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Synthesis of *N*-Acetylglucosamine Derivatives as Probes for Specificity of Chicken Hepatic Lectin

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Syntheses of the following compounds are described: 6-(Trifluoroacetylamino)hexyl 2-acetamido-2,6-dideoxy-β-D-glucopyranoside and 2-acetamido-2-deoxy-α-D-xylopyranoside, two allyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronic acid derivatives, and several allyl 2-acylamido-2-deoxy-β-D-glucopyranosides having different acyl groups. These and other compounds were used as inhibitors in the binding assay for the chicken hepatic lectin specific for *N*-acetylglucosamine. We found that: 1) The inhibitory potency of *N*-acylglucosamine derivatives decreased progressively with increase in the size of acyl group, 2) absence of either 3- or 4-OH group of *N*-acetylglucosamine lowered the binding affinity more than 100-fold, and 3) the presence of a negatively charged group (carboxylic acid) at the C-6 position did not lower the affinity. The first two items are similar to the mammalian hepatic galactose/*N*-acetylgalactosamine lectins, but the last item is in a strong contrast to the mammalian lectins.

Chicken hepatic lectin specific for *N*-acetylglucosamine is a trans-membrane protein structurally homologous to the mammalian hepatic galactose/*N*-acetylgalactosamine-specific lectins [1]. In spite of the difference in their sugar specificity, the chicken and mammalian lectins share many similarities in the general construct of their sugar binding sites [2]. In this paper two new aspects of binding specificity of the chicken hepatic lectin were examined and compared with the mammalian lectins. These are the effect of an *N*-acyl group at the C-2 of glucosamine and the requirement for the presence of the 3- and 4-OH groups of *N*-acetylglucosamine. To this end, we have synthesized, among others, various GlcN derivatives having different *N*-acyl groups and the 3- and 4-deoxy derivatives of *N*-acetylglucosamine, and used them as inhibitors in the binding assay.

Abbreviations: XylNAc, *N*-acetyl-D-xylosamine; BSA, bovine serum albumin; NeuAc, *N*-acetylneuraminic acid; GlcNAc34-BSA, amidino-type neoglycoprotein [6] containing on the average 34 *N*-acetylglucosaminyl residues per BSA molecule.

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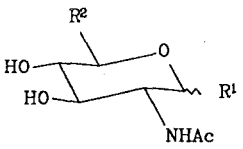
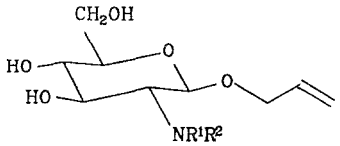


	R^1	R^2
1	β-O(CH$_2$)$_6$NHCOCF$_3$	CH$_3$
2	α-O(CH$_2$)$_6$NHCOCF$_3$	H
3	α-OCH$_2$CH=CH$_2$	CO$_2$H
4	α-OCH$_2$CH=CH$_2$	CO$_2$Me



	R^1, R^2
5	phthaloyl
6	H, H
7	CHO, H
8	COCH$_3$, H
9	COCH$_2$CH$_3$, H
10	CO(CH$_2$)$_2$CH$_3$, H
11	COCH(CH$_3$)$_2$, H
12	COPh, H

Materials and Methods

Materials

Sephadex G-25, UDP, UDP-Glc, UDP-GlcNAc, *N*-acetylneuraminic acid, tin(IV) chloride, and azoisobutyronitrile were purchased from Sigma (St. Louis, MO, USA.). Na^{125}I was obtained from Amersham Corp. (Arlington Heights, IL, USA.). 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose was prepared as described [3]. The preparation of 6-(benzyloxycarbonylamino)hexanol [4], 6-(trifluoroacetylamino)hexanol [4], allyl α- and β-*N*-acetylglucosamine [5], and GlcNAc$_{34}$-BSA [6] have been reported. The preparation of allyl 2-acetamido-2,3-dideoxy-β-D-glucopyranoside and allyl 2-acetamido-2,4-dideoxy-β-D-glucopyranoside will be published elsewhere.

General Methods

Melting points (uncorrected) were determined with a Fisher-Johns apparatus. Elemental analyses were performed by Galbraith Lab., Inc. (Knoxville, TN, USA). When needed, solvent extracts were dried over anhydrous sodium sulfate and solutions were evaporated under diminished pressure. Nuclear magnetic resonance spectra were recorded with a Varian XL-400 spectrometer in C^{2}HCl$_3$ solutions unless otherwise specified. Proton chemi-

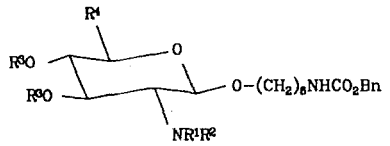
cal shift are in p.p.m. relative to an internal reference of tetramethylsilane (δ 0.00) or **H**O^{2}H in ^2H_2O at 24°C (δ 4.778). Unless otherwise stated, the column chromatography was carried out with Silica gel 60, 15-40 μm (Merck, Darmstadt, W. Germany).

The binding affinity of various glucosamine derivatives to the chicken hepatic lectin was estimated with an inhibition assay using isolated chicken hepatocytes. The isolation of chicken hepatocytes was by the collagenase digestion method of Seglen [7] as described [8]. As the reference ligand, $GlcNAc_{34}$-BSA was iodinated by the Chloramine T method [9] to a specific activity of ≈2 x 10^6 cpm/pmol. The inhibition assay was carried out by a scaled-down version of the previously described method [10]. Assay mixtures contained in 0.5 ml of the modified Dulbecco's Eagles medium ≈7 x 10^{-11} M of ^{125}I-$GlcNAc_{34}$-BSA, a test compound at various concentrations, and 3-4 million chicken hepatocytes. The mixture was incubated at 2°C for 2 h after which time an aliquot of each incubation mixture (duplicate) was centrifuged through an oil layer to separate the cells from the medium. The cell pellets were counted in a gamma-counter (Packard MINAXI 5000) to determine the amount of ^{125}I-$GlcNAc_{34}$-BSA bound to the cells. The percent inhibition was then plotted against log[inhibitor] and the concentration of the inhibitor that causes 50% inhibition (I_{50}) was obtained from each sigmoidal curve.

