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Note

Synthesis of *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-N-tosyl-L-serine

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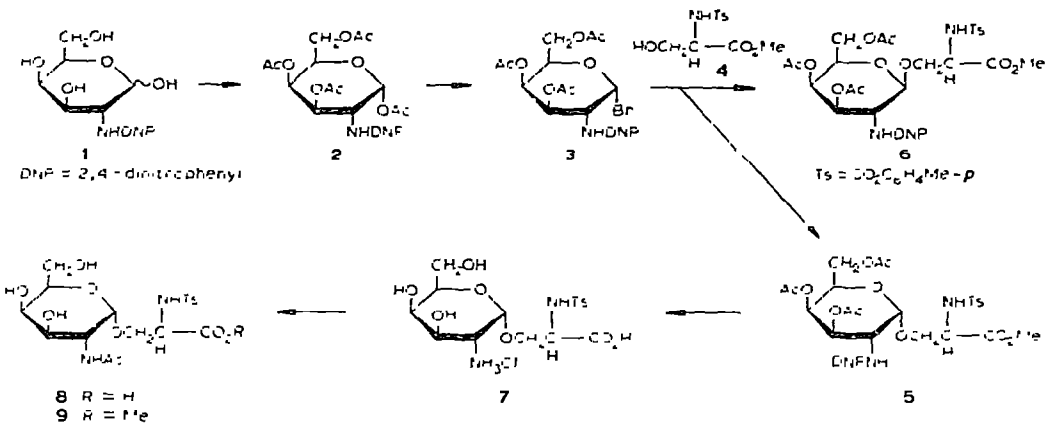
Mucin-type glycoproteins, including submaxillary mucins as well as numerous other glycoproteins, are characterized by an *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine linkage between their carbohydrate chains and the protein backbone[1]. This linkage has been shown to occur also in various cell-membrane glycoproteins[2]. As a standard compound for the determination of the specificity of lectins for which the serine residue may be a part of the immunodeterminant, and as a starting compound for the chemical synthesis of mucin-type glycopeptides, *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine (**8**) has been synthesized.

Earlier studies of Lloyd *et al.*[3–6] established that condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with simple alcohols usually furnishes a mixture of the α- and β-glycosides, the proportions of which are dependent on the solvent and catalyst employed. Therefore, the Koenigs–Knorr condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-galactopyranosyl bromide (**3**) with *N*-tosyl-L-serine methyl ester (**4**) was investigated.

2-Deoxy-2-(2,4-dinitroanilino)-D-galactose[7] (**1**) was acetylated with acetic anhydride in pyridine to give 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-galactopyranose (**2**). The α configuration of **2** was confirmed by n.m.r. spectroscopy (doublet at τ 3.70, $J_{1,2}$ 3.5 Hz). Compound **2** was converted into the α-D-galactosyl bromide (**3**) with hydrogen bromide in acetic acid, according to the method of Lloyd and Stacey[4]. Treatment of **3** with *N*-tosyl-L-serine methyl ester[8] (**4**), in the presence of mercuric cyanide as the acid acceptor, at 80° gave an anomeric mixture of *O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-galactopyranosyl]-*N*-tosyl-L-serine methyl ester. This condensation reaction proceeded, as expected, in favor of the formation of the α anomer. Thus, column chromatography of the anomeric mixture on silica gel gave pure α and β anomer (**5** and **6**) in 53 and 11% yield (based on **3**), respectively. Assignment of the anomeric configurations of **5** and **6** was based on the optical rotation, as well as on the n.m.r.-spectral data which showed, in addition to other characteristic signals, an anomeric proton as a one-proton doublet at τ 4.95 with $J_{1,2}$ 3.0 Hz for **5**, and at τ 5.21 with $J_{1,2}$ 8.0 Hz for **6**. Treatment of **5** with



Dowex-1 (OH^-) ion-exchange resin removed all of the acetyl, dinitrophenyl, and methyl ester groups of **5**, to give *O*-(2-amino-2-deoxy-α-D-galactopyranosyl)-*N*-tosyl-L-serine, which was adsorbed to the ion-exchange resin as soon as it was formed and could subsequently be eluted, as the hydrochloride (**7**), with 30% aqueous ethanol containing 0.1M hydrochloric acid. No appreciable amounts of carbohydrate residues were released from **5** *via* β-elimination by the alkaline treatment; this can be explained by the assumption that the methyl ester group of **5** is hydrolyzed before any appreciable liberation of the carbohydrate residues takes place, because it has been shown[9] that blocking of the carboxyl group is required for β-elimination. Acetylation of **7** with acetic annydride in methanol gave the crystalline title compound (**8**), and treatment of **8** with diazomethane gave the crystalline methyl ester (**9**).

