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SYNTHESES OF *O*-β-D-GALACTOPYRANOSYL-(1→3)-*O*-(2-ACETAMIDO-2-DEOXY-α(AND -β)-D-GALACTOPYRANOSYL)-*N*-TOSYL-L-SERINE AND THEIR INTERACTION WITH D-GALACTOSE-BINDING LECTINS*

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ABSTRACT

In order to use, as hapten inhibitors against various galactose-binding lectins, the derivatives of *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine, which is the common core structure of sugar chains of most mucins, the synthesis of these compounds was investigated. Koenigs–Knorr condensation of the 4,6-*O*-benzylidene derivative of *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine methyl ester with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide gave *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine. Deacetylation, followed by acid hydrolysis of the benzylidene group, gave *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine. A β anomer at the glycosidic linkage of the 2-acetamido-2-deoxy-D-galactose residue was also synthesized by the same procedure. *Agaricus bisporus* (mushroom) hemagglutinin was found to recognize the *O*-α-glycosyl linkage between 2-acetamido-2-deoxy-D-galactose and L-serine, in addition to the *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose sugar sequence, for which *Arachis hypogaea* (peanut) and *Bauhinia purpurea* hemagglutinins were found to be specific. *Ricinus communis* hemagglutinin is more specific for the *O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose (commonly found in serum glycoproteins) than for the *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose sequence. With *Wistaria floribunda* hemagglutinin, not much difference in the inhibitory activities of these two sugar sequences was observed, and *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine was the strongest inhibitor against this lectin.

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INTRODUCTION

In most mucins as well as in numerous other glycoproteins, the structure *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine occurs as the core structure of the oligosaccharide chains[1, 2]. On the cell surface of human erythrocytes, this structure was found to serve as the receptor site for various D-galactose-binding lectins, including the lectins of *Agaricus bisporus*, *Arachis hypogaea*, and *Bauhinia purpurea*[3, 4]. However, it seems that there are subtle differences of specificity between these D-galactose-binding lectins. Thus, the *A. bisporus* lectin was assumed to recognize even the *O*-glycosyl linkage between 2-acetamido-2-deoxy-D-galactose and L-serine, in addition to the *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose sugar sequence, because the glycopeptide material released from human erythrocytes with trypsin showed much greater inhibition against the lectin than the disaccharide *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose[4].

The present paper describes the synthesis of *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine (**7**) and related compounds, the inhibitory activities of these well-characterized synthetic compounds toward hemagglutination, and the binding to human erythrocytes of various galactose-binding lectins in order to establish the binding specificities of those lectins. These synthetic compounds may also serve as model compounds for comparison with the derivatives of natural products.

RESULTS AND DISCUSSION

The starting material, *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine methyl ester (**2**), was prepared by the method previously described[5]. The Koenigs–Knorr condensation of the 4,6-*O*-benzylidene derivative (**3**) of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**) in the presence of mercuric cyanide gave crystalline *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine methyl ester (**5**) in 39% yield. Treatment of **5** with Dowex-1 (OH^-) ion-exchange resin removed the acetyl and methyl ester groups to give an intermediate (**6**) that had a free carboxyl group, was adsorbed to the ion-exchange resin as soon as it was formed, and could subsequently be eluted with 30% aqueous ethanol containing 0.1M hydrochloric acid. Compound **6** was not isolated, and the benzylidene group was removed by keeping the acidic eluate overnight at room temperature to give amorphous *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine (**7**) in 52% yield from **5**. No appreciable amounts of carbohydrate residues were released through β-elimination, by this alkaline treatment of **5**. This can be explained by the assumption that the methyl ester group of **5** is hydrolyzed before any appreciable liberation of the carbohydrate residue takes place, because it has been shown that blocking of the carboxyl and amino groups is required for

