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Chemical Synthesis of UDP-Glc*p*A from N-Acetyl Chondrosine and UDP-[β-D-Gal-(1→4)-α-D-Glu] from Lactose

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Further studies on the chemistry of the natural chondrosine as starting material are presented in this paper. Acetylation reactions of either 2-NHAc-**3** or 2-NPhth-**3** furnished the corresponding peracetylated α-anomer as a sole product in a moderate to fair yields, respectively. Direct MacDonald phosphorylation attempt of acetylated chondrosine ester **4a** followed by *in situ* coupling with uridine-5-monophosphomorpholidate led to isolation of UDP-Glc*p*A **12** (32 %, 1:1 α/β) among other unreacted phosphates. The expected UDP-chondrosine **11** was not formed as indicated by MS analysis of the crude product. The decomposition of the oxazolidine intermediate **4c** under such conditions might account for the failure of preparing **11** in agreement with some literature methods that even used milder phosphorylating agents to add phosphate to carbohydrate-derived oxazolines. For comparison reasons, conducting the same reactions on lactose octaacetate **13** proved to be feasible and gave the corresponding UDP-Lac **16** in good yield and purity. Direct chemical synthesis of the nucleotide **16** represents a simple pathway that is added to the reported enzymatic procedure for finding oligosaccharide transferases.

Key Words: Transferases, Oligosaccharide, Chondrosine, Nucleotide, Glucuronic acid.

INTRODUCTION

Chondroitin sulfate (CS) **1** is a class of glycosaminoglycans (GAGs) and exists mainly in the extracellular and pericellular matrices of most animal species, including vertebrates, insects, mollusks and nematodes[1]. Structural studies[2] showed that **1** (Fig. 1) consists of a disaccharide repeating unit of the type D-glucuronic acid-(1→3)-2-acetamido-2-deoxy D-galactose [4)-β-D-Glc*p*A-(1→3)-β-D-GalNAc-(1→]$_n$. Chondrosine **2** is obtained from **1** by acid-catalyzed hydrolysis[3] or enzymatic degradations. Despite considerable interest in chondroitin sulfate and hyaluronic acid[4], little reports on the chemistry of chondrosine as starting material were published[5].

The biosynthesis of oligosaccharides usually occurs by glycosyltransferases that transfer a carbohydrate residue from an uridinediphosphate-sugar (UDP-sugar) as donor to an unprotected saccharide acceptor with complete regio- and stereoselectivity. These types of reactions have proved to be precious tools in preparative

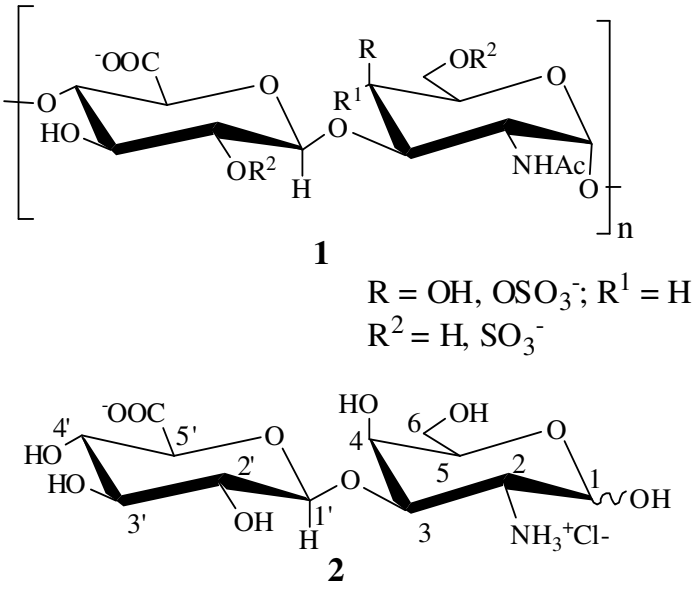


Fig. 1. Chemical structures of chondroitin **1** and chondrosine **2**

oligosaccharide synthesis and nowadays the number of glycosyltransferases cloned in research laboratories is increasing rapidly and much improvement has been achieved in this area for synthetic purposes[6,7]. Nevertheless, very little is known about employing unnatural UDP-disaccharides as nucleotide donors[8] in glycosyltransferases reactions may be because of the rigidity of these substrates. In continuation of our synthetic efforts toward natural carbohydrates[9] such as sulfated disaccharides[10], tetrasaccharides[11] or their mimetic analogues[12], we report here our efforts toward direct synthesis of UDP-chondrosine [UDP-[β-D-Glc*p*A)-(1→3)-β-D-GalNAc] and UDP-lactose [UDP-[β-D-Gal*p*-(1→4)-α-D-Glu] as sugar nucleotide donors for the biosynthesis of oligosaccharide fragments with different chain lengths.

EXPERIMENTAL

All chemical reagents were obtained from commercial suppliers and used without further purification. Melting points were measured using Electrothermal apparatus and were uncorrected. Reactions were monitored by TLC on Silica Gel $60F_{254}$ with detection by charring with 5 % H_2SO_4 in EtOH. ^1H and ^{13}C NMR spectra were recorded using JEOL at 500 MHz and 125 MHz, respectively in either $CDCl_3$ or D_2O with the chemical shifts are expressed on the δ scale ppm. Both positive-ion and negative-ion modes of mass spectral analysis were performed on an Agilent MSD Trap-SL mass spectrometer equipped with an electrospray ion source. Samples were dissolved in 1:1 water:methanol and introduced into ion source at a flow rate of 6 μL/min. Nebulizer pressure was set to 15 psi and the dry nitrogen gas was used at a flow rate at 5 L/min and the dry temperature was at 325 °C.