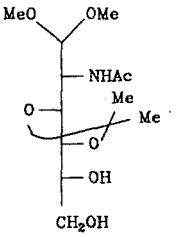
Preparation of a 6-Deoxy-β-GlcNAc Glycoside

*6-(Benzyloxycarbonylamino)hexyl 3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-gluco-pyranoside (**13**):* Tin(IV) chloride [11] (9.35 g, 35.9 mmol; 4.2 ml) was added dropwise during a 40 min period to a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (6.3 g, 13.2 mmol) and 6-(benzyloxycarbonylamino)hexanol (3.94 g, 15.7 mmol) in dichloromethane (200 ml) at room temperature, and the reaction mixture was stirred for 10 h. The mixture was poured into ice-cold aqueous sodium hydrogencarbonate and the precipitate was filtered off with the aid of Celite. The filtrate was extracted with dichloromethane, dried, and concentrated. The residual syrup was purified by column chromatography (eluted with toluene/methanol, 9/1 by vol) to give **13** (3.86 g, 44%), m.p. 102-103°C (from chloroform-ether-hexane). NMR data: ^{1}H, δ 1.04-1.50 (m, aminohexyl), 1.87, 2.03, 2.11 (s, 3 x OAc), 2.96-3.05 (m, C**H**$_2$NHCO$_2$Bn), 3.39-3.47 (m, O-C**H**H-CH$_2$-), 3.80-3.88 (m, H-5, O-CH**H**-CH$_2$-), 4.18 (dd, *J* 2.32 and 12.23 Hz, H-6a), 4.28-4.37 (m, H-2,6b), 4.64-4.70 (br s, N**H**CO$_2$Bn), 5.09 (s, 2 H, benzylic), 5.18 (dd, *J* 9.06 and 10.10 Hz, H-4), 5.34 (d, *J* 8.54 Hz, H-1), 5.79 (dd, *J* 9.06 and 10.74 Hz, H-3).
Analysis. Calculated for $C_{34}H_{40}N_2O_{12}$: C, 61.07; H, 6.03; N, 4.19. Found: C, 61.42; H, 6.11; N, 4.25.

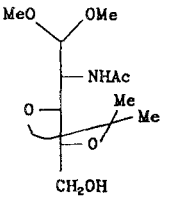
*6-(Benzyloxycarbonylamino)hexyl 3,4-Di-O-acetyl-2-deoxy-2-phthalimido-6-O-tolylsulfonyl-β-D-glucopyranoside (**14**):* A solution of **13** (3.20 g, 4.79 mmol) in 4 mM sodium methoxide in methanol (50 ml) was stirred for 3 h at room temperature, and the mixture was neutralized with Dowex 50W-X8 [H$^+$]. The resin was filtered off and the filtrate was evaporated. To a solution of the residue in dichloromethane (20 ml) and pyridine (20 ml) was added dropwise a solution of tolylsulfonyl chloride (1.57 g, 8.24 mmol) in dichloromethane (10 ml) and pyridine (10 ml) over 20 min at 0-5°C, and the mixture was stirred for one day at room temperature. The mixture was poured into ice-water and extracted with chloroform. The organic extracts were successively washed with ice-cold



	R^1, R^2	R^3	R^4
13	phthaloyl	Ac	CH_2OAc
14	phthaloyl	Ac	CH_2OTs
15	phthaloyl	Ac	CH_2I
16	phthaloyl	Ac	CH_3
17	Ac, H	H	CH_3



18



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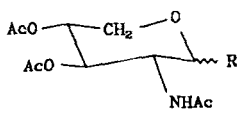
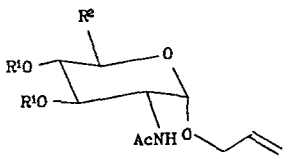


	R
20	OAc
21	α-$O(CH_2)_6NHCOCF_3$



	R^1	R^2
22	H	CH_2OH
23	Ac	CH_2OTr

dilute hydrochloric acid, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. After the 6-*O*-tolylsulfonylation product was partially purified by column chromatography (eluted with chloroform/ethyl acetate, 5/2 by vol), it was per-*O*-acetylated with acetic anhydride (20 ml) and pyridine (20 ml), and the mixture was evaporated. The residual syrup was purified by column chromatography (eluted with toluene/ethyl acetate, 9/1 by vol) to give **14** (2.17 g, 58%) as a syrup. NMR data: ^1H, δ 1.84, 2.00 (s, 2 x OAc), 2.46 (s, aromatic methyl of tolylsulfonyl), 3.89 (ddd, *J* 3.52, 5.10, and 10.14 Hz, H-5), 4.11-4.17 (m, H-6a, 6b), 4.22 (dd, *J* 8.45 and 10.77 Hz, H-2), 4.66 (br s, NH), 4.99 (dd, *J* 9.03 and 10.13 Hz, H-4), 5.09 (br s, benzylic), 5.28 (d, *J* 8.42 Hz, H-1), 5.75 (dd, *J* 9.03 and 10.74 Hz, H-3).

Analysis. Calculated for $C_{39}H_{44}N_2O_{13}S$: C, 59.99; H, 5.68; N, 3.59. Found: C, 59.70; H, 5.90; N, 3.95.