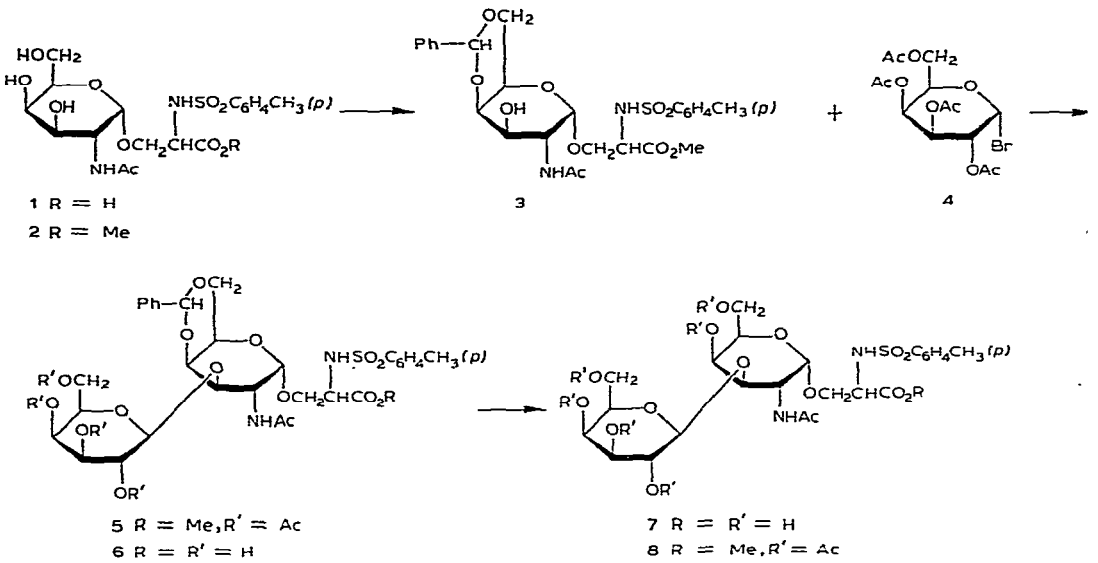


TABLE I

HEMAGGLUTINATION INHIBITION OF LECTINS

Compound	*Lectin*[a]				
	A. bisporus	A. hypogaea	B. purpurea	W. floribunda	R. communis
7	3.1	3.1	0.4	1.6	50
14	>100	3.1	0.2	1.6	50
1	>100	>100	3.1	0.01	>100
9	>100	>100	0.4	0.1	100
O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose	6.3	1.6	0.4	3.1	50
Benzyl O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside	3.1	1.6	0.4	3.1	50
Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside	[b]	[b]	3.1	0.1	[b]
D-Galactose	>100	50	6.3	6.3	50
2-Acetamido-2-deoxy-D-galactose	>100	>100	0.8	0.1	>100
Lactose	>100	25	1.6	3.1	6.3
Melibiose	>100	>100	6.3	13	100
O-β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose	>100	>100	1.6	1.6	3.1
O-β-D-Galactopyranosyl-(1→6)-2-acetamido-2-deoxy-D-glucose	[b]	[b]	[b]	[b]	6.3

