected as the benzylidene acetal with benzaldehyde dimethyl acetal and *p*-TSA in DMF giving a mixture **21**(α:β = 1:4) in quantitative yield. The chromatographic separation in small scale gave the compound **21*β*** in 25% yield, which was treated with benzyl bromide, $Ba(OH)_2$, and BaO mixture in DMF to protect the C3 hydroxyl group as the benzyl ether[23] affording compound **22** in 80% yield. We next explored the use of rare earth metal triflates in the formation of the β-benzyl glycoside to enhance the yield of the β-selective compound. Despite their expense, metal triflates have proven to be a highly valuable tool in a wide range of Lewis acid mediated reactions. The advantage of using these metals as catalysts is their high level of acidity and their stability toward moisture. In our revised Scheme 2, we began the synthesis with the commercially available β-D-glucosamine pentaacetate (β-derivative) **23**. Refluxing **23** with benzyl alcohol and scandium triflate in dichloroethane at 90 °C provided the β-benzyl glycoside of D-glucosamine tetraacetate **24** in good yield and with good selectivity.[24] Zemplen deacetylation reaction with sodium methoxide in MeOH afforded triol[24] **20*β***, which was treated with benzaldehyde dimethyl acetal and *p*-toluenesulfonic acid in DMF at room temperature afforded the corresponding 4,6-*O*-benzylidene protected benzyl-*N*-acetyl D-glucosamine derivative **21*β***. The C3-hydroxyl group was protected as benzyl ether to obtain **22** in good yield. Selective opening of the 4,6-*O*-benzylidene ring with triethylsilane in the presence of catalytic $BF_3 \cdot Et_2O$ furnished the selective 6-*O*-benzyl protected compound **25** in quantitative yield[25] and exposing the 4-OH. Propargylation of the C4 hydroxyl group with propargyl bromide in the presence of $Ba(OH)_2$ and BaO mixture afforded monosaccharide building block **6** in good yield.

The synthesis of the 3-*O*-propargyluted GalNAc β-benzyl glycoside building block is presented in Scheme 3. The 4,6-*O*-benzylidene protected glucosamine intermediate **21*β***, prepared in Scheme 2, was used to begin this synthesis. The C3 hydroxyl group was first protected as propargyl ether using propargyl bromide in the presence of $Ba(OH)_2$ and BaO affording the

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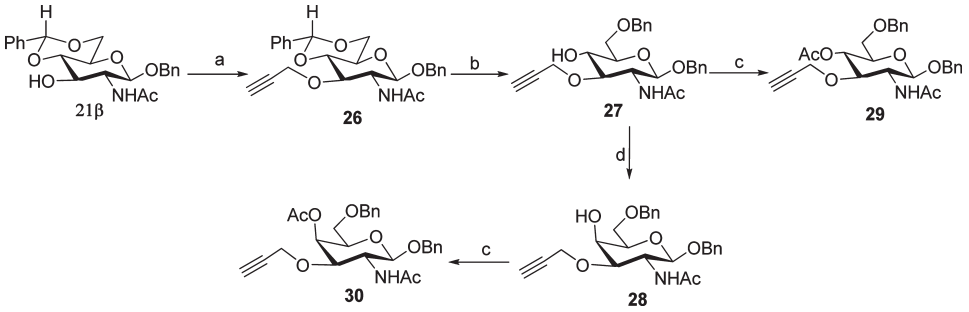
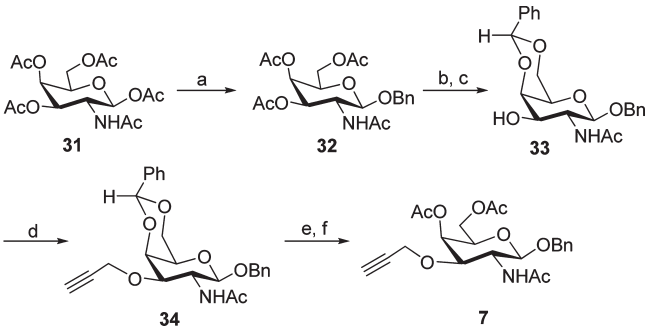
Scheme 3[a]



[a] Reagent and conditions: (a) $Ba(OH)_2$, BaO, Propargyl bromide, DMF, rt; (b) Et_3SiH, CH_2Cl_2, $BF_3 \cdot Et_2O$, 0 °C; (c) Ac_2O, pyridine, rt; (d) (i) Tf_2O, pyridine, CH_2Cl_2, −18 °C; (ii) $NaNO_2$, DMF, rt.

Scheme 4[a]



[a] Reagent and conditions: (a) $Sc(OTf)_3$, BnOH, DCE, 90 °C; (b) 0.5 M NaOMe, MeOH, 0 °C; (c) $PhCH(OMe)_2$, *p*-TSA, DMF, rt; (d) $Ba(OH)_2$, BaO, propargyl bromide, DMF, 0 °C−rt; (e) *p*-TSA, MeOH, rt; (f) Ac_2O, pyridine, rt.

propargylated derivative **26**. Selective reductive ring opening using triethylsilane in the presence of acid catalyst, afforded the 6-*O*-benzyl protected glucosamine derivative **27**.[25] Epimerization of the C-4 hydroxyl group was accomplished through its conversion to triflate by treatment with trifluoromethane sulfonic anhydride followed by overnight stirring at room temperature with sodium nitrite ($NaNO_2$) affording the galactosamine derivative **28** in 40% yield.[26] For better identification, both of the C-4 hydroxyl group of compounds **27** and **28** were acetylated with acetic anhydride in pyridine to afford compounds **29** ($^3J_{H4,H3}$ = 9.4 Hz) and **30** ($^3J_{H4,H3}$ = 2.7 Hz).

Scheme 3 was revised to synthesize galactoside derivative more efficiently. Commercially available galactosamine pentaacetate **31** was converted into the β-benzyl galactoside **32** in good yield and high selectivity with scandium triflate and benzyl alcohol in dichloroethane at 90 °C (Scheme 4).[24] Zemplen deacetylation reaction with sodium methoxide followed by treatment with benzaldehyde dimethyl acetal and *p*-toluenesulfonic acid in DMF at room temperature afforded the corresponding 4,6-*O*-benzylidene protected benzyl-*N*-acetyl galactosamine derivative **33** in quantitative yield. The free C3-hydroxyl group was propargyluted with propargyl bromide in the presence of BaO and $Ba(OH)_2$ to obtain **34** in 85% yield. Cleavage of 4,6-*O*-benzylidene ring with *p*-TSA in MeOH followed by acetylation with Ac_2O in pyridine provided the modified compound **7** in quantitative yield.

Synthesis of Heparosan Disaccharide Analogue. A 1,3-dipolar cycloaddition reaction was carried out to couple monosaccharide **5** and **6** in the presence of CuI and Hünig's base in acetonitrile at room temperature for 2 h. The reaction was monitored by TLC, and the reaction was quenched with satd NH_4Cl.[27] After standard workup, the desired disaccharide analogue **1a** was obtained in quantitative yield (Scheme 5). The structure of **1a** was confirmed by ^1H NMR, which showed a peak at 7.80 ppm of triazole proton of the linker and 3.85 ppm of methyl ester of glucuronic acid and the acetyl group at 1.78 ppm from glucosamine indicating the presence of two monosaccharide units in the disaccharide motif. The high-resolution mass spectrometric data at 971.3717 (M + H) support the structure of compound **1a**

Synthesis of Chondroitin Disaccharide Analogue. Similarly, a 1,3-dipolar cycloaddition reaction was carried out to couple **5** and **7** in the presence of CuI and Hünig's base in acetonitrile and CH_2Cl_2 (2:2) mixture at room temperature for 2 h. After standard workup, the disaccharide analogue **2a** was recovered in 85% yield (Scheme 6). The structure of **2a** was confirmed by NMR spectroscopy where the triazole proton appeared at 7.90 ppm, the methyl proton at 3.89 ppm, and acetate at 1.96 ppm, which indicates the presence of two monosaccharide unit. The high resolution mass spectrometric data support the structure of compound **2a**.

Synthesis of Heparosan and Chondroitin Tetrasaccharide Analogues. After successful synthesis of disaccharide analogues our next goal was to synthesize the tetrasaccharide analogues. To synthesize heparosan tetrasaccharide analogue **3**, the disaccharide analogue building block **1a** first was needed for transformation to two disaccharide analogue building blocks, one an anomeric azido derivative and the other a 4-*O*-propargyl derivative. These will then be subjected to click reaction to form the protected tetrasaccharide analogue that would be ready for global deprotection to provide the desired tetrasaccharide analogue **3**. The propargylation of the C-4 position of **1a** was undertaken using several propargylation conditions including (1) propargyl bromide, silver oxide in DMF (resulting in a very sluggish and low yielding reaction);[28] (2) $Ba(OH)_2$ and BaO in DMF from 0 °C to room temperature (resulting in benzoate ester cleavage);[29] and (3) NaHMDS and propargyl bromide at −78 °C (resulting in no reaction).[30] Ultimately, we were able to install the propargyl group in acidic medium using propargyl trichloroacetimidate, prepared from trichloroacetonitrile and propargyl alcohol in the presence of catalytic amount of sodium followed by distillation to obtain the fresh acetimidate.[31] Treatment of compound **1a** with propargyl trichloroacetimidate in the presence of catalytic trifluoromethane sulfonic acid in dichloromethane at room temperature afforded the partial conversion of **1a**

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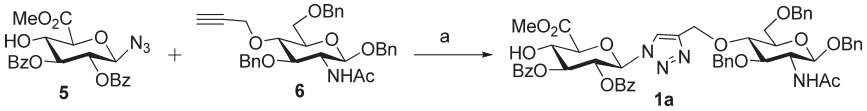
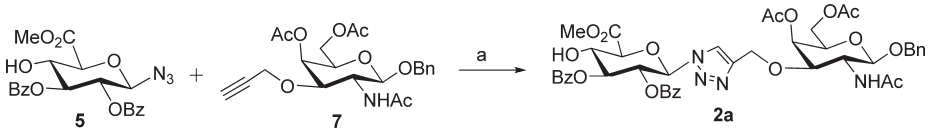
Scheme 5[a]



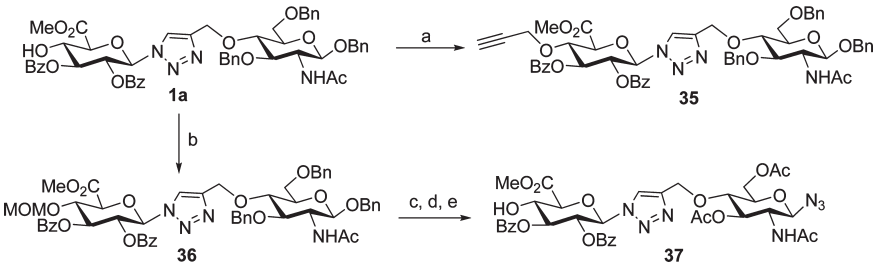
[a] Reagent and conditions: (a) CuI, DIPEA, CH_3CN, rt.

