Convenient Synthesis of Glucosamine and Mannosamine Starting from Glucose

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Glucosamine and mannosamine are 2-amino-2-deoxyhexoses that contain an amino group replacing 2-hydroxyl group of glucose and mannose, respectively. Glucosamine is the building unit of chitin, a major component in the exoskeletons of arthropods, and an important precursor in the glycosylation of proteins and lipids. Mannosamine, 2-epimer of glucosamine, is a biosynthetic precursor of sialic acid; enzymatic condensation of *N*-acetyl mannosamine and pyruvate produces sialic acid. Increased interest in the biochemical function of glucosamine and mannosamine raised the need for the L-isomers or isotope-labeled forms of these aminosugars, and led us to study the convenient synthetic route for those.[1–4]

Glucosamine is mostly obtained by hydrolysis of naturally occurring chitin. Mannosamine can be obtained by enzymatic cleavage of sialic acid in a small quantity.[5,6] Mannosamine can also be obtained by alkaline epimerization of 2-hydroxyl group of a glucosamine derivative.[7] A classical strategy for the synthesis of mannosamine is to replace 2-hydroxyl group of mannitol derivatives with an amino group followed by selective oxidation to obtain an aldose.[8,9] Chemical approaches to prepare 2-aminohexose include introduction of an azido function followed by reduction of the azido group into amine. Xue and Guo used 2,3-epoxide of a glucose derivative for nucleophilic ring opening with an azide.[10] Another widely used strategy is azidonitration of tri-acetyl glycal, which was found more convenient for our purpose and adopted in this study.[11,12]

Tri-*O*-acetyl-D-glucal is commercially available and the synthetic protocol is well established.[9] The literature procedure was reproduced in this study to start from L-glucose the synthesis of L-isomers of glucosamine and mannosamine. Isotope-labeled D-glucose could also be the starting point for the labeled forms of these aminosugars. Preparation of tri-*O*-acetyl-D-glucal can be accomplished in three sequential reaction steps, including peracetylation of glucose, substitution of 1-acetoxy group with bromide, and reductive elimination of acetoxy bromide. These reactions can be performed in one pot without separation of the intermediate products and we obtained the tri-acetyl glucal (**2**) in a 72% overall yield.[13]

The overall synthetic strategy is as shown in Figure 1. Azidonitration of tri-acetyl glucal with sodium azide and ceric ammonium nitrate (CAN) produced a mixture of **3** and **4**, which appear as a single spot on thin-layer chromatography (TLC). After acetolysis of the nitrates, the products **5** and **6** were separable on TLC. Equatorial orientation of all of the acetoxy groups of tri-acetyl glucal (**2**) did not discriminate the two faces of the double bond of **2** and resulted in the formation of **5** and **6** in a comparable ratio. ^{1}H NMR spectrum of the crude product shows the presence of both α and β anomers of **5** and **6** (Figure 2). The peaks at δ 5.57, 5.86, 6.14, and 6.31 correspond to 1-H of the pyranosides as assigned in Figure 2(a). Difficulties were found in the chromatographic separation of **5** and **6** due to low solubility of **6** in chromatographic solvent systems and it was circumvented by isolating part of **6** by recrystallization prior to column purification. The crystallized form of **6** contained only the α anomer of **6** (Figure 2(b)). When the mother liquor was purified by column chromatography, **5** and **6** was separated from each other, both as mixtures of α and β anomers. When the α/β mixture of **6** in MeOH was kept on a shelf for days, the β anomer isomerizes to α form with crystallization of the α isomer. Compound **6** can be thus obtained as an α form, but **5** as a α/β mixture. Isolated yields of **5** and **6** were 37 and 31%, respectively. Separation of **5** and **6** affords the divergence point for the two isomeric aminosugars. Removal of acetoxy groups followed by reduction of the azido function afforded glucosamine and mannosamine as hydrochloride salts.