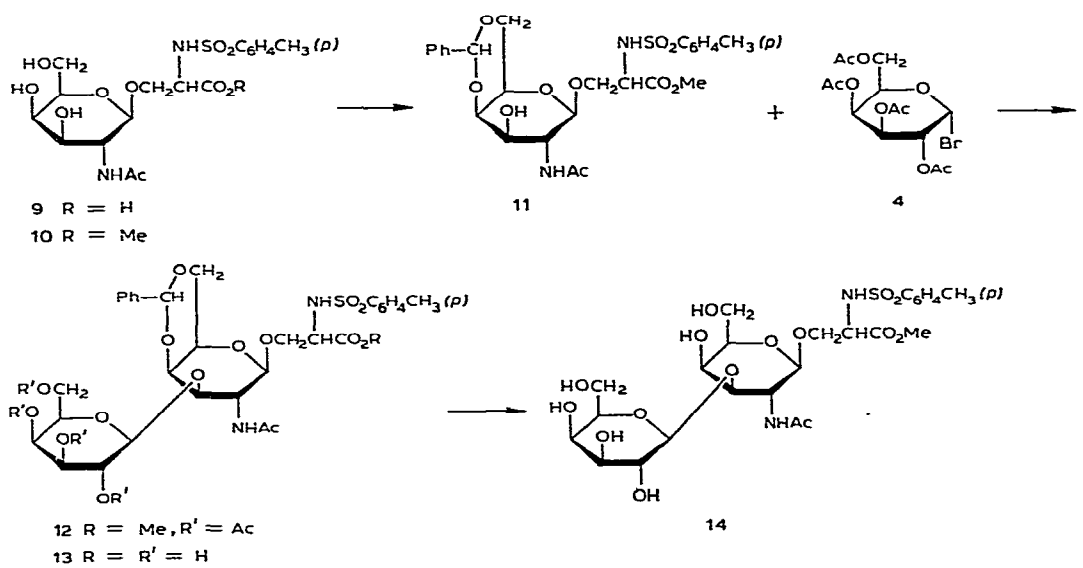
[a]Minimum concn. (mM) completely inhibiting 4 hemagglutinating doses. [b]Not determined.

TABLE II

INHIBITION OF BINDING OF ^{125}I-LABELED LECTINS

Compound	*Lectin*[a]			
	A. bisporus[b]	A. hypogaea[c]	B. purpurea[d]	R. communis[d]
7	20	8	20	82
14	85	15	20	76
1	100	94	48	91
9	96	89	25	98
O-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose	47	15	25	77
Benzyl *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside	18	15	22	78
D-Galactose	92	44	55	70
2-Acetamido-2-deoxy-D-galactose	100	95	23	100
Lactose	100	26	44	23
Melibiose	100	60	57	69
O-β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose	100	80	58	14

[a]Binding of labeled lectin (% of control). Average value of triplicate experiments. [b]Lectin concn.: 5 μg/ml, sugar concn.: 6.3 μmol/ml. [c]Lectin concn.: 2 μg/ml, sugar concn.: 3.1 μmol/ml. [d]Lectin concn.: 5 μg/ml, sugar concn.: 1.6 μmol/ml.



β-elimination[6,7]. Treatment of **7** with diazomethane followed by acetylation with acetic anhydride in pyridine gave a crystalline, fully acetylated methyl ester (**8**) of **7**.

O-β-D-Galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-*N*-tosyl-L-serine (**14**) was synthesized by exactly the same procedure as that just described for the synthesis of **7**, starting from *O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl-*N*-tosyl)-L-serine (**9**), which was prepared from the *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-galactopyranosyl)-*N*-tosyl-L-serine methyl ester previously described[5].

The hemagglutination inhibition assays of **7** and other related sugars against various D-galactose-binding lectins were carried out with neuraminidase-treated human erythrocytes, and the results are listed in Table I. The effects of these sugars on the binding of radio-iodinated D-galactose-binding lectins to neuraminidase-treated human erythrocytes are given in Table II. Good agreement was observed between the results given in both Tables.

The hemagglutinating and binding activities of *A. bisporus* hemagglutinin were most inhibited by **7**, whereas **14** (β anomer of **7**) was not inhibitory. Furthermore, since benzyl *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside has an inhibitory activity of almost the same degree as that of **7** against *A. bisporus* hemagglutinin, the α-glycosidic linkage of the 2-acetamido-2-deoxy-D-galactose residue is an important part of the receptor for *A. bisporus* hemagglutinin. The same conclusion has previously been reached by Presant and Kornfeld[4] in hemagglutination-inhibition assays using glycopeptides isolated from human erythrocytes as inhibitors.

On the other hand, no contribution of the glycosidic linkage of the 2-acetamido-2-deoxy-D-galactose residue to the receptor activity was observed for the *A. hypogaea* hemagglutinin. Coinciding with the results of other workers[3,8,9], this lectin was found to be most specific to the sugar sequence *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose, because this oligosaccharide was much more inhibitory than D-galactose.