EXPERIMENTAL

General. — Melting points were determined on a hot stage equipped with a microscope, and are not corrected. Specific rotations were measured in a semimicro polarimeter tube (length 1 dm) with a Zeiss polarimeter having a scale reading to 0.01°. N.m.r. spectra were recorded with a JEOL JNM-PS-100 spectrometer, with tetramethylsilane 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 diameter of the column to its length was 1:20. 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 stated otherwise. Microanalyses were performed by the Central Analyses Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-galactose (**2**). — A solution of 2-deoxy-2-(2,4-dinitroanilino)-D-galactose[7] (**1**; 5.0 g) in pyridine (10 ml)

was treated with acetic anhydride (50 ml) overnight at room temperature. The mixture was poured into ice-water (1.5 liters). The resulting, crystalline substance was recrystallized from ethanol, to give 5.7 g (76%) of yellow plates, m.p. 169–171°, $[\alpha]_D^{29}$ −7.7° (*c* 1.1, chloroform): n.m.r. data (100 MHz, chloroform-*d*): τ 0.98, 1.48, 1.75 (3 protons, dinitrophenyl), 2.94 (1-proton doublet, *J* 9 Hz, NH), 3.70 (1-proton doublet, $J_{1,2}$ 3.5 Hz, H-1 of GalN), 7.75, 7.77, 7.96, and 8.11 (12 protons, 4 Ac).

Anal. Calc. for $C_{20}H_{23}N_3O_{13}$: C, 46.79; H, 4.52; N, 8.18. Found: C, 46.89; H, 4.52; N, 8.08.

3,4,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-galactopyranosyl bromide (**3**). — To a solution of **2** (1 g) in chloroform (8 ml) was added 30% hydrogen bromide in acetic acid (15 ml), and the solution was kept for 2 h at room temperature, and poured into ice-water. The mixture was extracted with chloroform, and the extract was successively washed with cold, saturated sodium hydrogencarbonate solution, and water, dried (magnesium sulfate), and evaporated. The residue was crystallized from acetone–petroleum ether, to give 0.9 g (86%) of **3** as yellow needles, m.p. 158–159° (dec.), $[\alpha]_D^{29}$ +28° (*c* 1.1, chloroform); n.m.r. data (100 MHz, chloroform-*d*): τ 0.94, 1.32, 1.74 (3 protons, dinitrophenyl), 2.95 (1-proton doublet, *J* 10 Hz, NH), 3.36 (1-proton doublet, $J_{1,2}$ 3.5 Hz, H-1 of GalN), 7.76, 7.93, and 8.12 (12 protons, 4 Ac).

Anal. Calc. for $C_{18}H_{20}BrN_3O_{11}$: C, 40.48; H, 3.77; N, 7.87. Found: C, 40.78; H, 3.92; N, 7.66.

O-[*3,4,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α- and -β-D-galactopyranosyl*]-N-*tosyl-L-serine methyl ester* (**5** *and* **6**). — Compound **3** (5.6 g) and *N*-tosyl-L-serine methyl ester[9] (**4**, 3.0 g) in nitromethane (20 ml) were shaken with mercuric cyanide (1.2 g) and mercuric bromide (1.8 g) for 20 h at 80°. The mixture was diluted with chloroform (150 ml), treated with a small amount of activated charcoal, filtered, and the filtrate evaporated. The residue was taken up in chloroform (50 ml), the suspension kept for 3 h at room temperature, and the deposited, crystalline mercuric salt filtered off. The filtrate was evaporated to a syrup which was chromatographed on a column of silica gel (300 g) with 97:3 (v/v) chloroform–ethanol.

Fractions having R_F 0.30 in t.l.c. in 1:1 (v/v) chloroform–ether were combined and evaporated, to give 4.0 g (53%) of **5**; after rechromatography on a column of silica gel with 1:2 (v/v) chloroform–ether, it was obtained as a yellow, amorphous material, $[\alpha]_D^{28}$ +18° (*c* 1.1, chloroform); n.m.r. data (100 MHz, chloroform-*d*): τ 1.00, 1.38, 1.80 (3 protons, dinitrophenyl), 2.31, 2.74 (4 protons, tosyl), 2.98 (1-proton doublet, *J* 9 Hz, NH-Ph), 4.05 (1-proton doublet, *J* 7.5 Hz, $NH-SO_2$), 4.95 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1 of GalN), 6.37 (3 protons, CO_2CH_3), 7.60 (3 protons, $Ph-CH_3$), 7.79, 7.91, and 8.20 (12 protons, 4 Ac).

