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

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th December 2014,
Accepted 00th December 2014

DOI: 10.1039/x0xx00000x

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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 L-iduronate donor. This L-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.[1b,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 L-iduronate from diacetone D-glucose.[10] Moreover, the availability of L-iduronate donor (IdoA) is rare and commercially it is expensive. Therefore, development of an efficient process to synthesize L-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 L-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.

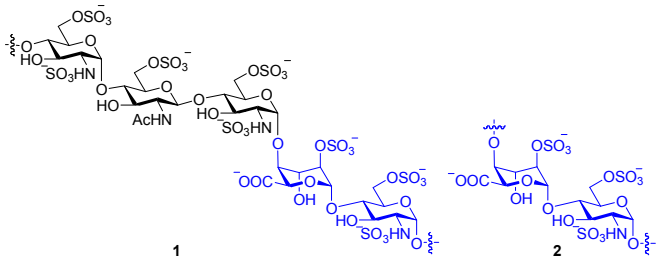

Figure 1. Structure of Heparin Sulfate **1** and it's disaccharide repeating unit (IdoA-GlcN) **2**.

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 L-idose derivative **6** which would be inverting from diacetone D-glucose **7**.

While designing the chemical synthesis of L-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

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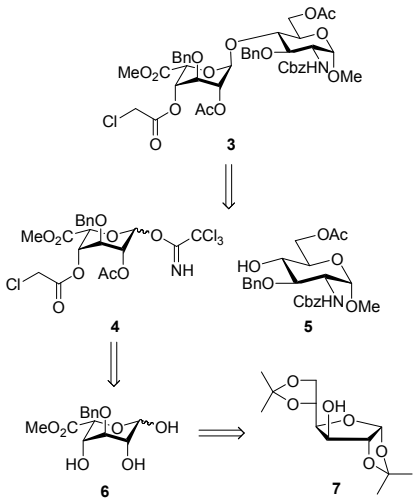
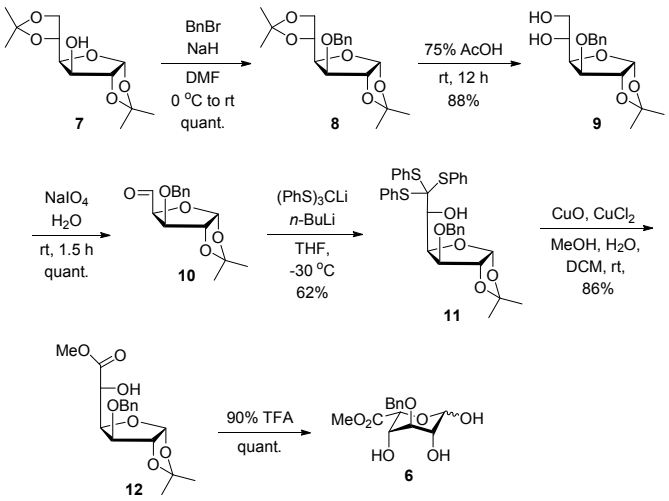


Scheme 1. Retrosynthetic plan for disaccharide repeating unit (IdoA-GlcN) **3**.



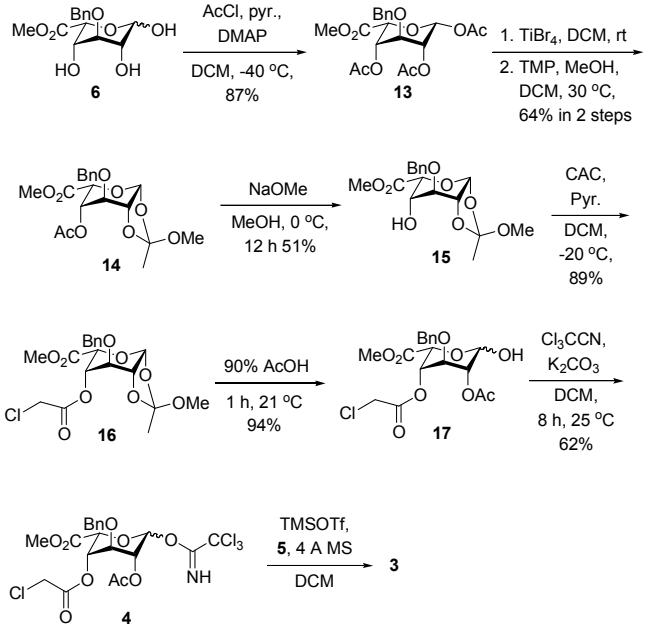
Scheme 2. Preparation of L-idopyranose derivative **6**.