(Methyl-β-D-glucopyranosyluronate)-(1→3)-2-amino-2-deoxy-D-galactopyranose hydrochloride salt (3)[5a]: Acetyl chloride (0.75 mL, 10.5 mmol) was added to a stirred suspension of chondrosine **2** (3.00 g, 8.44 mmol) in MeOH (90 mL) at 0 °C then left for 4 days at - 5 °C. *tert*-BuOH (20 mL) was added and the solution evaporated to dryness. The crude product was dissolved in water (150 mL), filtered through Celite and the solvent was evaporated. Recrystallization by dissolution in MeOH (20 mL) and 1:1 MeOH/*i*-PrOH (40 mL) was added followed by the addition of more *i*-PrOH (120 mL). The solids were removed by filtration and discarded and the filtrate was evaporated to dryness. The resulting solid was dissolved in MeOH and it was re-precipitated by addition of Et_2O. The amorphous white solid was filtered off and dried *in vacuo* to give 1.4 g of **3**, (40 %). For ^{1}H NMR (500 MHz, D_2O) and ^{13}C NMR (125 MHz, D_2O) see ref. 13. MS (ES): Calcd. for $C_{13}H_{24}NO_{11}Cl$ (405.5); Found: (+ve) MS m/z 370.1 $(M\text{-}Cl)^+$ (100 %), (-ve) MS m/z 404.5 $(M\text{-}1)^+$ (100 %).

Acetyl-O-(methyl-2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-4,6-di-O-acetyl-2-deoxy-2-acetamido-α-D-galactopyranose (4a): A solution of the ester **3** (1.5 g, 3.69 mmol) dissolved in pyridine/Ac_2O mixture (60 mL, 1:1 v/v) was stirred at room temperature for 12 h. Solvent and volatiles were removed under high vacuum and the residue was further co-evaporated with toluene (15 mL, 2x). Purification by silica gel column chromatography (gravity) using hexane/EtOAc (5:1) furnished 1.20 g (49 %) of the titled compound **4a** as a white crystalline solid; m.p. 115-116 °C. The following data were recorded: ^{1}H NMR ($CDCl_3$): δ 6.36 (d, 1H, H-1, $J_{1,2}$ = 3.5 Hz), 4.53 (dd, 1H, H-2, $J_{2,1}$ = 3.5, $J_{2,3}$ = 11.0 Hz), 4.56 (dd, 1H, H-3, $J_{3,2}$ = 11.0, $J_{4,3}$ = 3.4 Hz), 5.34 (dd, 1H, H-4, $J_{4,3}$ = 3.4, $J_{4,5}$ = 3.2 Hz), 4.20 (dd, 1H, H-5, $J_{5,4}$ = 3.2, $J_{5,6}$ = 6.8 Hz), 4.16-400 (m, 2H, 2x H-6), 4.86 (d, 1H, $J_{1',2'}$ = 8.3, H-1'), 5.90 (dd, 1H, $J_{2',1'}$ = 8.3, $J_{2',3'}$ = 8.8 Hz, H-2'), 5.20 (dd, 1H, $J_{3',2'}$ = 8.8, $J_{3',4'}$ = 9.3 Hz, H-3'), 5.15 (1H, $J_{4',3'}$ = 9.3, $J_{4',5'}$ = 9.8, H-4'), 5.17 (d, 1H, $J_{5',4'}$ = 9.8 Hz, H-5'), 3.75 (s, 3H, $-CO_2CH_3$), 2.19, 2.13, 2.12, 2.11, 2.07, 2.06 (CH_3). ^{13}C NMR ($CDCl_3$): δ 171.00, 170.73, 170.71, 170.22, 169.97, 169.82, 91.71 (C-1), 72.09 (C-2), 69.14 (C-3), 68.16 (C-4), 72.42 (C-5),167.68 (C-6), 53.49 (MeO), 97.84 (C-1'), 48.32 (C-2'), 72.17 (C-3'), 68.28 (C-4'), 69.28 (C-5'), 61.34 (C-6'), 23.10, 21.25, 21.19, 21.11, 21.05, 20.90 (CH_3). MS (ES): Calcd. for $C_{27}H_{37}NO_{18}$ (663); Found: +MS m/z 664 $(M^+\text{+}1)$ (40 %), 686 $(M^+\text{+}Na)$ (60 %), 604 $(M^+\text{-}AcOH)$ (100 %).

Acetyl-O-(methyl-2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-4,6-di-O-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranose (5): To a stirred suspension of methyl chondrosine **3** (0.5 g, 1.23 mmol) in MeOH (10 mL), Et_3N (250 µL, 2.5 mmol) and finely ground phthalic anhydride (300 mg, 2.0 mmol) were subsequently added. The mixture was stirred at 60 ºC for 1 h then at room temperature overnight before removing the solvent and volatiles under vacuum. To the white foam crude residue was added pyridine (20 mL) then Ac_2O (10 mL) and the solution was stirred at room temperature overnight. The solvents were concentrated to dryness by rotary evaporation and the clear sticky residue was subjected to gravity silica gel

chromatography (EtOAc/hexane 1:2) to furnish (0.3 g, 33 %) of compound **5** as a white solid; m.p. 129-130 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.88-7.73 (m, 4H, ArH), 6.24 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 5.65 (d, $J_{3,4}$ = $J_{4,5}$ = 2.8 Hz, H-4), 5.35 (dd, $J_{3,4}$ = 3.2, $J_{2,3}$ = 11.6 Hz, H-3), 5.17-5.13 (m, 2H, H-1', H-4'), 4.92 (dd, 1H, $J_{1,2}$ = 3.2, $J_{2,3}$ = 11.6 Hz, H-2), 4.80-4.73 (m, 2H, H-2', H-3'), 4.42 (d, J = 6.4 Hz, H-5'), 4.20 (dd, $J_{5,6a}$ = 5.2, $J_{6a,6b}$ = 12.0 Hz, 1H, H-6_a), 4.04-3.99 (m, 2H, H-5, H-6_b), 3.76 (s, 3H, CO_2CH_3), 2.16, 2.08, 2.05, 1.99, 1.95, 1.69 (6x s, 6 CH_3). ESMS calculated for $C_{33}H_{37}NO_{19}$ M^+ = 751; Found 775 (M^+ + Na).

