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Design and synthesis of unnatural heparosan and chondroitin building blocks

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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 Cycloadditions (CuAAC) reaction of appropriately substituted azido-glucuronic acid and propargylated *N*-acetyl glucosamine and *N*-acetyl galactosamine derivative respectively. The resulting suitably substituted tetrasaccharide analogs can be easily converted into azide and alkyne unit for further synthesis of higher oligosaccharide analogs.

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 and 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 proteins¹¹ 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, chondroitin 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 towards 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.

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

The goal of this study is 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 analogs of the disaccharide and tetrasaccharide 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 neoglycoconjugates¹⁴ and the bioconjugation study of glycosides.¹⁵ The 1,3-DCR utilizes relatively low-cost, non-toxic 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 tetrasaccharide analog 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 $\rightarrow$ 4) linkage, [$\rightarrow$ 4] β -GlcUA (1 $\rightarrow$ 4) α -GlcNAc 1 $\rightarrow$]_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 $\rightarrow$ 3)) and (α (1 $\rightarrow$ 4)) linkages, [$\rightarrow$ 4] β -GlcUA (1 $\rightarrow$ 3) α -GalNAc 1 $\rightarrow$]_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 propargylated 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 analogs 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 analog was transformed into either an acceptor by selective deprotection or a glycosyl donor through aglycone adjustment. Selective deprotection, aglycone adjustment and additional glycosylation steps leads to synthesis of a GAG oligosaccharide. In the second, at first disaccharide analog would be synthesized from two monosaccharide unit with orthogonally protecting group then this disaccharide analog would be used as an donor as well as acceptor for next tetrasaccharide analog and so on.

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

Retrosynthetic analysis suggests that heparosan tetrasaccharide analog **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 analog **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 analog **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 analog **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 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 glycoside¹⁸ with NIS, TMSN_3 and TMSOTf failed to give the desired product. Conversion of the thioglycoside to acetate **12** (α : β :7) was accomplished in quantitative yield with NIS in 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 $\text{H}_2/\text{Pd}(\text{OH})_2$ and $\text{H}_2/\text{Pd-C}$ in MeOH and catalytic amount of HCl, but the reaction failed to proceed. We ascribe the failure of this reaction steric hindrance at the C-4 position. At this point we decided to revise Scheme 1 modifying at benzylidene 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 re-protected with *p*-methoxybenzylidene dimethyl acetal at 50 °C in 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 triethylsilane¹⁶ 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** ($\alpha:\beta/2:8$) in 61% yield with NIS in 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 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 analog.

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 derivatives²¹ 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 mixture of $\alpha:\beta$ anomer (1: 4).²² To facilitate separation, the C-4 and C-6 hydroxyl group was protected as benzylidene acetal with benzaldehyde dimethyl acetal and *p*-TSA in DMF giving a mixture **21**($\alpha:\beta = 1:4$) in quantitative yield. The chromatographic separation in small scale gave the compound **21** β in 25% yield which was treated with benzyl bromide, $\text{Ba}(\text{OH})_2$ and BaO mixture in DMF to protect the C3 hydroxyl group as the benzyl ether²³ 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 towards 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 triol²⁴ **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 presence of catalytic $\text{BF}_3 \cdot \text{Et}_2\text{O}$ furnished the selective 6-*O*-benzyl protected compound **25** in quantitative yield²⁵ and exposing the 4-OH. Propargylation of C4 hydroxyl group with propargyl bromide in presence of $\text{Ba}(\text{OH})_2$ and BaO mixture afforded monosaccharide building block **6** in good yield.

The synthesis of the 3-*O*-propargylated 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 presence of $\text{Ba}(\text{OH})_2$ and BaO

affording the propargylated derivative **26**. Selective reductive ring opening using triethylsilane in 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 (NaNO₂) 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.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 propargylated with propargyl bromide in 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 Ac₂O in pyridine provided the modified compound **7** in quantitative yield.

