Notes

Preparation of *N*-Acryloyl and *N*-(*N*-Acryloylglycinyl) Derivatives of 2-Acetoamido-2-deoxy-β-D-glucopyranosylamine

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Received March 2, 2001

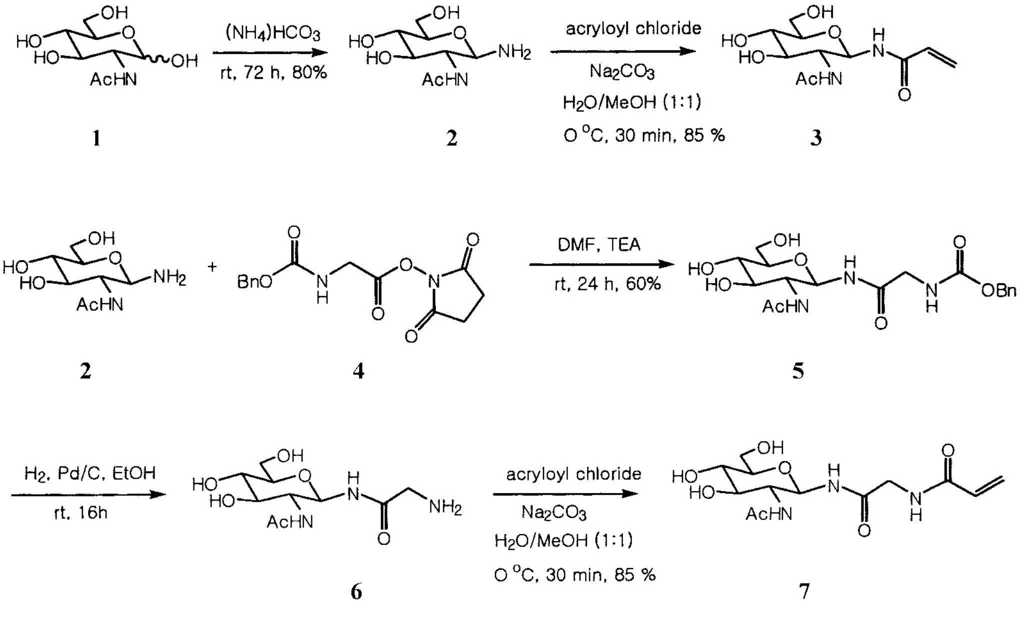
Keywords : *N*-Glycoside, 2-Acetamido-2-deoxy-D-glucopyranosylamine, 2-Acetamido-*N*-acryloyl-2-deoxy-β-D-glucopyranosylamine, Acryloyl chloride.

The β-*N*-glycosidic lingkage can be frequently found in large number of natural glycoconjugates. *N*-glycoside linkages between 2-acetamido-2-deoxy-D-glucopyranosylamine and L-asparagine are most commonly found, but D-galactose and D-glucose attached to glutamine or asparagine with *N*-glycosidic lingkage were also reported.[1] Because altered carbohydrate components of membrane glycoproteins and glycolipids were known to induce neoplastic or transformed cells, enormous attentions have been focused on the development of synthetic analogues of natural glycosides to control the biosynthesis of glycoproteins or glycolipids.[2] Lots of synthetic sugar analogues containing *N*-glycosidic lingkage have been prepared as potent modifiers or inhibitors of membrane glycoconjugates.[3] Some of them were immobilized with agarose gel or synthetic polymers and applied for the separation of various proteins or natural glycoprotiens.[4]

This note describes the convenient synthesis of 2-acetamido-*N*-acryloyl-2-deoxy-β-D-glucopyranosylamine (**3**) and 2-acetamido-*N*-(*N*-acryloylglycinyl)-2-deoxy-β-D-glucopyranosylamine (**7**) starting from 2-acetamido-2-deoxy-D-glucopyranose (**1**) without the protection of hydroxyl groups (Scheme 1).

Various *N*-acyl derivatives of 2-acetamido-2-deoxy-D-glucopyranosylamine have been already proved to be strong metabolic inhibitors of cellular membrane glycoconjugates.[5] The main purpose of introducing acryloyl group is to form glycoconjugates by Michael addition of amino side chains of proteins. Furthermore, it can be polymerized to the new synthetic glycopolymers mimicking natural polysaccharides.

Stereoselective *O*-glycosylations of 2-acetamido-2-deoxy-D-glucopyranose derivatives are usually perfomed with great care because of the neighboring group participation of *N*-acetyl group and various side reactions.[6] However, *N*-glycoside can be prepared much easily by treating ammonium carbonate even without the protection of hydroxyl groups.