Scheme 6[a]



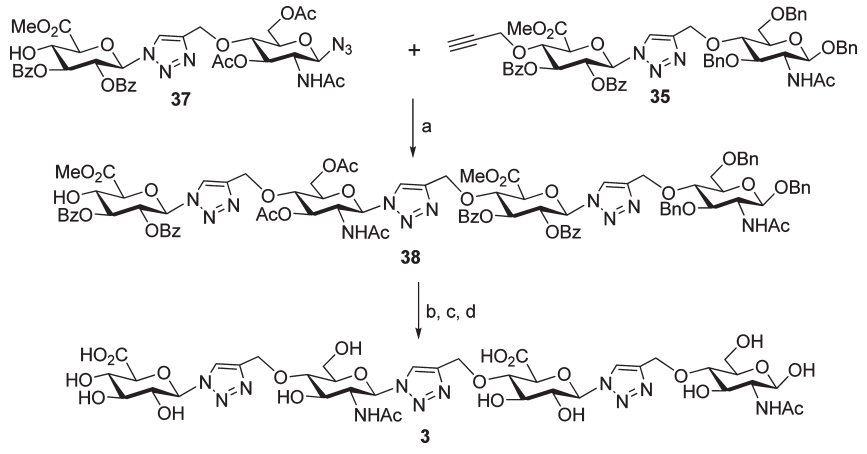
[a] Reagents and conditions: (a) CuI, DIPEA, CH_3CN, CH_2Cl_2, rt.

Scheme 7[a]



[a] Reagents and conditions: (a) propargyl trichloroacetimidate, trifluoromethane sulfonic acid, CH_2Cl_2, mol sieves powder 4 Å, rt; (b) MOMCl, DIPEA, DMAP, CH_2Cl_2, 35 °C; (c) H_2,10% Pd/C, MeOH/EtOAC, 1 N HCl (cat.); (d) Ac_2O, pyridine, rt; (e) $SnCl_4$, $TMSN_3$, CH_2Cl_2, 0 °C.

Scheme 8[a]



[a] Reagents and conditions: (a) CuI, DIPEA, CH_3CN, rt; (b) LiOOH, THF; (c) 2 N NaOH, MeOH; (d) H_2, 10% Pd/C, MeOH.

to compound **35**. After chromatographic purification afforded the desired propargylated compound **35** in 45% yield (Scheme 7).

Next, the azido group at anomeric position was installed in disaccharide **1a**. For this, at first the C4 hydroxyl was protected as its MOM ether derivative (**36**) using MOMCl and Hünig's base with catalytic DMAP. Then, reductive removal of the benzyl groups under hydrogenation over Pd/C at atmospheric pressure, followed by acetylation with acetic anhydride and treatment with trimethylsilyl azide in the presence of stannic chloride at 0 °C furnished the β-azido compound **37** in 61% yields with *in situ* removal of MOM group (Scheme 7). The compound **37** was confirmed by mass spectrometry and the structure was analyzed by NMR spectroscopy. The compound is confirmed with a mass of 809.2502 (M^+) by the high-resolution mass spectrometry.

Disaccharide analogues **35** and **37** were coupled under 1, 3-dipolar cycloaddition reaction with CuI in acetonitrile for 10 h to form the tetrasaccharide analogue **38** in 87% yield. The compound was confirmed with a mass of 1840.6193 ($M + Na)^+$. Finally, basic hydrolysis with LiOOH and 2 N NaOH and hydrogenation over Pd/C[16h] produced a tetrasaccharide

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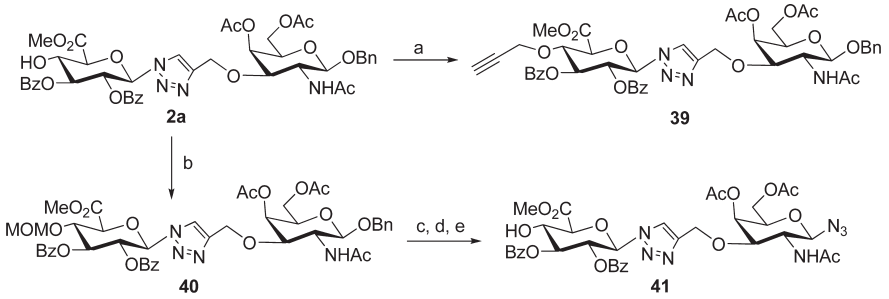
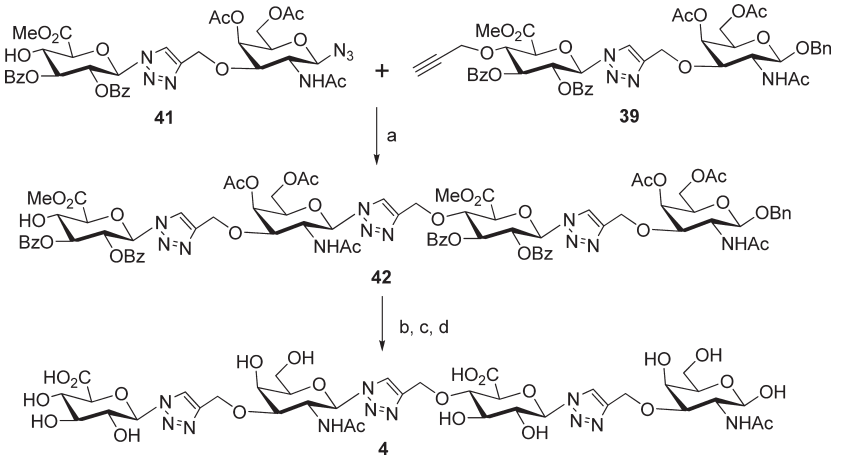
Scheme 9[a]



[a] Reagents and conditions: (a) propargyl trichloroacetimidate, trifluoromethane sulfonic acid, CH_2Cl_2, mol sieves powder 4 Å, rt; (b) MOMCl, DIPEA, DMAP, CH_2Cl_2, 35 °C; (c) H_2,10% Pd/C, MeOH/EtOAC, 1 N HCl (cat.); (d) Ac_2O, pyridine, rt; (e) $SnCl_4$, $TMSN_3$, CH_2Cl_2, 0 °C.

Scheme 10[a]



[a] Reagents and conditions: (a) CuI, DIPEA, CH_3CN, rt; (b) LiOOH, THF; (c) 2 N NaOH, MeOH; (d) H_2, 10% Pd/C, MeOH.

analogue **3**. Complete spectral characterization of **3**, including mass spectral analysis confirmed the synthesis of heparosan tetrasaccharide analogue (Scheme 8).

In a similar way, the construction of chondroitin tetrasaccharide analogue was undertaken from the chondroitin disaccharide analogue **2a**, which was first transformed into two disaccharide analogues **39** and **41** (Scheme 9). Treatment of compound **2a** with propargyl trichloroacetimidate in the presence of catalytic trifluoromethane sulfonic acid in CH_2Cl_2 at room temperature afforded compound **39**. Next, for 1-azido derivative of disaccharide analogue **2a**, the C4 hydroxyl was protected with MOMCl and catalytic DMAP in diisopropylethylamine as solvent to offer the MOM-protected disaccharide **40**. Reductive removal of the anomeric benzyl groups upon hydrogenation over Pd/C, followed by acetylation and treatment with trimethylsilyl azide in the presence of stannic chloride at 0 °C provided β-azido derivatives **41** as confirmed by mass spectrometry and NMR spectroscopy.

Disaccharide analogues **39** and **41** were coupled under 1,3-dipolar cycloaddition reaction with CuI in acetonitrile for 14 h at room temperature to form the tetrasaccharide **42** in 77% yield (Scheme 10). The compound was confirmed by high-resolution mass spectrometry based on a peak for [M + Na] at 1744.5462 amu. Finally, basic hydrolysis with LiOOH in THF and 2 N NaOH followed by debenzylation under hydrogenation condition with Pd/C in MeOH, produced tetrasaccharide analogue **4**. Complete spectral characterization of **4**, including mass spectral analysis confirm the structure of chondroitin tetrasaccharide analogue.

CONCLUSION

In conclusion, a highly effective approach for the preparation of heparosan and chondroitin disaccharide analogues and tetrasaccharide analogues bearing the triazole ring as the interglycosidic linker has been described. The key reaction for the assembly of these oligomers consists of very efficient copper catalyzed azide−alkyne cycloadditions (*CuAAC*). The efficiency and fidelity of this reaction were maintained regardless of the structural complexity of building blocks to be assembled. Very likely the 1,4-disubstituted triazole ring spacers contribute substantially to the overall length of these non-natural glycosaminoglycans. This tetrasaccharide building block could be extended to the higher oligosaccharide. Our experimental observation could be valuable for designing and synthesizing a novel drug in a rapid, cost-efficient manner. Future studies are planned for the chemical sulfonation of these tetrasaccharide analogues and their biological evaluation.

EXPERIMENTAL SECTION

General Methods. NMR spectra were recorded on 500 MHz spectrometer (^{1}H NMR and ^{13}C NMR). Optical rotation was measured at a concentration of g/100 mL (accuracy (0.002°). Analytical thin-layer

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chromatography was performed on precoated silica gel plates. Visualization was performed by ultraviolet light and/or by staining with anisaldehyde solution in ethanol. Chromatographic separations were performed on a silica gel column by flash chromatography. Yields are given after purification, unless differently stated. When reactions were performed under anhydrous conditions, the mixtures were maintained under nitrogen. Compounds were named following IUPAC rules as applied by Beilstein-Institute AutoNom software for systematic names in organic chemistry.

Phenyl 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-*β*-D-glucopyranoside (9). To a solution of compound **8** (2.0 g, 5.54 mmol) in pyridine (20 mL), benzoyl chloride (1.54 mL, 13.31 mmol) was added at 0 °C, and the mixture was stirred for 3 h at room temperature. After completion of the reaction by TLC, water was added and extracted with CH_2Cl_2. Organic layer was washed with $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4 and concentrated to get dibenzoyl derivative as solid, which was recrystallized from ethylacetate and hexane to obtain the pure dibenzoyl derivative **9**. Yield = 92%; R_f 0.6 (EtOAc/hexane, 1/4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, 2 H, J = 7.9 Hz), 7.93 (d, 2 H, J = 7.4 Hz), 7.52 (t, 1 H, J = 7.4 Hz), 7.48 (m, 3 H), 7.43 (m, 4 H), 7.38−7.30 (m, 8 H), 5.81 (t, 1 H, J = 9.5 Hz), 5.45 (m, 1 H), 5.48 (t, 1 H, J = 9.2 Hz), 5.05 (d, 1 H, J = 10.1 Hz), 4.47 (dd, 1 H, J = 5.0, 10.4 Hz), 3.98 (q, 2 H, J = 9.2 Hz), 3.76 ((dt, 1 H, J = 4.7, 9.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6, 165.2, 136.6, 133.3, 129.9−126.1, 101.4, 87.0, 78.6, 73.2, 71.0, 70.9, 68.5; HRMS-FAB: [M + Na] calcd for $C_{33}H_{28}O_7NaS^+$ 591.1453, found 591.1446.