Synthesis of heparosan disaccharide analog

A 1,3-dipolar cycloaddition reaction was carried out to couple monosaccharide **5** and **6** in 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 sat. NH₄Cl.²⁷ After standard work up, the desired disaccharide analog **1a** was obtained in quantitative yield (Scheme 5). The structure of **1a** was confirmed by ¹H NMR, which showed 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 analog

Similarly, a 1,3-dipolar cycloaddition reaction was carried out to couple **5** and **7** in presence of CuI and Hünig's base in acetonitrile and CH₂Cl₂ (2:2) mixture at room temperature for 2 h. After standard work up, the disaccharide analog **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 analogs

After successful synthesis of disaccharide analogs our next goal was to synthesize the tetrasaccharide analogs. To synthesize heparosan tetrasaccharide analog **3**, the disaccharide analog building block **1a** first was needed for transformation to two disaccharide analog 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 analog that would be ready for global deprotection to provide the desired tetrasaccharide analog **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 presence of catalytic amount of sodium followed by distillation to obtain the fresh acetimidate.³¹ Treatment of compound **1a** with propargyl trichloroacetimidate in presence of catalytic trifluoromethane sulfonic acid in dichloromethane at room temperature afforded the partial conversion of **1a** 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 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 analogs **35** and **37** were coupled under 1,3-dipolar cycloaddition reaction with CuI in acetonitrile for 10 h to form the tetrasaccharide analog **38** in 87% yield. The compound was confirmed with a mass of 1840.6193 ($M+Na$)⁺. Finally, basic hydrolysis with LiOOH and 2N NaOH and hydrogenation over Pd/C,^{16h} produced a tetrasaccharide analog **3**. Complete spectral characterization of **3**, including mass spectral analysis confirmed the synthesis of heparosan tetrasaccharide analog (Scheme 8).

In a similar way, the construction of chondroitin tetrasaccharide analog was undertaken from the chondroitin disaccharide analog **2a**, which was first transformed into two disaccharide analogs **39** and **41** (Scheme 9). Treatment of compound **2a** with propargyl trichloroacetimidate in presence of catalytic trifluoromethane sulfonic acid in CH₂Cl₂ at room temperature afforded compound **39**. Next, for 1-azido derivative of disaccharide analog **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 presence of stannic chloride at 0 °C provided β -azido derivatives **41** as confirmed by mass spectrometry and NMR spectroscopy.

Disaccharide analogs **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 2N NaOH followed by debenzoylation under hydrogenation condition with Pd/C in MeOH, produced tetrasaccharide analog **4**. Complete spectral characterization of **4**, including mass spectral analysis confirm the structure of chondroitin tetrasaccharide analog.

Conclusion

In conclusion, a highly effective approach for the preparation of heparosan and chondroitin disaccharide analogs and tetrasaccharide analogs 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 analogs and their biological evaluation.