Scheme 1

When 2-acetamido-2-deoxy-D-glucopyranose (**1**) was treated with excess ammonium carbonate in water at room temperature for 72 hours, 2-acetamido-2-deoxy-β-D-glucopyranosylamine (**2**) was obtained in 80% yield. One anomer was produced predominantly, and it should have a β-configuration as the literature described.[7]

N-acryloyl group can be introduced by adding acryloyl chloride dissolved in THF to the mixture of compound 2 and sodium carbonate in aqueous methanol at 0 °C. After stirring for 30 minutes, excess acryloyl chloride and sodium carbonate were removed, and the resulting solid was purified with chromatography to yield 2-acetamido-*N*-acryloyl-2-deoxy-β-D-glucopyranosylamine (**3**).

Glycine can be introduced as a spacer molecule between sugar and acryloyl group. *N*-acryloyl amino acid derivatives have been widely chosen as a spacer in the synthesis of biologically active polymers.[5(a),8] The glycine spacer is able to offer more flexibilities to the sugar moiety and reduce steric hindrances during polymerization.

Glycine group can be attached to the compound **2** by the reaction with *N*-(benzyloxycarbonyl)glycine *N*-hydroxysuccinimide ester (**4**) and triethylamine as a base in DMF. The benzyloxycarbonyl protection group of compound **5** was easily removed by hydrogenation. Finally, 2-acetamido-*N*-(*N*-acryloylglycinyl)-2-deoxy-β-D-glucopyranosylamine (**7**) was prepared by attaching acryloyl group to the compound **5** with the same method for the synthesis of compound **3**.

In summary, *N*-acryloyl derivatives of 2-acetamido-2-deoxy-β-D-glucopyranosylamine could be prepared by simple and effective method. Compound **3** and **7** must be considered as useful intermediates for preparing glycoconjugates and glycopolymers. One of the great advantages of *N*-glycoside over its *O*-glycoside counterpart is synthetic convenience. Especially, *O*-glycosidation of 2-acetamido-2-deoxy-D-glucopyranose usually gives low yield and poor stereoselectivities. Therefore, the development of efficient synthetic methodologies for various *N*-glycosides will be useful in designing and preparing synthetic analogues of natural glycoproteins.

Experimental Section

General. All chemicals and solvents were purchased from Sigma and Aldrich and purified before use by the method in the literature if necessary. *N*-(benzyloxycarbonyl)glycine *N*-hydroxysuccinimide ester (**4**) was purchased from Bachem, and 2-Acetamido-2-deoxy-β-D-glucopyranosylamine (**2**) is recently available in Sigma. TLC and column chromatography were done with Merck Kiesegel 60 F_{254} TLC plate and Merck 60, Art 7734 70-230 mesh silica gel. ^1H and ^{13}C NMR experiments were performed with Bruker Avance 400 MHz or Jeol JNM-LA400 MHz NMR.

2-Acetamido-2-deoxy-β-D-glucopyranosylamine (2). 2-Acetamido-2-deoxy-β-D-glucopyranose (**1**, 1 g, 4.45 mmol) was dissolved in water (40 mL), and excess amount of ammonium bicarbonate were added up to saturation. While stirring for 3 days at room temperature, more ammonium bicarbonate were added several times to maintain its saturation. After checking TLC to confirm no more starting material remained, 40 mL water was added, and the mixture was rotaevaporated until the total volume was reduced down to 40 mL. After repeating the same step for six more times, total volume was reduced to 10 mL. The remaining solution was neutralized with ion exchange resin (Dowex 50X, H^+ form), and water was removed. The remaining white solid was redissolved in methanol, and ethyl ether was added until precipitate was appeared. After the filtration, compound **2** was obtained as powder (0.6 g, 80%) and used for the next step without further purification. ^1H NMR (D_2O, ppm): 4.08 (1H, d, J = 8.8 Hz), 3.82 (1H, d, J = 12.0 Hz), 3.62 (1H, dd, J = 12.0 Hz), 3.54 (1H, t, J = 9.6 Hz), 3.43 (1H, m), 3.35 (2H, m), 1.91 (3H, s), ^{13}C-NMR (D_2O, ppm): 175.0, 84.6, 77.2, 74.9, 70.4, 61.2, 56.7, 22.6.