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 S_N2 substitution of alkyl sulfonate groups[14], stereoselective hydroboration of *exo*-glucals[15], and hydride mediated C5 inversion of the uronate derivative[16]. Alternatively, D-xylose[17] and the D-xylodialdose derived from D-glucose[18] were extended stereoselectively at C5, generating several IdoA derivatives.

Initially, we have prepared of L-iduranyl triol **6** starting from diacetone D-glucose **7** following the reaction sequence as shown in Scheme 2. The 3-OH group of diacetone D-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-D-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 Bonnaffé *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 trisphenylthiomethane, the *in-situ* generated trisphenylthiomethyl 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 $CuCl_2$ using a mixture of MeOH/H_2O/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, $TiBr_4$ *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 $TiBr_4$ was used, provided the anomeric bromination of triacetate **13** as sole product in TLC observation (R_f = 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 3. Formal synthesis of disaccharide repeating unit (IdoA-GlcN) 3 of Heparin sulfate.

Our efforts to enhance the yield of **15** were unsuccessful even after using different reaction conditions. The lower yield was attributed to

the formation of olefin as side product (R_f = 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-iduronate of disaccharide **3** for further elongation of chain assembly,[9] we installed the temporary protecting chloroacetyl group at C-4 OH of L-iduronate donor. However, deprotection of chloroacetyl group could be achieved using weak basic conditions without disturbing other acetates in the disaccharide **3**.[21] Thus, compound **15** was masked with chloroacetyl chloride group at C-4 OH in the presence of pyridine, provided fully protected iduronyl compound **16** in 89% yield. Next, we have cleaved the orthoester group under acidic conditions to furnish a hemiacetal compound **17** in 94% yield. The L-iduronate imidate **4** was generated by treating the hemiacetal **17** 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, L-iduronate imidate donor **4** was glycosylated with glucosyl acceptor 5[22] in the presence of TMSOTf, provided the key disaccharide repeating unit (IdoA-GlcN) **3** of heparin sulfate in 1/3 ratio of α/β mixture in respected yield.

Conclusion

We have accomplished formal synthesis of disaccharide repeating unit (IdoA-GlcN) **3** of heparin sulfate starting from diacetone α-D-glucose with glucosyl acceptor through simple synthetic route. Use of chloroacetyl group at C-4 OH of L-iduronate 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*-dimethyformamide were purified and dried from a safe purification system containing activated Al_2O_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 (0.25mm, E. Merck); detection was executed by spraying with a solution of $Ce(NH_4)_2(NO_3)_6$ (0.5 g), $(NH_4)_6Mo_7O_{24}$ (24 g) and H_2SO_4 (28 mL) in water (500 mL) and subsequent heating on a hot plate. Optical rotations were measured at 589 nm (Na) at ~ 27 °C. ^{1}H, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, and ^{1}H-^{1}H NOESY spectra were recorded with 400 and 600 MHz instruments. Chemical shifts are in ppm from Me_4Si, generated from the $CDCl_3$ lock signal at δ 7.26 ppm. IR spectra were taken with a FT-IR spectrometer using KBr plates. Mass spectra were analyzed on a Finnigan LTQ-OrbitrapxL instrument with an ESI source.

