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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 4337–4340

Synthesis of a *S*-linked heparan sulfate trisaccharide as the substrate mimic of heparanase

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Received 7 March 2005; revised 22 April 2005; accepted 25 April 2005

Abstract—An approach to the construction of the β-(1→4)-*S*-linkage between a glucuronic and a glucosamine unit, and then to the synthesis of a heparan sulfate trisaccharide containing such a linkage (**1**) as a nonhydrolyzable substrate mimic of heparanase was developed.

Heparan sulfate (HS) proteoglycans, occurring at cell surface and in the extracellular matrix (ECM), have been attributed significant biological functions that are generally mediated via interactions between the constituent HS fragments and various proteins.[1] Heparanase, an *endo*-β-D-glucuronidase responsible for cleavage of HS polysaccharides, thus contributes to the integrity and functional state of cell surface and ECM, and plays important roles in the control of normal and pathological processes, such as morphogenesis, tissue repair, neurite outgrowth, inflammation, autoimmunity, and tumor metastasis and angiogenesis.[2] However, only recently, the substrate specificity of heparanase has been convincingly characterized by employing purified recombinant human heparanase and a series of structurally defined HS/heparin oligosaccharides.[3,4] The minimum recognition HS backbone was deduced to be the trisaccharide: GlcN(NS,6S)-α-(1→4)-GlcUA-β-(1→4)-GlcN(NS,6S) (where GlcN, GlcUA, NS, 6S represent D-glucosamine, D-glucuronic acid, 2-*N*-sulfate, and 6-*O*-sulfate, respectively).[3] Replacing the native *O*-glycosidic linkage between the GlcUA and the GlcN unit with a *S*-linkage in the HS trisaccharide, leading to trisaccharide **1**, might provide a nonhydrolyzable substrate mimic, thus a competitive inhibitor of heparanase to facilitate the study of the function of this important enzyme. Such a *O*→*S* strategy has been successfully applied in the study of the interaction of oligosaccharides with proteins.[5] And effective approaches to the synthesis of the *S*-linked oligosaccharides have been developed.[5] However, construction of the *S*-linked oligosaccharide involving a glucuronic acid residue and the synthesis of a *S*-linked HS/heparin oligosaccharide have not been reported. Here we describe an effective approach to the synthesis of the *S*-linked HS trisaccharide **1**.

Chemical synthesis of HS/heparin oligosaccharides has been a topic of intensive research.[6] Nevertheless, in planning the synthesis of a *S*-analog, that is, trisaccharide **1**, the feasibility of two quite effective tactics for HS/heparin oligosaccharide synthesis is questionable: (1) The sulfated hydroxyl groups are used to be protected with the persistent benzyl group (Bn), which could be released cleanly via hydrogenolysis for sulfation at a later stage; while in the *S*-analog synthesis, the sulfur (or the contaminant of sulfur containing reagents) might deteriorate the catalyst for hydrogenolysis. (2) The carboxyl function on the GlcUA residue is preferably introduced after assembly of the oligosaccharide backbone via oxidation of the corresponding 6-OH groups; such an oxidation might affect the *S*-glycosidic linkage. To avoid these possible problems, we planed to use *p*-methoxybenzyl group (PMB) for protection of the hydroxyl groups requiring sulfation. Protecting the remaining hydroxyl groups with acyl groups and surrogating the 2-amino function of the GlcN residues with N_3, that are common practice in HS/heparin synthesis,[6] lead to the orthogonally protected trisaccharide **2** as the precursor to the synthesis of **1** (Scheme 1). The carboxyl function on the GlcUA residue should be introduced

Keywords: Heparan sulfate; *S*-Linked oligosaccharide; Glucuronic acid; Glucosamine; Glycosylation.
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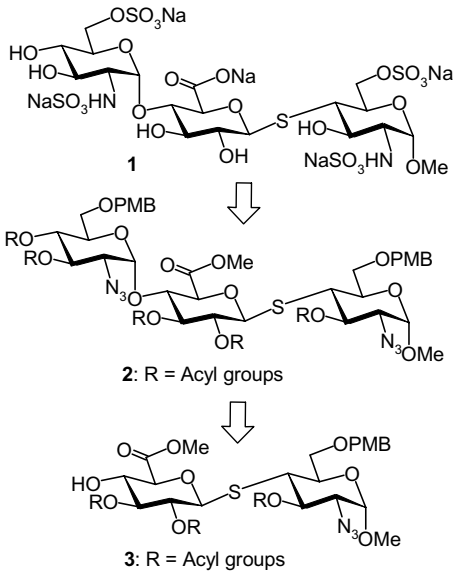
doi:10.1016/j.tetlet.2005.04.088