Anal. Calc. for $C_{28}H_{34}N_4O_{16}S$: C, 47.93; H, 4.71; N, 7.71. Found: C, 47.32; H, 4.62; N, 7.31.

Fractions having R_F 0.21 in t.l.c. in 1:1 (v/v) chloroform–ether were combined and evaporated, to give 0.87 g (11%) of **6**; after rechromatography on a column of silica gel with 1:2 (v/v) chloroform–ether, it was obtained as a yellow, amorphous

material, $[\alpha]_D^{28}$ −26° (*c* 1.13, chloroform): n.m.r. data (100 MHz, chloroform-*d*): τ 0.95, 1.43, 1.82 (3 protons, dinitrophenyl), 2.48, 2.77 (4 protons, tosyl), 2.88 (1-proton doublet, *J* 10 Hz, NH–Ph), 4.96 (1-proton doublet, *J* 7 Hz, NH–SO_2), 5.21 (1-proton doublet, $J_{1,2}$ 8 Hz, H-1 of GalN), 6.61 (3 protons, CO_2CH_3), 7.62 (3 protons, Ph–CH_3), 7.82, 7.95, and 8.08 (12 protons, 4 Ac).

Anal. Calc. for $C_{29}H_{34}N_4O_{16}S$: C, 47.93; H, 4.71; N, 7.71. Found: C, 47.90; H, 4.73; N, 7.68.

O-(*2-Amino-2-deoxy-α-*D*-galactopyranosyl*)-N-*tosyl*-L-*serine methyl ester*, *hydrochloride* (**7**). — A solution of **5** (2 g) in a mixture of acetone (30 ml) and water (15 ml) was stirred with Dowex-1 (OH^-) ion-exchange resin (40 ml) for 15 min. The resin was then packed into a column, washed with water (100 ml), and eluted with 0.1M hydrochloric acid in 30% aqueous ethanol. The fractions having R_F 0.66 in t.l.c. in 4:5:3 (v/v) butanol–acetone–water were combined, and evaporated to a syrupy residue. The residue was dissolved in a small amount of ethanol, and precipitated with petroleum ether, to give 930 mg (71%) of **7** as an amorphous powder, $[\alpha]_D^{28}$ +142° (*c* 1.2, water). This compound exhibited only one spot (R_F 0.33) in t.l.c. with 7:5:2 (v/v) propanol–ethyl acetate–water.

Anal. Calc. for $C_{16}H_{24}N_2O_9S \cdot HCl \cdot H_2O$: C, 40.47; H, 5.73; N, 5.90. Found: C, 40.41; H, 5.85; N, 5.63.

O-(*2-Acetamido-2-deoxy-α-*D*-galactopyranosyl*)-N-*tosyl*-L-*serine* (**8**). — A solution of **7** (930 mg) in 80% aqueous methanol (6 ml) was made neutral with sodium (48 mg) in methanol (48 ml), and treated with acetic anhydride (1 ml). After 2 h at room temperature, the solution was evaporated. The residue was chromatographed on a column of silica gel (40 g) with 30:10:10:1 (v/v) butanol–acetic acid–ether–water; the fractions having R_F 0.21 in t.l.c. in the same solvent mixture were combined, and treated with a small amount of Dowex-50 (H^+) ion-exchange resin. The resin was filtered off, and the filtrate was evaporated. The residue was crystallized from ethanol, to give 550 mg (58%) of **8** as needles, m.p. 193–195° (dec.), $[\alpha]_D^{28}$ +130° (*c* 1.0, water).

Anal. Calc. for $C_{18}H_{26}N_2O_{10}S \cdot 0.5H_2O$: C, 45.86; H, 5.77; N, 5.94. Found: C, 45.83; H, 5.51; N, 5.82.

O-(*2-Acetamido-2-deoxy-α-*D*-galactopyranosyl*)-N-*tosyl*-L-*serine methyl ester* (**9**). — To a solution of **8** (120 mg) in methanol (4 ml) was added a slight excess of diazomethane in ether. The solution was evaporated to a syrup, and this was crystallized from a mixture of acetone and ether to give needles, 88 mg (69%), m.p. 184–186°, $[\alpha]_D^{28}$ +86° (*c* 1.3, ethanol); n.m.r. data (100 MHz, dimethyl sulfoxide-d_6): τ 1.82 (1-proton doublet, *J* 9 Hz, NH–SO_2), 2.36, 2.68 (4 protons, tosyl), 2.80 (1-proton doublet, *J* 9 Hz, NH–Ph), 5.55 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1 of GalN), 6.60 (3 protons, CO_2CH_3), 7.65 (3 protons, Ph–CH_3), and 8.20 (3 protons, N–Ac).

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, 46.18; H, 5.74; N, 5.56.

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