In a previous paper[10], we observed that the so-called Mäkelä's group 2 sugars, particularly 2-acetamido-2-deoxy-D-galactose, are the most potent inhibitors for *B. purpurea* hemagglutinin. From Tables I and II, it is apparent that this lectin is more specific for the β-glycosides of D-galactose and 2-acetamido-2-deoxy-D-galactose than for the α-glycosides of these sugars from the comparison of the inhibitory activity between lactose and melibiose, and between the α and β anomers (**1** and **9**) of *O*-(2-acetamido-2-deoxy-D-galactopyranosyl)-*N*-tosyl-L-serine; the inhibitory activity of D-galactose was markedly enhanced when this sugar was β-glycosidically linked to C-3 of a 2-acetamido-2-deoxy-D-galactose residue. We previously assumed, from hemagglutination inhibition assays using various glycopeptides as inhibitors, that *B. purpurea* hemagglutinin binds preferentially to such sugar chains as those of mucins on the cell surface[3,10,11]. This assumption is also supported by the results in Tables I and II, because *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose was more inhibitory than *N*-acetyllactosamine [*O*-β-D-galactopyranosyl-(1→4)-2-acet-

amido-2-deoxy-D-glucose], which is usually a part of such sugar chains as those of serum glycoproteins.

In contrast, *R. communis* hemagglutinin appears to be much more specific to the serum glycoprotein-type sugar chains than to the mucin-type sugar chains on the cell surface, because *O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose (*N*-acetyllactosamine) is the strongest inhibitor among the sugars tested. This is in good agreement with our previous results[3, 11] obtained by inhibition assays using various glycoproteins and glycopeptides, and their sequential enzymic degradation products as hapten inhibitors.

As shown in Table I, *W. floribunda* hemagglutinin is quite specific for *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine (**1**) and the inhibitory activity of the β anomer (**9**) is much weaker than that of the α anomer **1**. Furthermore, the L-serine residue seems to be a part of the receptor for this lectin, because **1** is much more inhibitory than benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside. Although inhibitory activities of many other derivatives of 2-acetamido-2-deoxy-D-galactose should be tested to reach a final conclusion, these results may indicate that the most favorable cell-surface receptors for this lectin are incomplete, mucin-type sugar chains, where they exist on the cell surface. However, since we cannot see much difference in inhibitory activity between *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose and *N*-acetyllactosamine (Table I), *W. floribunda* hemagglutinin might have a rather wide specificity in regard to the type of sugar chains with which it interacts on the cell surface.

EXPERIMENTAL

General methods. — Melting points were determined on a hot stage equipped with a microscope, and are not corrected. Specific rotations were measured in a semimicropolarimeter tube (length 1 dm) with a Zeiss polarimeter having a scale reading to 0.01°. The silicic acid used for chromatography was Wakogel C-100 (100 mesh; Wako Pure Chemical, Tokyo), used without pretreatment. The activated charcoal for column chromatography was Shirasagi activated charcoal (Wako Pure Chemical, Tokyo). The ratio of the diameter of the column to its length was 1:20. Thin-layer chromatography (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. sulfuric acid–ethanol. Evaporations were conducted *in vacuo*, with a bath temperature below 40°, unless otherwise stated. Microanalyses were performed by the Central Analyses Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo.

Lectins and enzymes. — *A. hypogaea* hemagglutinin was obtained from peanuts (from a local market) according to the method of Terao *et al.*[8], *B. purpurea* hemagglutinin from *B. purpurea* alba seeds (purchased from F. W. Schumacher, Sandwich, MA, U.S.A.) by the method previously described[10], *R. communis* hemagglutinin

from *R. communis* seeds (from a local market) by the method of Tomita *et al.*[12], and *W. floribunda* hemagglutinin from *W. floribunda* seeds (purchased from F. W. Schumacher) according to the method previously described[13]. Purified *A. bisporus* hemagglutinin was kindly provided by Dr. S. Kornfeld, and *Vibrio cholerae* neuraminidase was purchased from Calbiochem (La Jolla, CA 92037, U.S.A.).