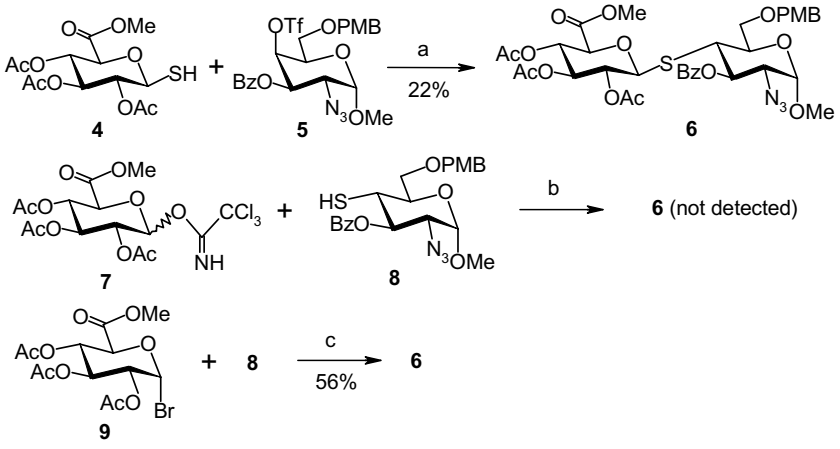


Scheme 1.

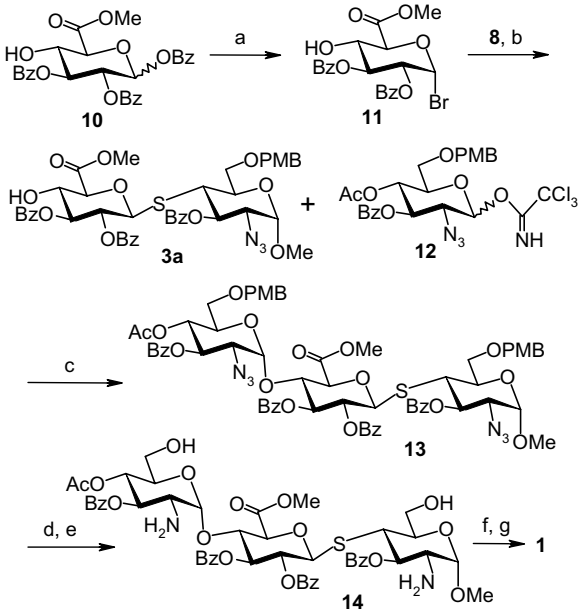
before assembly of the *S*-linkage, therefore construction of the *S*-glycosidic linkage between a GlcUA and a GlcN residue (e.g., **3**) is required. Two major strategies have been employed in assembly of the (1→4)-*S*-glycosidic linkage:[5] (1) S_N2-type displacement of a 4-*O*-leaving group (mostly a triflate) of a glycosyl acceptor with a sugar 1-thiolate or of a glycosyl halide with a sugar 4-thiolate; (2) glycosylation-type condensation between a glycosyl donor and a sugar 4-thiol. Both strategies might be problematic when applied to the synthesis involving a GlcUA residue, considering the violability of the GlcUA derivatives toward elimination under S_N2-type conditions[7] and the inertness of the GlcUA donors in glycosylation.[8] Therefore we tried a variety of conditions for the construction of the *S*-linkage between a GlcUA and a GlcN residue (Scheme 2).

Condensation of GlcUA 1-β-thiol **4**[9] with GalN 4-*O*-triflate **5** in the presence of NaH in DMF afforded the desired β-(1→4)-*S*-linked disaccharide **6** in a poor 22% yield. Comparably, coupling of thiol **4** with undec-10-enyl bromide under similar conditions gave the corresponding 1-*S*-alkylglucuronate in 30% yield.[9] Although glycosidic coupling with glycosyl trichloroacetimidates as donors has been successful in the synthesis of the *S*-glycosidic linkages,[10] glycosylation of the GlcN 4-thiol derivative **8** with GlcUA trichloroacetimidate **7**[11] under the promotion of TMSOTf or BF_3OEt_2 failed to provide the *S*-linked disaccharide **6**. Fortunately, the S_N2 displacement of the GlcUA bromide **9** with GlcN 4-thiol **8** proceeded smoothly in the presence of Cs_2CO_3,[12] affording the desired disaccharide **6** in a satisfactory 56% yield. The β-linkage was confirmed by the $J_{1',2'}$ value of 11.4 Hz (4.68 ppm, H-1′) and the upfielded C-1′ signal at 82.4 ppm.