*6-(Benzyloxycarbonylamino)hexyl 3,4-Di-O-acetyl-2,6-dideoxy-6-iodo-2-phthalimido-β-D-glucopyranoside (**15**):* A mixture of **14** (1.17 g, 1.50 mmol) and sodium iodide (674 mg, 4.50 mmol) in 2-butanone (50 ml) was heated under reflux for 10 h, then cooled and evaporated. The residue was partitioned between chloroform (100 ml) and water (10 ml), and the organic layer was successively washed with aqueous sodium hydrogensulfite, aqueous sodium hydrogencarbonate, and water, dried, and then evaporated. The residue was purified by column chromatography (eluted with toluene/ethyl acetate, 9/1 by vol) to give **15** (1.06 g, 96%), m.p. 82-83.5°C (from ethyl acetate-hexane). NMR data: ^{1}H, δ 1.86, 2.06 (s, 2 x OAc), 3.19 (dd, *J* 8.66 and 10.86 Hz, H-6a), 3.32 (dd, *J* 2.95 and 10.87 Hz, H-6b), 3.69 (ddd, *J* 2.66, 8.76, and 9.86 Hz, H-5), 4.30 (dd, *J* 8.55 and 10.77 Hz, H-2), 4.68-4.69 (br s, NH), 4.95 (br t, *J* 9.67 Hz, H-4), 5.09 (br s, benzylic), 5.36 (d, *J* 8.54 Hz, H-1), 5.78 (dd, *J* 8.91 and 10.77 Hz, H-3).
Analysis. Calculated for $C_{32}H_{37}N_2O_{11}I$: C, 52.18; H, 5.06; N, 3.80; I, 17.23. Found: C, 52.44; H, 5.23; N, 3.81; I, 17.12.

*6-(Benzyloxycarbonylamino)hexyl 3,4-Di-O-acetyl-2,6-dideoxy-2-phthalimido-β-D-glucopyranoside (**16**):* A solution of **15** (864 mg, 1.17 mmol), tri-*n*-butyltin hydride (1.02 g, 3.52 mmol; 0.95 ml), and azoisobutyronitrile (327 mg, 1.99 mmol) in toluene (20 ml) was heated at 80°C for 30 min and the mixture was concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 9/1 by vol) to give **16** (764 mg, 93%) as a syrup. NMR data: ^{1}H, δ 1.29 (d, *J* 7.22 Hz, 6-methyl), 1.87, 2.05 (s, 2 x OAc), 3.72-3.78 (m, H-5), 4.28 (dd, *J* 8.45 and 10.77 Hz, H-2), 4.91 (t, *J* 9.25 Hz, H-4), 5.09 (br s, benzylic), 5.31 (d, *J* 8.45 Hz, H-1), 5.75 (dd, *J* 9.25 and 10.75 Hz, H-3).
Analysis. Calculated for $C_{32}H_{38}N_2O_{10}$: C, 62.94; H, 6.27; N, 4.59: Found: C, 63.03; H, 6.53; N, 4.55.

*6-(Benzyloxycarbonylamino)hexyl 2-Acetamido-2,6-dideoxy-β-D-glucopyranoside (**17**):* A mixture of **16** (190 mg, 0.31 mmol) and butylamine [12] (3 ml) in methanol (15 ml) was heated under reflux for 10 h, then cooled and concentrated. The residue was evaporated with toluene twice. Acetic anhydride (2 ml) was added to a solution of the residue in methanol (10 ml) at 0-5°C, and the mixture was stirred for 2 h at room temperature, then concentrated. The residue was purified by column chromatography (eluted with chloroform/ethyl acetate/methanol, 50/20/2 by vol) to give **17**, which crystallized out from methanol-ether to give the desired product (64 mg, 48%), m.p. 161-162°C. NMR data: ^{1}H, δ($C^2H_3O^2H$) 1.28 (d, *J* 6.08 Hz, 6-methyl), 1.96 (s, NHAc), 3.03 (dd, *J* 8.95 and 9.22 Hz, H-4), 3.28 (dq, *J* 9.22 and 6.10 Hz, H-5), 3.39 (dd, *J* 8.30 and 10.10 Hz, H-2), 3.61 (dd, *J* 8.95 and 10.10 Hz, H-3), 4.34 (d, *J* 8.30 Hz, H-1), 5.06 (s, benzylic).
Analysis. Calculated for $C_{22}H_{34}N_2O_7$: C, 60.26; H, 7.82; N, 6.39: Found: C, 60.18; H, 7.70; N, 6.28.

*6-(Trifluoroacetylamino)hexyl 2-Acetamido-2,6-dideoxy-β-D-glucopyranoside (**1**):* Compound **17** (64 mg, 0.15 mmol) was hydrogenated with 10% Pd-C (45 mg) in 60% acetic acid (5 ml). After hydrogen uptake ceased, the suspension was filtered with the aid of Celite, then the filtrate was concentrated, and evaporated with toluene twice. The residue was treated with ethyl trifluoroacetate (1 ml) and triethylamine (5 drops) in methanol (15 ml) for 10 h at room temperature, and the mixture was concentrated. The residue was purified on a column of Sephadex G-25 (1.2 x 30 cm) with water as the eluant to give **1**, which was

freeze-dried (54 mg, 90%). NMR data: ^{1}H, δ(^2H_2O) 1.30 (d, *J* 6.24 Hz, 6-methyl), 1.15-1.63 (m, aminohexyl), 2.02 (s, NHAc), 3.20 (br t, *J* 9.30 Hz, H-4), 3.31-3.33 (m, -C**H**$_2$NHCOCF$_3$), 3.43-3.51 (m, H-5), 3.47 (dd, *J* 8.52 and 10.42 Hz, H-2), 3.56 (dt, *J* 10.27 and 6.39 Hz, O-C**H**H-CH$_2$-), 3.66 (dd, *J* 8.53 and 10.42 Hz, H-3), 3.84 (dt, *J* 10.27 and 6.03 Hz, O-CH**H**-CH$_2$-), 4.47 (d, *J* 8.52 Hz, H-1).

Preparation of an N-Acetylxylosamine Glycoside

*2-Acetamido-2-deoxy-3,4-O-isopropylidene-aldehydo-D-xylose Dimethyl Acetal (**19**):* A solution of sodium metaperiodate (343 mg, 1.61 mmol) in water (7 ml) was added dropwise to a cooled solution of 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-aldehydo-D-glucose dimethyl acetal (**18**) [13] (470 mg, 1.53 mmol) in water (5 ml) at 0-5°C, and the mixture was stirred for 30 min with cooling. A solution of barium chloride dihydrate [14] (198 mg, 0.81 mmol) in water (2 ml) was added to the reaction mixture, and the mixture was stirred for 5 min, after which time the precipitate was filtered off with Celite.