Some of the previous procedures leading to 2-amino functionality in hexose required tedious processes or, in some cases, they were hardly reproducible in our laboratory. The present procedure is a combination of known reactions and offers an expedient route to glucosamine and mannosamine producing good yields consistently. With the procedures developed in this study, we synthesized L-isomers of glucosamine and mannosamine starting from L-glucose. Current procedure also provides a convenient route for isotope-labeled forms of the aminosugars which would be valuable tools for biochemical research.

Experimental

Tri-*O*-acetyl-D-glucal (2).[13] D-Glucose (5.0 g, 28 mmol) was added to a stirred mixture of acetic anhydride (20 mL) and 60% $HClO_4$ (0.14 mL) at 40 °C during 20 min and maintained

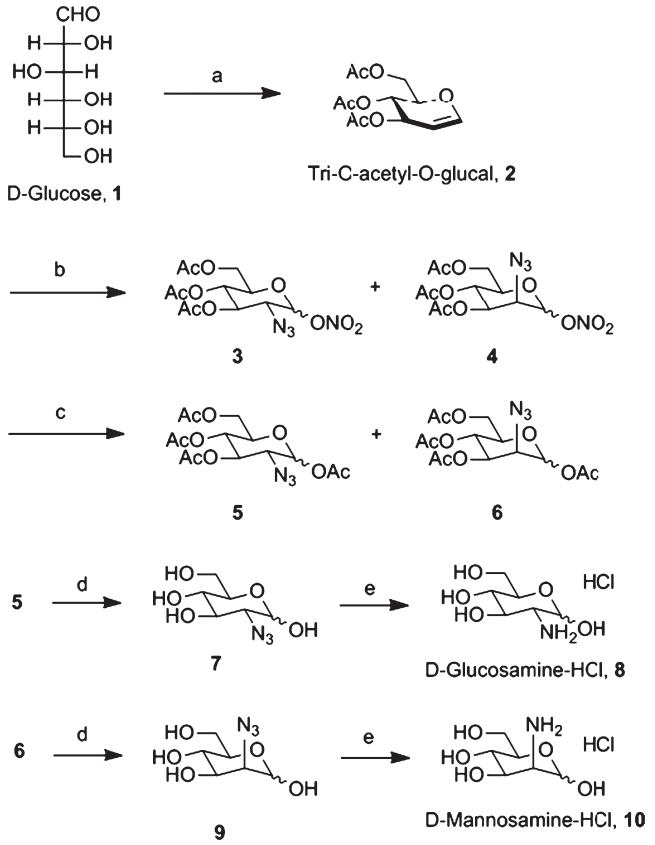

Figure 1. Strategy for the synthesis of D-glucosamine and D-mannosamine. Reagents and conditions: (a) (i) Ac_2O, cat. $HClO_4$, 40 °C, 20 min, (ii) Br_2, P, H_2O, room temperature, 3 h, (iii) Zn powder, AcOH/NaOAc, $CuSO_4{\cdot}5H_2O$, H_2O, 0 °C, 3 h, 72% in three steps; (b) NaN_3, CAN, CH_3CN, −15 °C, overnight; (c) AcOH, NaOAc, 100 °C, 1.5 h, recrystallization and column chromatography, 37% **5** and 31% **6** from **2**; (d) 4 M HCl, 40 °C, overnight; (e) H_2 (1 atm), 10% Pd/C, MeOH-H_2O-4 M HCl = 27:2:1, room temperature, 1 day, 61% **8** from **5**, 61% **10** from **6**.