Phenyl 2,3-Di-*O*-benzoyl-4-*O*-benzyl-1-thio-*β*-D-glucopyranoside (10). To a solution of compound **9** (1.8 g, 3.16 mmol) in dry CH_2Cl_2 (35 mL) were added $PhBCl_2$ (0.66 mL, 5.06 mmol) and Et_3SiH (0.70 mL, 4.42 mmol) at −78 °C, and the reaction was allowed to warm to room temperature. After 30 min, reaction mixture was quenched with Et_3N and MeOH. The reaction was diluted with CH_2Cl_2 and washed with aq $NaHCO_3$, and the organic layer was dried over anhydr Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by column chromatography to afford compound **10**. Yield = 87%; R_f 0.5 (EtOAc/hexane, 3/7); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (m, 2 H), 7.95 (m, 2 H), 7.50−7.43 (m, 4 H), 7.38 (m, 4 H), 7.31−7.29 (m, 3 H), 7.16 (m, 5 H), 5.79 (t, 1 H, J = 9.4 Hz), 5.40 (t, 1 H, J = 9.2 Hz), 5.01 (d, 1 H, J = 10.1 Hz), 4.60 (m, 2 H), 3.97 (dd, 1 H, J = 2.6, 12.1 Hz), 3.93 (t, 1 H, J = 9.6 Hz), 3.81 (dd, 1 H, J = 4.1, 12.1 Hz), 3.66 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 165.3, 137. 1, 133.3−127.9, 86.1, 79.6, 76.2, 75.4, 74.8, 70.8, 61.7; HRMS-FAB: [M + Na] calcd for $C_{33}H_{30}O_7NaS^+$ 593.1610, found 593.1604.

Methyl-phenyl 2,3-Di-*O*-benzoyl-4-*O*-benzyl-1-thio-*β*-D-glucuronate (11). To a vigorously stirred solution of thioglycoside **10** (1.5 g, 2.62 mmol) in CH_2Cl_2 (20 mL) and H_2O (20 mL) were added TEMPO (0.082 g, 0.524 mmol) and BAIB (2.11 g, 6.57 mmol). After 1 h the reaction mixture was quenched by the addition of aqueous $Na_2S_2O_3$ solution (50 mL) and aqueous satd $NaHCO_3$ (50 mL). The mixture was then extracted twice with EtOAc (2 × 30 mL), and the combined organic phase was dried ($MgSO_4$), filtered, and concentrated. Flash column chromatography using EtOAc/MeOH (9:1) afforded the glycuronic acid. To a solution of acid (1.4 g, 2.39 mmol) in DMF (20 mL) was added Cs_2CO_3 (3.11 g, 9.57 mmol), and the mixture was cooled to 0 °C. Then was added CH_3I (0.223 g, 3.58 mmol), and the mixture was stirred for 45 min, quenched with water, extracted with EtOAc, washed with brine, dried over anhydr Na_2SO_4, and concentrated *in vacuo*. The crude reaction mixture was purified by column chromatography to get **11** as thick liquid. Yield = 60% (2-steps); R_f 0.5 (EtOAc/hexane, 1/4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (m, 2 H), 7.91 (m, 2 H), 7.52 (m, 2 H), 7.49 (m, 2 H), 7.46 (m, 4 H), 7.31 (m, 3 H), 7.16 (m, 3 H), 7.09 (m, 2 H), 5.74 (m, 1 H), 5.39 (t, 1 H, J = 9.3, 10.0 Hz), 4.99 (d, 1 H, J = 9.9 Hz), 4.58 (d, 1 H, J = 11.0 Hz), 4.53 (d, 1 H, J = 11.9 Hz), 4.16 (m, 2 H), 3.73 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 168.4, 165.4, 165.1, 133.3−127.9, 87.0, 77.2, 75.4, 74.7, 70.2 (× 2), 52.8; $[\alpha]^{25}_D$ = +40.0 (c 0.11, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{34}H_{30}O_8NaS^+$ 621.1559, found 621.1556.

Methyl-1-*O*-acetyl-2,3-di-*O*-benzoyl-4-*O*-benzyl-1-thio-*β*-D-glucuronate (12). To a vigorously stirred solution of thioglycoside **11** (0.11 g, 0.183 mmol) in CH_2Cl_2 (10 mL) were added NIS (0.041 g, 0.183 mmol) and acetic acid (0.014 mL, 0.183 mmol) at 20 °C. After TLC analysis showed complete consumption of starting material, the reaction was quenched with satd aq $Na_2S_2O_3$ and washed with satd aq $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude reaction mixture was purified by column chromatography to get corresponding 1-*O*-acetyl glycosides **12**. Yield = 63%; R_f 0.6 (EtOAc/hexane, 3/7); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (m, 4 H), 7.51 (m, 2 H), 7.38 (m, 4 H), 7.16 (m, 3 H), 7.03 (m, 2 H), 5.99 (d, 1 H, J = 7.5 Hz), 5.74 (dd, 1 H, J = 8.8, 8.9 Hz), 5.50 (dd, 1 H, J = 8.8, 8.9 Hz), 4.50 (m, 2 H), 4.33 (d, 1 H, J = 8.9 Hz), 4.20 (dd, 1 H, J = 8.8, 8.9 Hz), 3.78 (s, 3 H), 2.05 (s, 3 H), 1.54 (br s, 1 H); ^{13}C NMR (125 MHz, CD_3OD) δ 168.6, 165.5, 136.8−128.2, 91.2, 76.7, 74.9, 74.6, 73.5, 70.6, 52.8, 20.8; $[\alpha]^{25}_D$ = +46.0 (c 0.13, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{30}H_{28}NaO_{10}^+$ 571.1580, found 571.1581.

Phenyl 2,3-Di-*O*-benzoyl-1-thio-*β*-D-glucopyranoside (14). To a solution of dibenzoylated compound **9** (3.0 g, 5.27 mmol) in MeOH and CH_2Cl_2 (4:1) (30 mL) was added *p*-TSA (0.50, 2.63 mmol), and the mixture was stirred at 35 °C for 8 h. The reaction mixture was neutralized with $NaHCO_3$, solvent was removed under reduced pressure, and the reaction mixture was partitioned between water (50 mL) and CH_2Cl_2 (80 mL). The aqueous layer was washed with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and concentrated to get the diol which was purified through column chromatography eluting with EtOAc/hexane (1/1) to get pure diol **14**. Yield = 83%; R_f 0.1 (EtOAc/hexane, 4/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (m, 2 H), 7.95 (m, 2 H), 7.53−7.50 (m, 2 H), 7.46−7.44 (m, 2 H), 7.40−7.34 (m, 4 H), 7.31−7.29 (m, 3 H), 5.43 (m, 2 H), 4.97 (d, 1 H, J = 9.6 Hz), 4.01 (dd, 1 H, J = 3.2, 12.0 Hz), 3.94 (t, 1 H, J = 9.2 Hz), 3.90 (dd, 1 H, J = 4.8, 12.0 Hz), 3.64−3.61 (m, 1 H), 3.14−3.11 (m, 1 H), 2.33 (br s, 1 H); ^{13}C NMR (125 MHz, CD_3OD) δ 167.4, 165.2, 133.5−127.4, 87.0, 80.0, 78.1, 70.1, 69.6, 62.3; $[\alpha]^{25}_D$ = +96.0 (c 1.7, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{26}H_{24}O_7NaS^+$ 503.1140, found 503.1142.

Phenyl 2,3-Di-*O*-benzoyl-4,6-*O*-*p*-methoxy-benzylidene-1-thio-*β*-D-glucopyranoside (15). To a solution of compound **14** (2.0 g, 4.16 mmol) in dry DMF (25 mL) were added anisaldehyde dimethyl acetal (1.13 mL, 6.24 mmol) and 10-camphorsulfonic acid (0.19 g, 0.832 mmol), and the mixture was stirred at 50 °C for 1 h. After completion of the reaction by TLC, the mixture was neutralized with $NaHCO_3$, water was added, the mixture was extracted with EtOAc, the organic layer was washed with water and brine, and the solution was dried over anhydr Na_2SO_4 and concentrated to obtain the *p*-methoxybenzylated derivative **15** as solid, which was purified by column chromatography. Yield = 89%; R_f 0.6 (EtOAc/hexane, 1/4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (m, 2 H, J = 1.2 Hz), 7.94 (m, 2 H), 7.54 (m, 2 H), 7.48−7.46 (m, 3 H), 7.39 (m, 3 H), 7.33 (m, 5 H), 6.84 (m, 2 H), 5.81 (t, 1 H, J = 9.0 Hz), 5.50 (s, 1 H), 5.48 (dd, 1 H, J = 9.2, 10.1 Hz), 5.05 (d, 1 H, J = 9.6 Hz), 4.47 (dd, 1 H, J = 5.0 Hz), 3.96−3.85 (m, 3 H), 3.78 (s, 3 H; ^{13}C NMR (125 MHz, CD_3OD) δ 165.6, 165.2, 160.1, 133.3−127.4, 113.5, 101.4, 87.0, 78.6, 73.2, 71.0, 70.9, 68.4, 55.2; $[\alpha]^{25}_D$ = +64.0 (c 0.8, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{35}H_{32}O_8NaS^+$ 635.1716, found 635.1719.

Phenyl 2,3-Di-*O*-benzoyl-4-*O*-*p*-methoxybenzyl-1-thio-*β*-D-glucopyranoside (16). Compound **15** (1.95 g, 3.18 mmol) and flame activated AW-300 molecular sieves were suspended in dry CH_2Cl_2 (30 mL) for 1 h at rt under argon and then cooled to −78 °C. $PhBCl_2$ (0.66 mL, 5.09 mmol) and Et_3SiH (0.71 mL, 4.45 mmol) were added, and the reaction was stirred at −78 °C for 30 min. After that the reaction mixture was quenched with Et_3N and MeOH, the reaction was diluted with CH_2Cl_2 and washed with $NaHCO_3$, the organic layer was dried

over anhydr Na_2SO_4, and the product was concentrated *in vacuo*. The resulting residue was purified by column chromatography to give corresponding compound **16**. Yield = 71%. R_f 0.4 (EtOAc/hexane, 3/7); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (m, 2 H), 7.88 (m, 2 H), 7.52−7.49 (m, 2 H), 7.45−7.43 (m, 2 H), 7.38−7.34 (m, 4 H), 7.31−7.29 (m, 3 H), 7.06 (d, 2 H, J = 8.6 Hz), 6.84 (d, 2 H, J = 8.7 Hz), 5.71 (t, 1 H, J = 9.0 Hz), 5.34 (t, 1 H, J = 9.2 Hz), 4.96 (d, 1 H, J = 9.6 Hz), 4.50 (ABq, 2 H, J = 10.9 Hz), 3.97 (dd, 1 H, J = 2.6, 12.1 Hz), 3.88 (t, 1 H, J = 9.6 Hz), 3.77 (dd, 1 H, J = 4.1, 12.1 Hz), 3.70 (s, 3 H), 3.63 (m, 1 H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.6, 165.2, 159.3, 133.3−113.7, 86.1, 79.6, 76.2, 74.0, 74.4, 70.8, 61.7, 55.2; $[\alpha]^{25}_D$ = +62.0 (*c* 1.8, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{34}H_{32}O_8NaS^+$ 623.1716, found 623.1710.

