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Formal Synthesis of Disaccharide Repeating Unit (IdoA-GlcN) of Heparin and Heparan Sulfate

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A concise route to access the key disaccharide repeating unit (IdoA-GlcN) of heparin sulfate is described. The synthesis was accomplished with commercially available diacetone α-d-glucose to functional group transformations which lead to the formation of 1-iduronate donor. This 1-iduronate donor was subsequently coupled with glucosyl acceptor to form corresponding key disaccharide repeating unit (IdoA-GlcN) of heparin sulfate in good overall yield.

Introduction

Heparin sulfate (HS) is a member of glycosaminoglycans (GAGs) family which perform a variety of crucial biological functions and have been broadly employed as therapeutic agents.1 It is a complex polysaccharide that has shown influential biological activities by mediating the action of numerous proteins such as growth factors, cytokines, chemokines, viral proteins, and coagulation factors.2 It also mediates various physiologically important processes such as viral and bacterial infection, angiogenesis, tumor metastasis, cell adhesion, and lipid metabolism.3 Due to wide range of biological applications, heparin sulfate structural framework has attracted significant interest in the development of new medicines. Recently, a possible role of HS in Alzheimer’s disease and Parkinson’s disease has been also found.4 Moreover, heparin was discovered in 1916 and has been used as drugs for the treatment of thrombotic disorders for nearly 10 decades.5 The discovery of heparin contributed extensively towards development of numerous advanced medical and surgical procedures.6 Heparin, a specialized highly sulfated form of HS is not only widely used as anticoagulant but also prevent and treat arterial and venous thrombosis.6,7

Heparin (HP) and HS have similar disaccharide repeating units. HS consists of a disaccharide repeating unit of either iduronic acid (IdoA) or glucuronic acid (GlcA), and glucosamine (GlcN) residues, each of them are capable of carrying sulfate groups (Fig. 1). However, nearly 90% of the disaccharide units in HP contain IdoA, while only 20% of the disaccharide units in HS contain IdoA. HS can be isolated from many cell types, whereas heparin is an exclusive product of mast cells.8 Due to the versatile nature of heparin and heparin sulfate, the syntheses of these molecules have attracted much attention in recent years.9 Recently, Hung et al developed facile methodologies for the synthesis of heparin/HS-like oligosaccharides and then using same strategy also synthesized heparin based anticoagulant drug fondaparinux in the acquisition of 1-iduronate from diacetone α-d-glucose.10 Moreover, the availability of 1-iduronate donor (IdoA) is rare and commercially it is expensive. Therefore, development of an efficient process to synthesize 1-iduronate donor (IdoA) is the need of hours. However, among the monosaccharide units of HS, the analogue that represents IdoA and GlcN require particular attention. Herein, we report facile protocol for the synthesis of 1-iduronate donor by using diacetone α-d-glucose and its application towards formal synthesis of disaccharide repeating unit (IdoA-GlcN) of heparin sulfate by glycosylation with suitable glucosyl acceptor.

Results and discussion

Accordingly, first we proposed retrosynthetic strategy for the synthesis of disaccharide repeating unit (IdoA-GlcN) 3 is shown in Scheme 1. Disaccharide repeating unit (IdoA-GlcN) 3 could be obtained from the glycosylation reaction between imidate 4 and glucosyl acceptor 5. The adduct imidate 4 could be prepared from the 1-idose derivative 6 which would be inverting from diacetone α-d-glucose 7.

While designing the chemical synthesis of 1-iduronate donor (IdoA), a choice was made on whether the carboxyl function of the uronic acid units would be generated before or after chain assembly. In general, carboxylate group reduces the reactivity at the anomeric center during glycosylation and it also renders the C5 position more susceptible to unwanted epimerization especially when protected as an ester. Several groups demonstrated that uronic acids can function as effective glycosyl donors.11 The IdoA residue is a crucial part of
most protein binding sites in HP and HS.12 Numerous synthetic efforts were put forward for its acquisition.13 A common approach involves the chemical manipulation of the more abundant D-glucose-based compounds, which differ from L-idose only by the C5 stereochemistry. The transformation has been achieved through S2 substitution of alkyl sulfonate groups14, stereoselective hydroboration of exo-glucals15, and hydride mediated C5 inversion of the uronate derivative16. Alternatively, δ-xylidade17 and the δ-xyloaldose derived from δ-glucose18 were extended stereoselectively at C5, generating several IdoA derivatives.

