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Note

Synthesis of 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactopyranose from 2-acetamido-2-deoxy-D-glucose through a trifluoromethylsulfonyl group displacement

André Lubineau* and Hugues Bienaymé
Laboratoire de Chimie Organique Multifonctionelle (URA CNRS 462), Institut de Chimie Moléculaire d'Orsay, Université Paris-Sud, Bt. 420, F-91405 Orsay (France)

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The carbohydrate sequence β-D-Gal*p*-(1→3)-D-GalNAc is widely distributed in nature, either in *O*-glycoproteins or in glycolipids such as the *ganglio*-gangliosides. In *O*-glycoproteins, this disaccharide is linked α-D-(1→3) to serine or threonine, and usually further glycosylated. On the contrary, alteration in glycosylation, often associated with malignancy, results in its appearance in glycoconjugates. Such structure, the socalled T-antigen, which is present in many carcinoma cells is now considered as an important tumor marker[1]. Moreover, this disaccharide is also found to a large extent in antifreeze glycoprotein, where it has been shown to be essential for antifreeze properties[2]. The α configuration of the 2-acetamido-2-deoxy-D-galactopyranosyl residue linked to serine or threonine is absolutely necessary for the biochemical properties. Recently as an alternative to azidonitration, we described[3] a new method that allows the introduction of a linking arm having the α configuration at the reducing end of 2-acetamido-2-deoxy-D-glucose or *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose (**8**), which have already the naturally occurring *N*-acetyl protecting group. Thus, artificial antigens useful for immunochemical evaluation could be prepared. This encouraged us to devise a direct access to the T-antigen structure starting from *N*-acetyl-D-glucosamine, a 200-fold less expensive sugar than *N*-acetyl-D-galactosamine.

The key step in the synthesis of **8** was the inversion of configuration at C-4 of the protected disaccharide **4**. The conversion of a suitably protected glucosamine into a galactosamine derivative has been the subject of several previous reports[4–11]. Since the pionnering work of Brendel *et al.*[4] who used a 4,6-di-*O*-methylsulfonyl derivative, various conditions have been used, including the use of the 4-*O*-bromotolylsulfonyl leaving group[9,11]. All the conditions reported, up to now, required high temperature (up to 150°), and the use of *N*,*N*-dimethylformamide or *N*,*N*,*N*,*N*′,*N*′,*N*′-hexamethylphosphoric triamide as solvent. Curiously, the trifluoromethylsulfonyl group, which is known as one of the best leaving groups, has not yet been used for this essential transformation. We chose to achieve the inversion step after the coupling reaction, by

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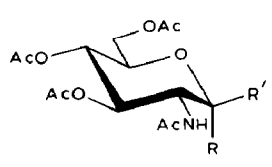
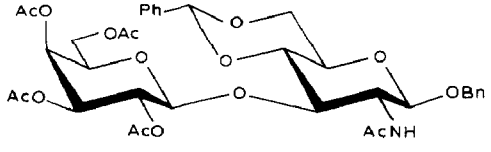
use of a per-*O*-acetyl-D-galactosyl group linked at O-3 of the 2-acetamido-2-deoxy-D-glucopyranosyl residue as permanent protecting group to avoid several protection–deprotection steps.

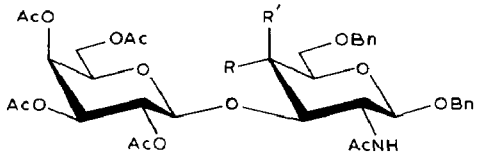
Starting from chloride **1**, the use of tin(II) trifluoromethylsulfonate[12,13] as promotor allowed the preparation of the benzyl β-D-glycoside **2** in 87% yield, a notable improvement in comparison to previous results[6,14]. The protected disaccharide **4** was prepared as previously described[15,16]. Condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside in the presence of mercuric cyanide gave disaccharide[15] **3** in 90% yield. Reductive opening of the benzylidene acetal group gave **4** identical in every respect with the previously reported[16] compound; during this last step, the yield improved from 55 to 78% without noticeable variations in the experimental procedures[16]. In order to introduce a triflyl group in the presence of an acetamido group, it was necessary to use triflic anhydride in pure pyridine at room temperature or below. When the reaction was conducted in dichloromethane solution in the presence of triethylamine, even at low temperature (0°), extensive side-reactions involving the acetamido group (as shown by the disappearance of the NH-n.m.r. signal) took place. Under the present conditions, after 6 h at 0°, **4** afforded **5**, which was only briefly purified before the inversion step. Benzoate displacement proceeded readily at room temperature with tetrabutylammonium benzoate as nucleophile to give the protected disaccharide **6** in 66% yield (84% based on recovery of the starting material). When the inversion step was performed at room temperature under these conditions, a large part (21%) of starting **4** was recovered without the formation of any degradation products. The unprotected disaccharide **7** was obtained by Zemplén deacetylation, followed by catalytic hydrogenation. The overall yield was essentially quantitative for these steps. Although all physical and spectroscopic properties were found identical with those previously reported[17,18], no