General method for the preparation of 1-phosphosugars: Crystalline phosphoric acid (500 mg, 5.1 mmol) was dried *in vacuo* over phosphorus pentoxide for 12 h. Solid of the peracetylated sugar (0.75 mmol) was added and the mixture was heated at 60 ºC *in vacuo* for 2 h. The heating was ceased and the resulting dark black mixture was dissolved in anhydrous THF (5.0 mL). The solution was cooled to 0 ºC and NH_4OH (0.5 mL) was added. The precipitate of ammonium phosphate was filtered off and washed with THF (20 mL). The filtrate was evaporated to give a syrupy residue that was purified by column chromatography (silica gel) using $CHCl_3$/MeOH (4:1) then $CHCl_3$/MeOH/H_2O (10:10:1) to give the phosphor sugar.

General method for the de-O-acetylation of 1-phosphosugars: The prepared 1-phosphosugar was dissolved in an excess of a mixture of NH_4OH/MeOH (2:1) (~ 0.5 g in 15 mL 10:5 v/v) and the mixture was stirred at room temperature for 15 h. The solvent was evacuated and the solid residue was passed over Dowex 50W-X8 resin (Py form and previously washed with MeOH) using distilled water as eluent.

General procedure for UMP morpholidate coupling with glycosyl phosphates: The phosphate (0.18 mmol) obtained by the general procedure was dissolved in water (1 mL) and passed through a column (1 cm × 5 cm) of Dowex 50W-X8 (Py form) to give the pyridinum salt in quantitative yield. The well dried phosphate was then mixed with uridine-5-monophosphomorpholidate (4-morpholine-N,N-dicyclohexylcarboxamidinium salt, 2.0 equiv, 0.30-0.40 mmol) and co-evaporated with pure pyridine (3 × 10 mL). The resulting syrup-like residue was dissolved in Py/DMF (3 mL, 1:2) and stirred for 5 days at room temperature in a sealed flask under argon atmosphere. Removal of the solvents under reduced pressure followed by purification on Bio-gel P2 using 0.25 M NH_4HCO_3 solution as eluent and then desalting with water to furnish the ammonium salt of the UDP-sugar.

Uridine diphosphoryl-α,β-D-glucopyranosyluronic acid, ammonium salt (12): Acetate **4a** (500 mg, 0.754 mmol) was heated with phosphoric acid following the general method to furnish a mixture of (2,3,4-tri-O-acetyl-β-D-glucopyranosyluronic acid)-(1→3)-4,6-di-O-acetyl-2-deoxy-2-acetamido-α/β-D-galactopyranosyl-1-phosphoric acid **6**, [ESMS: Calcd. for $C_{24}H_{34}NO_{20}P$ (687.14): Found; (m/z 686, M^+-1) (100 %)], two additional fragments at m/z 644 (M^+-Ac) (80 %) and m/z 602 (M^+-2 × Ac) (50 %) and 2,3,4-tri-O-acetyl-α,β-D-glucopyranosyluronylphosphoric acid anhydride-1-phosphoric acid **7** [ESMS: Calcd. for $C_{12}H_{18}O_{14}P_2$ (480.01): Found; + MS: m/z 503.1 (M^++Na) (50 %); - MS: m/z 515.3 (M^++Cl) (50 %)].

Without purification the mixture was de-O-acetylaed using the reported general method to furnish β-D-glucopyranosyluronate)-(1→3)-2-deoxy-2-acetamido-α/β-D-galacto-pyranosyl-1-phosphoric acid **7** [calculated for $C_{14}H_{24}NO_{15}P$; (477.09); found: -MS: 476 (M-1) (100 %)], N-acetylchondrosine **9** [calculated for $C_{14}H_{23}NO_{12}$ (397.12); Found: +MS: m/z 421 (M^++Na) (100 %) and α,β-D-glucopyranosyl-uronylphosphoric acid anhydride-1-phosphoric acid **10** [Calcd. for $C_6H_{12}O_{13}P_2$ (354.10); Found: +MS: (m/z 356 (M^++2) (95 %)]. It was passed to the next step without purification.

The crude phosphate dissolved in 3 mL (Py/DMF, 1:2 v/v) was stirred with uridine-5-monophosphomorpholidate (2.0 equiv. based on **4a**) and following the described general coupling procedure to subsequently furnish the following:

1-**β-D-Glucopyranosyluronate)-(1→3)-2-deoxy-2-acetamido-α/β-D-galactopyranosyl-1-phosphoric acid (8):** White solid (highly hygroscopic); 40 mg (11 %, α/β 2:1) isolated material from the Bio-gel column. ^{1}H NMR (D_2O at δ 4.66): δ 5.27 (d, J = 3.4 Hz, 1H, H-1α), 4.46 (d, J = 7.8 Hz, 1H, H-1' β), 4.19 (dd, $J_{4,3}$ = 11.0, $J_{4,5}$ = 11.0 Hz, 1H, H-4), 4.09 (dd, $J_{3',2'}$ = 11.0, $J_{3',4'}$ = 9.5 Hz, 1H, H-3'), 3.99 (dm, $J_{3,2}$ = 7.5 Hz, 1H, H-3), 3.87 (dd, $J_{5,4}$ = 11.0, $J_{5,6}$ = 2.9 Hz, 1H, H-5), 3.71 (d, J = 9.3 Hz, 1H, H-5'), 3.61 (dd, $J_{2',1'}$ = 7.8, $J_{2',3'}$ = 11.0 Hz, 1H, H-2'), 3.58 (dd, $J_{2,1}$ = 3.4, $J_{2,3}$ = 7.5 Hz, 1H, H-2), 3.54 (ddd, J = 11.7, 11.7, 2.9 Hz, 2H, 2 × H-6), 3.33 (dd, $J_{4',3'}$ = 9.5, $J_{4',5'}$ = 9.3 Hz, 1H, H-4'), 1.93 (s, 3H, $NHCOCH_3$). Additional signals: δ 4.05 (dd, J = 9.0, J = 8.3 Hz, 1H), 3.37 (dd, J = 9.5, J = 9.0 Hz, 1H), 3.28 (dd, J = 9.5, J = 4.6 Hz, 1H), 3.18 (dd, J = 9.0, J = 9.0 Hz, 1H), 1.91 (s, 3H, $NHCOCH_3$). ESMS: Calcd. for $C_{14}H_{24}NO_{15}P$ (477.31): Found; m/z 476 (M^+-1, -MS) (100%).

2-Uridine diphosphoryl-α,β-D-glucopyranosyluronic acid, ammonium salt (12): White solid (highly hygroscopic),140 mg (32 %, α/β 1:1) isolated solid from the Bio-gel column. The following data were recorded: ^{1}H NMR: 7.80 (d, J = 8.4 Hz, 1H), 5.84 (d, J = 3.8 Hz, 1H, H-1α), 5.81 (d, J = 7.6 Hz, 1H, H-1β), 5.47 (2d, J = 4.6 Hz, J = 8.4 Hz, 1H), 4.26-4.21 (m, 2H), 4.16-4.12 (m, 1H), 4.11-4.07 (m, 1H), 4.05-4.02 (m, 1H, H-2), 3.99 (d, $J_{5,4}$ = 9.9 Hz, 1H, H-5), 3.63 (dd, $J_{4,5}$ = 9.9, $J_{4,3}$ = 9.1 Hz, 1H, H-4), 3.42 (d br, J = 11.4 Hz, 1H, H-5'), 3.35 (dd, $J_{3,2}$ = 9.9, $J_{3,4}$ = 9.1 Hz, 1H, H-3). ^{13}C NMR: 176.42 (CO), 166.28 (CO), 151.87 (CO), 141.57 (CH=CH), 102.75 (CH=CH); β-Anomer: 102.66 (C-1), 73.08 (C-2), 72.60 (C-3), 69.65 (C-4), 88.31 (C-5), 83.29 (C-1'), 73.82 (C-2'), 71.85 (C-3'), 71.41 (C-4'), 64.99 (C-5'). α-Anomer: 95.35 (C-1), 72.98 (C-2), 72.57 (C-3), 69.63 (C-4), 88.23 (C-5), 73.78 (C-2'), 71.35 (C-4'), 64.94 (C-5'). ESMS: Calcd. for $C_{15}H_{22}N_2O_{18}P_2$ (580.05): Found; m/z 599 (M^+ + NH_4^+, -MS), m/z 623 (M^++Na, +MS).

β-D-Galactopyranosyl-(1→4)-α-D-glucopyranosyl-1-phosphoric acid mono-pyridinum salt (15): The known peracetylated lactose **13** (1.0 g, 0.678 mmol) was heated with phosphoric acid following the general method described above to furnish crude 2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl-1-phosphoric acid **14** (ESMS: Calcd. for $C_{26}H_{37}O_{21}P$ (716); Found: + MS 717 (M^++1), - MS 716 (M^+). It was passed to the next step