3-*O*-Benzyl-1,2:5,6-*O*-di-isopropylidene-α-D-glucofuranose (8). Commercial available diacetone D-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. NaH (3.75 g, 95.18 mmol) was added in portions (1.25 g × 3) to a cooled solution (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 mL) at 0 °C. The resulting mixture was extracted with EtOAc (100 mL × 3). The organic phase was concentrated under reduced pressure, dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (EtOAc/Hexane, 1:3 to 1:1) to afford **8** (20.21 g) in quantitative yield as colourless oil. R_f = 0.6 (EtOAc/Hexane = 1/2); $[\alpha]^{24}_D$ = -0.3 (*c* 1.0, CH_2Cl_2); IR (KBr) *v* 3050, 3018, 1467, 1394 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35–7.27 (m, 5H, Ph), 5.89 (d, *J* = 4.2 Hz, 1H, H-1), 4.69-4.63 (dd, *J* = 11.4 Hz, 2H, CH_2Ph), 4.58 (d, *J* = 4.2 Hz, 1H, H-2), 4.38-4.35 (m, 1H, H-4), 4.15 (dd, *J* = 7.8, 3.0 Hz, 1H, H-5), 4.11 (dd, *J* = 8.4, 6.0 Hz, 1H, CH_2), 4.24 (d, *J* = 3.0 Hz, 1H, H-3), 4.00 (dd, *J* = 8.4, 6.0 Hz, 1H, CH_2), 1.49 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.31 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6 (CH), 128.3 (CH × 2), 127.8 (CH), 127.6 (CH × 2), 11.7 (C), 108.9 (C), 105.2 (CH), 82.6 (CH), 81.6 (CH), 81.2 (CH), 72.5 (CH), 72.3 (CH_2), 67.3 (CH_2), 26.8 (CH_3), 26.7 (CH_3), 26.2 (CH_3), 25.4 (CH_3) ppm. HRMS (M+Na$^+$) calcd for $C_{19}H_{26}O_6Na$ 373.1622, found: 373.1620.

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (9). Acetic acid (70 mL) and water (30 mL) were added to the **8** (10 g, 28.23 mmol) in a round bottom flask. After the reaction mixture was stirred at 55 °C for 3 hours, the combined layers were concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/Hexane, 1:3 to 1:1) to afford the **9** (7.83 g, 88%) as colourless oil. R_f = 0.2 (EtOAc/Hexane = 1/2); $[\alpha]^{24}_D$ = -1.6 (*c* 1.0, CH_2Cl_2); IR (KBr) *v* 3510, 3079, 1487, 1444 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35–7.28 (m, 5H, Ph), 5.90 (d, *J* = 3.6 Hz, 1H, H-1), 4.69 (dd, *J* = 11.4 Hz, 1H, H-7'), 4.60 (d, *J* = 3.6 Hz, 1H, H-2), 4.57 (d, *J* = 11.4 Hz, 1H, H-7'), 4.18-4.11 (m, 2H, H-4, H-5), 4.02-3.99 (m, 1H, H-3), 3.78 (dd, *J* = 11.4 Hz, 1H, CH_2Ph), 3.66 (dd, *J* = 11.4 Hz, 1H, CH_2Ph), 2.92 (m, 2H, 2-OH), 1.46 (s, 3H, CH_3), 1.29 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.2 (C), 128.3 (CH × 2), 127.6 (CH), 127.5 (CH × 2), 111.4 (C), 104.8 (CH), 81.9 (CH), 81.6 (CH), 79.7 (CH), 71.9 (CH_2), 68.7 (CH), 64.0 (CH_2), 26.4 (CH_3), 25.9 (CH_3) ppm; HRMS (M+Na$^+$) calcd for $C_{16}H_{22}O_6Na$ 333.1309, found: 333.1308.

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-dialdose (10). The **9** (0.43 g) was dissolved in water (3 mL), DCM (3 mL) and $NaIO_4$ (0.63 g, 2.13 mmol) were added to the reaction mixture portion wise over 20 min. The stirring was continued at 30 °C for 1.5 h and EtOH (5 mL) was added. Salts were filtered off, washed with water, and the filtrate was extracted with DCM (10 mL × 3) and dissolved in Et_2O. The resulting residue was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated to give **10** as colourless syrup (0.36 g, 95%) the ^{1}H and ^{13}C data for which were identical to those previously reported.[18a] This was used in the next step without further purification.