To the cooled filtrate was added a solution of sodium borohydride (69 mg, 1.84 mmol) in water (2 ml) and the mixture was stirred for 15 min with cooling. After dropwise addition of acetic acid (1 ml) to the reaction mixture, the mixture was stirred for 5 min, and evaporated. The residue was purified by column chromatography (eluted with toluene/ethyl acetate, 1/2 by vol) to give **19** (410 mg, 97%) as a syrup. NMR data: ^{1}H, δ 1.40, 1.41 (s, methyl groups of isopropylidene), 2.06 (s, NHAc), 3.33, 3.41 (s, **Me**O- of dimethylacetal), 3.72-3.80 (m, H-3,4,5a,5b), 4.16-4.19 (m, OH), 4.25 (ddd, *J* 1.62, 6.41, and 9.49 Hz, H-2), 4.43 (d, *J* 6.41 Hz, H-1), 5.93 (br d, *J* 9.29 Hz, N**H**Ac).
Analysis. Calculated for $C_{12}H_{23}NO_6$: C, 51.98; H, 8.36; N, 5.05. Found: C, 52.26; H, 8.16; N, 4.92.

*1,3,4-Tri-O-acetyl-2-acetamido-2-deoxy-D-xylopyranose (**20**):* A solution of **19** (410 mg, 1.48 mmol) in 2 M sulfuric acid (6 ml) and glacial acetic acid (12 ml) was heated for 1 h at 100°C, cooled, and neutralized by careful addition of a saturated aqueous sodium hydroxide, and concentrated. The residue was evaporated with pyridine twice and acetylated with acetic anhydride (5 ml) and pyridine (10 ml). The mixture was concentrated, and purified by column chromatography (toluene/ethyl acetate, 1/1 by vol) to give **20** (368 mg, 81%) as the α-isomer: m.p. 204-206°C (from ethyl acetate-hexane). NMR data: ^{1}H, δ 1.95, 2.06, 2.08, 2.19 (s, 3 x OAc, NHAc), 3.60 (dd, *J* 10.68 and 11.11 Hz, H-5ax), 3.96 (dd, *J* 5.55 and 11.11 Hz, H-5eq), 4.41 (br dt, *J* 3.42 and 9.45 Hz, H-2), 5.06 (ddd, *J* 5.55, 10.04, and 10.68 Hz, H-3), 5.61(br d, *J* 9.19 Hz, NH), 6.10 (d, *J* 3.42 Hz, H-1).
Analysis. Calculated for $C_{12}H_{19}NO_8$: C, 49.20; H, 6.04; N, 4.41. Found: C, 49.27; H, 6.03; N, 4.37.

*6-(Trifluoroacetylamino)hexyl 2-Acetamido-2-deoxy-α-D-xylopyranoside (**2**):* A solution of **20** (220 mg, 0.72 mmol), 6-(trifluoroacetylamino)hexanol (184 mg, 0.86 mmol), and borontrifluoride etherate (102 mg, 0.72 mmol; 89 µl) in nitromethane (20 ml) was heated under reflux for 20 min, cooled, and concentrated [15]. The residue was partially purified by column chromatography (2:3 (v/v) eluted with toluene/ethyl acetate, 2/3 by vol) to give **20** which contained a small amount of starting material **19**. This material was treated with 0.1 M methanolic sodium methoxide (1.0 ml) in methanol (10 ml) for 45 min at room

temperature. After neutralizing with Dowex 50W-X8 [H^+], the resin was filtered off, and the filtrate was concentrated. The residue was purified by column chromatography (eluted with chloroform/ethyl acetate/methanol, 55/17/8 by vol) and then crystallized from methanol-ether to give **2** (964 mg, 24% overall yield), m.p. 145-146°C. NMR data: ^1H, $\delta(^2H_2O)$ 1.32-1.70 (m, 6-aminohexyl), 1.98 (s, NHAc), 3.26-3.30 (m, C**H**$_2$NHCOCF$_3$), 3.37 (dt, *J* 9.79 and 6.32 Hz, O-C**H**H-CH$_2$-), 3.45-3.61 (m, H-3,4,5ax,5eq), 3.66 (dt, *J* 9.79 and 6.41 Hz, O-CH**H**-CH$_2$-), 3.84 (dd, *J* 3.47 and 10.16 Hz, H-2), 4.71 (d, *J* 3.47 Hz, H-1).
Analysis. Calculated for $C_{15}H_{25}F_3N_2O_6$: C, 46.63; H, 6.52; N, 7.25. Found: C, 46.65; H, 6.60; N, 7.11.

Preparation of Uronic Acid Derivatives of N-Acetylglucosamine

*Allyl 2-Acetamido-2-deoxy-α-D-glucopyranosiduronic Acid (**3**):* A solution of allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**22**) [5] (1.5 g, 5.74 mmol) and trityl chloride (1.92 g, 6.89 mmol) in pyridine (50 ml) was heated at 80°C for 10 h. After cooling, acetic anhydride (10 ml) was added to the mixture, and the mixture was stirred for 3 h at room temperature. The mixture was concentrated and evaporated with toluene three times. A solution of the residue in 100 ml of 80% acetic acid [16] was left at room temperature for 10 h. The precipitate was filtered off, then the filtrate was concentrated, and the residue was evaporated with toluene twice.

To a cooled solution of the residue in acetone (100 ml) was added dropwise chromium trioxide solution (8 M; 5 ml) over 5 min at 0-5°C, and the mixture was stirred for 4 h at room temperature. The mixture was poured into ice-water and extracted with chloroform. The extracts were washed with water, dried, and concentrated. The residue was purified by column chromatography (5:2:1 (v/v) eluted with chloroform/ethyl acetate/methanol, 5/2/1 by vol) to give a uronic acid, which was treated with 0.1 M sodium methoxide in methanol (20 ml) for 3 h at room temperature. The mixture was carefully neutralized by addition of Dowex 50W-X8 [H^+], then the resin was filtered off, and the filtrate was concentrated.