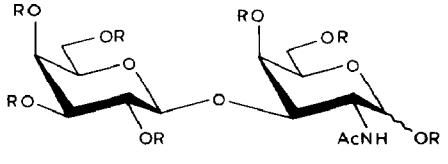
1 R = Cl, R′ = H
2 R = H, R′ = OBn



3



4 R = OH, R′ = H
5 R = OTfl, R′ = H
6 R = H, R′ = OBz



7 R = H
8 R = Ac

Tfl = F_3CSO_3

correct elemental analysis was obtained for **7**, and it was fully characterized as its per-*O*-acetyl derivative **8**, obtained in the usual way in a 94% yield.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured with a Roussel–Jouan electronic digital micropolarimeter. ^{1}H- and ^{13}C-n.m.r. spectra were recorded with a Bruker AM-250 spectrometer. The chemical shifts are given relative to the signal of tetramethylsilane as internal standard for solution in $CDCl_3$, and as external reference for solutions in D_2O. Reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck) with detection by charring with H_2SO_4. Silica gel chromatography was performed with Chromagel 6-35μ (S. D. S. Chemical Co.). Preparative h.p.l.c. was carried out on a home-made 15 μ silica gel column with a Jobin–Yvon chromatospac using a LKB UV detector. Solvents were dried and distilled just before use, pyridine and dichloromethane from CaH_2, ether and oxolane from sodium–benzophenone.

*Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**2**). — Tetramethylurea (2.58 g, 2.66 mL, 22.6 mmol) and benzyl alcohol (20 g, 19 mL, 185 mmol) were added to a stirred suspension of Sn(II) triflate (11.56 g, 27.7 mmol) and 3A molecular sieves (1 g) in dichlormethane (100 mL), and then chloride **1** (6.76 g, 18.5 mmol) was added. After 16 h at room temperature, the mixture was diluted with dichloromethane, and washed successively with cold 5% aqueous $NaHCO_3$ and water. The organic phase was evaporated under high vacuum until most of the benzyl alcohol had been removed. Crystallization of the residue 10:1 (v/v) from water–methanol, gave **2** (7.02 g, 87%) as white needles, m.p. 170° $[\alpha]_D$ − 40° (*c* 0.7, methanol); ^{1}H-n.m.r. ($CDCl_3$, 250 MHz): δ 7.25–7.40 (m, 5 H, arom.), 5.64 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH), 5.25 (t, 1 H, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5 Hz, H-4), 5.09 (t, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.5 Hz, H-3), 4.90 (d, 1 H, J_{gem} 12 Hz, CH_2Ph), 4.65 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.60 (d, 1 H, J_{gem} 12 Hz, CH_2Ph), 4.28 (dd, 1 H, $J_{5,6a}$ 4.5 $J_{6a,6b}$ 12 Hz, H-6′), 4.15 (dd, 1 H, $J_{5,6a}$ 2.5, $J_{6a,6b}$ 12 Hz, H-6), 3.98 (ddd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9.5, $J_{2,NH}$ 9.5 Hz, H-2), 3.68 (ddd, 1 H, $J_{5,6a}$ 4.5, $J_{5,6b}$ 2.5, $J_{4,5}$ 9.5 Hz, H-5), and 1.91, 2.02, 2.11 (s each, 12 H, 3 OAc, 1 NHAc); lit.[6] m.p. 166–167°; lit.[14] m.p. 170°, $[\alpha]_D$ −43° (*c* 1, methanol).

*Benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**4**). — A saturated solution of HCl in ether (10 mL) was added dropwise to a cooled (0°) and stirred suspension of compound[15] **3** (1.095 g, 1.5 mmol), $NaBH_3CN$ (1 g, 15 mmol), and 3A molecular sieves (5 g) in oxolane (20 mL). The reaction was monitored by t.l.c. (4:1, v/v toluene–acetone), and, after complete disappearance of **3** (20 min), the mixture was worked up as described[16]. Preparative h.p.l.c. (4:1, v/v, toluene–acetone) afforded **4** (0.86 g, 78%), m.p. 148° (dichloromethane–ether–hexane), $[\alpha]_D$ −16° (*c* 1, dichloromethane); ^{1}H-n.m.r. ($CDCl_3$, 250 MHz): δ 7.2–7.4 (m, 10 H, arom.), 5.64 (d, 1 H, $J_{NH,2}$ 7 Hz, NH), 5.37 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4′), 5.22 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 11 Hz, H-2′), 5.00 (dd, 1 H, $J_{2',3'}$ 11, $J_{3',4'}$ 3.5 Hz, H-3′), 4.97 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.89 (d, 1 H, *J* 12 Hz, CH_2Ph), 4.63 (s, 2 H, CH_2Ph), 4.58 (d, 1 H, *J*

12 Hz, CH_2Ph),) 4.56 (d, $J_{1',2'}$ 8 Hz, H-1'), 4.39 (dd, 1 H, $J_{2,3}$ 8, $J_{3,4}$ 10 Hz, H-3), 3.65–4.15 (m, 6 H, OH, H_2-6, H_2-6', H-5'), 3.45–3.55 (m, 2 H, H-4,5), 3.12 (dt, 1 H, $J_{NH,2}$ 7, $J_{1,2}$ 8, $J_{2,3}$ 8 Hz, H-2), and 1.92, 1.96, 2.01, 2.05, 2.16 (s, each, 15 H, 4 OAc, 1 NHAc); lit.[16] m.p. 145–146°, $[\alpha]_D$ −12.6° (*c* 1, chloroform).

Anal. Calc. for $C_{36}H_{45}NO_{15}$: C, 59.09; H, 6.20; O, 32.79. Found: C, 58.35; H, 6.36; O, 32.57.

Benzyl 2-acetamido-4-O-benzoyl-6-O-benzyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**6**). — Trifluoromethylsulfonyl anhydride (250 μL, 1.5 mmol) was added to a cooled (−30°) and stirred solution of **4** (0.732 g, 1 mmol) in pyridine (15 mL). The mixture was warmed to 0° and the reaction was monitored by t.l.c. (2:1, v/v, toluene–acetone); trifluoromethylsulfonate **5**, R_F 0.50; **4**, R_F 0.40). After 6 h at 0°, the mixture was diluted with dichloromethane (100 mL) and washed successively with cold phosphate buffer (pH 7) and cold water. Several coevaporations with toluene to remove pyridine gave a residue that was dissolved in toluene (30 mL). Tetrabutylammonium benzoate (1.81 g, 5 mmol) was added and the mixture kept at room temperature for 48 h. At this time, t.l.c. (2:1, v/v, toluene–acetone) indicated complete conversion of **5** into a compound showing a slower moving spot (R_F 0.45). The solution was diluted with dichloromethane, washed with water, and concentrated. Chromatography (3:2, v/v, dichloromethane–ether) of the residue gave amorphous **6** (0.55 g, 66%; 84% based on recovery of **4**), $[\alpha]_D$ +17° (*c* 1, dichloromethane); ^{1}H-n.mr. ($CDCl_3$, 250 MHz): δ 5.72 (d, 1 H, $J_{3,4}$ 3,5 Hz, H-4), 5.68 (d, 1 H, $J_{NH,2}$ 8.5 Hz, NH), 5.28 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.20 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.08 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2'), 4.94 (d, 1 H, *J* 12 Hz, CH_2Ph), 4.92 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.5 Hz, H-3'), 4.80 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 3.50 Hz, H-3), 4.66 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'), 4.61 (d, 1 H, *J* 12 Hz, CH_2Ph), 4.53 (s, 2 H, CH_2Ph), 3.57–4.03 (m, 6 H, H-5,5', H_2-6, H_2-6'), 3.53 (dt, 1 H, $J_{2,3}$ 11, $J_{1,2}$ 8.5, $J_{NH,2}$ 8.5 Hz, H-2), and 1.90, 1.92, 1.95, 2.00, 2.01 (s each, 15 H, 4 OAc, 1 NHAc).

