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SYNTHESIS OF *O*-β-D-GALACTOPYRANOSYL-(1→4)-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→2)-D-MANNOSE AND ITS INTERACTION WITH VARIOUS LECTINS

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ABSTRACT

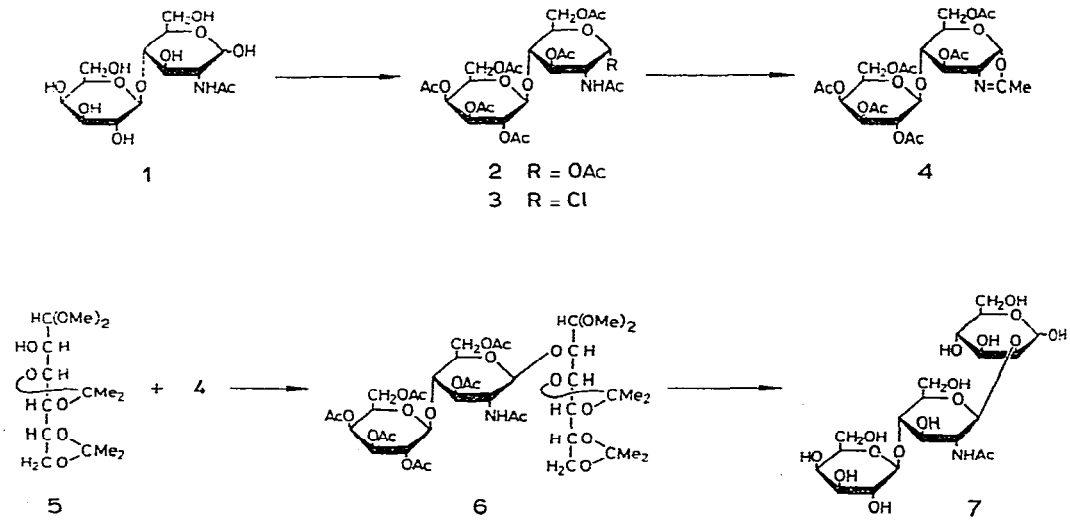
2-Methyl-[3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (**4**) was prepared from 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride. Condensation of 3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal with **4** in the presence of a catalytic amount of *p*-toluenesulfonic acid afforded *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal (**6**) in 8.6% yield. Catalytic deacetylation of **6** with sodium methoxide, followed by hydrolysis with dilute sulfuric acid, gave *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-D-mannose (**7**). The inhibitory activities of **7** and related sugars against the hemagglutinating activities of various lectins were assayed, and **7** was found to be a good inhibitor against *Phaseolus vulgaris* hemagglutinin.

INTRODUCTION

Recent investigations have revealed that two types of sugar chains are present in the major sialoglycoprotein of human erythrocytes[1–3]: One is the "mucin-type"[1] having an *O*-glycosyl linkage to a serine or threonine residue and the other is the "serum glycoprotein-type"[2] having a glycosylamine linked to an asparagine residue. The latter sugar chain has been found to contain the *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-D-mannopyranose sequence[4]. This report describes the synthesis of the trisaccharide *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-D-mannose (**7**) and the use of this trisaccharide and related mono- and disaccharides as hapten inhibitors against various lectins.

RESULTS AND DISCUSSION

2-Acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (**1**) can be easily prepared from lactose and 2-acetamido-2-deoxy-D-galactose by means of a crude enzyme from *Bifidobacterium bifidum* var. pennsylvanicus by the method of Zilliken *et al.*[5], and the condensation of the fully acetylated oxazoline derivative of this disaccharide with a partially protected D-mannose derivative was attempted. 2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose[6] (**2**) was treated with acetic acid–acetic anhydride saturated with hydrogen chloride, and the resulting 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride (**3**) was converted into 2-methyl-[3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (**4**) by the method of Khorlin *et al.*[7]. Condensation of **4** with 3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal[8] (**5**) in the presence of a catalytic amount of *p*-toluenesulfonic acid afforded *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal (**6**) in 8.6% yield. The β configuration of the linkage formed by this reaction was confirmed by n.m.r. spectroscopy (doublet at τ 5.37, $J_{1,2}$ 9 Hz). The *O*-acetyl, isopropylidene, and methyl acetal groups of **6** were removed by catalytic deacetylation with sodium methoxide, followed by hydrolysis with dilute sulfuric acid to give amorphous *O*-β-D-galactopyranosyl-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-D-mannose (**7**).