The residue was purified by column chromatography of Sephadex G-10 (2 x 90 cm) with 0.1 M acetic acid, followed by crystallization from methanol-ether to give **3** (420 mg, 29%), m.p. 173-176°C. NMR data: ^1H, $\delta(^2H_2O)$ 2.03 (s, NHAc), 3.65 (dd, *J* 10.12 and 10.91 Hz, H-4), 3.79 (dd, *J* 10.52 and 10.91 Hz, H-3), 3.98 (dd, *J* 3.68 and 10.52 Hz, H-2), 4.23 (d, *J* 10.12 Hz, H-5), 4.25 (d, *J* 3.68 Hz, H-1).
Analysis. Calculated for $C_{11}H_{17}NO_7$: C, 47.99; H, 6.22; N, 5.09. Found: C, 47.91; H, 6.22; N, 4.93.

*Allyl 2-Acetamido-2-deoxy-α-D-glucopyranosiduronic Acid Methyl Ester (**4**):* A suspension of **3** (120 mg, 0.44 mmol) and Dowex 50W-X8 [H^+] (200 mg) in methanol (5 ml) was stirred for 10 h at room temperature, and the mixture was filtered. The filtrate was concentrated and fractionated on a column of Sephadex G-25 (1.5 x 40 cm) with water. The fractions containing **4** were freeze-dried to give **4** as a solid (120 mg, 94%). NMR data: ^1H, $\delta(^2H_2O)$ 2.04 (s, NHAc), 3.66 (dd, *J* 9.04 and 10.00 Hz, H-4), 3.79 (dd, *J* 9.04 and 10.69 Hz, H-3), 3.84 (s, CO$_2$C**H**$_3$), 3.99 (dd, *J* 3.64 and 10.69 Hz, H-2), 4.30 (d, *J* 10.00 Hz, H-5), 4.99 (d, *J* 3.64 Hz, H-1).

*Allyl 2-Deoxy-2-phthalimido-β-D-glucopyranoside (**5**):* Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside [11] (1.0 g, 2.10 mmol) was treated with sodium methoxide in methanol (3.3 mM, 30 ml) for 3 h at room temperature. After neutralization with Dowex 50W-X8 [H^+], the resin was filtered off, and the filtrate was concentrated. The residue was recrystallized from methanol-ether to give **5** (700 mg, 95%), m.p. 188-189°C. NMR data: ^1H, δ($C^2H_3O^2H$) 5.19 (d, *J* 8.51 Hz, H-1).
Analysis. Calculated for $C_{17}H_{19}NO_7$: C, 58.46; H, 5.48; N, 4.01. Found: C, 58.47; H, 5.66; N, 4.04.

*Allyl 2-Amino-2-deoxy-β-D-glucopyranoside (**6**):* A solution of allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (100 mg, 0.21 mmol) and butylamine (2 ml) in methanol (10 ml) was heated under reflux for 10 h, cooled, and concentrated. This was used in the *N*-acylation reactions without further purification. For the binding study, a portion of the residue was purified by chromatography on a column of Sephadex G-25 (1.2 x 30 cm) with water and then freeze-dried to give **6** as a solid (36.9 mg, 81%).

*Allyl 2-Deoxy-2-formamido-β-D-glucopyranoside (**7**):* Compound **6** (46 mg, 0.21 mmol) was treated with 15 ml of methyl formate [17] at room temperature for 10 h, and the mixture was concentrated. The residue was treated with acetic anhydride (2 ml) and pyridine (3 ml) for 3 h at room temperature, followed by concentration. The residue was purified by column chromatography (eluted with toluene/ethyl acetate, 1/1 by vol) to give the acetylated **7** (90.8 mg), which was *O*-deacetylated with 5 mM methanolic sodium methoxide (10 ml). After neutralization with Dowex 50W-X8 [H^+], the resin was filtered off, and the filtrate was concentrated. Crystallization from methanol-ether gave **7** (32.5 mg, 63%), m.p. 165-167°C.
Analysis. Calculated for $C_{10}H_{17}NO_6$: C, 48.59; H, 6.93; N, 5.67. Found: C, 48.73; H, 7.03; N, 5.43.

*Allyl 2-Deoxy-2-propionamido-β-D-glucopyranoside (**9**):* Propionic anhydride (273 mg, 2.1 mmol; 0.27 ml) was added to a cooled solution of **6** (46 mg, 0.21 mmol) in methanol (10 ml) at 0-5°C, and the mixture was stirred at room temperature for 10 h, then concentrated, and the product was crystallized from methanol-ether to give **9** (27.7 mg, 48%), m.p. 192-192.5°C. NMR data: ^1H, δ(^2H_2O) 1.13 (t, *J* 7.65 Hz, $NHCOCH_2C\mathbf{H}_3$), 2.30 (q, *J* 7.65 Hz, $NHCOC\mathbf{H}_2CH_3$), 3.41-3.47 (m, H-4,6a), 3.50-3.57 (m, H-3), 3.71 (dd, *J* 8.47 and 10.37 Hz, H-2), 3.71-3.77 (m, H-5), 3.93 (dd, *J* 1.76 and 12.48 Hz, H-6b), 4.12-4.18 (m, allylic proton of allyl group), 4.30-4.37 (m, allylic proton of allyl group), 4.57 (d, *J* 8.47 Hz, H-1), 5.23-5.34 (m, vinylic protons of allyl group), 5.85-5.96 (m, vinylic proton of allyl group).
Analysis. Calculated for $C_{12}H_{21}NO_6$: C, 52.35; H, 7.69; N, 5.09. Found: C, 51.56; H, 7.69; N, 4.86.