Tris(thiophenyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-β-L-orthoido-furanuronate (11). A solution of tris(phenylthio)methane (289 mg, 0.85 mmol) in anhydrous THF (2 mL) was cooled to -30 °C and *n*-BuLi (531 µL, 0.85 mmol) was added slowly. The mixture was kept stirring at -30 °C for 1 h. Then a solution of aldehyde **10** (200 mg, 0.71 mmol) in anhydrous THF (2 mL) was injected into the reaction mixture at -30 °C. The resulting mixture was stirred at -30 °C for 2 h and the reaction was quenched with NH_4Cl solution (2 mL). The aqueous phase was extracted with EtOAc (10 mL × 3); the combined organic phases were washed with brine (2 mL), dried over anhydrous $MgSO_4$, filtered, concentrated, and purified by column chromatography (EtOAc/Hex, 1:3 to 1:1) to give product **11** (270 mg) in 62% yield as white solid. R_f = 0.4 (EtOAc/Hexane = 1/2); mp

= 101-103 °C; IR (KBr) *v* 3525, 3059, 1735, 1472 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.67–7.66 (m, 6H, Ph), 7.35–7.23 (m, 12H, Ph), 7.07–7.06 (m, 2H, Ph), 6.00 (d, *J* = 3.6 Hz, 1H, H-1), 4.81 (t, *J* = 3.0, 2.4 Hz, 1H, H-4), 4.52 (d, *J* = 4.2 Hz, 1H, H-2), 4.50 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.24 (d, *J* = 3.6 Hz, 1H, H-5), 4.21 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 3.60 (d, *J* = 3.6 Hz, 1H, H-3), 3.25 (s, 1H, OH), 1.48 (s, 3H, CH_3), 1.31 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) 136.9 (C), 136.6 (CH × 6), 131.3 (C × 2), 129.1 (CH × 4), 128.43 (CH × 3), 128.39 (CH × 5), 127.8 (CH), 127.6 (CH × 2), 112.2 (C), 104.9 (C), 83.1 (CH), 81.9 (CH), 79.9 (C), 77.7 (CH), 76.7 (CH), 72.8 (CH), 71.7 (CH_2), 27.2 (CH_3), 26.6 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{34}H_{34}O_5NaS_3$ 641.14606, found: 641.14616.

Methyl 3-*O*-benzyl-1,2-*O*-isopropylidene-α-L-idofuranosyluronate (12). Methanol (114 mL), CuO (730 mg, 9.19 mmol), $CuCl_2$ (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 CH_2Cl_2 (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 CH_2Cl_2 (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 CH_2Cl_2 (50 mL × 2). The combined organic layers were washed with a satd. aqueous $NaHCO_3$ solution (20 mL) and water (20 mL), filtered through a silicon-treated filter, and concentrated. Flash chromatography of the residue (EtOAc/Hex, 1:3 to 1:1) gave **12** as colourless oil (1.56 g, 86%). R_f = 0.3 (EtOAc/Hexane = 1/3); $[\alpha]^{28}_D$ = -24.3 (*c* 1.0, CH_2Cl_2); IR (KBr) *v* 3525, 3062, 1735, 1497, 1376 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.37-7.30 (m, 5H, Ph), 6.00 (d, *J* = 3.6 Hz, 1H, H-1), 4.71 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.67 (d, *J* = 4.2 Hz, 1H, H-2), 4.54-4.50 (m, 3H, H-5, H-4, CH_2Ph), 4.18 (d, *J* = 4.2 Hz, 1H, H-3), 3.72 (s, 3H, $COOCH_3$), 3.43 (s, 1H, 5-OH), 1.47 (s, 3H, CH_3), 1.33 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.7 (C), 136.6 (C), 128.3 (CH × 2), 127.9 (CH), 127.7 (CH × 2), 112.2 (C), 105.0 (CH), 82.8 (CH), 80.1 (CH), 72.1 (CH_2), 69.6 (CH), 53.3 (CH), 52.4 (CH), 26.8 (CH_3), 26.4 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{17}H_{22}O_7Na$ 361.1258, found: 361.1255.

Methyl 3-*O*-benzyl-L-idopyranosyluronate (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 coevaporated with water (5 mL × 3). The residue was crystallized from EtOAc, to which the minimum pyridine necessary to reach neutrality has been added, giving a quantitative yield of **6** (1.05 g) as white solid. R_f = 0.1 (EtOAc/Hexane = 1/1); mp = 111-112 °C. IR (KBr) *v* 3573, 3033, 2954, 1742, 1445 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ7.37-7.28 (m, 5H, Ph), 5.07 (s, 1H, H-1), 4.62 (s, 2H, CH_2Ph), 4.56 (s, 1H, H-5), 4.02 (s, 1H, H-4), 3.96 (t, *J* = 3.0 Hz, 1H, H-3), 3.90 (s, 1H, H-2), 3.76 (s, 3H, $COOCH_3$) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4 (C), 137.2 (CH), 128.4 (CH × 2), 128.0 (CH), 127.5 (CH × 2), 93.1 (CH), 75.5 (CH), 74.3 (CH), 72.2 (CH_2), 68.1 (CH), 67.5 (CH), 52.6 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{14}H_{18}O_7Na$ 321.0945, found: 321.0948.

Methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-β-L-idofuranoate (13). *N,N*-Dimethylaminopyridine (156 mg, 1.28 mmol), pyridine (10.34 mL, 128.5 mmol) and acetyl chloride (5.48 mL, 77.1 mmol) were added to a cooled (-40°C) suspension of crystalline **6** (3.83 g, 12.85 mmol) in CH_2Cl_2 (80 mL). After 10 h stirring at this temperature, the mixture was diluted with dichloromethane (150 mL) and the resulting organic phase was washed with saturated $NaHCO_3$ solution (50 mL × 3), water (50 mL × 2), 10 % H_2SO_4 (50 mL × 3), and water (50 mL × 3), filtered through a phase separator filter, and concentrated. The residue was purified by flash chromatography (EtOAc/Hex, 1:3 to 1:1) gave **13** (4.78 g, 87%) as white solid. R_f = 0.5 (EtOAc/ Hexane = 1/2); mp = 111-113 °C; IR (KBr) *v* 3692, 3570, 2955, 1750, 1443, 1373, 1227, 1145 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.38-7.30 (m, 5H, Ph), 6.00 (d, *J* = 1.8 Hz, 1H, H-1), 5.14 (dd, *J* =4.2, 2.4 Hz, 1H, H-4), 5.02 (dd, *J* =2.4, 1.8 Hz, 1H, H-2), 4.78 (d, *J* = 2.4 Hz, 1H, H-5), 4.76-4.71 (dd, *J* = 11.4 Hz, 2H, CH_2Ph), 3.95 (t, *J* = 3.0 Hz, 1H, H-3), 3.76 (s, 3H, CO_2Me), 2.10 (bs, 9H, Ac) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) 169.7 (C), 169.6 (C), 168.4 (C), 167.2 (C), 136.5 (C), 128.5 (CH × 2), 128.2 (CH), 127.8 (CH × 2), 89.8 (CH), 73.2 (CH), 72.9 (CH_2), 72.7 (CH), 67.0 (CH), 65.0 (CH), 52.5 (CH), 20.7 (CH_3), 20.7 (CH_3), 20.5 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{20}H_{24}O_{10}Na$ 447.1262, found: 447.1276.

Methyl 4-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate-1,2-(methyl-orthoacetate) (14). $TiBr_4$ (106 mg, 0.23 mmol) was added to a solution of **13** (100 mg, 0.18 mmol) in CH_2Cl_2 (2 mL). The resulting mixture was stirred at room temperature (30 °C) for 2 h and then diluted with CH_2Cl_2 (10 mL), and washed with ice-cold water (5 mL). The organic layer was filtered through a Celite 545 pad, and the filtrate was filtered through a phase silicon-treated filtered, concentrated, giving methyl 1-bromo-2,4-di-*O*-acetyl-3-*O*-benzyl-L-idofuranoate. Then, a solution of methyl 1-bromo-2,4-di-*O*-acetyl-3-*O*-benzyl-L-idofuranoate in anhydrous DCM (2 mL) containing freshly distilled 2,4,6-trimethyl pyridine (150 μL) and methyl alcohol (140 μL) was stirred for 2 days at room temperature (30 °C). The mixture was diluted with dichloromethane (10 mL) and the resulting organic phase was washed with saturated $NaHCO_3$ solution (2 mL), filtered, and concentrated. The residue was purified by flash chromatography (EtOAc/Hexane, 1:3 to 1:1) gave **14** (58 mg, 64% in two steps) as light yellow oil. R_f = 0.5 (EtOAc/Hexane = 1/2); $[\alpha]^{28}_D$ = -2.0 (*c* 1.0, CH_2Cl_2); IR (KBr) *v* 3552, 3031, 1763, 1472, 1505, 1440 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.37-7.30 (m, 5H, Ph), 5.55 (d, *J* = 3.0 Hz, 1H, H-1), 5.18-5.17 (m, 1H, H-4), 4.79 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.67 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.54 (d, 1H, H-5), 4.12 (t, *J* = 2.4, 1.8 Hz, 1H, H-3), 4.07-4.06 (m, 1H, H-2), 3.77 (s, 3H, CO_2CH_3), 3.23 (s, 3H, OCH_3), 2.02 (s, 3H, $OCOCH_3$), 1.72 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0 (C), 168.0 (C), 136.7 (C), 128.5 (CH × 2), 128.2 (CH), 127.9 (CH × 2), 124.1 (C), 96.5 (CH), 76.0 (CH), 72.8 (CH_2), 71.2 (CH), 69.5 (CH), 66.7 (CH), 52.5 (CH_3), 49.0 (CH_3), 24.9 (CH_3), 20.6 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{19}H_{24}O_9Na$ 419.1313, found: 419.1316.