Methyl-phenyl 2,3-Di-*O*-benzoyl-4-*O*-*p*-methoxybenzyl-1-thio-*β*-D-glucuronate (17). To a vigorously stirred solution of thioglycoside **16** (1.32 g, 2.19 mmol) in CH_2Cl_2 (15 mL) and H_2O (5 mL) were added TEMPO (0.171 g, 1.09 mmol) and BAIB (1.7 g, 5.49 mmol). The reaction mixture was quenched by the addition of $Na_2S_2O_3$ solution (10% in H_2O, 25 mL) and $NaHCO_3$ (satd aq 20 mL). The mixture was then extracted twice with EtOAc (20 mL), and the combined organic phase was dried ($MgSO_4$), filtered, and concentrated. Flash column chromatography using EtOAc/petroleum ether afforded the acids. To a solution of acid (1.18 g, 1.91 mmol) in DMF (25 mL) was added Cs_2CO_3 (2.49 g, 7.67 mmol), the mixture was cooled to 0 °C, CH_3I (0.178 mL, 2.86 mmol) was added, and the mixture was stirred for 1 h, quenched with water, extracted with EtOAc, washed with brine, dried over anhydr Na_2SO_4, and concentrated *in vacuo*. The crude reaction mixture was purified by column chromatography to get **17** as thick liquid. Yield = 88%; R_f 0.36 (EtOAc/hexane, 3/7); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, 2 H, J = 7.2 Hz), 7.80 (d, 2 H, J = 7.2 Hz), 7.42 (m, 2 H), 7.38 (m, 2 H), 7.29 (m, 4 H), 7.21 (m, 3 H), 6.91 (m, 2 H), 6.56 (m, 2 H), 5.61 (t, 1 H, J = 9.0 Hz), 5.28 (t, 1 H, J = 9.5 Hz), 4.87 (d, 1 H, J = 9.9 Hz), 4.42 (d, 1 H, J = 10.8 Hz), 4.35 (d, 1 H, J = 10.8 Hz), 4.06 (m, 2 H), 3.73 (s, 3 H), 3.62 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2, 165.4, 165.2, 159.3, 133.4−113.7, 86.9, 78.1, 76.6, 75.4, 74.3, 70.4, 55.2, 52.9; $[\alpha]^{25}_D$ = +51.0 (*c* 2.2, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{35}H_{32}O_9NaS^+$ 651.1665, found 651.1667.

Methyl-1-*O*-acetyl-2,3-di-*O*-benzoyl-4-*O*-*p*-methoxybenzyl-1-thio-*β*-D-glucuronate (18). To a vigorously stirred solution of thioglycoside **17** (0.96 g, 1.52 mmol) in CH_2Cl_2 (15 mL) were added *N*-iodosuccinimide (NIS) (343 mg, 1.52 mmol) and acetic acid (0.117 mL, 1.52 mmol) at 0 °C. After TLC analysis showed complete consumption of starting material, the reaction was quenched with satd aq $Na_2S_2O_3$ and washed with satd aq $NaHCO_3$. The organic layer was dried over anhydr $MgSO_4$ and concentrated *in vacuo*. The crude reaction mixture was treated with pyridine and Ac_2O and stirred for overnight. After removal of pyridine the crude reaction mixture was purified by column chromatography to obtain the *β*-acetyl glycoside **18**. Yield = 61%; R_f 0.4 (EtOAc/hexane, 3/7); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (m, 2 H), 7.52 (m, 2 H), 7.38 (m, 6 H), 7.02 (m, 2 H), 6.67 (m, 1 H), 6.56 (m, 1 H), 5.98 (d, 1 H, J = 7.5 Hz), 5.71 (dd, 1 H, J = 8.9, 9.0 Hz), 5.48 (dd, 1 H, J = 8.9, 9.0 Hz), 4.53 (m, 2 H), 4.44 (d, 1 H, J = 10.9 Hz) 4.32 (d, 1 H, J = 9.1 Hz), 3.73 (s, 3 H), 3.62 (s, 3 H), 2.04 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 168.5, 165.4, 159.3, 133.4−113.7, 91.2, 76.4, 74.9, 74.2, 73.5, 70.4, 55.2, 52.8, 20.9; $[\alpha]^{25}_D$ = +35.0 (*c* 0.9, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{35}H_{32}O_9NaS^+$ 651.1665, found 651.1664.

Methyl-2,3-di-*O*-benzoyl-1-azido-*β*-D-glucuronate (5). $SnCl_4$ (0.14 mL, 1.24 mmol) and $TMSN_3$ (0.17 mL, 3.11 mmol) were added to a solution of **18** (900 mg, 1.55 mmol) in dry CH_2Cl_2 (6 mL), and the mixture was stirred at room temperature for 3 h. The mixture was washed successively with aqueous $NaHCO_3$ and water and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford **5** as colorless oil. Yield = 66%; R_f 0.4 (EtOAc/hexane, 2/3); 8.01 (m, 4 H), 7.52 (m, 2 H), 7.43 (m, 4 H), 5.65 (dd, 1 H, J = 9.1, 9.3 Hz), 5.43 (dd, 1 H, J = 9.1, 9.3 Hz), 4.95 (d, 1 H, J = 8.7 Hz), 4.21 (m, 2 H), 3.73 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4, 166.4, 165.0, 133.5−129.4, 88.6, 76.2, 74.6, 72.4, 71.7, 70.5, 70.2, 53.1; $[\alpha]^{25}_D$ = +45.0 (*c* 1.3, $CHCl_3$); HRMS-FAB: [M + H] calcd for $C_{21}H_{20}N_3O_8^+$ 442.1250, found 442.1247.

Benzyl-*N*-acetyl D-Glucosamine (20). *N*-Acetyl D-glucosamine **19** (0.5 g, 2.31 mmol) was suspended in DMF (5 mL) and successively treated with sodium hydride (0.12 g, 3.01 mmol) and benzyl bromide (0.824 g, 6.93 mmol) at room temperature for 4 h. After standard workup, the compound was dried and concentrated to obtain compound **20** as a mixture of α:*β* in a ratio of 1:4. Yield = 77%; R_f 0.6 (MeOH/EtOAc 1/4); HRMS-FAB: [M + Na] calcd for $C_{15}H_{21}NNaO_6^+$ 334.1267, found 334.1264.

Benzyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranoside (21*β*). Glucosamine pentaacetate **23** (2.46 g, 6.3 mmol) and benzyl alcohol (2.0 mL, 0.0189 mol) were coupled with $Sc(OTf)_3$ (0.466 g, 0.094 mmol) in dry dichloroethane (25 mL) at 90 °C for 90 min. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with water, dried over Na_2SO_4, and purified by column chromatography to give benzyl glycoside **24**. Yield = 78%. This compound (2.00 g, 4.57 mmol) was dissolved in dry CH_3OH (20 mL) under a N_2-atmosphere, and 0.5 M NaOMe in MeOH (9.1 mL, 4.57 mmol) was added to it. After 3 h the reaction mixture was neutralized with Amberlite (IR-120 H^+) and filtered, and the resulting mixture was concentrated under vacuum to give the crude triol. A solution of the triol (1.00 g, 3.21 mmol), which was dissolved in dry DMF (30 mL), was treated with *p*-TsOH (0.018 mg, 0.096 mmol) and benzaldehyde dimethyl acetal (0.721 mL, 4.81 mmol). After 12 h at room temperature, the mixture was treated with $NaHCO_3$ (1 mL) and evaporated under reduced pressure. The residue was purified by column chromatography to afford **21*β*** as a white solid. Yield = 81%; R_f 0.41 (EtOAc/hexane, 3/2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.32 (m, 2 H), 7.20−7.13 (m, 8 H), 5.41 (s, 1 H), 4.72 (d, 1 H, J = 12.0 Hz), 4.46 (d, 1 H, J = 8.2 Hz), 4.43 (d, 1 H, J = 12.0 Hz), 4.20 (dd, 1 H, J = 4.9, 10.4 Hz), 3.68−3.59 (m, 3 H), 3.40 (t, 1 H, J = 9.2 Hz), 3.29 (dt, 1 H, J = 4.9, 9.5 Hz), 1.85 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 172.4, 136.9−126.0, 101.6, 100.2, 81.3, 70.8, 70.6, 68.4, 66.1, 56.8, 23.5; HRMS-FAB: [M + Na] calcd for $C_{22}H_{25}NNaO_6^+$ 422.1580, found 422.1581.

Benzyl 2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranoside (22). To a mixture of BaO (1.26 g, 8.25 mmol) and $Ba(OH)_2$ (1.12 g, 3.57 mmol) in *N*,*N*-dimethylformamide (20 mL) containing benzyl bromide (0.68 mL, 4.13 mmol) was added compound **21*β*** (1.1 g, 2.75 mmol) at 0 °C. The mixture was stirred at room temperature for 18 h, diluted with water, and neutralized with aqueous formic acid (10%). The white precipitate obtained was filtered, washed with water, and dried to give compound **22**. Yield = 90%; R_f 0.5 (EtOAc/hexane, 3/2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (m, 2 H), 7.46 (m, 2 H), 7.38−7.29 (m, 11 H), 5.61 (s, 1 H), 4.86 (dd, 2 H, J = 10.6 Hz), 4.71 (d, 1 H, J = 8.0 Hz), 4.64 (d, 1 H, J = 12.0 Hz), 4.58 (d, 1 H, J = 11.6 Hz), 3.92 (t, 1 H, J = 9.2 Hz), 3.84 (t, 2 H, J = 9.5 Hz), 3.74 (t, 2 H, J = 7.5 Hz), 3.48 (m, 2 H), 1.87 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4, 142.0, 141.0 (× 3), 132.9−129.8, 105.1, 104.1, 86.0, 85.4, 78.0, 74.8, 72.5, 69.8, 59.8, 26.5; HRMS-FAB: [M + Na] calcd for $C_{29}H_{31}NNaO_6^+$ 512.2049, found 512.2046.