Experimental Section

General Methods

NMR spectra were recorded on 500 MHz spectrometer (^1H NMR, and ^{13}C NMR). Optical rotation was measured at a concentration of g/100 mL (accuracy (0.002°)). Analytical thin-layer chromatography was performed on pre-coated 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 stirred for 3h 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); ^1H 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: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{33}\text{H}_{28}\text{O}_7\text{NaS}^+$ 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), PhBCl_2 (0.66 mL, 5.06 mmol) and Et_3SiH (0.70 mL, 4.42 mmol) were added 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 , washed with aq NaHCO_3 , 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); ^1H 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: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{33}\text{H}_{30}\text{O}_7\text{NaS}^+$ 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) was added TEMPO (0.082 g, 0.524 mmol) and BAIB (2.11 g, 6.57 mmol). After 1h the reaction mixture was quenched by the addition of aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution (50 mL) and aqueous satd. NaHCO_3 (50 mL). The mixture was then extracted twice with EtOAc (2 $\times$ 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 acid (1.4 g, 2.39 mmol) in DMF (20 mL), Cs₂CO₃ (3.11 g, 9.57 mmol) was added and cooled to 0 °C and then added CH₃I (0.223 g, 3.58 mmol) and stirred for 45 min, quenched with water, extracted with EtOAc, washed with brine, dried over anhydr Na₂SO₄ 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); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CD₃OD): δ 168.4, 165.4, 165.1, 133.3-127.9, 87.0, 77.2, 75.4, 74.7, 70.2 (x 2), 52.8; [α]_D²⁵ = +40.0 (c 0.11, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₃₄H₃₀O₈NaS⁺ 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 CH₂Cl₂ (10 mL) was 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 Na₂S₂O₃ and washed with satd aq NaHCO₃. The organic layer was dried over MgSO₄ 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); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CD₃OD): 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; [α]_D²⁵ = +46.0 (c 0.13, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₃₀H₂₈NaO₁₀⁺ 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 CH₂Cl₂ (4:1) (30 mL), *p*-TSA (0.50, 2.63 mmol) was added and stirred at 35 °C for 5 h. The reaction mixture was neutralized with NaHCO₃, solvent was removed under reduced pressure, and the reaction mixture was partitioned between water (50 mL) and CH₂Cl₂ (80 mL). The aqueous layer was washed with CH₂Cl₂ and the organic layers was dried over Na₂SO₄ and concentrated to get 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); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CD₃OD): δ 167.4, 165.2, 133.5-127.4, 87.0, 80.0, 78.1, 70.1, 69.6, 62.3; [α]_D²⁵ = +96.0 (c 1.7, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₂₆H₂₄O₇NaS⁺ 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), anisaldehyde dimethyl acetal (1.13 mL, 6.24 mmol) and 10-camphorsulfonic acid (0.19 g, 0.832 mmol) was added and stirred at 50 °C for 1 h. After completion of the reaction by TLC, neutralized with NaHCO₃ and water was added, the mixture was extracted with EtOAc, the organic layer was washed with water and brine, the solution was dried over anhydr Na₂SO₄ 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); ¹H NMR (500 MHz, CDCl₃): 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; $[\alpha]_{\text{D}}^{25} = +64.0$ (c 0.8, CHCl_3); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{35}\text{H}_{32}\text{O}_8\text{NaS}^+$ 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 20 min. After that the reaction mixture was quenched with Et_3N and MeOH, the reaction was diluted with CH_2Cl_2 , 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); ^1H 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; $[\alpha]_{\text{D}}^{25} = +62.0$ (c 1.8, CHCl_3); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{34}\text{H}_{32}\text{O}_8\text{NaS}^+$ 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) was 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 $\text{Na}_2\text{S}_2\text{O}_3$ solution (10% in H_2O , 25 mL) and NaHCO_3 (sat., 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), Cs_2CO_3 (2.49 g, 7.67 mmol) was added, cooled to 0°C , CH_3I (0.178 mL, 2.86 mmol) was added, 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); ^1H 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; $[\alpha]_{\text{D}}^{25} = +51.0$ (c 2.2, CHCl_3); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{35}\text{H}_{32}\text{O}_9\text{NaS}^+$ 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) was 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 $\text{Na}_2\text{S}_2\text{O}_3$ 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); ^1H 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; $[\alpha]_{\text{D}}^{25} = +35.0$ (c 0.9, CHCl_3); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{35}\text{H}_{32}\text{O}_9\text{NaS}^+$ 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; $[\alpha]_{\text{D}}^{25} = +45.0$ (c 1.3, CHCl_3); HRMS-FAB: $[\text{M}+\text{H}]$ calcd. for $\text{C}_{21}\text{H}_{20}\text{N}_3\text{O}_8^+$ 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 work-up and 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: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{15}\text{H}_{21}\text{NNaO}_6^+$ 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 $\text{Sc}(\text{OTf})_3$ (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 0.5M 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^+), filtered and the resulting mixture concentrated under vacuum to give the crude triol. To a solution of 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); ^1H 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: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{22}\text{H}_{25}\text{NNaO}_6^+$ 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 $\text{Ba}(\text{OH})_2$ (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); ^1H 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 (x 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: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{29}\text{H}_{31}\text{NNaO}_6^+$ 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 $\text{BF}_3/\text{Et}_2\text{O}$ (0.614 mL, 4.98 mmol). After stirring the reaction mixture 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 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); ^1H 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 (x 2), 70.6, 56.8, 23.5; HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{29}\text{H}_{33}\text{NNaO}_6^+$ 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), $\text{Ba}(\text{OH})_2$ (1.3 g, 1.31 mmol) and BaO (0.046 g, 3.03 mmol) was added and cooled to 0°C and then benzyl 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); ^1H NMR (500 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): 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; $[\alpha]_{\text{D}}^{25} = +35.0$ (c 0.3, CHCl_3); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{32}\text{H}_{35}\text{NNaO}_6^+$ 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 **21b** as a white solid. Yield = 83%. R_f 0.3 (EtOAc/hexane, 3/2); ^1H 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; $[\alpha]_{\text{D}}^{25} = +40.0$ (c 0.09, CHCl_3); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{25}\text{H}_{27}\text{NNaO}_6^+$ 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 $\text{BF}_3/\text{Et}_2\text{O}$ (0.52 mL, 4.23 mmol). After stirring the reaction mixture 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 Na₂SO₄ 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); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CD₃OD): 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; [α]_D²⁵ = +31.0 (c 0.2, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₂₅H₂₉NNaO₆⁺ 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 CH₂Cl₂ (10 mL) at −18°C was added dropwise pyridine (0.09 mL) diluted with CH₂Cl₂ (1 mL), and then a solution of compound **27** (0.125 g, 0.284 mmol) in CH₂Cl₂ (45 mL). After 30 min, the mixture was diluted with CH₂Cl₂, washed with 2 M HCl, satd aq NaHCO₃ and with water, dried (MgSO₄), and evaporated. The residue containing the 4-*O*-triflate derivative was treated with NaNO₂ (0.195 g, 2.84 mmol) in DMF (12 mL) under stirring at room temperature for 1 h. The crude mixture was diluted with CH₂Cl₂, washed with water, dried (Na₂SO₄), and evaporated. The residue was purified to give **28**. Yield = 40%. *R*_f 0.2 (EtOAc/hexane, 4/6); ¹H NMR (500 MHz, CDCl₃:CD₃OD (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); ¹³C NMR (125 MHz, CD₃OD): 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 C₂₅H₂₉NNaO₆⁺ 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), acetic anhydride (0.02 mL, 0.22 mmol) was added at 0 °C and stirred for 3 h. After completion of the reaction by TLC, water was added and extracted with CH₂Cl₂. Organic layer was washed with NaHCO₃, water and brine. The solution was dried over Na₂SO₄ and concentrated to get 4-acetyl derivative **29** as oil. Yield = 85%; *R*_f 0.5 (EtOAc/hexane, 1/1); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): δ 169.2 (x 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 C₂₇H₃₁NNaO₇⁺ 504.1998, found: 504.1996.