Methyl 3-*O*-benzyl-β-L-idopyranuronate 1,2-(methylorthoacetate) (15). Compound **14** (1.59 g, 0.55 mmol) was dissolved in methanol (20 mL) and cooled to -0 °C. A 0.5 M solution NaOMe (6.40 mL) was added, and the reaction mixture was stirred at 0 °C for 2 h and at 5 °C overnight. The solution was diluted with CH_2Cl_2 (20 mL) at 5 °C, quenched with aqueous $NaHCO_3$ and H_2O (10 mL each), and then extracted with (25 mL × 3). The organic fractions were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel flash chromatography [EtOAc:Hexanes (1:4 to 1:1) + 1% Et_3N] yielded **15** (971 mg, 68%) as a colourless oil. R_f = 0.3 (EtOAc/Hexane = 1/2); $[\alpha]^{28}_D$ = -0.5 (*c* 1.0, CH_2Cl_2); IR (KBr) *v* 3570, 3030, 1762, 1737, 1511, 1441 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.37-7.30 (m, 5H, Ph), 5.50 (d, *J* = 1.8 Hz, 1H, H-1), 4.71 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.64 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.48 (s, 1H, H-4), 4.15-4.08 (m, 3H, H-2, H-5), 3.81 (s, 3H, CO_2CH_3), 3.29 (s, 3H, OCH_3), 2.78 (d, *J* = 11.4 Hz, 1H, H-3), 1.75 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.8 (C), 136.7 (C), 128.6 (CH × 2), 128.3 (CH), 127.8 (CH × 2), 123.4 (CH), 96.7

(CH), 75.7 (CH), 72.9 (CH), 72.8 (CH_2), 71.7 (CH), 66.9 (CH), 52.4 (CH_3), 50.2 (CH_3), 24.3 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{17}H_{22}O_8Na$ 377.1207, found: 377.1211.

Methyl 3-*O*-benzyl-4-*O*-chloroacetyl-β-L-idopyranuronate 1,2-(methylorthoacetate) (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 chloroacetyl 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 $NaHCO_3$ 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 faint yellow solid (401 mg, 89%). R_f = 0.4 (EtOAc/Hexane = 1/2); mp = 120-121 °C; IR (KBr) *v* 3607, 3089, 3002, 1752, 1497, 1329 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.39-7.32 (m, 5H, Ph), 5.56 (d, *J* = 2.4 Hz, 1H, H-l), 5.24 (t, *J* = 2.4 Hz, 1H, H-4), 4.67 (AB system, *J* = 12.0 Hz, 2H, CH_2Ph), 4.57 (d, *J* = 1.2 Hz, 1H, H-5), 4.15 (t, *J* = 2.4 Hz, 1H, H-3), 4.07 (s, 1H, H-2), 4.06-3.99 (m, 2H, $ClCH_2CO$), 3.79 (s, 3H, COOMe), 3.24 (s, 3H, OCH_3), 1.71 (s, 3H, CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) 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_2), 71.0 (CH), 69.3 (CH), 68.3 (CH), 52.7 (CH_3), 49.2 (CH_3), 40.4 (CH_2), 24.9 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{19}H_{23}O_9ClNa$ 453.0923, found: 453.0941.

Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(chloroacetyl)-2-*O*-acetyl-L-idopyranuronate (17). Compound **16** (100 mg) was dissolved in the solution of AcOH/H_2O (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_f = 0.2 (EtOAc/Hexane = 1/2). IR (KBr) *v* 3607, 3089, 3002, 1752, 1497, 1329 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.38-7.32 (m, 5H, Ph), 5.31 (d, *J* = 7.2 Hz, 1H, H-1β), 5.26 (t, *J* = 2.4 Hz, 1H, H-2α), 5.19 (dd, *J* = 2.0 Hz, 1H, H-1α), 5.03 (d, *J* = 1.8 Hz, 1H, H-4α), 4.92-4.91 (m, 0.6 H, H-5α), 4.83 (m, 1H, H-5β), 4.77-4.75 (m, 1H, CH_2Ph), 4.74 (s, 0.6 H, H-4β), 4.39 (d, *J* = 8.4 Hz, 1H, H-2β), 4.03–3.99 (m, 2H, H-3b, CH_2Cl), 3.97 (d, *J* = 1.8 Hz, 1H, CH_2Cl), 3.95 (d, *J* = 1.2 Hz, 1H, 0.6H, H-3β), 3.94-3.92 (dt, *J* = 4.2, 3.0 Hz, 1-H, H-3α), 3.77-3.76 (m, 3H, 2 x CH_3 OMe), 2.11 (s, 3H, CH_3OAc), 2.05 (s, 3H, CH_3OAc); ^{13}C NMR (150 MHz, $CDCl_3$) 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), 128.4 (CH), 128.0 (CH × 2), 127.8 (CH), 92.8 (CH), 91.9 (CH), 73.4 (CH_2), 73.1 (CH_2), 72.6 (CH), 72.2 (CH), 71.7 (CH), 68.5 (CH), 68.5 (CH), 67.6 (CH), 66.5 (CH), 65.3 (CH), 52.6 (CH), 40.2 (CH_3), 40.2 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{18}H_{21}O_9NaCl$ 439.0766, found: 439.0783.

Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(chloroacetyl)-2-*O*-acetyl-L-idopyranuronate trichloroacetimidate (4). Trichloroacetonitrile (173 μL, 1.72 mmol) and K_2CO_3 (166 mg, 1.15 mmol) were added to a solution of **17** (96 mg, 0.23 mmol) in CH_2Cl_2 (4 mL). After stirring for 12 hours at room temperature (22 °C), the reaction mixture was quenched with water and $NaHCO_3$ and dried over $MgSO_4$, filtered, and concentrated to give crude residue which was purifies by column chromatography [EtOAc/Hexane (1:3 to 1:1) + 1 % NEt_3] to afford **4** as 1:1 α/β mixture (80 mg, 62%). R_f 0.6 (EtOAc/Hexane = 1/2). The imidate **19** were used directly in the next step.