Initially, we have prepared of L-iduranyl triol 6 starting from diacetone δ-glucose 7 following the reaction sequence as shown in Scheme 2. The 3-OH group of diacetone δ-glucose 7 was initially protected by benzyl group in the presence of sodium hydride in DMF, followed by usual aqueous workup and column chromatography provided the resultant product 8 in quantitative yield with expected purity.18a 5,6-O-isopropylidene group of diacetone α-L-glucose 8 was then hydrolyzed regioselectively using 75% acetic acid to provide the diol 9 in 88% yield. Oxidative cleavage of diol 9 was accomplished by sodium periodate in water furnished aldehyde 10 in quantitative yield which was pure enough to use for sequential step without any further purification.

In order to generate the pyranose ring of L-iduronic acid, we have followed the Bonnafé et al.19 procedure where bulky trisphenylthiomethane group was installed at C-5 position to afford compound 11. In this regard, first we have treated n-BuLi with trisphenylmethane, the in-situ generated trisphenylmethyl lithium was then treated with aldehyde 10 at -30 °C to afforded compound 11 in 62% yield. The synthesis of 11 was necessitate for achieving exact configuration of L-iduronyl sugar. Although for this inversion reaction of aldehyde 10 to compound 11, we have tried several reaction conditions while changing the temperature, relative equivalent and time, the best result was obtained at higher temperature (-30 °C) which provided 11 62% yield as compared with the literature report (-78 °C, 92% yield).19 The cleavage of thioether functionality was carried out using CuO with CuCl2 using a mixture of MeOH/H2O/DCM as solvent, provided compound 12 in 86% yield by retaining the methyl ester group at C-6 position. Finally acid hydrolysis of the 1,2-O-isopropylidene of 12 provided the L-iduranyl ester 6 in quantitative yield.

To obtain the disaccharide repeating unit 3 from the L-iduranyl ester 6 various synthetic steps were carried out as shown in Scheme 3. Initially, acetylation of L-idopyranose 6 was accomplished by treating acetyl chloride in the presence of pyridine and catalytic amount of DMAP at -40 °C which afforded the β-form of triacetate 13 in 87% yield. The preparation of orthoester 14 was achieved through one-pot bromination and cyclization of triacetate 13.20 In an attempt for bromination of 13 various brominating reagents such as TMSBr, TiBr4 etc. were used. When TMSBr was used, several spots were observed on TLC, we then subjected the crude for cyclization using 2,4,6-collidine in methanol solution but we could not obtained the expected orthoester 14. However, when TiBr4 was used, provided the anomeric bromination of triacetate 13 as sole product in TLC observation (RF = 0.4, EtOAc/Hexane: 1:2) which on subsequent treatment with 2,4,6-collidine in methanol provided orthoester 14 in overall 64% yield for two steps. De-acetylation of orthoester 14 was achieved using 0.5 N NaOMe in MeOH at 0 °C which delivered 4-hydroxy compound 15 in 51% yields.