*Allyl 2-Butyramido-2-deoxy-β-D-glucopyranoside (**10**):* Butyric anhydride (967 mg, 6.30 mmol; 1.0 ml) was added to a cooled solution of **6** (46 mg, 0.21 mmol) in methanol (10 ml) at 0-5°C, and the mixture was stirred at room temperature for 10 h. The mixture was evaporated and the residue was dissolved in a mixture of toluene and water. The aqueous layer was washed with ether twice, and evaporated. The product was crystallized from methanol-ether-hexane to give **10** (26.6 mg, 44%), m.p. 201-203.5°C. NMR data: ^1H,

δ(^2H_2O) 0.93 (t, *J* 7.45 Hz, $NHCOCH_2CH_2C\mathbf{H}_3$), 1.57-1.67 (m, $NHCOCH_2C\mathbf{H}_2CH_3$), 3.51-3.60 (m, H-3), 3.72 (dd, *J* 8.50 and 10.31 Hz, H-2), 3.72-3.77 (m, H-5), 3.93 (dd, *J* 1.85 and 12.36 Hz, H-6b), 4.57 (d, *J* 8.50 Hz, H-1).
Analysis. Calculated for $C_{13}H_{23}NO_6 \cdot H_2O$: C, 50.81; H, 8.20; N, 4.56. Found: C, 50.44; H, 8.09; N, 4.43.

*Allyl 2-Deoxy-2-(2-methylpropion)amido-β-D-glucopyranoside (**11**):* To a cooled solution of **6** (46 mg, 0.21 mmol) in methanol (10 ml) was added 2-methyl-propionic anhydride (333 mg, 2.10 mmol; 0.35 ml) at 0-5°C, and the mixture was stirred at room temperature for 10 h. The mixture was concentrated and the residue was dissolved in a mixture of toluene and water. The aqueous layer was washed with ether and concentrated. The product was crystallized from methanol-ether-hexane to give **11** (44.5 mg, 73%), m.p. 223-224°C. NMR data: ^1H, δ(^2H_2O) 1.11 (d, *J* 6.85 Hz, methyl group of isopropyl), 1.12 (d, *J* 6.95 Hz, methyl group of isopropyl), 2.51 (dq, *J* 6.93 and 6.95 Hz, $NHCOC\mathbf{H}Me_2$), 3.40-3.46 (m, H-4,6a), 3.51-3.56 (m, H-3), 3.70 (dd, *J* 8.54 and 10.32 Hz, H-2), 3.92 (dd, *J* 1.82 and 12.34 Hz, H-6b), 4.57 (d, *J* 8.54 Hz, H-1).
Analysis. Calculated for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84. Found: C, 53.26; H, 8.03; N, 4.65.

*Allyl 2-Benzamido-2-deoxy-β-D-glucopyranoside (**12**):* Benzoic anhydride (476 mg, 2.10 mmol) was added to a cooled solution of **6** (46 mg, 0.21 mmol) in methanol (10 ml) at 0-5°C, and the mixture was stirred at room temperature for 10 h. The mixture was evaporated and the residue was dissolved in a mixture of toluene and water. The aqueous layer was washed with ether, and concentrated. The residue was purified on a column of Sephadex G-25 (1.5 x 35 cm) with water as eluant. The product was freeze-dried to give 12 (43.4 mg, 64%) as an amorphous powder.
Analysis. Calculated for $C_{16}H_{21}NO_6$: C, 59.44; H, 6.55; N, 4.33. Found: C, 58.65; H, 6.67; N, 4.52.

Results

Synthesis

Deoxygenation of the 6-hydroxyl group of **13** was performed by the sequential modification: $CH_2OH \rightarrow CH_2OTs \rightarrow CH_2I \rightarrow CH_3$ [18]. A triol obtained by *O*-deacetylation of **13** was treated with tolylsulfonyl chloride followed by reacetylation to give 6-*O*-tolylsulfonyl derivative (**14**) in 58% overall yield. Displacement of tolylsulfonyloxy group of **14** with iodine, and reduction of the resulting iodide (**15**) with tri-*n*-butyltin hydride gave the 6-deoxy derivative (**16**) in high yield. *N*-Dephthaloylation of **16** with butylamine [12] and subsequent *N*-acetylation gave **17** in 40% yield after crystallization. In order to reduce the hydrophobicity of the aglycon, the *N*-benzyloxycarbonyl group of **17** was replaced with a trifluoroacetyl group by catalytic hydrogenolysis followed by reaction with ethyl trifluoroacetate to give **1** in 90% yield.

Only two reports [14, 19] on the synthesis of xylosamine derivatives have appeared to the best of our knowledge. Wolfrom and Thompson [19] prepared D-xylosamine derivatives

Table 1. Inhibition of the binding of ^{125}I-GlcNAc$_{34}$-BSA to chicken hepatocytes by *N*-acyl-glucosamine derivatives.

Compounds	*N*-Acyl group[a]	I_{50} (mM)
6	none	35[b]
7	CHO	0.32
8	$COCH_3$	0.18
9	$COCH_2CH_3$	0.26
10	$CO(CH_2)_2CH_3$	0.43
11	$COCH(CH_3)_2$	0.80
12	COC_6H_5	0.90
5	phthaloyl	N.I.[c]

[a] Substituent on the 2-amino group of allyl 2-amino-2-deoxy-β-D-glucopyranoside.

[b] 47% inhibition at 35 mM.

[c] Not inhibitory at 0.4 mM. Due to its low solubility, this compound could not be tested above 0.4 mM.

from diethylthioacetal of *N*-acetylglucosamine. However, both the oxidative cleavage of vicinal diol and the deprotection of thioacetal group in their synthesis gave a poor chemical yield (18%). Presumably, the thioacetal moiety was also oxidized under the periodate oxidation conditions. Therefore, in our preparation of *N*-acetylxylosamine, we used a dimethylacetal to avoid such side reactions.

Our synthesis started with **18** [13], in which both aldehyde and 3,4-hydroxyl groups of the *N*-acetylglucosamine were protected with dimethylacetal and isopropylidene group, respectively. Oxidative cleavage of the diol **18** with sodium metaperiodate and subsequent reduction of the resulting 5-oxo group with sodium borohydride gave **19** in 97% yield. Deprotection of **19**, however, required vigorous conditions; in fact, treatment of **19** with CF_3COOH-Ac_2O or 90% CF_3COOH could not remove dimethylacetal group. The compound **19** was treated with AcOH and 2 M H_2SO_4 at 100°C [20] for 1 h, followed by acetylation to give **20** in 81% yield. The glycoside formation between **20** and 6-(trifluoroacetyl-amino)hexanol with BF_3·OEt_2 as a catalyst [15] afforded the α-glycoside (**21**) as a sole product, which was subjected to *O*-deacetylation to yield **2**.