Benzyl 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (25). To a solution of compound **22** (0.815 g, 1.66 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C was added Et_3SiH (0.53 mL, 3.32 mmol) followed by BF_3/Et_2O (0.614 mL, 4.98 mmol). After the reaction mixture stirred at 0 °C for 1 h, it was quenched with saturated $NaHCO_3$ solution (20 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified through flash silica gel column chromatography to get compound **25** as white solid. Yield = 73%; R_f 0.4 (EtOAc/hexane, 1/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.28 (m, 15 H), 5.47 (d, 1 H, J = 5.6 Hz), 4.87 (d, 1 H, J = 6.0 Hz), 4.85 (d, 1 H, J = 9.6 Hz), 4.77 (d, 1 H, J = 11.7 Hz), 4.69 (d, 1

H, J = 11.7 Hz), 4.58 (m, 3 H), 3.96 (dd, 1 H, J = 8.7, 10.1 Hz), 3.72 (d, 2 H, J = 4.9 Hz), 3.69 (t, 2 H, J = 4.9 Hz), 3.53 (penta, 1 H, J = 4.7 Hz), 3.41 (m, 1 H), 2.75 (br s, 1 H), 1.85 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.4, 138.5−127.7, 99.2, 80.3, 74.1, 73.7, 73.2, 70.9 (× 2), 70.6, 56.8, 23.5; HRMS-FAB: [M + Na] calcd for $C_{29}H_{33}NNaO_6^+$ 514.2206, found 514.2211.

Benzyl 2-Acetamido-3,6-di-*O*-benzyl-4-*O*-propargyl-2-deoxy-*β*-D-glucopyranoside (6). To a solution of compound **25** (0.5 g, 1.01 mmol) in dry DMF (20 mL) were added $Ba(OH)_2$ (1.3 g, 1.31 mmol) and BaO (0.046 g, 3.03 mmol), the mixture was cooled to 0 °C, and then propargyl bromide (0.20 mL, 1.53 mmol) was added and stirred at rt for 10 h. After standard workup compound **6** was obtained as white solid. Yield = 88%; R_f 0.4 (EtOAc/hexane, 2/3); ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$) δ 7.30−7.23 (m, 15 H), 4.80 (d, 1 H, J = 12.0 Hz), 4.73 (d, 1 H, J = 11.3 Hz), 4.64 (d, 1 H, J = 8.1 Hz), 4.60 (m, 4 H), 4.29 (dd, 1 H, J = 2.5, 15.2 Hz), 4.21 (dd, 1 H, J = 2.5, 15.2 Hz), 3.85 (dd, 1 H, J = 8.4, 10.1), 3.69 (dd, 1 H, J = 1.9, 10.1 Hz), 3.56 (dd, 1 H, J = 8.2, 9.9 Hz), 3.49 (dd, 1 H, J = 8.3, 9.5 Hz), 3.45 (dd, 1 H, J = 1.8, 4.9 Hz), 2.40 (t, 1 H, J = 2.4 Hz), 1.78 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.2, 138.1−127.6, 99.2 (anomeric), 80.9, 79.6, 78.0, 74.6, 74.5, 74.4, 73.4, 70.5, 69.1, 59.6, 56.0, 22.9; $[\alpha]^{25}_D$ = +35.0 (c 0.3, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{32}H_{35}NNaO_6^+$ 552.2362, found 552.2356.

Benzyl 2-Acetamido-3-*O*-propargyl-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranoside (26). Following the procedure used for compound **6**, compound **26** was obtained from compound **21*β*** as a white solid. Yield = 83%. R_f 0.3 (EtOAc/hexane, 3/2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (m, 2 H), 7.14−7.10 (m, 8 H), 5.37 (s, 1 H), 4.67 (d, 1 H, J = 12.0 Hz), 4.60 (d, 1 H, J = 8.3 Hz), 4.39 (d, 1 H, J = 12.0 Hz), 4.20−4.14 (m, 4 H), 3.78 (t, 1 H, J = 9.5 Hz), 3.67 (t, 1 H, J = 10.3 Hz), 3.45 (q, 1 H, J = 8.5 Hz), 3.30 (m, 1 H), 2.34 (m, 1 H), 1.75 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.7, 136.9−125.7, 101.6, 100.0, 81.9, 79.7, 76.8, 74.2, 70.8, 68.4, 65.6, 59.2, 55.5, 22.4; $[\alpha]^{25}_D$ = +40.0 (c 0.09, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{25}H_{27}NNaO_6^+$ 460.1736, found 460.1733.

Benzyl 2-Acetamido-3-*O*-propargyl-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (27). To a solution of compound **26** (0.62 g, 1.41 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C was added Et_3SiH (0.452 mL, 2.8 mmol) followed by BF_3/Et_2O (0.52 mL, 4.23 mmol). After the reaction mixture stirred at 0 °C for 1 h, it was quenched with saturated $NaHCO_3$ solution (20 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The compound was purified through flash column chromatography to get pure compound **27**. Yield = 77%; R_f 0.26 (EtOAc/hexane, 4/6); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (m, 5 H), 7.23 (m, 5 H), 4.80 (d, 1 H, J = 12.0 Hz), 4.62 (d, 1 H, J = 8.3 Hz), 4.54 (m, 3 H), 4.31 (m, 2 H), 3.75 (dd, 1 H, J = 2.9, 10.7 Hz), 3.66 (q, 1 H, J = 5.2 Hz), 3.67 (m, 1 H), 3.47 (m, 1 H), 3.42 (m, 1 H), 3.40 (m, 1 H), 2.34 (d, 1 H, J = 2.3 Hz), 1.87 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.4, 137.8−127.6, 99.3, 80.7, 80.3, 74.7, 74.3, 73.6, 71.5, 70.5, 69.6, 59.5, 55.5, 23.0; $[\alpha]^{25}_D$ = +31.0 (c 0.2, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{25}H_{29}NNaO_6^+$ 462.1893, found 462.1887.

Benzyl 2-Acetamido-3-*O*-propargyl-6-*O*-benzyl-2-deoxy-*β*-D-galactopyranoside (28). To a stirred solution of trifluoromethanesulfonic anhydride (0.096 mL, 0.568 mmol) in CH_2Cl_2 (10 mL) at −18 °C were added dropwise pyridine (0.09 mL) diluted with CH_2Cl_2 (1 mL) and then a solution of compound **27** (0.125 g, 0.284 mmol) in CH_2Cl_2 (45 mL). After 30 min, the mixture was diluted with CH_2Cl_2, washed with 2 M HCl, satd aq $NaHCO_3$ and with water, dried ($MgSO_4$), and evaporated. The residue containing the 4-*O*-triflate derivative was treated with $NaNO_2$ (0.195 g, 2.84 mmol) in DMF (12 mL) under stirring at room temperature for 1 h. The crude mixture was diluted with CH_2Cl_2, washed with water, dried (Na_2SO_4), and evaporated. The residue was purified to give **28**. Yield = 40%. R_f 0.2 (EtOAc/hexane, 4/6); ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$ (2:1)) δ 7.33 (m, 10 H), 4.96 (d, 1 H, J = 8.3 Hz), 4.88 (d, 1 H, J = 11.9 Hz), 4.59 (m, 2 H), 4.57 (d, 1 H, J = 11.5 Hz), 4.23 (d, 1 H, J = 2.3 Hz), 4.20 (t, 1 H, J = 3.2 Hz), 4.14 (d, 1 H, J = 2.9 Hz), 3.82 (dd, 1 H, J = 5.8, 9.9 Hz), 3.76 (dd, 1 H, J = 5.8, 9.9 Hz), 3.70 (t, 1 H, J = 5.8 Hz), 4.53 (dd, 1 H, J = 8.3, 10.4 Hz), 2.43 (t, 1 H, J = 2.3 Hz), 1.92 (s, 3 H), 1.62 (br s, 1 H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.8, 137.8−127.5, 99.7, 79.4, 77.2, 74.7, 73.6, 73.3, 70.3, 69.4, 65.2, 56.3, 51.9, 22.7. HRMS-FAB: [M + Na] calcd for $C_{25}H_{29}NNaO_6^+$ 462.1893, found 462.1884.

Benzyl 2-Acetamido-3-*O*-propargyloxy-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranoside (29). To a solution of compound **27** (0.075 g, 0.17 mmol) in pyridine (1 mL) was added acetic anhydride (0.02 mL, 0.22 mmol) at 0 °C, and the mixture was stirred for 3 h. After completion of the reaction by TLC, water was added and extracted with CH_2Cl_2. Organic layer was washed with $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4 and concentrated to get 4-acetyl derivative **29** as oil. Yield = 85%; R_f 0.5 (EtOAc/hexane, 1/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 10 H), 5.76 (s, 1 H), 5.06 (d, 1 H, J = 8.3 Hz), 4.93 (d, 2 H, J = 9.3 Hz), 4.90 (d, 1 H, J = 11.8 Hz), 4.59 (d, 1 H, J = 11.8 Hz), 4.54 (m, 2 H), 4.27 (m, 4 H), 3.68 (q, 1 H, J = 9.7 Hz), 3.28 (m, 1 H), 2.41 (t, 1 H, J = 2.3 Hz), 1.98 (s, 3 H); 1.94 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2 (× 2), 137.9−127.5, 99.8, 79.9, 74.4, 73.6, 73.2, 72.4, 71.8, 64.8, 60.2, 57.7, 54.3, 21.0, 20.7; HRMS-FAB: [M + Na] calcd for $C_{27}H_{31}NNaO_7^+$ 504.1998, found 504.1996.

Benzyl 2-Acetamido-3-*O*-propargyloxy-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-*β*-D-galactopyranoside (30). Using the same procedure as described form the synthesis of compound **29**, compound **30** was obtained from compound **28**. Yield = 88%; R_f 0.55 (EtOAc/hexane, 1/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 10 H), 5.60 (s, 1 H), 5.59 (d, 1 H, J = 2.7 Hz), 5.05 (d, 1 H, J = 8.2 Hz), 4.90 (d, 1 H, J = 12.0 Hz), 4.60 (dd, 2 H, J = 10.6, 11.2 Hz), 4.47 (d, 1 H, J = 11.9 Hz), 4.28 (dd, 1 H, J = 2.9, 10.9 Hz), 4.20 (dd, 1 H, J = 2.9, 10.9 Hz), 4.19 (d, 1 H, J = 1.8, 5.9 Hz), 3.83 (t, 1 H, J = 6.3 Hz), 3.58 (dd, 1 H, J = 7.1, 9.3 Hz), 3.46 (q, 1 H, J = 10.3 Hz), 3.47 (m, 1 H), 2.40 (t, 1 H, J = 2.3 Hz), 2.42 (s, 3 H), 1.93 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.4, 137.6−127.9, 99.2, 79.5, 74.8, 73.6, 72.0, 71.2, 67.9, 65.4, 56.7, 54.3, 23.5, 20.7; HRMS-FAB: [M + Na] calcd for $C_{27}H_{31}NNaO_7^+$ 504.1998, found 504.1995.