Scheme 1. Retrosynthetic plan for disaccharide repeating unit (IdoA-GlcN) 3.
the formation of olefin as side product (\( R_1 = 0.4 \), EtOAc/Hexane: 1/2) via removal of acidic C-5 proton followed by removal of C-4 hydroxyl group. Due to requirement of free C-4 OH group in l-iduronic acid, disaccharide 3 was further elongation of chain assembly,3 we installed the temporary protecting chloroacetyl group at C-4 OH of 1-iduronic acid disaccharide 3 to provide access to other structurally related analogues for disaccharide repeating unit (IdoA1GlcN) of heparin sulfate in 1/3 ratio of IdoA-GlcN 3 of heparin sulfate. However, chloroacetyl group could be achieved using weak basic conditions without disturbing other acetates in the disaccharide disaccharide 3.5 Thus, compound 5 was obtained from chloroacetyl chloride group at C-4 OH in the presence of pyridine, provided fully protected iduronic compound 6 in 89% yield. Next, we cleaved the orthoester group under acidic conditions to furnish a hemiacetal compound 7 in 94% yield. The 1-iduronic imidate 4 was generated by treating the hemiacetal 7 with trichloroacetonitrile under basic conditions. This well-developed concise strategy was then successfully applied for the preparation of disaccharide repeating unit (IdoA-GlcN) 3 of heparin sulfate. However, 1-iduronic imidate donor 4 was glycosylated with glucosyl acceptor 5 in the presence of TMSOTf, provided the key disaccharide repeating unit (IdoA-GlcN) 3 of heparin sulfate in 1/3 ratio of \( \alpha / \beta \) mixture in respected yield.

**Conclusions**

We have accomplished formal synthesis of disaccharide repeating unit (IdoA-GlcN) 3 of heparin sulfate starting from diacetone \( \alpha / \beta \)-glucose with glucosyl acceptor through simple synthetic route. Use of chloroacetyl group at C-4 OH of 1-iduronic moiety could provide direct access to the chain elongation on disaccharide 3 to furnish trisaccharide moiety by coupling with appropriate donor. Our strategy disclosed the simple route for the synthesis of key disaccharide repeating unit (IdoA-GlcN) of heparin sulfate and it is expected to provide access to other structurally related analogues for exploring their biological activities.

**Experimental section**

**General Information**

Some reactions were conducted in flame-dried glassware, under nitrogen atmosphere. Dichloromethane, tetrahydrofuran, toluene, methanol, and \( N,N \)-dimethylformamide were purified and dried from a safe purification system containing activated Al\( _2 \)O\( _3 \). All reagents obtained from commercial sources were used without purification, unless otherwise mentioned. Flash column chromatography was carried out on Silica Gel 60 (230-400 mesh, E. Merck). TLC was performed on pre-coated glass plates of Silica Gel 60 F254 (0.25 mm). TLC was obtained from commercial sources were used without purification, unless otherwise mentioned. Flash column chromatography (EtOAc/Hexane, 1:3 to 1:1) afforded 8 (20.21 g, 82.23 mmol) in quantitative yield as colourless oil. The reaction mixture was cooled to 0 °C, 0.85 mmol) was added to the reaction mixture and stirred for 5 min. The reaction mixture was cooled to 0 °C, and reaction mixture was stirred for 2 hours at room temperature (24 °C). The reaction was quenched with IPA (6 mL) at 0 °C followed by slow addition of \( H_2O (115 \text{ mL}) \) at 0 °C. The resulting mixture was extracted with EtOAc (100 mL \( \times 3 \)). The organic phase was concentrated under reduced pressure, dried over MgSO\( _4 \), filtered, concentrated, and purified by flash column chromatography (EtOAc/Hexane, 0.85:1.5:1) to afford 9 (32.23 g, 82.23 mmol) in quantitative yield as colourless oil.