Benzyl 2-acetamido-3-O-propargyloxy-4-O-acetyl-6-O-benzyl-2-deoxy-β-Dgalactopyranoside (30)

—Using the same procedure as described from the synthesis of compound **29**, compound **30** was obtained from compound **28**. Yield = 88%; *R*_f 0.55 (EtOAc/hexane, 1/1); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): 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 C₂₇H₃₁NNaO₇⁺ 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), benzyl alcohol (0.66 mL, 0.006 mmol) and Sc(OTf)₃ (0.15 mg, 0.031 mmol) were added and heated to reflux at 90 °C for 90 min. After cooling, the reaction mixture was washed with sat aq NaHCO₃, dried with anhydr MgSO₄, concentrated, and purified by column chromatography to obtain the benzyl galactoside **32** as a white powder. Yield = 81%; *R*_f = 0.37 (EtOAc); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): δ 170.4, 170.4, 170.3 (x 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 C₂₁H₂₇NNaO₉⁺ 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** , benzylidene galactoside **33** was obtained from the β -D-benzyl-*N*-acetyl-galactosamine triacetate **31**. Yield = 77%; *R*_f 0.2 (EtOAc/hexane, 7/3); ¹H NMR (500 MHz, CDCl₃:CD₃OD (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); ¹³C NMR (125 MHz, CDCl₃:CD₃OD (2:1)): δ 172.2, 137.4–126.2, 101.1, 99.7, 75.3, 70.2 (x 2), 68.9, 66.4, 52.8, 22.5; HRMS-FAB: [M+Na] calcd. for C₂₂H₂₅NNaO₆⁺ 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); ¹H NMR (500 MHz, CDCl₃:CD₃OD (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); ¹³C NMR (125 MHz, CDCl₃:CD₃OD (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; [α]_D²⁵ = +25.0 (c 0.1, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₂₅H₂₇NNaO₆⁺ 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, *p*-TSA (0.053 g, 0.028 mmol) was added and stirred for 5 h. The product was neutralized with triethylamine, concentrated, 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); ¹H NMR (500 MHz, CDCl₃): 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), 5.11 (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); ¹³C NMR (125 MHz, CDCl₃): 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; [α]_D²⁵ = +31.0 (c 0.35, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₂₂H₂₇NNaO₈⁺ 456.1634, found: 456.1632.