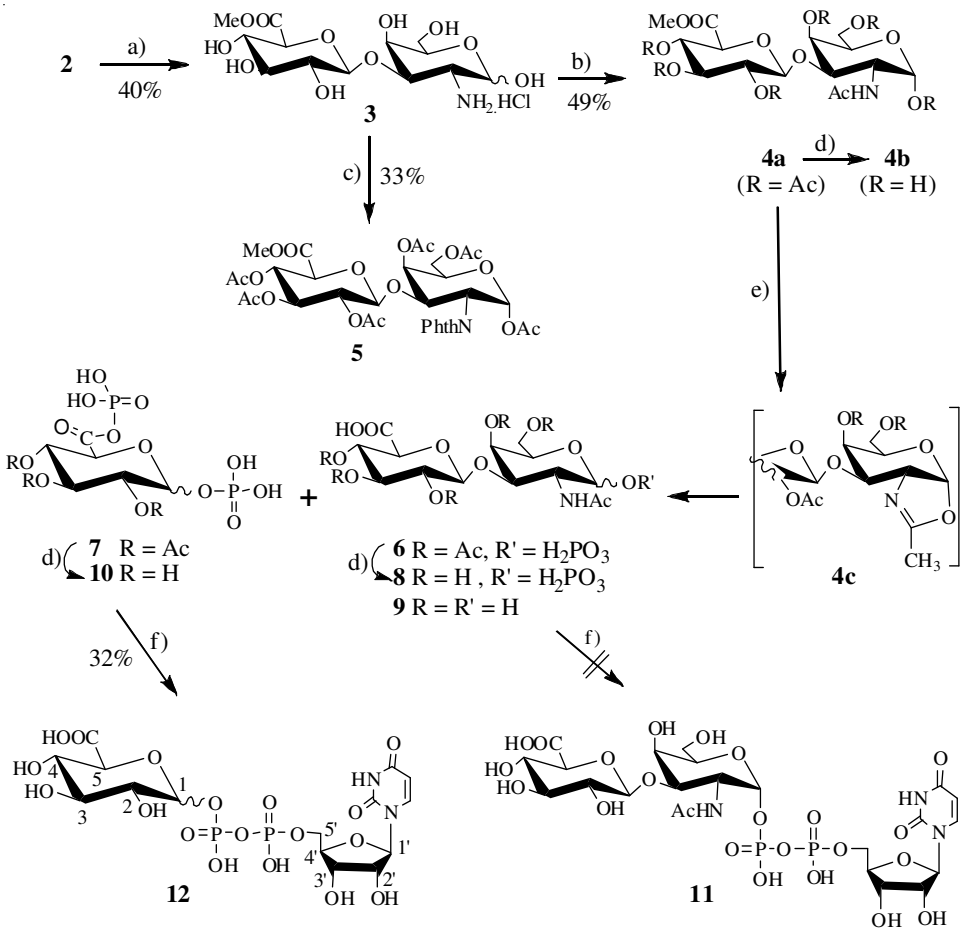
without purification. De-O-acetylation of **14** using the described general method furnished 550 mg (93 %) of **15** as white solid (hygroscopic). The following spectral data were recorded: ^{1}H NMR: δ 8.80-8.60 (m, 6H, Py), 8.30-8.20 (m, 4H, Py), 5.25 (dd, $J_{1,P}$ = 7.5, $J_{1,2}$ = 3.0, 1H, H-1α), 4.23 (d, $J_{1,2}$ = 7.5 Hz, H-1 β), 3.74-3.64 (m, 4H), 3.53-3.43 (m, 4H). 3.40-3.32 (m, 4H). ^{13}C NMR: 147.32 (C-2, C-6 Py), 141.23 (C-4 Py), 127.65 (C-3, C-5 Py), 103.03 (β-C-1), 94.84 (α-C-1), 78.04 (C-5'), 75.47 (C-5), 72.64 (C-3', C-3), 71.41 (C-4'), 71.22, 71.06, 68.68, 61.16 (C-6'), 59.75 (C-6). ESMS: Calcd. for $C_{12}H_{20}O_{14}P.2C_5H_5N$ (m/z 578): -MS m/z 421 (M^+-$2C_5H_5N$+1).

Uridine diphosphoryl β-D-galactopyranosyl-(1→4)-α-D-glucopyranose, ammonium salt (16): Pyridinium salt of the phosphate **15** (500 mg, 1.23 mmol) dissolved in 3 mL (Py/DMF, 1:2 v/v) was stirred with uridine-5-monophosphomorpholidate (1.34 g, 1.96 mmol) and following the described general coupling procedure to furnish 300 mg (33.5 %) of the titled compound **16** as a white solid (highly hygroscopic). The following spectral data were recorded: ^{1}H NMR (500 MHz, D_2O): 7.69 (d, 1H, *J* = 7.6 Hz, CH=CH), 5.91 (d, $J_{1'',2''}$ = 5.2 Hz, 1 H, H-1''), 5.76 (d, 1H, *J* = 7.6 Hz, CH=CH), 4.57 (dd, 1H, $J_{1,2}$ = 3.3, $J_{1,P}$ = 5.0 Hz H-1), 4.42 (d, 1H, *J* = 7.8 Hz, H-1'), 4.23 (dd, $J_{3'',2''} = J_{3,4}$ = 5.2 Hz, 1 H, H-3''), 4.20 (dd, $J_{2'',1''} = J_{2,3}$ = 5.2 Hz, 1H, H-2''), 4.11-4.00 (m, 3 H, 2 × H-5'', H-4''), 3.98 (dd, 1H, *J* = 3.3, 9.5 Hz, H-2), 3.90 (dd, 1H, *J* = 7.8, 8.5 Hz, H-2'), 3.81-3.60 (m, 4H, 2 × H-6, 2 × H-6'), 3.52 (m, 2H, H-4, H-4'), 3.40-3.33 (m, 4H, H-3, H-3', H-5, H-5'). ^{13}C NMR (D_2O, 125 MHz): 166.68 (CO), 151.33 (CO), 141.14 (CH=CH), 103.15 (CH=CH), 103.03 (C-1'), 96.30 (C-1), 83.29 (C-1'', C-4), 78.30 (C-4'', C-5'), 75.45 (C-2''), 74.81 (C-3', C-3), 74.53 (C-2), 73.82 (C-2'), 72.62 (C-5), 71.85, 71.46 (C-4'), 71.41, 71.05, 68.65 (C-3''), 64.99 (C-5''), 61.12 (C-6'), 60.15 (C-6). ESMS: Calculated for $C_{21}H_{33}N_2O_{22}P_2$ (727.12); Found: 728 (M + 1), 746 (M + NH_4^+).

RESULTS AND DISCUSSION

As shown in **Scheme-I**, methyl estrification is carried out by slow addition of equivalent amount of acetyl chloride to a cold (0 °C) suspension of **2** in MeOH and the mixture was then stored for 4 days at -5 °C to give the ester **3** in 40 % yield. The NMR spectroscopic studies[13] of **3** in D_2O revealed the presence of two disaccharides anomers namely methyl [β-D-Glc*p*A-(1→3)-α,β-D-GalNH_2] and the α/β ratios 2:1 were in agreement with the literature value[14] of equilibrated solution of chondrosine **2** in D_2O.