Sugars. — *O*-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose, benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside, and benzyl *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside were synthesized according to the method of Flowers and Shapiro[14]. *O*-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine was synthesized by the method previously described[5]. *O*-β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose (*N*-acetyllactosamine) was enzymically synthesized according to the method of Zilliken *et al.*[15]. *O*-β-D-Galactopyranosyl-(1→6)-2-acetamido-2-deoxy-D-glucose (*N*-acetylallolactosamine) was synthesized by the method of Okuyama[16]. *D*-Galactose, 2-acetamido-2-deoxy-D-galactose, lactose, and melibiose were purchased from Nakarai Chemical Co. (Tokyo).

Iodination of lectins. — Lectins were iodinated with ^{125}I by the lactoperoxidase method of Hubbard and Cohn[17]. The labeled lectins were freed from an excess of reagents by passage over Biogel P-60. This procedure did not affect the hemagglutinating activity of the lectins. The specific radioactivity was 0.6–6 × 10^5 c.p.m./μg of protein.

Preparation of erythrocytes for lectin-binding assays and hemagglutination assays. — Human group *O* venous blood was taken with syringes previously treated with heparin. The heparinized blood was transferred to glass cylinders, and the erythrocytes were allowed to sediment by gravity. The erythrocyte layer, after removal of the leukocyte-rich plasma and buffy coat, was washed three times by centrifugation with 5mM phosphate-buffered saline (pH 7.0), each time carefully removing the top layer of cells. The erythrocytes thus isolated were treated with *V. cholerae* neuraminidase, to remove sialic acid, by the method previously described[10].

Binding inhibition assays. — Binding reactions were carried out according to the method previously described[18]. To a mixture containing 4 × 10^6 neuraminidase-treated erythrocytes and ^{125}I-labeled lectin (0.2–0.5 μg) in 5 mM sodium phosphate buffered saline (pH 7.0)–0.25% bovine serum albumin (0.2 ml) was added an inhibitor (0.16–0.63 μmol) in the same buffer. After incubation for 90 min at 23° with occasional mixing, the cells were washed twice with chilled phosphate–buffered saline (pH 7.0)–0.25% bovine serum albumin (3 ml), and the amount of bound ^{125}I was determined in an Aloka autogamma counter.

Hemagglutination inhibition assays. — The titration and inhibition assays were carried out according to the method previously described[19].

O-(2-*Acetamido-4,6*-O-*benzylidene-2-deoxy*-α-D-*galactopyranosyl*)-N-*tosyl*-L-*serine methyl ester* (**3**). — Compound **2** (530 mg) was shaken for 20 h at room temperature with benzaldehyde (5 ml) and freshly fused zinc chloride (0.5 g). The reaction mixture was poured into water, and the precipitate was filtered off, washed succes-

sively with water and hexane, and dried to give 860 mg (86%) of **3** as an amorphous powder. Crystallization from 80% ethanol gave needles, m.p. 208–210°, $[\alpha]_D^{18}$ +122° (*c* 0.7, chloroform).

Anal. Calc. for $C_{26}H_{32}N_2O_{10}S$: C, 55.31; H, 5.71; N, 4.96. Found: C, 55.03; H, 5.71; N, 4.85.

O-(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-(1→3)-O-(*2-acetamido-4,6*-O-*benzylidene-2-deoxy*-α-D-*galactopyranosyl*)-N-*tosyl*-L-*serine methyl ester* (**5**). — A solution of **3** (540 mg) in a mixture of nitromethane (15 ml) and benzene (15 ml) was evaporated until 6 ml of the solvent mixture had been distilled off. After cooling to room temperature, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4** ,700 mg) and mercuric cyanide (420 mg) were added, and the solution was stirred at room temperature. After 20 h, further amounts of **4** (450 mg) and mercuric cyanide (250 mg) were added, and the reaction was allowed to proceed for a further 20 h. After filtration, the clear solution obtained was diluted with benzene, washed three times with a saturated solution of sodium hydrogencarbonate, then with water, dried (magnesium sulfate), and evaporated. The residue was chromatographed on a column of silica gel (130 g) with 1:1 (v/v) ethyl acetate–ether; the fractions having R_F 0.41 in t.l.c. in ethyl acetate were combined and evaporated to give 333 mg (39%) of **5** as an amorphous powder, $[\alpha]_D^{13}$ +95° (*c* 1.0, methanol).