Taking advantage of the higher nucleophilicity of the thiolate over alkyloxide, we expected to prepare the *S*-linked disaccharide bearing a free 4′-OH (i.e., **3a**) via direct condensation of a 4-OH-GlcUA 1-bromide derivative (i.e., **11**) with the 4-SH-GlcN derivative **8** (Scheme 3). Thus, treatment of methyl 1,2,3-tri-*O*-benzoyl-glucuronate **10**[13] with 33% HBr/HOAc at 0 °C provided the desired GlcUA bromide **11** in 71% yield. Meanwhile, the corresponding 4-*O*-acetylated byproduct (methyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-glucuronate 1-bromide) was isolated in 12% yield. Condensation of the GlcUA 1-α-bromide **11**, bearing a free 4-OH, with GlcN 4-SH **8** in the presence of Cs_2CO_3 in DMF afforded the desired β-(1→4)-*S*-linked disaccharide **3a** in good yield (67%). Glycosylation of the hindered 4′-OH in **3a** with 2-azido-glucopyranosyl trichloroacetimidate **12** was achieved in the presence of *tert*-butyldimethylsilyl triflate (TBSOTf, 0.25 equiv), providing the desired trisaccharide **13** in a satisfactory 74% yield. The final elaboration of the HS trisaccharide *S*-mimic **1** from the fully protected **13** was succeeded expectedly. The 6,6″-di-*O*-PMB group on trisaccharide **13** was removed cleanly with dichlorodicyanobenzoquinone (DDQ)[14] without affecting the *S*-glycosidic linkage. Subsequent reduction of the 2,2″-azido group was realized with 1,3-dithiopropanol in the presence of triethylamine in a mixture solvent of pyridine and water,[15] furnishing trisaccharide **14** in quantitative yield (for two steps). Simultaneous sulfation of the resulting 6,6″-di-OH and



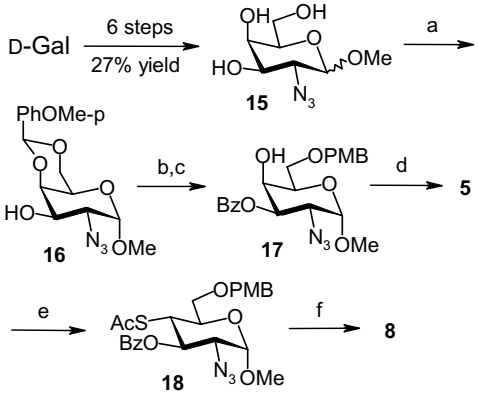
Scheme 2. Reagents and conditions: (a) NaH, DMF, rt; (b) TMSOTf or BF_3OEt_2, CH_2Cl_2, −78 °C to rt; (c) Cs_2CO_3, DMF, rt.



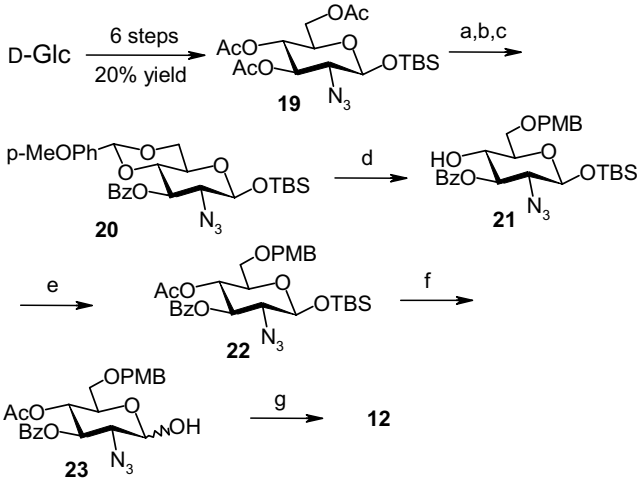
Scheme 3. Reagents and conditions: (a) 33% HBr/HOAc, 0 °C, 71%; (b) Cs_2CO_3, DMF, rt, 67%; (c) TBSOTf, 4Å MS, toluene, −20 °C, 74%; (d) DDQ, CH_2Cl_2–H_2O (10:1, v/v), rt, 99%; (e) $HS(CH_2)_3SH$, Et_3N, pyridine/H_2O, rt, 100%; (f) SO_3·pyridine, Et_3N, CH_2Cl_2, rt; (g) NaOH (2 N), rt; followed by neutralization with IR-120 [H^+], ion exchange with IR-120 [Na^+], and then desalting with G10, and lyophilization, 23%.