**3-O-Benzyl-1,2,5,6-O-di-isopropylidene-\( \alpha / \beta \)-glucosuranate (8).** Commercial available diacetone \( \alpha / \beta \)-glucose 7 (15 g, 57.69 mmol) was dissolved in DMF (144 mL), benzyl bromide (10.09 mL, 86.53 mmol) was added to the reaction mixture and stirred for 5 min. The reaction mixture was cooled to 0 °C, 0.85 mmol) was added to the reaction mixture and stirred for 5 min. The reaction mixture was cooled to 0 °C, and reaction mixture was stirred for 2 hours at room temperature (24 °C). The reaction was quenched with IPA (6 mL) at 0 °C followed by slow addition of \( H_2O (115 \text{ mL}) \) at 0 °C. The resulting mixture was extracted with EtOAc (100 mL \( \times 3 \)). The organic phase was concentrated under reduced pressure, dried over MgSO\( _4 \), filtered, concentrated, and purified by flash column chromatography (EtOAc/Hexane, 0.85:1.5:1) to afford 9 (32.23 g, 82.23 mmol) in quantitative yield as colourless oil.
Methyl 3-O-benzyl-1,2,3-O-isopropylidene-α-L-idofuranosyluronate (12). Methanol (114 mL), CuO (730 mg, 9.19 mmol), CuCl (2.76 g, 20.55 mmol), and water (10 mL) were successively added to a solution of 11 (3.34 g, 5.41 mmol) in CHCl₃ (10 mL). The reaction mixture was vigorously shaken for 2 h, filtered through a Celite 545 and concentrated without warming above 30 °C. The residue was dissolved in CHCl₃ (50 mL), and water (50 mL) was added, giving a Cu salt precipitate that was eliminated by filtration through a Celite 545. After decantation, the aqueous layer was extracted with CHCl₃ (50 mL × 2). The combined organic layers were washed with a satd. aqueous NaHCO₃ solution (20 mL) and water (20 mL), filtered through a silicon-treated filter, and concentrated. Flash chromatography of the residue (EtOAc/Hexane, 1:3) gave 12 as colourless oil (1.96 g, 86%).  It was purified by flash chromatography (EtOAc/Hexane = 1/2; [α]D = -24.3 (c 1.0, CHCl₃); IR (KBr) ν 3570, 3030, 1762, 1737, 1511, 1441 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.37–7.30 (m, 5H, Ph), 5.18–5.17 (m, 1H, H₁₄), 4.79 (d, J = 11.4 Hz, 1H, C₁₅), 4.67 (d, J = 4.2 Hz, 1H, C₁₂), 4.54 (d, J = 2.4 Hz, 1H, C₁₁), 4.52 (d, J = 4.2 Hz, 1H, C₂), 4.21 (d, J = 12.0 Hz, 1H, C₁₆), 4.24 (d, J = 3.6 Hz, 1H, C₁₅), 4.02 (s, 3H, CH₃), 3.96 (t, J = 3.0 Hz, 1H, C₁₁), 3.90 (s, 1H, H₁₂), 3.76 (s, 3H, COOCH₃) ppm; ¹³C NMR (150 MHz, CDCl₃) δ 171.7 (C), 136.6 (C), 128.3 (CH₂), 127.9 (CH₂ × 2), 127.2 (CH₃), 71.7 (CH₃), 71.2 (CH₂), 26.6 (CH₃) ppm. HRMS (M+Na⁺) calcd for C₁₉H₂₀O₇Na₃ 361.14606, found: 361.14616.14616.

Methyl 4-O-acetyl-3-O-benzyl-1,2,3-O-isopropylidene-α-L-idofuranosyluronate (13). Compound 12 (1.2 g, 3.54 mmol) was dissolved in a mixture of trifluoroacetic acid (6.66 mL) and water (720 μL). After 20 min stirring at room temperature (24 °C) the solvents were evaporated and the resulting solution was evaporated with water (5 mL × 3). The residue was crystallized from EtOAc, to which the minimum pyridine necessary to reach neutrality had been added, giving a quantitative yield of 0.73 g (91%) as white solid. Rf = 0.1 (EtOAc/Hexane = 1/1); [α]D = -23.8 (c 1.0, CHCl₃); IR (KBr) ν 3573, 3033, 2954, 1742, 1445 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.37–7.30 (m, 5H, Ph), 5.07 (s, 1H, H₁₂), 4.62 (s, 2H, CH₂Ph), 4.56 (s, 1H, H₁₅), 4.02 (s, 1H, H₁₄), 4.02 (s, 1H, H₁₄), 3.96 (t, J = 3.0 Hz, 1H, C₁₁), 3.90 (s, 1H, H₁₂), 3.76 (s, 3H, COOCH₃) ppm; ¹³C NMR (150 MHz, CDCl₃) δ 170.4 (C), 136.7 (C), 128.4 (CH × 3), 128.3 (CH × 5), 127.8 (CH), 127.6 (CH × 2), 127.3 (CH), 72.8 (CH₂), 72.2 (CH₂), 26.6 (CH₃) ppm. HRMS (M+Na⁺) calcd for C₁₉H₂₀O₇Na₃ 361.1258, found: 361.1255.