Anal. Calc. for $C_{40}H_{50}N_2O_{19}S \cdot H_2O$: C, 52.63; H, 5.74; N, 3.07. Found: C, 52.02; H, 5.50; N, 2.94.

O-β-*Galactopyranosyl*-(*1→3*)-O-(*2-acetamido-2-deoxy*-α-D-*galactopyranosyl*)-N-*tosyl*-L-*serine* (**7**). — A solution of **5** (387 mg) in a mixture of acetone (10 ml) and water (3 ml) was stirred with Dowex-1 (OH^-) anion-exchange resin (5 ml) for 3 h at room temperature. The resin was then packed into a column and washed with water (10 ml), and the fractions were eluted with 0.1M hydrochloric acid in 50% aqueous ethanol. The fractions having R_F 0.67 in t.l.c. in 4:5:3 (v/v) 1-butanol–acetone–water were combined, and the eluate was allowed to stand overnight at room temperature. The solution was neutralized with M sodium hydroxide and evaporated. The residue was dissolved in a small amount of methanol, and the solution was clarified by filtration. The residue obtained after evaporation was dissolved in a small amount of water and applied to a column of activated charcoal (1 × 6 cm). The column was eluted successively with water (10 ml) and 50% ethanol (40 ml). Fractions eluted with the latter solvent were treated with IR-120 B (H^+) cation-exchange resin (1 g). The resin was filtered off, and the filtrate evaporated. The residue was dissolved in a small amount of absolute ethanol and precipitated with ether to give 150 mg (52%) of pure **7** as a hygroscopic, amorphous powder showing, on examination by t.l.c. on silica gel with 4:5:3 (v/v) 1-butanol–acetone–water, only one spot (R_F 0.59), $[\alpha]_D^{18}$ +88° (*c* 0.6, water).

Anal. Calc. for $C_{24}H_{36}N_2O_{15}S \cdot 2H_2O$: C, 43.63; H, 6.10; N, 4.24. Found: C, 43.74; H, 5.70, N, 3.97.

O-(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-O-(*2-acetamido-4,6-di*-O-*acetyl-2-deoxy*-α-D-*galactopyranosyl*)-N-*tosyl*-L-*serine methyl ester* (**8**). — To a

solution of **7** (10 mg) in methanol (1 ml) was added a slight excess of diazomethane in ether. The solution was evaporated, and the residue was treated with acetic anhydride (0.4 ml) and pyridine (0.1 ml) overnight at room temperature. Evaporation of the reaction mixture and crystallization of the residue from ethanol gave 6 mg (44%) of **8** as needles, m.p. 139–141°, $[\alpha]_D^{25}$ +49° (*c* 1.5, chloroform).

Anal. Calc. for $C_{37}H_{50}N_2O_{21}S$: C, 49.88; H, 5.66; N, 3.14. Found: C, 49.60; H, 5.54; N, 3.01.

O-(*2-Acetamido-2-deoxy-β*-D-*galactopyranosyl*)-N-*tosyl*-L-*serine* (**9**). — A solution of *O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-*β*-D-galactopyranosyl]-*N*-tosyl-L-serine methyl ester[5] (1.3 g) in a mixture of acetone (30 ml) and water (15 ml) was stirred with Dowex-1 (OH^-) anion-exchange resin (30 ml) for 2.5 h. The resin was then packed into a column, washed with water (50 ml), and eluted with 0.1M hydrochloric acid in 30% aqueous ethanol. The fractions having R_F 0.52 in t.l.c. in 4:5:3 (v/v) 1-butanol–acetone–water were combined and evaporated to give a syrupy residue. The residue was dissolved in methanol (10 ml), and the solution was neutralized with sodium (9 mg) in methanol (3 ml), and treated with acetic anhydride (0.2 ml). After 2 h at room temperature, the solution was evaporated. The residue was crystallized from ethanol to give 300 mg (35%) of **9** as needles, m.p. 124–126°, $[\alpha]_D^{25}$ +15.6° (*c* 1.1, water).