2,2″-di-NH_2 groups in **14** with SO_3·pyridine in the presence of triethylamine afforded the corresponding tetrasulfated trisaccharide, which was directly subjected to saponification to remove the Ac and Bz protective groups. The final product **1** was successfully obtained after ion exchange with IR-120 [Na^+] resin, desalting with G10 gel, and lyophilization.

The hither required glycosamine derivatives **5**, **8**, and **12** were conveniently prepared as depicted in Schemes 4 and 5. Methyl 2-azido-2-deoxy-galactopyranoside **15** was readily synthesized from D-galactose in six steps



Scheme 4. Reagents and conditions: (a) *p*-MeOPhCH(OMe)$_2$, *p*-TsOH, DMF, 40 °C, 33% for **16** and 48% for the β isomer; (b) BzCl, DMAP, pyridine, rt, 99%; (c) $NaBH_3CN$, TFA, 4 Å MS, DMF, 0 °C, 82%; (d) Tf_2O, DMAP, pyridine, 0 °C; (e) KSAc, DMF, 0 °C, 87%; (f) NaOMe, MeOH, rt.



Scheme 5. Reagents and conditions: (a) NaOMe/MeOH, rt; (b) *p*-MeOPhCH(OMe)$_2$, *p*-TsOH, DMF, 40 °C; (c) BzCl, DMAP, pyridine, rt, 85% (for three steps); (d) $NaBH_3CN$, TFA, 4 Å MS, DMF, 0 °C, 97%; (e) Ac_2O/pyridine, DMAP, rt, 100%; (f) 1 M TBAF in THF, THF, rt, 87%; (g) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 96%.

and in 27% overall yield employing a literature procedure.[16] Blocking the 4,6-di-OH in **15** with *p*-methoxybenzylidene led to the isolation of **16** and its β-anomer, in 33% and 48% yield, respectively. Protection of the 3-OH in **16** with a benzoyl group, followed by reductive opening of the 4,6-*O*-*p*-methoxybenzylidene acetal with a modified protocol using $NaBH_3CN$–TFA in DMF[17] led to the desired 6-*O*-PMB-galactopyranoside **17** in excellent regioselectivity (97% yield). The released axial 4-OH was then transformed into a 4-*O*-triflate (Tf) by treatment with trifluoromethanesulfonic anhydride (Tf_2O) in the presence of pyridine at 0 °C, providing **5**, which was directly used in the next substitution reaction. Thus treatment of **5** with potassium thioacetate in DMF afforded 4-*S*-acetyl-glucopyranoside **18** (in 87% yield).[18] Selective removal of the *S*-acetate, in the presence of the 3-*O*-benzoate, was achieved with NaOMe in methanol, providing 4-SH-glucopyranoside **8**, which was utilized in the subsequent transformation without purification.

3,4,6-Tri-*O*-Ac-2-azido-1-*O*-TBS-β-D-glucopyranoside **19** was readily prepared from glucose in six steps and 20% overall yield following a literature procedure.[19] Removal of the 3,4,6-tri-*O*-acetyl groups in **19**, followed by subsequent blocking of the resulting 4,6-OH with a *p*-methoxybenzylidene acetal and the 3-OH with a benzoate, provided **20** conveniently in 85% yield (three steps). Regioselective opening of the glucopyranose 4,6-*O*-*p*-methoxybenzylidene acetal with $NaBH_3CN$/TFA afforded the desired 6-*O*-PMB derivative **21** in an excellent 97% yield. The resulting 4-OH was then protected with a acetate, leading to **22**. Cleavage of the 1-*O*-TBS group with tetrabutylammonium fluoride (TBAF) in the presence of acetic acid furnished **23**, which was converted readily into the desired glycosyl trichloroacetimidate **12**.

In conclusion, the β-(1→4)-*S*-linkage between the GlcUA and the GlcN unit was successfully constructed via substitution of a GlcUA 1-α-bromide with a GlcN

4-thiolate. The synthesis of a HS trisaccharide containing such a *S*-linkage (i.e., **1**), as a nonhydrolyzable substrate mimic of heparanase, was achieved. The present synthesis represents the first synthesis of a HS/heparin oligosaccharide with a *S*-glycosidic linkage, and shall be informative for the preparation of the diverse congeners of the HS/heparin oligosaccharide *S*-mimics. Synthesis of longer congeners (of **1**) and measurement of their heparanase inhibition activities are undergoing and will be reported in due course.

Acknowledgements

Financial support from the Committee of Science and Technology of Shanghai (04DZ19213, 02QMA1401, and 03DZ19203) is gratefully acknowledged.

Supplementary data

Experimental procedures and spectroscopic data for new compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.04.088.

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