2-Acetamido-*N*-acryloyl-2-deoxy-β-D-glucopyranosylamine (3). Compound **2** (1 g) and sodiunm carbonate (3.3 g) were dissolved in 60 mL aqueous methanol (H_2O : MeOH = 1 : 1). To the reaction flask stirring in ice bath was slowly dropwised acryloyl chloride (1.94 mL, 27.24 mmol) dissolved in THF (16 mL). After stirring 30 min in ice bath, 60 mL water was added and solvent was rotaevaporated until total volume was reduced to 30 mL. Small amount of 2,6-Di-*t*-butyl-4-methylphenol (6 mL of 0.5% solution in THF) and more water (30 mL) were added, and mixture was rotaevaporated again until the total volume was reduced to 15 mL. After pouring 40 mL of methanol, white precipitate of salt was appeared. After the filteration, the collected filtrate was freeze-dried. The remaining solid was redissolved in methanol and purified by chromatography to yield compound **3**. (1.05 g, 85%) ^1H NMR (CD_3OD, ppm): 6.14 (2H, m), 5.64 (1H, dd, J = 2.8, 9.2 Hz), 4.96 (1H, d, J = 10.0 Hz), 3.72 (1H, dd, J = 12.0, 1.6 Hz), 3.69 (1H, t, J = 10.0 Hz), 3.58 (1H, dd, J = 12.0, 4.8 Hz), 3.45 (1H, t, J = 8.8 Hz), 3.27-3.31 (2H, m), 1.86 (3H, s). ^{13}C-NMR (CD_3OD, ppm): 174.9, 168.9, 130.7, 132.2, 128.8, 80.9, 80.2, 76.6, 72.3, 63.1, 56.5, 23.2.

2-Acetamido-*N*-[*N*-(benzyloxycarbonyl)glycinyl]-2-deoxy-β-D-glucopyranosylamine (5). To the Compound **2** (854 mg, 3.9 mmol) dissolved in dry DMF were added *N*-(benzyloxycarbonyl)glycine *N*-hydroxysuccinimide ester (**4**, 1.19 g, 3.9 mmol) and triethylamine (0.54 mL, 3.9 mmol). After stirring for 1 day, solvent was rotaevaporated, and the remaining syrup was purified with chromatography to yield compound **5** (957 mg, 60%). ^1H NMR (CD_3OD, ppm): 7.42 (5H, m), 5.26 (1H, d, J = 12.4 Hz), 5.21 (1H, d, J = 12.4 Hz), 5.06 (1H, d, J = 10 Hz), 3.96-3.84 (3H, m), 3.80 (1H, dd, J = 11.2, 3.6 Hz), 3.59 (1H, m), 3.45 (1H, m), 3.42 (2H, m), 2.09 (3H, s). ^{13}C-NMR (CD_3OD, ppm): 174.8, 173.2, 159.2, 138.2, 129.6, 129.2, 129.1, 80.9, 79.9, 76.2, 72.0, 68.1, 62.8, 56.0, 45.2, 22.9.

2-Acetamido-*N*-(*N*-acryloylglycinyl)-2-deoxy-β-D-glucopyranosylamine (7). The reaction mixture of compound **5** (402.3 mg, 0.98 mmol) and palladium on charcoal (200 mg) in ethanol (30 mL) was stirred for 16 hours under the atmospheric H_2 gas. After filtering with short celite column, the filtrate was collected and rotaevaporated. The remaining syrup of crude compound **6** was dissolved in aqueous methanol (18 mL, H_2O : MeOH = 1 : 1), and 500 mg of sodium carbonate was added. To the reaction flask stirring in ice bath was slowly dropwised acryloyl chloride (4.5 mL, 7.02 mmol) dissolved in THF (4.5 mL). After stirring 30 min in ice bath, 60 mL water was added, and the solvent was rotaevaporated until total volume was reduced to 30 mL. Small amount of 2,6-Di-*t*-butyl-4-methylphenol (6 mL of 0.5% solution in THF) and more water (30 mL) were added, and mixture was rotaevaporated again until the total volume was reduced to 15 mL. After pouring 40 mL of methanol, white precipitate of salt was appeared. White salt was removed by filteration, and the filtrate was freeze-dried. The remaining solid was redissolved in methanol and purified by chromatography to yield compound **7** (1.00 g, 87%). ^{1}H NMR (CD_3OD, ppm): 6.04 (2H, m), 5.41 (1H, dd, J = 10.0, 2.0 Hz), 4.87 (1H, d, J = 9.6 Hz), 3.76 (2H, m), 3.58-3.81 (4H, m), 3.49 (1H, m), 3.31 (1H, m), 3.25 (2H, m), 1.88 (3H, s). ^{13}C-NMR (CD_3OD, ppm): 179.8, 175.9, 173.3, 136.9, 125.9, 79.9, 76.0, 72.2, 69.3, 62.9, 56.2, 52.9, 26.9.

Acknowledgment. This work was supported by the Brain Korea 21 Project in 2000.

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