Acetylation of **3** (Ac_2O/Py, 0 °C, 20 h) furnished a mixture of peracetylated anomers separable by careful silica gel column chromatography. The desired α-anomer **4a** was obtained in 49 % yield as a white crystalline product. The indicated stereochemistry at C-1 and C-1' was confirmed by NMR spectral analysis. ^{1}H NMR ($CDCl_3$) of **4a** showed two doublet signals at δ 6.36 ppm (d, $J_{1,2}$ = 3.5 Hz) correspond to H-1_{eq} and at δ 4.86 (d, $J_{1',2'}$ = 8.3 Hz) assigned to H-1_{ax} while their corresponding carbons resonances in ^{13}C NMR spectrum appeared at δ 97.84 (C-1'b) and 91.71 ppm (C-1α). In order to obtain a better assignment of ^{1}H NMR of a pure anomer of **3**,



Key: a) AcCl, MeOH, 4 d, 0 ºC; b)Ac_2O, Py, 15 h, rt; c) Phthalic anhydride, Et_3N, MeOH, then Ac_2O, Py; d) MeOH / NH_4OH 1:2, 15h, rt; e) H_3PO_4, 60 ºC, vacuum, 3h; f) uridine-5 monophospho-morpholidate, Py / DMF (3:1 v/v), 5 d, rt

Scheme-I

de-O-acetylation of a sample of **4a** was considered a solution. Thus, **4a** was stirred in a mixture of NH_4OH/MeOH (2:1) for 6 h, monitored by TLC (hexane/EtOAc 2:1), to give **4b** quantitatively after removing the solvent and volatiles by rotary evaporation. Signals of ^{1}H NMR spectrum of **4b** (D_2O, 500 MHz) indicated the presence of α/β anomers with 2:1 populations. For instance, a strong doublet displayed at δ 5.31 ppm (d, $J_{1,2}$ = 3.5 Hz) and two broad doublets displayed at δ 4.72 ppm ($J_{1,2}$ = 8.4 Hz) and 4.54 ppm ($J_{1',2'}$ = 8.7 Hz) clearly indicate the presence of anomeric α- and β-linkages as indicated by the *J* coupling constant and integration values. Apparently, ^{1}H NMR spectrum revealed that **4b** in D_2O undergoes mutarotation in the $GalNH_2$

residue and the equilibrium state at 25 °C is a 2:1 ratio of α- to β-anomers. Interestingly, these assignments are consistent with literature data of 2-deoxy-N-acetylamino sugars in aqueous solution[15].

As a trial to improve the reaction yield of the desired α-anomer **4a**, protection of the amino group at C-2 with a non-participating group such as phthaloyl group was considered a solution. Thus, **3** was directly treated with finely powdered phthalic anhydride and Et_3N in MeOH. The crude product and salts were carefully dried under reduced pressure then suspended in pyridine and treated with acetic anhydride. Solvents and volatiles were removed by rotary evaporation to minimize problems associated with waste remediation and loss of product by insufficient partitioning between the aqueous and the organic phases. Nevertheless, the desired peracetylated N-phthaloyl chondrosine **5** was obtained in 33 % yield as a sole product after purification on silica gel (EtOAc/hexane 1:2)†.

Our next concern was the preparation of glycosyl-1-phosphate derived from compound **4b**. Generally, the synthesis of UDP-sugars relies on installing a phosphate group on the anomeric carbon of the sugar followed by coupling of the unmasked glycosyl monophosphate with an activated form of nucleotide monophosphate usually the morpholidate introduced by Moffatt and others[16].

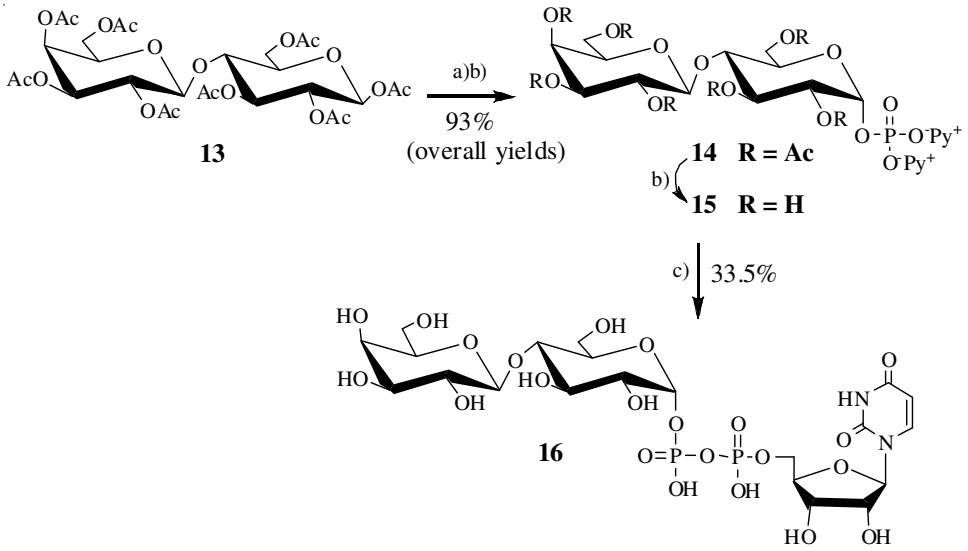
The synthesis of glycosyl monophosphates[17] falls into two categories wherein the sugar is either the electrophilic component (nucleophilic addition of phosphate anion) or the nucleophile (phosphorylation or phosphitylation/oxidation at the anomeric hydroxyl group). In the former case, carbohydrates bearing a neighboring participating group at C-2, favour the formation of 1,2-*trans* linked glycosyl phosphates (β-anomer in the case of GlcNAc). Although addition of phosphate diesters to carbohydrate derived oxazolines has been reported to deliver α-phosphates in many cases[18,19], decomposition has been reported in others[20]. Phosphorylation of **4a** was carried out by heating with crystalline H_3PO_4 at 60 °C in vacuum for 2 h as described by MacDonald[21] followed by purification of the crude residue over silica gel column using $CHCl_3$/MeOH (4:1) then $CHCl_3$/MeOH/H_2O (10:10:1) mixtures, respectively. The ESMS spectrum of the polar residue revealed the presence of a mixture of phosphorylated products. Thus, the strong peak at m/z 686 (M^+-1), corresponds to 1-phosphoperacetyl uronic acid derivative **6**, proved the deprotection of the methyl ester group under acidic condition. Two strong peaks at m/z 644 and m/z 602 diagnostic for the subsequent removal of one and two acetyl protecting groups, respectively, from **6**. The strong peak at m/z 503 refers, to our surprise, to the 1-phosphoglucuronic acid anhydride tri-O-acetate **7** which may be formed upon degradation of either the starting material **4a** or its oxazolidine intermediate **4c**, which is formed by neighbouring group participation of 2-NHAc protecting group, under reaction