Anal. Calc. for $C_{14}H_{26}N_2O_{10}S \cdot H_2O$: C, 45.02; H, 5.87; N, 5.83. Found: C, 45.21; H, 5.58; N, 5.51.

O-(*2-Acetamido-2-deoxy-β*-D-*galactopyranosyl*)-N-*tosyl*-L-*serine methyl ester* (**10**). — To a solution of **9** (1.8 g) in methanol (15 ml) was added a slight excess of diazomethane in ether. The clear solution was evaporated, and the residue was chromatographed on a column of silica gel (45 g) with 9:1 (v/v) chloroform–methanol; the fractions having R_F 0.13 in t.l.c. in the same solvent mixture were combined and evaporated. The residue was crystallized from ethanol to give 1.2 g (64%) of **10** as needles, m.p. 186–188, $[\alpha]_D^{25}$ +4.7° (*c* 0.1, 50% ethanol).

Anal. Calc. for $C_{19}H_{28}N_2O_{10}S \cdot H_2O$: C, 46.15; H, 6.11; N, 5.66. Found: C, 46.32; H, 5.66; N, 5.14.

O-(*2-Acetamido-4,6-O-benzylidene-2-deoxy-β*-D-*galactopyranosyl*)-N-*tosyl*-L-*serine methyl ester* (**11**). — Treatment of **10** (1.1 g) with benzaldehyde (10 ml), as described for the preparation of **3** from **2**, gave 1.1 g (89%) of **11** as an amorphous powder. Crystallization from ethanol gave needles, m.p. 135–137°, $[\alpha]_D^{25}$ +4.1° (*c* 0.9, chloroform).

Anal. Calc. for $C_{26}H_{32}N_2O_{10}S \cdot 0.5H_2O$: C, 54.44; H, 5.79; N, 4.88. Found: C, 54.46; H, 5.61; N, 4.71.

O-(*2,3,4,6-Tetra-O-acetyl-β*-D-*galactopyranosyl*)-(*1→3*)-O-(*2-acetamido-4,6-O-benzylidene-2-deoxy-β*-D-*galactopyranosyl*)-N-*tosyl*-L-*serine* (**12**). — A solution of **11** (550 mg) in a mixture of nitromethane (15 ml) and benzene (15 ml) was processed by exactly the same procedure as that for the synthesis of **5** from **3**. After chromatography of the reaction mixture on a column of silica gel (130 g) with 1:1 (v/v) ethyl acetate–ether, the fractions having R_F 0.37 in t.l.c. in ethyl acetate were combined

and evaporated to give 443 mg (50%) of **12** as an amorphous powder, $[\alpha]_D^{25}$ +19° (*c* 0.7, methanol).

Anal. Calc. for $C_{40}H_{50}N_2O_{19}S \cdot H_2O$: C, 52.63; H, 5.74; N, 3.07. Found: C, 52.75; H, 5.57; N, 3.36.

O-β-D-Galactopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-N-tosyl-L-serine (**14**). — Compound **14** (180 mg, 56%) was obtained from **12** (433 mg) by the same procedure as that described for the synthesis of **7** from **5**, as a hygroscopic amorphous powder showing on examination by t.l.c. on silica gel with 4:5:3 (v/v) butanol–acetone–water only one spot (R_F 0.59), $[\alpha]_D^{25}$ +16° (*c* 0.8, water).

Anal. Calc. for $C_{24}H_{36}N_2O_{15}S \cdot 0.5H_2O$: C, 45.49; H, 5.89; N, 4.10. Found: C, 45.47; H, 6.09; N, 4.42.

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