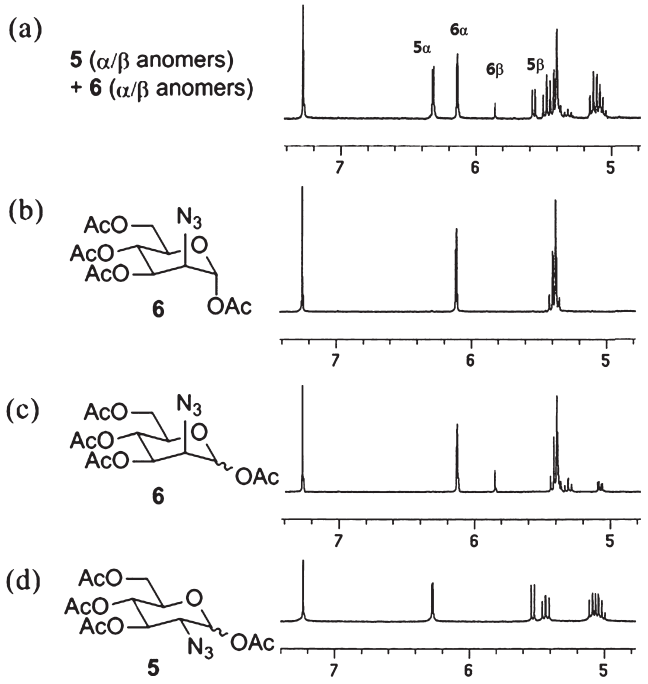

Figure 2. ^{1}H NMR spectra of (a) a mixture of **5** and **6** (both α/β anomers) in the crude product, (b) **6** obtained by recrystallization, (c) **6** obtained from column chromatography, and (d) **5** from column chromatography.

at that temperature for 40 min. After addition of red phosphorus (1.5 g, 48 mmol), the reaction flask was cooled to 0 °C and Br_2 (9 g, 56 mmol) was added during 40 min. H_2O (1.5 mL) was then added during 20 min. The reaction mixture was stirred at room temperature for 3 h. The mixture was filtered with suction through a celite pad, which was washed with CH_3CO_2H (2 mL). The filtered solution containing tetra-*O*-acetyl-α-D-glucopyranosyl bromide was saved to be used in the next step.

$CH_3CO_2Na{\cdot}3H_2O$ (20 g) was dissolved in H_2O (29 mL) and CH_3CO_2H (20 mL) in 500 mL Erlenmeyer flask and the solution was cooled in an ice-salt bath. Zinc dust (11 g, 168 mmol) and then a solution of $CuSO_4{\cdot}5H_2O$ (1.1 g) in H_2O (4 mL) were added into the solution and stirred for 5 min. Into the resulting suspension was added the bromide solution saved previously over a period of 2 h while cooling in an ice-salt bath. After stirring for an additional 3 h at 0 °C, the mixture was filtered and the filter paper was washed with 50% acetic acid (20 mL). H_2O (50 mL) was added into the filtrate, and the solution was extracted six times with $CHCl_3$ (10 mL). The $CHCl_3$ extracts were combined and washed with cold H_2O (20 mL), cold half-saturated Na_2CO_3 solution (20 mL), and cold H_2O (20 mL). It was then dried, concentrated, and purified with column chromatography (SiO_2, hexane:EtOAc = 2:1) to obtain tri-*O*-acetyl-D-glucal as a syrup (5.6 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 4.19 (dd, 1H, J = 12.0 Hz, 3.2 Hz), 4.22–4.27 (m, 1H), 4.39 (dd, 1H, J = 6.4 Hz, 3.6 Hz), 4.84 (dd, 1H, J = 6.4 Hz, 3.6 Hz), 5.22 (dd, 1H, J = 7.6 Hz, 6.0 Hz), 4.32–4.35 (m, 1H), 6.46 (dd, J = 6.4 Hz, 1.2 Hz).

Compounds 5 and 6.[12] A solution of tri-*O*-acetyl-D-glucal (5.65 g, 20.8 mmol) in anhydrous CH_3CN (70 mL) was cooled at −15 °C and added into a solution of NaN_3 (2.02 g, 31.1 mmol) and CAN (34.1 g, 62.2 mmol) in anhydrous CH_3CN (40 mL) at −15 °C. After stirring overnight at −15 °C, the reaction mixture was mixed with cold EtOAc (150 mL) and H_2O (100 mL). The separated organic layer was washed with cold H_2O (3 × 35 mL), dried over Na_2SO_4, and concentrated to obtain a mixture of **3** and **4** as a syrup (7.5 g). The mixture of **3** and **4** was mixed with CH_3CO_2H (50 mL) and sodium acetate (3.3 g), and heated at 100 °C for 1.5 h. The reaction mixture was poured into cold EtOAc (260 mL) and H_2O (200 mL). The organic layer was separated, washed with cold saturated $NaHCO_3$ solution (3 × 50 mL) and cold H_2O (100 mL) successively, dried over Na_2SO_4 and concentrated to obtain a mixture of **5** and **6** as a thick syrup (8.6 g). The syrup was dissolved in MeOH (20 mL) and stored at −20 °C overnight to