†The ester **3** or its N-Phth derivative **5** was obtained as deliquescent amorphous solid and both changed to white solids under vacuum. During work-up, we were surprised to collect appreciable amounts (white foam) of **3** or **5** from the rotary evaporator parts and this observation might account for the reported low yields.

harsh conditions. In addition, two small peaks at m/z 461 and m/z 413 diagnostic for the subsequent de-O-acetylation of **7**. Unfortunately, it was not possible to separate these products on silica gel or Dowex 50W-X8 resins.

Removal of the acetyl groups was our next step as a possible solution for the fractionation of the crude phosphate products. Treatment of **6** with a mixture of NH_4OH/MeOH (2:1 v/v) at room temperature for 15 h followed by purification on Dowex 50W-X8 resin (Py-form) using H_2O as eluent furnished **8** among some other inseparable products as indicated by examining the ESMS of the crude product. The base peak at m/z 421 (M^++Na) was diagnostic for the 2-NHAc-chondrosine **9**. The peak at m/z 356 (90 %) refers to the de-O-acetylated 1-phosphoglucuronic acid **10**. Without further purification, crude phosphates dissolved in a small volume of distilled water was passed through a short Dowex 50W-X8 (Py form) column then the well-dried salt was mixed with an excess of uridine-5-monophosphomorpholidate (4-morpholine-N,N-dicyclo-hexylcarbox-amidinium salt) and co-evaporated with pyridine. The resulting syrup-like residue was stirred in pyridine/DMF (1:2 v/v) for 5 days at room temperature under argon. Separation on Bio-gel P2 with 0.25 M NH_4HCO_3 solution then with distilled water furnished, respectively, UDP-Glc*p*A **12**, unreacted phosphates **8** and **6**. Surprisingly, the target UDP-chondrosine **11** was not one of the products as indicated by examining the mass spectrum (ESMS) of the crude product. The structure of **12** was fully characterized by spectral measurements. ^{1}H NMR (D_2O) spectrum displayed two doublet signals of equal intensities at δ 5.84 (d, J = 3.8 Hz, H-1_{eq}) and 5.81 (d, J = 7.6 Hz, H-1_{ax}) assigned to the anomeric proton. ^{13}C NMR (D_2O) spectrum displayed signals correspond to the uridine moiety of one anomer at δ 176.42, 166.28, 151.87 ppm (3 × CO); δ 141.57, 102.75 ppm (C=C), 83.29 (C-1'), 73.82 (C-2'), 71.85 (C-3'), 71.41 (C-4') and 64.99 ppm (C-5') whereas the additional signals resonated at δ 73.78 (C-2'), 71.35 (C-4'), 64.94 ppm (C-5') were assigned to a second anomer. The carbon atoms in GlcA ring resonated at δ 102.66 (C-1, β-anomer), 73.08 (C-2), 72.60 (C-3), 69.65 (C-4) and 88.31 ppm (C-5) while other signals of equal intensities correspond to the second anomer appeared at δ 95.35 (C-1, α-anomer), 72.98 (C-2), 72.57 (C-3), 69.63 (C-4), 88.23 ppm (C-5), respectively. These data confirm the formation of **12** in a 1:1 α/β ratio anomeric population. The electron spray mass spectra (ESMS) calculated for $C_{15}H_{22}N_2O_{18}P_2$ (M^+ = 580.05) revealed two peaks at m/z 599 ($M^+ + NH_4^+$, -MS) and m/z 623 (M^++Na, +MS) (**Scheme-II**).

Isolation of UDP-Glc*p*A **12** in relatively good yield raised the general question about the reactivity of the peracetylaed disaccharides during MacDonald phosphorylation step and therefore conducting the reactions sequence on a mimic diasaccharide such as **13** was considered a solution. As demonstrated in **Scheme-II**, heating the readily available β-lactose octaacetates **13** with phosphoric acid gave **14** in high yield and purity as judged by ESMS [+MS; 717 (M^++1), -MS; 716 (M^+)] of the crude product. Nevertheless, ^{1}H and ^{13}C NMR spectral data of **14** showed broad signals, probably due to the presence of the polar 1-O-H_2PO_3 group attached to the anomeric



Key: a) H_3PO_4, 60°C, vacuum ; b) NH_4OH/MeOH 2:1, 15h, rt;
c) uridine-5-monophosphomorpholidate, Py / DMF (3:1v/v), 5 d, rt