Anal. Calc. for $C_{43}H_{49}NO_{16}$: C, 61.79; H, 5.91; H, 5.91; O, 30.63. Found: C, 61.43; H, 5.89; O, 31.06.

Further elution gave **4** (0.157 g. 21%).

2-Acetamido-2-deoxy-3-O-β-D-galactopyranosyl α, β-D-galactopyranose (**7**). — To a solution of **6** (1.56 g, 1.87 mmol) in methanol (50 mL) was added a 22mM solution of sodium methoxide in methanol (1 mL). The mixture was stirred for 24 h at room temperature, the acid was neutralized with Amberlite IR-120 (H^+), filtered, and the filtrate concentrated. A solution of the residue in methanol (100 mL) was shaken under H_2 at 600 kPa in the presence of 10% Pd–C (500 mg) at room temperature. After 16 h, the suspension was filtered, and the filtrate contentrated to give pure **7** (0.71 g, 99%), as judged by t.l.c. (3:2:1 ethyl acetate–2-propanol–water) and ^{1}H- and ^{13}C-n.m.r. spectroscopy, m.p. 160° (dec., ethanol), $[\alpha]_D$ +55 → +32° (*c* 1, water); ^{13}C-n.m.r. (D_2O): δ 105.12 (C-1'β), 104.69 (C-1'α), 95.19 (C-1β), 91.20 (C-1α), 80.09 (C-3β), 77.08 (C-3α), 74.98–74.80 (C-5'β,5β), 72.55 (C-3'β), 71.04 (C-2'β), 70.65 (C-5α), 68.74 (C-4α), 68.65 (C-4'β), 68.09 (C-4β), 61.20–60.99 (C-6α,6β,6'β), 52.46 (C-2β), and 48.98 (C-2α), similar to the ^{13}C-n.m.r. spectrum described by Matta *et al.*[18]; ^{1}H- n.m.r. (D_2O, 250 MHz): δ 5.20 (d, 0.7 H, $J_{1,2}$ 4 Hz, H-1α), 4.68 (d, 0.3 H, $J_{1,2}$ 8.5 Hz, H-1β), 4.49 (d, 0.7 H, $J_{1',2'}$ 7.5 Hz, H-1'),

4.42 (d, 0.3 H, $J_{1',2'}$ 7.5 Hz, H-1'), and 2.02 (s, 3 H, HNAc); lit.[17] m.p. 160° (dec., ethanol), $[\alpha]_D$ + 52 → +31° (*c* 1, water).

2-Acetamido-1,4,6-tri-O-*acetyl-2-deoxy-3*-O-*(2,3,4,6-tetra*-O-acetyl-β-D-*galactopyranosyl)*-α,β-D-*galactopyranose* (**8**). — Compound **7** (0.3 g) was peracetylated in the usual manner with acetic anhydride–pyridine 1:1 (v/v) (40 mL). After 16 h at room temperature, the mixture was coevaporated several times with toluene. Chromatography (2:1, v/v, toluene–acetone) afforded **8** (0.499 g, 94%) as a mixture of the α and β anomers; ^{1}H-n.m.r. ($CDCl_3$, 250 MHz): δ 6.29 (d, 0.5 H, $J_{1,2}$ 2.5 Hz, H-1α), 6.02 (d, 0.5 H, $J_{1,2}$ 8.5 Hz, H-1β), 4.68 (d, 0.5 H, $J_{1',2'}$ 8 Hz, H-1'), and 4.65 (d, 0.5 H, $J_{1',2'}$ 8 Hz, H-1').

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 49.40; H, 5.97; N, 2.09; O, 42.64.

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