Uronic acid derivatives of *N*-acetylglucosamine were prepared from allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**22**) [5]. 6-*O*-Tritylation of **22** followed by acetylation gave **23**, which upon oxidation with chromic trioxide in acetone followed by treatment with sodium methoxide gave **3** in 29% overall yield. Methanolysis of **3** in the presence of Dowex 50-X8 [H^+] resin afforded the methyl ester **4**.

N-Acyl derivatives of allyl 2-amino-2-deoxy-β-D-glucopyranoside were synthesized by treating the 2-amino derivative, **5**, which was obtained by butylamine treatment of **12**, with various acid anhydrides such as propionic, butyric, isobutyric and benzoic anhydrides, and with methyl formate [17].

Table 2. Inhibition of the binding of ^{125}I-GlcNAc$_{34}$-BSA to chicken hepatocytes by other *N*-acetylglucosamine derivatives.

Compounds	I_{50} (mM)
allyl β-GlcNAc (**8**)	0.18
allyl 3-deoxy-β-GlcNAc	20[a]
allyl 4-deoxy-β-GlcNAc	N. I.[b]
TFAAH[c] 6-deoxy-β-GlcNAc (**1**)	0.28[d]
TFAAH α-XylNAc (**2**)	1.0[d]
allyl α-GlcNAc	0.34
3	1.1
4	1.1
UDP-GlcNAc	0.027
UDP-Glc	4.2
UDP	20[e]
NeuAc	N.I.[f]

[a] 35% inhibition at 20 mM.

[b] Not inhibitory at 20 mM.

[c] TFAAH = $-O(CH_2)_6NHCOCF_3$.

[d] Published results [2].

[e] 23% inhibition at 20 mM.

[f] Not inhibitory at 24 mM.

Inhibition Assay

Various glucosamine derivatives were tested as inhibitors in the binding assay and the I_{50} values are presented in Tables 1 and 2. Table 1 lists a series of allyl *N*-acyl-β-D-glucosaminides in the order of increasing size of the acyl group. The Table shows that the acetyl group was the optimal N-substituent (lowest I_{50}), and the increase in size of the acyl group brought about a gradual decrease in the binding affinity.

The importance of the 3- and 4-OH groups for the binding affinity is clearly shown, since the allyl β-glycoside of both 3- and 4-deoxy-GlcNAc had at least a 100-fold weaker affinity than allyl β-GlcNAc (Table 2). Compounds **1-4** represent various modifications at the C-6 position of *N*-acetylglucosamine. The replacement of CH_2OH with CH_3 (**1**) showed a barely discernible increase in I_{50}, while the replacement with H (**2**), COOH (**3**), or COOMe (**4**) all caused a slightly more lowering of affinity. Curiously, UDP-GlcNAc, with a bulky and negatively charged aglycon, was a 20-fold stronger inhibitor than *N*-acetylglucosamine. Similarly, the I_{50} of UDP-Glc (4.2 mM) was 30-fold lower than that of glucose (≈130 mM). This strong binding of UDP-GlcNAc is a sugar-mediated phenomenon, since the I_{50} of UDP itself was more than 1000-fold higher than that of UDP-GlcNAc.

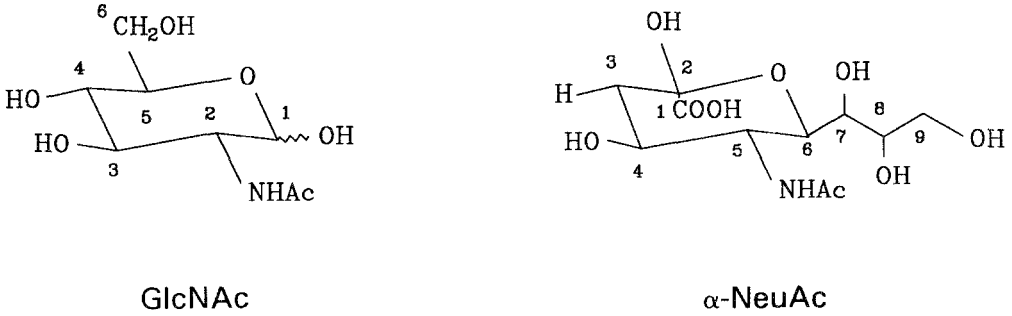
Discussion

The *N*-acetylglucosamine-specific chicken hepatic lectin and the galactose/*N*-acetylgalactosamine-specific mammalian hepatic lectins are structurally related trans-membrane proteins located on the respective hepatocyte surface as well as on the internal membranes. Their trans-membrane domain is located near the N-terminal end of the polypeptide and a larger C-terminal portion contains the carbohydrate-recognition domain [21]. Recently, we reported that the topography of the sugar-binding site is also similar between the two lectins [2]. The sugar-combining site of both lectins is small, interacting mainly with the non-reducing terminal monosaccharide, i.e., galactose or *N*-acetylgalactosamine for the mammalian lectins and *N*-acetylglucosamine for the chicken lectin. Both the C-1 and C-6 positions can be substituted with a fairly large group without causing a significant lowering of the binding affinity. This suggests that both lectins possess a short, trough-like binding area with a rather large opening on either end.

On the other hand, the presence of hydroxyl groups in the mid-section of the monosaccharide, namely 2-, 3-, and 4-OH groups, are of utmost importance in binding. In particular, we have shown for the galactose/*N*-acetylgalactosamine-specific lectins that the binding activity requires both 3- and 4-OH groups to be present and unsubstituted [22]. In the case of the chicken lectin, thus far we have only shown that a large substituent on the 3- and 4-OH of *N*-acetylglucosamine cannot be tolerated [2]. In this paper, we further showed that the absence of the 3- and 4-OH of *N*-acetylglucosamine is also detrimental to the binding affinity, again suggesting a strong similarity in this part of binding site of the two lectins. Decrease in affinity of more than 100-fold caused by the absence of a single OH group (3- or 4-OH) means a strong energetic contribution afforded by these OH groups. It may be that both OH groups are engaged in a bidentate or coordinated hydrogen bonding, which is stronger than a simple hydrogen bond and is often observed in the binding area of sugar-binding proteins, such as the L-arabinose-binding protein [23].