The results of hemagglutination inhibition assays of **7** and other related sugars against several lectins are listed in Table I. Compound **1** did not show significant

TABLE I

HEMAGGLUTINATION INHIBITION OF LECTINS

Sugars	*Lectins*[a]					
	L. culinaris	*Concanavalin A*	P. vulgaris	R. communis	W. floribunda	B. purpurea
β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→2)-D-Man	9.2	37	18	2.3	1.1	1.1
β-D-Gal*p*-(1→4)-D-GlcNAc	>52	>52	>104	3.3	1.6	1.6
β-D-GlcNAc*p*-(1→2)-D-Man	0.8	0.8	48	>48	>48	>48
D-Galactose			>111	28	14	1.7
D-Mannose	14	4	>100	>100	>100	>100
Phenyl α-D-galactopyranoside			>74	18	9.2	0.6
Phenyl β-D-galactopyranoside			>74	4.6	9.2	0.6
Methyl α-D-galactopyranoside			>103	52	6.4	0.8
Methyl β-D-galactopyranoside			>103	13	13	0.8
2-Acetamido-2-deoxy-D-galactose			>90	>90	0.4	0.4

[a]Minimum concentration (μmol/ml) completely inhibiting 4 hemagglutinating doses.

inhibitory activity against *Phaseolus vulgaris* hemagglutinin, but its linkage to O-2 of D-mannose to give **7** resulted in strong inhibitory activity. This finding shows that the D-mannose residue is essential for the inhibitory activity, although D-mannose itself is not an inhibitor against this lectin. The D-galactose residue at the nonreducing end of **7** is also important for the hapten inhibitory activity against *P. vulgaris* hemagglutinin, as 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose[9] has much weaker inhibitory activity (Table I). These results are in good agreement with previously reported results[10–12] obtained by hemagglutination inhibition assays with various glycopeptides and their sequential degradation products as hapten inhibitors, and they confirm that *P. vulgaris* hemagglutinin recognizes the *O*-β-D-galactopyranosyl-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-D-mannose sequence.

Ricinus communis hemagglutinin was more effectively inhibited by **1** or **7** than by both anomers of methyl or phenyl D-galactopyranoside (Table I). Contribution of the D-mannose residue of **7** to the inhibitory activity against this lectin may be small, as the inhibitory activity of **7** is almost the same as that of **1**. These results support the assumption[13] that this lectin primarily recognizes the 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose sequence at the cell surface.

In contrast, 2-acetamido-2-deoxy-D-galactose and both anomers of methyl or phenyl D-galactopyranoside were stronger inhibitors against *Bauhinia purpurea* hemagglutinin than **1** or **7**. The presence of a 2-acetamido-2-deoxy-D-glucose residue in penultimate position to the nonreducing, terminal β-D-galactopyranosyl group seems to decrease significantly the inhibitory activity against *B. purpurea* hemagglutinin.

Wistaria floribunda hemagglutinin was inhibited to the highest degree by 2-acetamido-2-deoxy-D-galactose, but **1** and **7** also showed an inhibitory activity stronger than that shown by both anomers of methyl or phenyl D-galactopyranoside. Therefore, the presence of a 2-acetamido-2-deoxy-D-glucose residue in penultimate position to the terminal β-D-galactopyranosyl group rather enhances the inhibitory activity against *W. floribunda* hemagglutinin.

It was previously reported[9] that 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose is a good inhibitor against both *Lens culinaris* hemagglutinin and concanavalin A. However, as shown in Table I, the substitution at *O*-4 of the 2-acetamido-2-deoxy-D-glucopyranose residue by a β-D-galactopyranosyl group significantly decreased the inhibitory activity against these D-mannose-binding lectins.