Heparosan disaccharide analog 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) and 10 mL of NH₄Cl 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 anhydrous Na₂SO₄, 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/CH₂Cl₂, 1/2.4); ¹H NMR (500 MHz, CDCl₃): 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, 11 H), 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); ¹³C NMR (125 MHz, CD₃OD): 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 (x 2), 73.4, 70.5, 70.3, 69.6, 68.6, 65.1, 55.6, 52.7, 22.6; [α]_D²⁵ = +37.0 (c 0.09, CHCl₃); HRMS-FAB: [M+H] calcd. for C₅₃H₅₅N₄O₁₄⁺ 971.3715, found: 971.3717.

Chondroitin disaccharide analog 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/CH₂Cl₂, 1/2.4); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): 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 (x 2); [α]_D²⁵ = +30.0 (c 0.06, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₄₃H₄₆N₄NaO₁₆⁺ 897.2807, found: 897.2805.

4-O-Propargyl-heparosan disaccharide analog 35—To a mixture of propargyl alcohol (1.00 g, 17.8 mmol) and trichloroacetonitrile (2 mL, 19.6 mmol), a catalytic amount of sodium was added 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 anhydrous MgSO₄. 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 CH₂Cl₂ (5 mL) a catalytic amount of trifluoromethanesulfonic acid and molecular sieves powder was added at room temperature. After stirring overnight, the mixture was filtered through a pad of celite, washed with NaHCO₃, dried over anhydrous MgSO₄ and evaporated. The residue was purified by flash chromatography to afford compound **35**. Yield = 45%; *R*_f 0.6 (MeOH/CH₂Cl₂, 1/2.4); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): 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; [α]_D²⁵ =

+27.0 (c 0.08, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₅₆H₅₆N₄NaO₁₄⁺ 1031.3691, found: 1031.3687.

4-O-Methoxymethyl ether of heparosan disaccharide analog 36—To a stirred solution of **1a** (75 mg, 0.077 mmol) in dry CH₂Cl₂ (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 NaHCO₃ (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 (MgSO₄), and concentrated to obtain compound **36**. Yield = 78%. *R*_f 0.5 (MeOH/CH₂Cl₂, 1/2.4); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): 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. [α]_D²⁵ = +33.0 (c 0.06, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₅₅H₅₈N₄NaO₁₅⁺ 1037.3796, found: 1037.3793.

1-Azido-heparosan disaccharide analog 37—Compound **36** (80 mg, 0.081 mmol) underwent hydrogenation over 10% Pd/C (10 mg) in MeOH (5 mL) in presence of 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 SnCl₄ (0.003 mL, 0.03 mmol) and TMSN₃ (0.008 mL, 0.06 mmol) were in dry CH₂Cl₂ (6 mL) and the mixture was stirred at room temperature for 3 h. The mixture was washed successively with aq NaHCO₃ and water and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to afford **37** as colorless oil. Yield = 61%, *R*_f 0.45 (MeOH/CH₂Cl₂, 1/2.4); ¹H NMR (600 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): δ 171.8, 170.7 (x 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; [α]_D²⁵ = +35.0 (c 0.07, CHCl₃); HRMS-FAB: [M] calcd. for C₃₆H₃₉N₇O₁₅⁺ 809.2504, found: 809.2502.