obtain 1.3 g of amorphous solids. The solids were recrystallized in MeOH to obtain **6** (1.0 g) as crystals. The mother liquors were combined and purified by SiO_2 column chromatography (hexane:EtOAc = 3:1) to obtain **5** (2.90 g, 36.5%) and **6** (2.44 g including the recrystallization product, 30.8%). Combined yield was 67% from tri-*O*-acetyl glucal.

Compound 5. $R_f = 0.40$ (hexane-EtOAc 2:1); mp 114–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s), 2.02 (s), 2.05 (s), 2.07 (s), 2.08 (s), 2.17 (s), 3.62–3.80 (m), 3.76–3.80 (m), 4.01–4.08 (m), 4.25–4.30 (m), 4.99–5.11 (m), 5.43 (dd, J = 10.4 Hz, J = 9.2 Hz), 5.53 (d, J = 8.4 Hz), 6.27 (d, J = 4.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.70, 20.81, 21.02, 21.07, 60.46, 61.59, 62.75, 67.97, 68.07, 69.92, 70.92, 72.84, 72.87, 90.13, 92.72, 168.66, 168.70, 169.68, 169.77, 169.92, 170.19, 170.67.

Compound 6 (α-anomer). $R_f = 0.25$ (hexane-EtOAc 2:1); mp 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05 (3H, s), 2.09 (3H, s), 2.11 (3H, s), 2.16 (3H, s), 4.00–4.03 (2H, m), 4.09 (1H, dd, J = 12.4 Hz, 2.4 Hz), 4.24 (1H, dd, J = 12.8 Hz, 4.8 Hz), 5.35–5.42 (2H, m), 6.11 (1H, d, J = 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.68, 20.78, 20.88, 21.05, 60.71, 61.98, 65.52, 70.74, 70.95, 91.56, 168.37, 169.55, 170.23, 170.87.

D-Glucosamine hydrochloride. The suspension of **5** (749 mg, 1.96 mmol) in 4 M HCl (8 mL) was stirred overnight at 40 °C. The solids dissolve slowly as the reaction proceeds. Activated carbon (200 mg) was added and stirred for 10 min. After filtration to remove carbon, 1-butanol was added and evaporated *in vacuo* to obtain **7** as a syrup (371 mg). Crude **7** was dissolved in a mixture of MeOH-H_2O-4 M HCl (27:2:1) and hydrogenated at room temperature and 1 atm of H_2 in the presence of 10% Pd/C. After 1 day, the reaction mixture was filtered over a celite pad, concentrated to ca. 1 g, mixed with EtOH (1.5 mL), and left at 4 °C overnight. Solids thus obtained were recrystallized again in a mixture of EtOH-H_2O (4:1) to obtain crystals of D-glucosamine hydrochloride (118 mg, 0.547 mmol, 28%). Combined mother liquor was used to harvest additional products (140 mg, 33%) by repeated recrystallization in EtOH.

D-Mannosamine hydrochloride. The procedure for D-glucosamine hydrochloride was repeated starting with **6** (435 mg, 1.14 mmol) except the recrystallization step. After hydrogenation, the reaction mixture was filtered and concentrated to obtain a syrup (370 mg). It was dissolved in 95% EtOH (1.5 mL) and kept at −20 °C. Scratching the surface of the flask helps crystal formation. The yield was 151 mg (61%).

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Supporting Information. ^{1}H and ^{13}C NMR spectra for compounds **5**, **6**, **8**, and **10** are available.

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