Methyl 3-O-benzyl-1,2,3-O-isopropylidene-α-L-idofuranosyluronate (6). Compound 12 (1.2 g, 3.54 mmol) was dissolved in a mixture of trifluoroacetic acid (6.66 mL) and water (720 μL). After 20 min stirring at room temperature (24 °C) the solvents were evaporated and the resulting solution was evaporated with water (5 mL × 3). The residue was crystallized from EtOAc, to which the minimum pyridine necessary to reach neutrality had been added, giving a quantitative yield of 0.73 g (91%) as white solid. Rf = 0.1 (EtOAc/Hexane = 1/1); [α]D = -24.3 (c 1.0, CHCl₃); IR (KBr) ν 3573, 3033, 2954, 1742, 1445 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.37–7.30 (m, 5H, Ph), 5.07 (s, 1H, H₁₂), 4.62 (s, 2H, CH₂Ph), 4.56 (s, 1H, H₁₅), 4.02 (s, 1H, H₁₄), 4.02 (s, 1H, H₁₄), 3.96 (t, J = 3.0 Hz, 1H, C₁₁), 3.90 (s, 1H, H₁₂), 3.76 (s, 3H, COOCH₃) ppm; ¹³C NMR (150 MHz, CDCl₃) δ 170.4 (C), 136.7 (C), 128.4 (CH × 3), 128.3 (CH × 5), 127.8 (CH), 127.6 (CH × 2), 127.3 (CH), 72.8 (CH₂), 72.2 (CH₂), 26.6 (CH₃) ppm. HRMS (M+Na⁺) calcd for C₁₉H₂₀O₇Na₃ 361.1258, found: 361.1255.
Methyl 3-O-benzyl-4-O-chloroacetyl-β-L-idopyranuronate 1,2- (methylthioacetate) (16). DCM (15 mL) and compound 15 (374 mg, 1.05 mmol) were charged into a round bottom flask under nitrogen atmosphere then cooled to 0 °C. Pyridine (422 μL, 5.25 mmol) was subsequently added into the reaction mixture then cooled to -20 °C. A solution of chloroacetic chloride (333 μL, 4.2 mmol) was slowly charged into the reaction mixture at -20 °C. After stirring the reaction mixture for 12 hrs, reaction mass was diluted with DCM (10 mL) and quenched into cold water (10 mL). The organic and aqueous layers were separated and the organic layer was washed with NaHCO₃ solution and dried over magnesium sulfate. After evaporation, the residue was purified in a silica gel column using the solvent system 20:80:1 (EA/Hex/TEA) to afford 16 as a faint yellow solid (401 mg, 89%). R<sub>f</sub> = 0.4 (EtOAc/Hexane = 1/2); mp = 120-121 °C; IR (KBr) v 3607, 3089, 3002, 1752, 1497, 1329 cm<sup>-1</sup>; ¹H NMR (600 MHz, CDCl₃) δ 7.39-7.32 (m, 5H, Ph), 5.56 (d, J = 2.4 Hz, 1H, H₁), 5.24 (t, J = 2.4 Hz, 1H, H₄), 4.67 (AB system, J = 12.0 Hz, 2H, Ph), 4.57 (d, J = 1.2 Hz, 1H, H₅), 4.15 (t, J = 2.4 Hz, 1H, H₃), 4.07 (s, 1H, H₂), 3.69-3.65 (m, 2H, C₂H₂COClO₃); ³C NMR (150 MHz, CDCl₃) δ 167.6 (C), 166.6 (C), 136.5 (C), 128.6 (CH × 2), 128.4 (CH), 128.0 (CH = 2), 124.1 (CH), 96.5 (CH), 75.8 (CH), 73.0 (CH), 71.0 (CH), 69.3 (CH), 68.3 (CH), 52.7 (CH₂), 49.2 (CH₃), 40.4 (CH₃), 24.9 (CH₃) ppm. HRMS (M+Na) calcd for C₁₉H₁₆O₄Na⁺: 343.0923, found: 343.0941.