Triazole containing heparosan tetrasaccharide analog 38—To a solution of alkyne **35** (10 mg, 10.0 μmol) and azide **37** (8 mg, 10.0 μmol) in CH₃CN, CuI (1 mg, 5.0 μmol) and DIPEA (1.0 μL, 60.0 μmol) were added and stirred for overnight. After a standard work up followed by purification desired heparosan triazole derivative **38** was obtained as a solid. Yield = 87%, *R*_f 0.4 (MeOH/CH₂Cl₂, 1/1.9); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz,

CDCl₃): 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 (x 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 (x 3); $[\alpha]_D^{25} = +31.0$ (c 0.27, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₉₂H₉₅N₁₁NaO₂₉⁺ 1840.6195, found: 1840.6193.

4-O-Propargyl-chondroitin disaccharide analog 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/CH₂Cl₂, 1/2.4); Yield = 49%; *R_f* 0.65 (MeOH/CH₂Cl₂ / 1:2.4); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): 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 (x 2); $[\alpha]_D^{25} = +37.0$ (c 0.09, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₄₆H₄₈N₄NaO₁₆⁺ 935.2963, found: 935.2954.

4-O-Methoxymethyl ether of chondroitin disaccharide analog 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/CH₂Cl₂, 1/2.4); ¹H NMR (500 MHz, CDCl₃): δ 7.97 (br s, 1 H), 7.95 (m, 2 H), 7.59 (m, 2 H), 7.48 (m, 1 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); ¹³C NMR (125 MHz, CDCl₃): 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; $[\alpha]_D^{25} = +36.0$ (c 0.078, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₄₅H₅₀N₄NaO₁₇⁺ 941.3069, found: 941.3045.

1-Azido-chondroitin disaccharide analog 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/CH₂Cl₂, 1/2.4); ¹H NMR (500 MHz, CDCl₃): 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); ¹³C NMR (125 MHz, CDCl₃): 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; $[\alpha]_D^{25} = +29.0$ (c 0.25, CHCl₃); HRMS-FAB: [M+Na] calcd. for C₃₆H₃₉N₇NaO₁₅⁺ 832.2402, found: 832.2391.

Triazole containing chondroitin tetrasaccharide analog 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/CH₂Cl₂, 1/1.9); ¹H NMR (500 MHz, CDCl₃): 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 (x 2), 22.3, 20.4-20.3; $[\alpha]_{\text{D}}^{25} = +36.0$ (c 0.08, CHCl_3); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{82}\text{H}_{87}\text{N}_{11}\text{NaO}_{31}^+$ 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 2N solution of NaOH (1.0 mL) was added until pH 14. The reaction mixture was stirring for 18 h at room temperature and then neutralized with Amberlite IR-120 (H^+) resin and the solvent was concentrated in vacuo. 10% Pd/C (1equiv.) was added to a solution of the starting material in MeOH (3 mL for 5 mg) and 1N 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, $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (20/5/1)). The mixture was filtered, and the residue was washed with H_2O (5 mL). The filtrate was freeze dried, the residue was passed through a short reversed phase chromatography using H_2O as the eluent, freeze dried to provide the final product.

Unnatural heparosan tetrasaccharide analog 3—Using the above mentioned procedure compound **3** was obtained from compound **38**. Yield = 55%. ^1H 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): 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; $[\alpha]_{\text{D}}^{25} = +23.0$ (c 0.5, water); HRMS-FAB: $[\text{M}+\text{Na}]$ calcd. for $\text{C}_{37}\text{H}_{53}\text{N}_{11}\text{NaO}_{23}^+$ 1042.3213, found: 1042.3197.

Unnatural chondroitin tetrasaccharide analog 4—Using the above mentioned procedure compound **4** was obtained from compound **42**. Yield = 52%. ^1H 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; $[\alpha]_{\text{D}}^{25} = +38.0$ (c 0.62, water); HRMS-FAB: $[\text{M}+\text{Na}+\text{H}]$ calcd. for $\text{C}_{37}\text{H}_{53}\text{N}_{11}\text{NaO}_{23}^+$ 1043.3292, found: 1043.3244.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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$R = \text{SO}_3^-$
 $X = \text{SO}_3^-, \text{COCH}_3 \text{ or H}$ Heparan sulfate/ Heparin

chondroitin sulfate/dermatan sulfate

hyaluronan and keratan sulfate

Figure 1.