***β*-D-Benzyl-*N*-acetyl-galactosamine Triacetate (32).** To a solution of *β*-D-*N*-acetyl-galactosamine tetraacetate **31** (820 mg, 2.3 mmol) in dichloroethane (5 mL) were added benzyl alcohol (0.66 mL, 0.006 mmol) and $Sc(OTf)_3$ (0.15 mg, 0.031 mmol), and the mixture was heated to reflux at 90 °C for 90 min. After cooling, the reaction mixture was washed with sat aq $NaHCO_3$, dried with anhydr $MgSO_4$, concentrated, and purified by column chromatography to obtain the benzyl galactoside **32** as a white powder. Yield = 81%; R_f = 0.37 (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.29 (m, 5 H), 5.36 (m, 1 H), 5.34 (dd, 1 H, J = 1.0, 3.3 Hz), 5.19 (dd, 1 H, J = 3.3, 11.2 Hz), 4.90 (dd, 1 H, J = 12.0 Hz), 4.65 (m, 2 H), 4.21−4.15 (m, 1 H), 4.10−4.09 (m, 1 H), 3.89 (dt, 1 H, J = 1.0, 6.5 Hz), 2.04 (s, 3 H), 1.98 (s, 3 H), 1.90 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.4, 170.3 (× 2), 136.9−128.0, 99.6, 70.7, 70.6, 69.9, 66.7, 61.5, 51.3, 23.5, 20.7, 20.6; HRMS-FAB: [M + Na] calcd for $C_{21}H_{27}NNaO_9^+$ 460.1584, found 460.1587.

Benzyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-*β*-D-galactopyranoside (33). Using the same procedure as described for the synthesis of glucosamine derivative **21*β***, benzylidine galactoside **33** was obtained from the *β*-D-benzyl-*N*-acetyl-galactosamine triacetate **32**. Yield = 77%; R_f 0.2 (EtOAc/hexane, 7/3); ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD (2:1)) δ 7.34 (m, 2 H), 7.16 (m, 8 H), 5.41 (s, 1 H), 4.74 (d, 1 H, J = 12.0 Hz), 4.44 (d, 1 H, J = 12.0 Hz), 4.40 (d, 1 H, J = 8.3 Hz), 4.15 (dd, 1 H, J = 1.9, 12.0 Hz), 3.68−3.59 (m, 3 H), 3.93 (dd, 1 H, J = 1.8, 12.4 Hz), 3.83 (dd, 1 H, J = 8.3, 10.8 Hz), 3.60 (dd, 1 H, J = 3.6, 10.8 Hz), 3.91 (m, 1 H), 1.77 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD (2:1)) δ 172.2, 137.4−126.2, 101.1, 99.7, 75.3, 70.2 (× 2), 68.9, 66.4,

52.8, 22.5; HRMS-FAB: [M + Na] calcd for $C_{22}H_{25}NNaO_6^+$ 422.1580, found 422.1583.

Benzyl 2-Acetamido-3-*O*-propargyl-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (34). Using the same procedure as described form the synthesis of glucosamine derivative **26**, compound **34** was obtained from compound **33**. Yield = 85%. R_f 0.35 (EtOAc/hexane, 7/3); ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD (2:1)) δ 7.44 (m, 2 H), 7.25 (m, 8 H), 5.49 (s, 1 H), 4.96 (d, 1 H, *J* = 8.3 Hz), 4.83 (d, 1 H, *J* = 11.9 Hz), 4.51 (d, 1 H, *J* = 11.8 Hz), 4.28 (dd, 1 H, *J* = 1.5, 3.2 Hz), 4.24 (dd, 1 H, *J* = 1.9, 9.3 Hz), 4.16 (t, 2 H, *J* = 1.8 Hz), 4.08 (dd, 1 H, *J* = 1.5, 12.2 Hz), 3.53 (t, 1 H, *J* = 9.6 Hz), 3.44 (m, 1 H), 3.30 (m, 1 H), 2.42 (t, 1 H, *J* = 2.3 Hz), 1.85 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD (2:1)) δ 171.7, 137.5−126.0, 101.6, 98.8, 79.7, 74.9, 74.2, 72.6, 70.7, 69.2, 66.3, 56.3, 53.1, 22.9; $[\alpha]^{25}_D$ = +25.0 (*c* 0.1, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{25}H_{27}NNaO_6^+$ 460.1736, found 460.1735.

Benzyl 2-Acetamido-3-*O*-propargyl-4,6-di-*O*-acetyl-2-deoxy-β-D-galactopyranoside (7). To a solution of compound **34** (0.62 g, 1.41 mmol) in MeOH was added *p*-TSA (0.053 g, 0.028 mmol), and the mixture was stirred for 5 h. The product was neutralized with triethylamine, concentrated, and purified through column chromatography to obtain the diol derivative, which was dissolved in pyridine (5 mL) and acetic anhydride (0.531 mL, 5.64 mmol). After stirring at rt for 4 h, pyridine was removed under reduced pressure and purified through column chromatography using EtOAc and hexane to obtain pure compound **7**. Yield = 86%; R_f 0.35 (EtOAc/hexane, 7/3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.26 (m, 5 H), 5.59 (d, 1 H, *J* = 7.2 Hz), 5.36 (d, 1 H, *J* = 3.2 Hz), 511 (d, 1 H, *J* = 8.3 Hz), 4.90 (d, 1 H, *J* = 12.0 Hz), 4.60 (d, 1 H, *J* = 11.8 Hz), 4.34 (dd, 1 H, *J* = 2.9, 10.9 Hz), 4.18- 4.15 (m, 4 H), 3.88 (t, 1 H, *J* = 6.5 Hz), 3.41 (m, 1 H), 2.42 (m, 1 H), 2.12 (s, 3 H), 2.08 (s, 3 H), 1.93 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.5, 170.4, 136.9−127.9, 98.9, 79.3, 74.9, 73.7, 71.2, 70.6, 65.4, 62.0, 56.7, 54.3, 23.5, 20.7, 20.6; $[\alpha]^{25}_D$ = +31.0 (*c* 0.35, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{22}H_{27}NNaO_8^+$ 456.1634, found 456.1632.

Heparosan Disaccharide Analogue 1a. To a solution of alkyne derivative **6** (0.143 g, 0.271 mmol) and azide derivative **5** (0.120 g, 0.271 mmol) in acetonitrile (10 mL) were added CuI (0.027 g, 0.5 mmol) and *N*,*N*-diisopropylethylamine (0.103 mL, 0.813 mmol) at rt, and the mixture was stirred for 1−2 h. The reaction mixture was diluted with EtOAc (20 mL), 10 mL of NH_4Cl was added and, the aqueous layer was extracted with EtOAc (3 × 15 mL), and the combined organic layer was washed with brine solution, dried over anhydr Na_2SO_4, and concentrated *in vacuo* to obtain a crude residue that was purified by flash chromatography to obtain the desired triazole derivative **1a** as a solid. Yield = 83%; R_f 0.5 (MeOH/CH_2Cl_2, 1/2.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (m, 2 H), 7.80 (br s, 1 H), 7.60 (m, 2 H), 7.41 (m, 1 H), 7.33 (m, 1 H), 7.28−7.26 (m, 3 H), 7.21 (m, 5 H), 7.18−7.15 (m, 11H), 6.10 (d, 1 H, *J* = 9.2 Hz), 5.71 (dd, 1 H, *J* = 9.1, 9.2 Hz), 5.60 (dd, 1 H, *J* = 9.1, 9.2 Hz), 4.75 (m, 1 H), 4.72 (m, 1 H), 4.65 (d, 1 H, *J* = 11.2 Hz), 4.52−4.40 (m, 6 H), 4.29 (d, 1 H, *J* = 9.7 Hz), 4.16 (dd, 1 H, *J* = 9.2, 9.6 Hz), 3.85 (s, 3 H), 3.64 (m, 1 H), 3.60 (m, 2 H), 3.49 (m, 2 H), 3.35 (m, 1 H), 1.78 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.2, 167.9, 165.9, 164.7, 145.2, 138.1−127.5, 121.4, 99.4, 85.6, 81.0, 78.3, 77.5, 74.7, 74.4 (× 2), 73.4, 70.5, 70.3, 69.6, 68.6, 65.1, 55.6, 52.7, 22.6; $[\alpha]^{25}_D$ = +37.0 (*c* 0.09, $CHCl_3$); HRMS-FAB: [M + H] calcd for $C_{53}H_{55}N_4O_{14}^+$ 971.3715, found 971.3717.

Chondroitin Disaccharide Analogue 2a. Using the same procedure as described for the synthesis of compound **1a**, compound **2a** was obtained from compound **5** and **7**. Yield = 85%; R_f 0.4 (MeOH/CH_2Cl_2, 1/2.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, 2 H, *J* = 1.2, 7.3 Hz), 7.90 (s, 1 H), 7.66 (dd, 2 H, *J* = 1.2, 7.3 Hz), 7.58 (m, 1 H), 7.51 (m, 1 H), 7.47 (m, 1 H), 7.43 (m, 2 H), 7.30 (m, 4 H), 7.23 (m, 2 H), 6.15 (d, 1 H, *J* = 9.3 Hz), 6.05 (d, 1 H, *J* = 8.7 Hz), 5.69 (dd, 1 H, *J* = 9.3, Hz), 5.50 (d, 1 H, *J* = 9.3, Hz), 4.92 (d, 1 H, *J* = 12.3 Hz), 4.68 (d, 2 H, *J* = 2.2 Hz), 4.62 (d, 1 H, *J* = 12.2 Hz), 4.56 (d, 1 H, *J* = 8.4 Hz), 4.39 (m, 2 H), 4.22 (d, 1 H, *J* = 6.4 Hz), 4.20 (d, 1 H, *J* = 6.9 Hz), 3.89 (s, 3 H), 3.69 (m, 1 H), 2.14 (s, 3 H), 2.10 (s, 3 H), 1.96 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.5, 170.4, 168.1, 165.6, 165.4, 145.3, 137.2−127.7, 121.6, 99.9, 85.8, 74.4, 71.6, 70.5, 70.4, 70.2, 70.0, 64.6, 62.2, 61.7, 53.1, 51.9, 22.9, 20.9, 20.7 (× 2); $[\alpha]^{25}_D$ = +30.0 (*c* 0.06, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{43}H_{46}N_4NaO_{16}^+$ 897.2807, found 897.2805.