Methyl 2-O-acetyl-3-O-benzyl-4-O-(chloroacetyl)-2-O-acetyl-1,2- (methylthioacetate) (17). Compound 16 (100 mg) was dissolved in the solution of AcOH/H₂O (2 mL, 9:1) and stirred for 30 min at 28 °C. After evaporation, the residue was purified in a silica gel column using the solvent system 1:3 (EtOAc/Toluene) to afford product 17 (90 mg, 94%) as colourless oil (β/α mixture). R<sub>f</sub> = 0.2 (EtOAc/Hexane = 1/2). IR (KBr) v 3607, 3089, 3002, 1752, 1497, 1329 cm<sup>-1</sup>; ¹H NMR (600 MHz, CDCl₃) δ 7.38-7.32 (m, 5H, Ph), 5.31 (d, J = 7.2 Hz, 1H, H₁-B), 5.26 (t, J = 2.4 Hz, 1H, H₂-a), 5.19 (dd, J = 2.0 Hz, 1H, H₃-a), 5.03 (d, J = 1.8 Hz, 1H, H₄-a), 4.92-4.91 (m, 0.6 Hz, H₅-a), 4.83 (m, 1H, H₅-B), 4.77-4.75 (m, 1H, CH₂Ph), 4.74 (s, 0.6 H, H₄-B), 4.39 (d, J = 8.4 Hz, 1H, H₂-B), 4.03-3.93 (m, 2H, H₃-B, CH₂OAc), 3.97 (d, J = 1.8 Hz, 1H, CH₂Ph), 3.95 (d, J = 1.2 Hz, 1H, H₂, 0.6H, H₃-B), 3.94-3.92 (dt, J = 4.2, 3.0 Hz, 1H, H₁-oa0), 3.77-3.76 (m, 3H, 2 x C₂H₂OAc, 2.11 (s, 3H, CH₃OAc), 2.05 (s, 3H, CH₃OAc); ³C NMR (150 MHz, CDCl₃) δ 170.1 (C), 169.6 (C), 168.2 (C), 167.3 (C), 166.3 (C), 136.4 (C), 128.6 (CH × 2), 128.5 (CH), 128.0 (CH × 2), 127.8 (C), 92.8 (CH), 91.9 (CH), 74.3 (CH₃), 73.1 (CH₃), 72.6 (CH₂), 71.7 (CH), 68.5 (CH), 68.5 (CH), 67.6 (CH), 67.6 (CH), 56.3 (CH₂), 52.6 (CH), 40.2 (CH₂OAc), 40.2 (CH₂OAc) ppm. HRMS (M+Na) calcd for C₁₉H₁₇O₅NaClO₃ 439.0766, found: 439.0783.

Methyl 2-O-acetyl-3-O-benzyl-4-O-(chloroacetyl)-2-O-acetyl-1,2- (methylthioacetate) trichloroacetimide (4). Trichloroacetanilide (175 μL, 1.72 mmol) and K₂CO₃ (166 mg, 1.15 mmol) were added to a solution of 17 (96 mg, 0.23 mmol) in CH₂Cl₂ (4 mL). After stirring for 12 hours at room temperature (27 °C), the reaction mixture was quenched with water and NaHCO₃ and dried over MgSO₄, filtered, and concentrated to give crude residue which was purified by column chromatography [EtOAc/Hexane (1:3 to 1:1) + 1% NEt₃] to afford 4 as 1:1 α/β mixture (80 mg, 62%). R<sub>f</sub> = 0.6 (EtOAc/Hexane = 1/2). The imidate 19 were used directly in the next step.