Structures of different glycosaminoglycans. Heparan sulfate contains primarily glucuronic acid while heparin contains primarily iduronic acid and *N*-sulfoglucosamine. Heparosan has the same basic structure as heparan sulfate with $R=\text{H}$, $X=\text{COCH}_3$ and chondroitin has the same basic structure as chondroitin sulfate with $R=\text{H}$.

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

Figure 3.
Retrosynthesis of heparosan disaccharide and tetrasaccharide analogs

Figure 4.
Retrosynthesis of chondroitin disaccharide and tetrasaccharide analogs

Scheme 1a.

^aReagents and Conditions: (a) Benzoyl chloride, pyridine, rt; (b) PhBCl₂, Mol Sieves powder 4Å, Et₃SiH, CH₂Cl₂, -78 °C-rt; (c) BAIB, CH₂Cl₂, H₂O, TEMPO, rt; (d) MeI, Cs₂CO₃, DMF; (e) NIS, AcOH, CH₂Cl₂, 20 °C; (f) H₂, 20% Pd(OH)₂/C, MeOH, 1N HCl or (g) H₂, 10% Pd/C, MeOH, 1N HCl; (i) *p*-TSA, MeOH, CH₂Cl₂; (j) *p*-OMe-PhCH(OMe)₂, 10-camphorsulfonic acid, 50 °C; (k) SnCl₄, TMSN₃, CH₂Cl₂, 0 °C.

Scheme 2a.

^aReagents 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) Et₃SiH, CH₂Cl₂, BF₃·Et₂O, 0 °C; (i) Ba(OH)₂, BaO, Propargyl bromide, DMF, 0 °C.

Scheme 3a.

^aReagent and conditions: (a) Ba(OH)₂, BaO, Propargyl bromide, DMF, rt; (b) Et₃SiH, CH₂Cl₂, BF₃·Et₂O, 0 °C; (c) Ac₂O, pyridine, rt; (d) (i) Tf₂O, pyridine, CH₂Cl₂, -18 °C; (ii) NaNO₂, DMF, rt.

Scheme 4a.

^a**Reagent and conditions:** (a) $\text{Sc}(\text{OTf})_3$, BnOH , DCE , $90\text{ }^\circ\text{C}$; (b) 0.5M NaOMe , MeOH , $0\text{ }^\circ\text{C}$; (c) $\text{PhCH}(\text{OMe})_2$, $p\text{-TSA}$, DMF , rt ; (d) $\text{Ba}(\text{OH})_2$, BaO , Propargyl bromide, DMF , $0\text{ }^\circ\text{C}$ - rt ; (e) $p\text{-TSA}$, MeOH , rt ; (f) Ac_2O , pyridine , rt .

Scheme 5a.

^aReagent and conditions: (a) CuI, DIPEA, CH₃CN, rt.

Scheme 6a.

^aReagents and conditions: (a) CuI, DIPEA, CH₃CN, CH₂Cl₂, rt.

Scheme 7a.

^aReagents and Conditions: (a) Propargyl trichloroacetimidate, trifluoromethane sulfonic acid, CH_2Cl_2 , Mol Sieves powder 4Å, 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 8a.

^a**Reagents and Conditions:** (a) CuI, DIPEA, CH₃CN, rt; (b) LiOOH, THF; (c) 2N NaOH, MeOH; (d) H₂, 10% Pd/C, MeOH.

Scheme 9a.

^aReagents and Conditions: (a) Propargyl trichloroacetimidate, trifluoromethane sulfonic acid, CH₂Cl₂, Mol Sieves powder 4Å, rt; (b) MOMCl, DIPEA, DMAP, CH₂Cl₂, 35 °C; (c) H₂, 10% Pd/C, MeOH/EtOAc, 1 N HCl (cat.); (d) Ac₂O, pyridine, rt; (e) SnCl₄, TMSN₃, CH₂Cl₂, 0 °C.

Scheme 10a.

^aReagents and Conditions: (a) CuI, DIPEA, CH₃CN, rt; (b) LiOOH, THF; (c) 2N NaOH, MeOH; (d) H₂, 10% Pd/C, MeOH.