There are examples of *N*-acetylglucosamine-binding lectins (e.g., wheat germ agglutinin) that also bind *N*-acetylneuraminic acid [24]. When the 4-OH and 5-NHAc on the ring of *N*-acetylneuraminic acid are lined up with the same groups of *N*-acetylglucosamine (3-OH and 2-NHAc) (Fig. 1), the similarity between the two sugars becomes quite evident [24]. However, the chicken hepatic lectin does not bind glycoproteins with sialylated terminals, such as α_1-acid glycoprotein [25]. In the present study, *N*-acetylneuraminic acid was also found to be totally ineffective as an inhibitor (Table 2). Since the bulkiness and the negative charge are tolerated well both at the C-1 and C-6 positions of *N*-acetylglucosamine, the fact that the chicken lectin does not bind *N*-acetylneuraminic acid must be due mainly to the absence of the OH group (at the 3-position of *N*-acetylneuraminic acid) that corresponds to the 4-OH of *N*-acetylglucosamine, again underscoring the importance of this group.

At the C-2 position, both lectins prefer the presence of an acetamido group to a hydroxyl group. For the mammalian lectins, it was shown that a moderate increase in the size of an N-substituent (e.g., propionyl vs acetyl) did not decrease the binding affinity appreciably. *N*-Benzoyl, a larger but planar, substitution decreased the affinity by 4-fold [26]. Interestingly, the effect of an N-substituent on the binding affinity of the chicken lectin was also similar in that a modest increase in size (from acetyl to propionyl) only slightly decreased the affinity,



GlcNAc α-NeuAc

Figure 1. Comparison of *N*-acetylglucosamine and α-*N*-acetylneuraminic acid.

while the *N*-benzoyl derivative had a 5-fold lower affinity (Table 1). In addition, Table 1 shows that a branched substituent is more obtrusive than a non-branched isomer (compare *n*-butyl, **10**, and isobutyl, **11**).

There are some differences in the binding characteristics of the two lectins, most notably their preference for the opposite configuration at C-4 (the axial OH for the mammalian lectin and the equatorial OH for the chicken lectin). Also, their tolerance or preference of a charged group is quite different. A negative charge on the C-6 (e.g., Gal-6-P) and C-1 (Gal-1-P) lowers the binding affinity more than 10 fold for the mammalian lectins [27]. On the contrary, the chicken lectin bound GlcNAc-6-P as well as *N*-acetylglucosamine, and bound GlcNAc-1-P 10-fold tighter than *N*-acetylglucosamine [2]. The fact that the chicken lectin tolerates the negative charge at the C-6-position, and prefers the presence of a negative charge at the C-1 area was further corroborated in the present study, since a uronic acid derivative (negatively charged) of *N*-acetylglucosamine (**3**) and its methyl ester (**4**) had the same binding affinity (Table 2). Likewise, the binding affinity of UDP-GlcNAc, with a negatively charged phosphate groups at C-1, was similar to that of GlcNAc-1-P, and both were approximately 10-fold stronger than *N*-acetylglucosamine itself.

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References

1 Drickamer K, Mamon JF, Binns G, Leung JO (1984) J Biol Chem 259:770-78.
2 Lee RT, Rice KG, Rao BNN, Ichikawa Y, Barthel T, Piskarev V, Lee YC (1989) Biochemistry 28:8351-58.
3 Lemieux RU, Takeda T, Chung BY (1976) Am Chem Soc Symp Ser 39:90-115.
4 Chipowsky S, Lee YC (1973) Carbohydr Res 31:339-46.
5 Lee RT, Lee YC (1974) Carbohydr Res 37:193-201.
6 Lee YC, Stowell CP, Krantz MJ (1976) Biochemistry 15:3956-63.
7 Seglen PO (1976) Methods Cell Biol 13:29-83.
8 Kuhlenschmidt MS, Schmell E, Slife CW, Kuhlenschmidt TB, Sieber F, Lee YC, Roseman S (1982) J Biol Chem 257: 3157-64.
9 Greenwood FC, Hunter WM, Glover JS (1963) Biochem J 89:114-23.
10 Connolly DT, Townsend RR, Kawaguchi K, Bell WR, Lee YC (1982) J Biol Chem 257:939-94
11 Campos-Valdes MT, Marino-Albernas, Verz-Bencomo V (1987) J Carbohydr Chem 6:509-13 and cited therein.
12 Durette PL, Meitzner EP, Shen TY (1979) Carbohydr Res 77:C1-C4.
13 Hasegawa A, Kiso M (1980) Carbohydr Res 79:265-70.
14 Wolfrom ML, Winkley MW (1966) J Am Chem Soc 31:1169-1173.
15 Lee RT, Wong TC, Lee YC (1986) J Carbohydr Chem 5:343-57.
16 Roy R, Jennings HJ (1983) Carbohydr Res 112:63-72.
17 Peters T, Bundle DR (1989) Can J Chem 67:491-96.
18 Spohr U, Lemieux RU (1988) Carbohydr Res 174:211-37.
19 Wolfrom ML, Thompson A (1962) Methods Carbohydr Chem 1:209-11.
20 Perrine TD, Glaudemans CPJ, Ness RK, Kyle J, Fletcher HG Jr (1967) J Org Chem 32:664-69.
21 Chiacchia KB, Drickamer K (1984) J Biol Chem 295:15440-46.
22 Ichikawa Y, Lee RT, Lee YC, J Carbohydr Chem, in press.
23 Quiocho FA (1988) Ann Rev Biochem 55: 287-315.
24 Peters BP, Ebisu S, Goldstein IJ, Flashner M (1979) Biochemistry 18:5505-11.
25 Kawasaki T, Ashwell G (1977) J Biol Chem 252:6536-43.
26 Wong TC, Townsend RR, Lee YC (1987) Carbohydr Res 170:27-46.
27 Lee RT, Myers RW, Lee YC (1984) Biochemistry 21:6292-98.