Methoxy [methyl 2-*O*-acetyl-3-*O*-benzyl-(4-chloroacetyl)-L-idofuranuranuate]-(1→4)-*O*-6-*O*-acetyl-2-benzylformate-2-deoxy-α,β-D-glucopyranoside (3). The imidate **4** (80 mg, 0.14 mmol) and acceptor **5**[22] (59 mg, 0.12 mmol) were azeotropically dried with toluene and dissolved in DCM (2 mL). A 4 Å molecular sieve (100 mg) was added and mixture was then stirred for 30 min at 21 °C. TMSOTf (8 μL, 0.045 mmol) was added and stirred for 5 minutes. The mixture was continuously stirred for another 1 hour. The residue was purified by column chromatography with (EtOAc/ Toluene, 1:4) to afford the colourless oil **3α** (11 mg) and white solid **3β** (33 mg) in 40% yield. **3α-form**: R_f = 0.3 (EtOAc/Hexane = 1/1.5); $[\alpha]^{24}_D$ = 0.5 (*c* 1.0, CH_2Cl_2); IR (KBr) *v* 3796, 3034, 2362, 1734, 1409, 1370, 1237 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.23 (m, 15H, Ph), 5.66 (d, *J* = 7.8 Hz, 1H, H-5′), 5.32 (s, 1H, H-1′), 5.22 (s, 1H, H-2′), 5.08-5.03 (m, 2H, CH_2Ph), 4.81 (m, 2H, N-H & CH_2Ph), 4.70 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.65 (m, 1H, H-4′), 4.62 (d, *J* = 3.0 Hz, 1H, H-1), 4.55 (d, *J* = 10.8 Hz, 1H, CH_2Ph), 4.41 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.31-4.23 (m, 2H, 6-Ha, 6-Hb), 4.11 (m, 1H, H-3′), 4.06-3.95 (m, 2H, OCH_2Cl), 3.93 (m, 1H, CH_2Ph), 3.77-3.63 (m, 3H, H-3, H-4, H-5), 3.61 (s, 3H, COOMe), 3.32 (s, 3H, OMe), 2.09 (s, 3H, CH_3OAc), 2.05 (s, 3H, CH_3OAc) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ 170.8 (C), 169.5 (C), 167.6 (C), 166.1 (C), 155.7 (C), 138.0 (C), 136.7 (C), 136.2 (C), 128.4 (CH), 128.4 (CH × 3), 128.3 (CH × 3), 128.2 (CH × 2), 128.2 (CH × 4), 128.0 (CH), 127.6 (CH), 107.8 (C), 98.8 (CH), 80.7 (CH), 79.6 (CH), 79.4 (CH), 78.9 (CH), 76.2 (CH), 74.7 (CH_2), 72.6 (CH_2), 68.6 (CH), 66.9 (CH_2), 62.4 (CH_2), 55.1 (CH_3), 54.6 (CH), 52.5 (CH_3), 40.5 (CH_2), 20.8 (CH_3), 20.7 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{42}H_{48}O_{16}NClNa$ 880.2554, found: 880.2530. **β-form**: R_f = 0.2 (EtOAc/Hexane = 1/1.4); mp = 116-118 °C; IR (KBr) *v* 3851, 3589, 2953, 1736, 1646, 1521, 1368, 1240 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38-7.12 (m, 15H, Ph), 5.16-5.05 (m, 3H,CH_2Ph, H-1′, H-5), 5.11 (t, *J* = 12.6 Hz, 1H, CH_2Ph), 5.05 (d, *J* = 12.6 Hz, 1H, CH_2Ph), 5.00 (d, *J* = 1.6 Hz, 1H, NH), 4.97 (d, *J* = 2.8 Hz, 1H, CH_2), 4.84 (d, *J* = 10.8 Hz, 2H, H-4, H-5′), 4.75 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.70 (dd, *J* = 4.8, 3.6 Hz, 1H, H-2), 4.65 (m, 1H, H-1), 4.61 (s, 1H, CH_2), 4.49 (d, *J* = 12.0 Hz, 2H, CH_2Ph), 4.22-4.20 (m, 1H, 6Ha′), 4.07-4.05 (m, 1H, 6Hb′), 3.96 (d, *J* = 4.0 Hz, 2H, CH_2Cl), 3.91 (dd, *J* = 5.6, 4.0 Hz, 1H, H-3′), 3.82 (dd, *J* = 6.0, 3.2 Hz, 1H, H-2′), 3.57-3.48 (m, 2H, H-4′, CH_2Ph), 3.40 (s, 3H, COOMe), 3.35 (s, 3H, OMe), 2.12 (s, 3H, CH_3OAc), 2.03 (s, 3H, CH_3OAc) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7 (C), 169.6 (C), 168.2 (C), 166.4 (C), 155.7 (C), 137.8 (C), 137.0 (C), 136.0 (C), 128.5 (CH), 128.4 (CH × 3), 128.1 (CH × 3), 128.1 (CH × 2), 128.1 (CH × 3), 127.9 (CH), 127.2 (CH), 98.8 (C), 97.5 (CH), 78.9 (CH), 74.8 (CH), 74.3 (CH), 72.8 (CH_2), 72.4 (CH_2), 69.4 (CH), 69.2 (CH_2), 67.1 (CH), 66.9 (CH), 66.4 (CH), 63.0 (CH_2), 62.1 (CH_2), 55.2 (CH_3), 54.5 (CH), 52.2 (CH_3), 40.3 (CH_2), 29.3 (CH_2), 20.9 (CH_3), 20.8 (CH_3) ppm. HRMS ($M+Na^+$) calcd for $C_{42}H_{48}O_{16}NClNa$ 880.2554, found: 880.2536.

Acknowledgments

The authors thank the Ministry of Science and Technology (MOST) in Taiwan (103-2113-M-005-010) and National Chung Hsing University for financial support.

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