4-*O*-Propargyl-heparosan Disaccharide Analogue 35. To a mixture of propargyl alcohol (1.00 g, 17.8 mmol) and trichloroacetonitrile (2 mL, 19.6 mmol) was added a catalytic amount of sodium at 0 °C. After stirring for 3 h at rt, the reaction mixture was quenched with 0.2 mL of acetic acid, diluted with ether, washed with brine, and dried over anhydr $MgSO_4$. The solvent was evaporated at reduced pressure, and the residue was distilled (100 °C, 5 mmHg) to afford the corresponding trichloroacetimidate (2.30 g, 65%, colorless oil). To a solution of this trichloroacetimidate (52 mg, 0.262 mmol) and compound **1a** (85 mg, 0.087 mmol) in CH_2Cl_2 (5 mL) were added a catalytic amount of trifluoromethanesulfonic acid and molecular sieves powder at room temperature. After stirring overnight, the mixture was filtered through a pad of Celite, washed with $NaHCO_3$, dried over anhydrous $MgSO_4$, and evaporated. The residue was purified by flash chromatography to afford compound **35**. Yield = 45%; R_f 0.6 (MeOH/CH_2Cl_2, 1/2.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, 1 H, *J* = 7.5 Hz), 7.87 (d, 1 H, *J* = 7.2 Hz), 7.80 (br s, 1 H), 7.60 (d, 1 H, *J* = 1.3 Hz), 7.74 (dd, 2 H, *J* = 8.4, 9.3 Hz), 7.53 (m, 2 H), 7.43 (m, 2 H), 7.38 (m, 3 H), 7.32−7.28 (m, 13 H), 6.10 (d, 1 H, *J* = 9.2 Hz), 5.99 (d, 2 H, *J* = 9.2 Hz), 5.85−5.79 (m, 3 H), 5.43 (d, 1 H, *J* = 7.7 Hz), 4.85- 4.79 (m, 2 H), 4.71 (m, 1 H), 4.65−4.50 (m, 4 H), 4.37−4.35 (m, 2 H), 4.25 (dd, 1 H, *J* = 2.5, 3.4 Hz), 3.95 (t, 1 H, *J* = 8.1 Hz), 3.81 (s, 3 H), 3.74 (m, 1 H), 3.60 (m, 1 H), 3.53 (m, 2 H), 2.28 (t, 1 H, *J* = 1.9 Hz), 1.78 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 167.4, 165.7, 164.7, 145.8, 138.0−129.4, 120.9, 98.4, 85.7, 80.4, 80.1, 79.3, 78.5, 76.6, 76.7, 75.7, 74.5, 74.1, 73.4, 71.3, 70.4, 68.4, 65.8, 64.3, 59.7, 53.0, 26.4; $[\alpha]^{25}_D$ = +27.0 (*c* 0.08, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{56}H_{56}N_4NaO_{14}^+$ 1031.3691, found 1031.3687.

4-*O*-Methoxymethyl Ether of Heparosan Disaccharide Analogue 36. To a stirred solution of **1a** (75 mg, 0.077 mmol) in dry CH_2Cl_2 (1 mL) was added *N*,*N*-diisopropylethylamine (0.195 mL, 1.54 mmol), and the mixture was cooled to 0 °C. MOMCl (0.11 mL, 0.154 mmol) was added dropwise, and the mixture was stirred overnight at 30 °C. Saturated aq $NaHCO_3$, (20 mL) was added, and the two phases were separated. The organic phase was washed with 5% hydrochloric acid (2 × 5 mL) and brine (2 × 5 mL), dried ($MgSO_4$), and concentrated to obtain compound **36**. Yield = 78%. R_f 0.5 (MeOH/CH_2Cl_2,1/2.4);^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (m, 2 H), 7.73 (br s, 1 H), 7.72 (m, 1 H), 7.71 (d, 2 H, *J* = 1.9 Hz), 7.52 (m, 1 H), 7.43−7.38 (m, 6 H), 7.32−7.28 (m, 13 H), 6.10 (d, 1 H, *J* = 9.2 Hz), 5.84−5.81 (m, 2 H, *J* = 9.0 Hz), 5.62 (d, 1 H, *J* = 7.7 Hz), 4.87 (d, 1 H, *J* = 12.0 Hz), 4.85 (d, 1 H, *J* = 12.0 Hz), 4.80 (d, 1 H, *J* = 4.4 Hz), 4.78 (s, 2 H), 4.66−4.60 (m, 3 H), 4.57−4.52 (m, 3 H, *J* = 12.5 Hz), 4.40−4.37 (m, 2 H), 3.95 (dd, 1 H, *J* = 8.1, 9.4 Hz), 3.79 (s, 3 H), 3.77 (d, 1 H, *J* = 5.4 Hz), 3.73 (m, 1 H), 3.65 (m, 1 H), 3.53 (m, 1 H), 3.18 (s, 3 H), 1.78 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 167.2, 165.4, 164.6, 145.6, 138.3−127.8, 121.0, 99.3, 97.8, 85.7, 80.1, 78.5, 77.1, 76.1, 74.6, 74.3, 73.8, 73.3, 70.6, 70.5, 68.8, 65.3, 56.4, 5.2, 53.0, 23.4. $[\alpha]^{25}_D$ = +33.0 (*c* 0.06, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{55}H_{58}N_4NaO_{15}^+$ 1037.3796, found 1037.3793.

1-Azido-heparosan Disaccharide Analogue 37. Compound **36** (80 mg, 0.081 mmol) underwent hydrogenation over 10% Pd/C (10 mg) in MeOH (5 mL) in the presence of a catalytic amount of 1 N HCl (0.1 mL) at room temperature for 2 days to produce triol, which was treated with acetic anhydride (0.045 g, 0.484 mmol) in pyridine (1 mL) and stirred for overnight to afford the triacetate. After chromatographic separation the triacetate (26 mg, 0.03 mmol) was treated with $SnCl_4$ (0.003 mL, 0.03 mmol) and $TMSN_3$ (0.008 mL, 0.06 mmol) were in dry

CH_2Cl_2 (6 mL), and the mixture was stirred at room temperature for 3 h. The mixture was washed successively with aq $NaHCO_3$ and water and dried over anhydr Na_2SO_4. The solvent was removed under reduced pressure to afford **37** as colorless oil. Yield = 61%, R_f 0.45 (MeOH/CH_2Cl_2, 1/2.4); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (m, 2 H), 7.96 (br s, 1 H), 7.75 (m, 2 H), 7.56 (m, 1 H), 7.50 (m, 1 H), 7.42 (m, 2 H), 7.30 (m, 2 H), 6.16 (d, 1 H, J = 9.3 Hz), 5.91 (dd, 1 H, J = 9.3, 9.5 Hz), 5.79 (m, 1 H), 5.61 (d, 1 H, J = 9.6 Hz), 5.07 (dd, 1 H, J = 8.9, 10.7 Hz), 4.76 (ABq, 2 H, J = 12.2 Hz), 4.50 (d, 1 H, J = 9.1 Hz), 4.45 (dd, 1 H, J = 2.2, 12.1 Hz), 4.39 (m, 2 H), 4.23 (dd, 1 H, J = 4.5, 7.6 Hz), 4.05 (dd, 1 H, J = 9.5, 19.4 Hz), 3.77 (s, 3 H), 3.71 (t, 1 H, J = 9.5 Hz), 3.65 (m, 1 H), 3.42 (br s, 1 H), 2.08 (s, 3 H), 2.00 (s, 3 H), 1.92 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 170.7 (× 2), 168.1, 166.2, 164.7, 144.6, 133.7−127.9, 121.7, 88.4, 85.6, 76.8, 75.8, 75.0, 74.7, 74.5, 70.3, 70.2, 65.6, 62.2, 53.4, 52.8, 23.0, 20.9, 20.8; $[\alpha]^{25}_D$ = +35.0 (c 0.07, $CHCl_3$); HRMS-FAB: [M] calcd for $C_{36}H_{39}N_7O_{15}^+$ 809.2504, found 809.2502.

Triazole-Containing Heparosan Tetrasaccharide Analogue 38. To a solution of alkyne **35** (10 mg, 10.0 μmol) and azide **37** (8 mg, 10.0 μmol) in CH_3CN were added CuI (1 mg, 5.0 μmol) and DIPEA (1.0 μL, 60.0 μmol), and the mixture was stirred for 10 h. After a standard workup followed by purification desired heparosan triazole derivative **38** was obtained as a solid. Yield = 87%, R_f 0.4 (MeOH/CH_2Cl_2, 1/1.9); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (br s, 1 H), 7.95 (m, 1 H), 7.91 (br s, 1 H), 7.90 (br s, 1 H), 7.87−7.81 (m, 3 H), 7.73−7.71 (m, 3 H), 7.68 (m, 3 H), 7.48 (m, 3 H), 7.33 (m, 5 H), 7.28−7.23 (m, 17 H), 6.16 (d, 1 H, J = 2.0, 9.2 Hz), 6.08 (m, 1 H), 6.05 (m, 1 H), 6.83 (m, 4 H), 5.45−5.68 (m, 2 H), 4.81 (dd, 2 H, J = 4.7, 12.0 Hz), 4.74−4.64 (m, 2 H), 4.62 (m, 2 H), 4.57−4.45 (m, 6 H), 4.35 (m, 4 H), 4.28 (m, 2 H), 3.78 (s, 3 H), 3.76 (m, 1 H), 3.70 (m, 4 H), 3.63 (s, 3 H), 3.58 (m, 3 H), 3.44 (m, 2 H), 2.07 (s, 3 H), 1.96 (s, 3 H), 1.77 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 171.3, 170.7, 170.6, 167.9, 167.4, 165.8, 165.3, 164.7, 164.4, 145.3, 144.5, 144.4, 138.0−127.4, 121.9 (× 2), 99.5, 85.6, 85.3, 81.3, 78.2, 77.7, 76.2, 76.1, 76.0, 75.6, 75.3, 74.6, 74.4, 74.3, 74.2, 74.0, 73.2, 65.4, 65.2, 65.0, 64.7, 62.2, 60.0, 55.5, 53.1, 53.0, 52.8, 52.6, 22.4, 22.0, 21.9, 20.4, 20.3 (× 3); $[\alpha]^{25}_D$ = +31.0 (c 0.27, $CHCl_3$); HRMS-FAB:[M + Na] calcd for $C_{92}H_{95}N_{11}NaO_{29}^+$ 1840.6195, found 1840.6193.

4-*O*-Propargyl-chondroitin Disaccharide Analogue 39. Using the same procedure as described for the synthesis of compound **35**, compound **39** was obtained from compound **2a**. Yield = 49%; R_f 0.65 (MeOH/CH_2Cl_2,1/2.4). Yield = 49%; R_f 0.65 (MeOH/CH_2Cl_2, 1/2.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (br s, 1 H), 7.93 (m, 2 H), 7.57 (m, 2 H), 7.41 (m, 3 H), 7.26−7.24 (m, 6 H), 7.14 (m, 2 H), 6.16 (d, 1 H, J = 9.2 Hz), 5.82 (t, 2 H, J = 8.7 Hz), 5.66 (t, 1 H, J = 9.0 Hz), 5.45 (br d, 1 H, J = 2.0 Hz), 4.87 (d, 2 H, J = 12.3 Hz), 4.63 (m, 2 H), 4.57 (d, 1 H, J = 12.2 Hz), 4.44 (d, 1 H, J = 8.4 Hz), 4.39 (d, 1 H, J = 8.2 Hz), 4.30 (t, 1 H, J = 2.3 Hz), 4.29 (dd, 2 H, J = 2.3, 5.4 Hz), 4.15 (m, 1 H), 3.80 (s, 3 H), 3.62 (m, 1 H), 3.54 (dd, 1H, J = 3.0, 10.3 Hz), 2.31 (t, 1 H, J = 2.5 Hz), 2.14 (s, 3 H), 2.05 (s, 3 H), 1.92 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.5, 170.4, 167.4, 165.3, 165.2, 145.6, 137.2−127.7, 121.5, 100.8, 85.4, 78.2, 76.9, 76.3, 75.8, 74.3, 73.5, 71.7, 70.5, 70.1, 64.6, 61.7, 61.3, 59.3, 53.1, 51.8, 23.2, 20.7 (× 2); $[\alpha]^{25}_D$ = +37.0 (c 0.09, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{46}H_{48}N_4NaO_{16}^+$ 935.2963, found 935.2954.