EXPERIMENTAL

General. — Melting points were taken on a hot stage equipped with a microscope, and are not corrected. Specific rotations were determined, in a semimicro polarimeter tube (length 1 dm), with a Zeiss polarimeter having a scale reading to 0.01°. I.r. spectra were recorded with a JASCO DS-402 G spectrophotometer, and n.m.r. spectra with a JEOL JNM-PS-100 spectrometer, with Me_4Si as the internal

standard. The silicic acid used for chromatography was Wakogel C-100 (100 mesh; Wako Pure Chemical, Tokyo), used without pretreatment. The ratio of weight of substance to weight of silica gel was 1:100. The ratio of diameter of the column to its length was 1:20. The activated charcoal for column chromatography was Shirasagi activated charcoal (Wako Pure Chemical, Tokyo). T.l.c. was performed on precoated Silica Gel G plates (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany); the solvent travel-distance was ~6 cm. The spots were detected by spraying the chromatogram with 1:1:18 (v/v) anisaldehyde–conc. H_2SO_4–ethanol. Evaporations were conducted *in vacuo*, with a bath temperature below 40°, unless stated otherwise. Microanalyses were performed by the Central Analyses Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo.

Lectins. — Concanavalin A from jack bean (Sigma Chemical Co., St. Louis, MO 63178) was purified according to the method of Agrawal and Goldstein[14]. The *P. vulgaris* hemagglutinin used in this study was the fraction E-PHA obtained from Bacto-phytohemagglutinin M (Difco Laboratories, Detroit, MI 48232) by the method previously described[15]. *L. culinaris* hemagglutinin[16], *R. communis* hemagglutinin[17], *B. purpurea* hemagglutinin[18], and *W. floribunda* hemagglutinin[19] were purified from the corresponding seeds according to the methods previously described.

Sugars. — 2-Acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose was enzymically synthesized according to the method of Zilliken *et al.*[5]. 2-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose was synthesized by the method previously described[9]. Methyl α- and β-D-galactopyranoside were synthesized by the method of Austin *et al.*[20]. D-Galactose, 2-acetamido-2-deoxy-D-galactose, and phenyl α- and β-D-galactopyranoside were purchased from Nakarai Chemical Co. (Tokyo, Japan).

Hemagglutination assays. — Titration and inhibition assays with human erythrocytes freshly obtained from a donor were performed according to the method previously described[21].

*2-Acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride* (**3**). — A solution of 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose[6] (**2**, 6 g) in 2:1 (v/v) acetic acid–acetic anhydride (100 ml) was saturated with dry HCl at 0°, and kept at room temperature for 24 h. The solution was saturated again with dry HCl at 0° and kept at room temperature for further 24 h. After dilution with dichloroethane (400 ml), the solution was washed once with water, twice with an ice-cold saturated solution of $NaHCO_3$, and finally with water. The solution was dried (Na_2SO_4), and the crystalline residue obtained after evaporation of the solvent was recrystallized from ethyl acetate–ether to give 4.9 g (85%) of **3**, m.p. 135–138°, $[\alpha]_D^{20}$ +62° (*c* 1.0, chloroform).

Anal. Calc. for $C_{26}H_{37}ClNO_{16}$: C, 47.7; H, 5.7; N, 2.1. Found: C, 47.8; H, 5.5; N, 2.2.

*2-Methyl-[3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano)]-[2,1-d]-2-oxazoline* (**4**). — A solution of **3** (4.9 g) in dry acetone (30 ml) was added dropwise to a suspension of $AgNO_3$ (2.8 g) in a mixture

of 2,4,6-trimethylpyridine (8 ml) and dry acetone (40 ml), and the solution was stirred for 1 h at room temperature. The solution was diluted with dichloroethane (200 ml), filtered, and the filtrate was evaporated. The residue was dissolved in dichloroethane (200 ml), successively washed with cold, saturated $NaHCO_3$ solution and water, dried (anhydrous K_2CO_3), and evaporated. The residue was treated with a mixture of ether and petroleum ether to give 3.9 g (85%) of **4** as an amorphous powder showing on examination by t.l.c. on silica gel with ethyl acetate only one spot (R_F 0.43), $[\alpha]_D^{20}$ +17° (*c* 1.1, chloroform); i.r.: ν_{max}^{KBr} 1672 (C=N) and 1748 cm^{-1} (C=O); lit.[22]: $[\alpha]_D^{21}$ +15° (*c* 1.0, chloroform).