Scheme-II

carbon. De-O-acetylation of **14** using methanolic ammonia mixture gave **15** in high yield and purity as judged by NMR spectroscopy. ^{1}H NMR spectrum displayed two doubles at δ 5.25 ppm (dd, $J_{1,P}$ = 7.5, $J_{1,2}$ = 3.0, 1H, H-1eq) and 4.23 ppm (d, $J_{1,2}$ = 7.5 Hz, H-1'ax) while the ^{13}C NMR spectrum showed two signals at δ 103.03 ppm (C-1'β) and 94.84 ppm (C-1α). This data clearly proved not only the indicated stereochemistry of 15 at C-1 and C-1' but also the formation of α-phosphate as a sole product which is consistent with literature data[19,20]. Other carbons resonances in sugar rings appeared at δ 78.04 ppm (C-5'), 75.47 (C-5), 72.64 (C-3', C-3), 71.41 (C-4'), 71.22, 71.06, 68.68, 61.16 (C-6'), 59.75 (C-6) and the pyidium salt carbons were resonated at 147.32 (C-2, C-6 Py), 141.23 (C-4 Py), 127.65 (C-3, C-5 Py), respectively. The mass spectrum (electron spray) calculated for $C_{12}H_{22}O_{14}P$ m/z 421; found (-ve MS) m/z 421 (M^+) and (+ve MS) m/z 445 (M^+ + Na) and m/z 483, respectively. Finally coupling of **15** with uridine-5-monophospho-morpholidate in pyridine/DMF mixture furnished **16** (in the form of its ammonium salt) in 33 % yield[22]. The structure of the disaccharide-conjugated UDP **16** was fully characterized by spectral measurements. Thus, ^{1}H NMR (D_2O solution) spectrum displayed protons signals corresonding to the uridine moeity at δ 7.69 (d, 1H, J = 7.6 Hz, CH=CH), 5.91 (d, $J_{1'',2''}$ = 5.2 Hz, 1 H, H-1''), 5.76 (d, 1H, J = 7.6 Hz, CH=CH), 4.23 (dd, $J_{3'',2''}$ = $J_{3'',4''}$ = 5.2 Hz, 1 H, H-3''), 4.20 (dd, $J_{2'',1''}$ = $J_{2'',3''}$ = 5.2 Hz, 1 H, H-2'') and 4.11-4.00 (m, 3 H, 2 × H-5'', H-4'') while protons resonances in sugar rings appeared at δ 4.57 (dd, 1H, $J_{1,2}$ = 3.3, $J_{1,P}$ = 5.0 Hz H-1), 4.42 (d, 1H, J = 7.8 Hz, H-1'), 3.98 (dd, 1H, J = 3.3, 9.5 Hz, H-2), 3.90 (dd, 1H, J = 7.8, 8.5 Hz, H-2'), 3.81-3.60 (m, 4H, 2 × H-6,

2 × H-6'), 3.52 (m, 2H, H-4, H-4') and δ 3.40-3.33 ppm (m, 4H, H-3, H-3', H-5, H-5'), respectively. The carbons signals of the uridine moeity resonated in the ^{13}C NMR spectrum of **16** at δ 166.68 ppm (CO), 151.33 (CO), 141.14 (CH=CH), 103.15 (CH=CH), 83.29 (C-1''), 78.30 (C-4''), 75.45 (C-2''), 68.65 (C-3'') and δ 64.99 ppm (C-5'') while the other carbons resonances in sugar rings appeared at δ 103.03 ppm (C-1'β), 96.30 (C-1α), 83.29 (C-4 superimposed with C-1''), 78.30 (C-5', superimposed with C-4''), 74.81 (C-3', C-3), 74.53 (C-2), 73.82 (C-2'), 72.62 (C-5), 71.46 (C-4'), 61.12 (C-6'), 60.15 (C-6). The mass spectrum calculated for $C_{21}H_{33}N_2O_{22}P_2$ (M^+ = 727.12) showed two peaks at m/z = 728 and m/z = 746 correspond to (M + 1) and (M + NH_4^+), respectively.

Conclusion

We report further studies on the chemistry of the natural chondrosine as starting material. Acetylation reactions of 2-NHAc-**3** or 2-NPhth-**3** furnished exclusively the corresponding peracetylated α-anomer as a sole product in a moderate to fair yields, respectively. ^1H NMR data of 2-NAc-chondrosine methyl ester **4b** in D_2O revealed that the compound undergoes mutarotation in the $GalNH_2$ residue and the equilibrium state is a 2:1 ratio of α- to β-anomers. An attempt to direct phosphorylation of acetylated chondrosine methyl ester **4a** as described by MacDonald followed by *in situ* coupling with excess of uridine-5-monophospho-morpholidate was conducted. Gradual separation on Bio-gel P2 of the mixture products led to isolation of UDP-Glc*p*A **12** (32 %, 1:1 α/β) among other unreacted starting phosphate derivatives. Unfortunately, the expected UDP-chondrosine **11** was not one of the products as indicated by examining the mass spectrum (ESMS) of the crude product. The failure of preparing UDP-[β-D-GlcA-(1→3)-α-D-GalNHAc] **11** may be attributed to the decomposition of the oxazolidine intermediate **4c**, which has been formed earlier by the typical neighboring 2-NHAc group participation, under the phosphorylation conditions. This result is consistent with some literature reports that even used milder phosphorylation methods to add phosphate group to carbohydrate derived oxazolines. For comparison reasons, conducting the same reactions on lactose octaacetate **13**, as a mimic, proved to be feasible and gave the corresponding UDP-Lac **16** in good yield and purity. Direct chemical synthesis of the nucleotide **16** represents a simple pathway that is added to the reported enzymatic procedure[22] for finding oligosaccharide transferases[23].

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