4-*O*-Methoxymethyl Ether of Chondroitin Disaccharide Analogue 40. Using the same procedure as described for the synthesis of compound **36**, compound **40** was obtained from compound **2a**. Yield = 81%; R_f 0.5 (MeOH/CH_2Cl_2, 1/2.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (br s, 1 H), 7.95 (m, 2 H), 7.59 (m, 2 H), 7.48 (m. One H), 7.41 (m, 1 H), 7.27 (m, 8 H), 7.14 (m, 1 H, J = 7.2 Hz), 6.16 (d, 1 H, J = 9.2 Hz), 5.84 (dd, 2 H, J = 8.0, 9.0 Hz), 5.75 (t, 1 H, J = 9.3 Hz), 5.48 (d, 2 H, J = 2.8 Hz), 4.88 (dd, 2 H, J = 12.3 Hz), 4.68 (d, 2 H, J = 11.8 Hz), 4.65 (s, 2 H), 4.58 (d, 1 H, J = 12.2 Hz), 4.48 (t, 3 H, J = 12.4 Hz), 4.39 (t, 1 H, J = 8.0 Hz), 4.18 (d, 1 H, J = 6.3 Hz), 4.14 (d, 2 H, J = 7.0 Hz), 3.80 (s, 3 H), 3.63 (t, 1 H, J = 6.5 Hz), 3.21 (s, 3 H), 2.14 (s, 3 H), 1.95 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.5, 170.4, 167.4, 165.3, 165.2, 145.1, 137.2−127.7, 121.5, 100.0, 97.7, 85.4, 76.9, 75.8, 74.2, 73.4, 71.8, 70.5, 70.1, 64.6, 61.7, 61.2, 56.3, 53.0, 51.7, 22.9, 20.9, 20.7; $[\alpha]^{25}_D$ = +36.0 (c 0.078, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{45}H_{50}N_4NaO_{17}^+$ 941.3069, found 941.3045.

1-Azido-chondroitin Disaccharide Analogue 41. Using the same procedure as described for the synthesis of compound **37**, compound **41** was obtained from compound **40**. R_f 0.3 (MeOH/CH_2Cl_2, 1/2.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (br s, 1 H), 7.86 (m, 2 H), 7.68 (m, 2 H), 7.44 (m, 2 H), 7.30−7.20 (m, 4 H), 6.39 (d, 1 H, J = 8.4 Hz), 6.18 (dd, 1 H, J = 9.2 Hz), 5.84 (dd, 2 H, J = 9.2, 9.4 Hz), 5.75 (dd, 1 H, J = 9.2, 9.4 Hz), 5.41 (d, 2 H, J = 2.8 Hz), 4.56 (m, 2 H), 4.43 (m, 2 H), 4.34 (t, 1 H, J = 9.4 Hz), 4.07 (dd, 1 H, J = 1.9, 6.3 Hz), 3.84 (dd, 2 H, J = 9.0, 19.4 Hz), 3.80 (s, 3 H), 3.14 (dd, 1 H, J = 3.1, 10.8 Hz), 2.09 (s, 3 H), 1.95 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 170.5, 170.4, 168.0, 165.9, 165.6, 145.2, 134.1−127.7, 121.8, 89.0, 85.8, 74.4, 74.3, 72.7, 71.5, 70.5, 70.1, 64.6, 61.7, 61.2, 53.0, 51.2, 22.9, 20.9, 20.7; $[\alpha]^{25}_D$ = +29.0 (c 0.25, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{36}H_{39}N_7NaO_{15}^+$ 832.2402, found 832.2391.

Triazole-Containing Chondroitin Tetrasaccharide Analogue 42. Using the same procedure as described for the synthesis of compound **38**, compound **42** was obtained from compound **39** and **41**. R_f 0.3 (MeOH/CH_2Cl_2, 1/1.9); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (br s, 1 H), 8.04 (br s, 1 H), 8.01 (br s, 1 H), 7.85−7.74 (m, 8 H), 7.55 (m, 2 H), 7.44 (m, 5 H), 7.31 (m, 5 H), 7.20 (m, 5 H), 7.12 (m, 1 H), 7.04 (m, 1 H), 6.15−6.11 (m, 2 H), 5.80 (m, 1 H), 5.73 (m, 2 H), 5.63 (m, 1 H), 5.58 (m, 1 H), 5.54 (m, 1 H), 5.53 (m, 1 H), 5.37 (m, 2 H), 4.77 (t, 1 H, J = 12.3 Hz), 4.71 (m, 2 H), 4.64−4.55 (m, 4 H), 4.49 (m, 2 H), 4.44 (dd, 1 H, J = 8.3, 8.6 Hz), 4.37 (dd, 1 H, J = 5.7, 5.8 Hz), 4.32 (m, 2 H), 4.21 (m, 2 H), 4.08 (m, 2 H), 3.99 (m, 2 H), 3.91 (m, 1 H), 3.84 (m, 1 H), 3.75 (s, 3 H), 3.63 (s, 3 H), 3.41 (m, 1 H), 2.06 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3 H), 1.95 (s, 3 H), 1.89 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$ and CD_3OD) δ 171.7, 170.8, 170.7, 170.6, 170.4, 170.0, 167.9, 167.7, 165.8, 165.6, 165.2, 165.0, 145.0, 144.2, 143.7, 136.9−127.4, 122.6, 122.5, 121.8, 99.9, 87.8, 85.7, 85.2, 77.6, 76.6, 74.8, 74.4, 74.2, 74.0, 73.7, 73.5, 71.6, 71.4, 70.4, 70.2, 69.5, 65.0, 64.5, 64.2, 61.8, 60.9, 52.8, 52.7, 51.7, 51.5, 49.5, 49.3, 22.5 (× 2), 22.3, 20.4−20.3; $[\alpha]^{25}_D$ = +36.0 (c 0.08, $CHCl_3$); HRMS-FAB: [M + Na] calcd for $C_{82}H_{87}N_{11}NaO_{31}^+$ 1744.5467, found 1744.5462.

General Procedure for Saponification of Methyl Esters, De-*O*-benzoylation, De-*O*-acetylation, and Debenzylation. LiOOH (prepared from 30% solution of H_2O_2 in water (100 equiv per CO_2Me) and 1 M LiOH (50 equiv per CO_2Me)) were added to a solution of the starting material in THF (0.02 M). The reaction mixture was stirred at room temperature for 16 h, and then a 2 N solution of NaOH (1.0 mL) was added until pH 14. The reaction mixture was stirred for 18 h at room temperature and then neutralized with Amberlite IR-120 (H^+) resin, and the solvent was concentrated in vacuo. Next, 10% Pd/C (1 equiv) was added to a solution of the starting material in MeOH (3 mL for 5 mg) and 1 N HCl (0.1 mL for 5 mg). The mixture was placed under an atmosphere of hydrogen, and the progress of the reaction was monitored by TLC (silica gel, $CHCl_3$/MeOH/H_2O (20/5/1). The mixture was filtered, and the residue was washed with H_2O (5 mL). The filtrate was freeze-dried, and the residue was passed through a short reversed phase chromatography using H_2O as the eluent and freeze-dried to provide the final product.

Unnatural Heparosan Tetrasaccharide Analogue 3. Using the above-mentioned procedure compound **3** was obtained from compound **38**. Yield = 55%. ^{1}H NMR (500 MHz, D_2O) δ 8.21 (br s, 1 H), 8.12 (br s, 2 H), 5.73−5.68 (m, 4 H), 4.91 (m, 1 H), 4.80 (m, 3 H), 4.70 (m, 2 H), 4.52 (m, 1 H), 4.23 (m, 1 H), 4.20−4.11 (m, 2 H), 3.97 (t, 2 H, J = 8.5 Hz), 3.70 (m, 2 H), 3.65−3.59 (m, 7 H), 3.54 (m, 3 H), 3.38 (m, 2 H), 1.89 (s, 3 H), 1.68 (s, 3 H); ^{13}C NMR (125 MHz, D_2O)

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δ 174.7, 174.5, 174.1, 171.3, 144.0, 143.7, 125.6, 124.6, 123.9, 94.9, 90.6, 86.8, 86.1, 77.9, 77.8, 76.9, 75.3, 74.8, 73.7, 73.5, 73.2, 71.6, 70.6, 70.2, 69.1, 64.4, 64.3, 60.3, 60.1, 59.9, 58.8, 56.6, 55.2, 54.0, 53.1, 21.8, 21.5; $[\alpha]^{25}_{D}$ = +23.0 (*c* 0.5, water); HRMS-FAB: [M + Na] calcd for $C_{37}H_{53}N_{11}NaO_{23}^{+}$ 1042.3213, found 1042.3197.

Unnatural Chondroitin Tetrasaccharide Analogue 4. Using the above-mentioned procedure compound **4** was obtained from compound **42**. Yield = 52%. ^{1}H NMR (800 MHz, D_2O) δ 8.21 (br s, 1 H), 8.18 (br s, 2 H), 5.77 (m, 2 H), 5.68 (m, 2 H), 4.80 (m, 1 H), 4.79 (m, 1 H), 4.65−4.60 (m, 3 H), 4.57 (m, 1 H), 4.33 (t, 1 H, *J* = 10.2 Hz), 4.26 (m, 3 H), 4.06 (m, 3 H), 3.89 (m, 2 H), 3.79 (t, 1 H, *J* = 8.5 Hz), 3.73 (m, 2 H), 3.69 (m, 7 H), 3.57 (m, 1 H), 1.89 (s, 3 H), 1.68 (s, 3 H); ^{13}C NMR (200 MHz, D_2O) δ 173.9, 171.3, 170.1, 169.3, 144.5, 144.5, 143.9, 124.7, 124.1, 123.7, 94.9, 90.9, 86.8, 86.8, 78.1, 76.7, 76.6, 75.3, 75.2, 74.9, 70.7, 65.1, 64.4, 64.3, 61.3, 61.2, 61.0, 60.8, 58.8, 56.9, 54.4, 53.2, 52.3, 50.6, 48.9, 21.8, 21.5; $[\alpha]^{25}_{D}$ = +38.0 (*c* 0.62, water); HRMS-FAB: [M + Na + H] calcd for $C_{37}H_{53}N_{11}NaO_{23}^{+}$ 1043.3292, found 1043.3244.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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