Anal. Calc. for $C_{26}H_{36}NO_{16}\cdot H_2O$: C, 49.1; H, 6.0; N, 2.2. Found: C, 49.1; H, 5.7; N, 2.1.

O-(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1→2*)-*3,4:5,6-di*-O-*isopropylidene*-D-*mannose dimethyl acetal* (**6**). — To a solution of 3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal[7] (**5**, 3.9 g) and **4** (3.9 g) in dry 1:1 (v/v) toluene–nitromethane (30 ml) was added sufficient *p*-toluenesulfonic acid to adjust the pH of the solution to 4. The solution was heated for 40 min at 110°, and then evaporated under diminished pressure. The residue was dissolved in chloroform (120 ml), successively washed with cold, saturated $NaHCO_3$ solution and water, dried (anhydrous Na_2CO_3), and evaporated. The brown syrup obtained was chromatographed on a column of silica gel with 97:3 (v/v) chloroform–ethanol; the fractions having R_F 0.21 in t.l.c. in ethyl acetate were combined and evaporated. The residue was rechromatographed on a column of silica gel with 9:1 (v/v) chloroform–ethanol; fractions containing **6** were combined and evaporated to a syrupy residue. This residue was dissolved in a small amount of absolute ethanol, and precipitated with ether to give 0.5 g (8.6%) of **6** as an amorphous powder, $[\alpha]_D^{20}$ −26° (*c* 0.5, chloroform); n.m.r. (100 MHz, chloroform-*d*): τ 4.19 (one-proton doublet, *J* 8 Hz, NH), 5.37 (one-proton doublet, *J* 9 Hz, H-1 of GlcNAc), 5.51 (one-proton doublet, *J* 8 Hz, H-1 of Gal), 6.56 (6 protons, 2 OMe), ~8 (21 protons, AcO), 8.60, 8.63, and 8.68 (12 protons, 2 Me_2C).

Anal. Calc. for $C_{40}H_{61}NO_{23}\cdot H_2O$: C, 51.0; H, 6.8; N, 1.5. Found: C, 51.0; H, 6.5; N, 1.2.

O-(β-D-*Galactopyranosyl*)-(*1→4*)-O-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(*1→2*)-D-*mannose* (**7**). — To a suspension of **6** (380 mg) in dry methanol (12 ml) was added 0.1M sodium methoxide (1.2 ml), and the mixture was kept for 20 h at room temperature. After the addition of 0.05M H_2SO_4 (50 ml), the solution was heated for 80 min at 80°, the acid neutralized with $BaCO_3$, the suspension filtered, and the filtrate evaporated under diminished pressure. The residue was chromatographed on a column of cellulose powder (10 g) with 4:5:3 (v/v) butanol–acetone–water; fractions having R_F 0.46 in t.l.c. in the same solvent were combined and evaporated. The residue was rechromatographed on a column of activated charcoal (1 g) with 3% aqueous ethanol. The effluent (300 ml) gave 70 mg (31%) of pure **6** as a hygroscopic, amorphous powder showing on examination by t.l.c. on silica gel with

5:5:1:3 (v/v) ethyl acetate–pyridine–acetic acid–water only one spot (R_F 0.30), $[\alpha]_D^{20}$ −13° (at equilibrium, *c* 0.86, water).

Anal. Calc. for $C_{20}H_{35}O_{16}N \cdot 2H_2O$: C, 41.3; H, 6.8; N, 2.4. Found: C, 40.7; H, 6.2; N, 2.4.

After hydrolysis of **6** with 3M HCl for 1 h at 80°, t.l.c. on silica gel with 4:5:3 (v/v) butanol–acetone–water showed mannose, galactose, and 2-amino-2-deoxyglucose.

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