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Note

The β-D-Gal*p*NAc-(1 → 3)-D-Gal*p* linkage through the oxazoline glycosylation method

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The disaccharide β-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p* is a component of some important naturally occurring oligosaccharides such as the Forssman pentasaccharide [1], the stage-specific embryonic antigen 3 (SSEA 3) pentasaccharide [2], and the tumor associated antigen Globo-H hexasaccharide [3].

Synthetic work has been performed [1,2,4] aimed towards the synthesis of such a disaccharide moiety, which requires as glycosyl donors, 2-amino-2-deoxy-galactose derivatives properly protected on their amino group. In these cases a 2-phthalimido or a 2-azido derivative was used, implying further synthetic steps after the formation of the β-glycosyl linkage, in order to restore the required 2-acetamido functionality.

Among the methods used in the synthesis of the related disaccharide β-D-Glc*p*NAc-(1 → 3)-α-D-Gal*p* is the oxazoline procedure [4], which results directly in the desired product. This methodology is now applied to obtain the β-D-Gal*p*NAc-(1 → 3)-D-Gal*p* linkage.

As we are currently engaged in the conformational analysis [5,6] of fragments of the Globo-H hexasaccharide, we planned to use the oxazoline method to obtain one such fragment, the disaccharide β-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p*-1-*O*-Pr (**8**).

The glycosyl donor **4** was obtained (Scheme 1) starting from allyl 2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (**1**) [7] through acetylation followed by catalytic isomerization [8] of the allyl group and treatment with iodine and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [9], in an overall yield of about 50%. The oxazoline **4**

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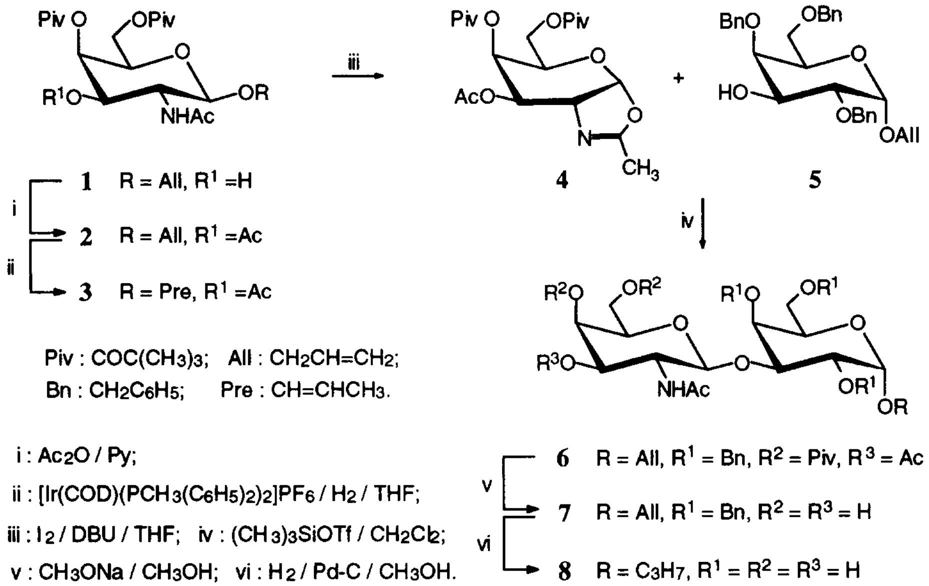

Scheme 1.

was then allowed to react with allyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranoside **5** [10] in dichloromethane at room temperature using trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) as the promoter [11].

In this way, a satisfactory 79% yield of the disaccharide **6** was obtained, so demonstrating the efficiency of the oxazoline procedure for the formation of this linkage.

Compound **6** was then conventionally deprotected by Zemplén deacylation and catalytic hydrogenolysis to give, through **7**, the disaccharide β-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p* as the propyl glycoside **8** suitable for conformational studies.

1. Experimental

General methods.—All reagents, except (cycloocta-1,5-diene)bis(methyl diphenylphosphine)iridium hexafluorophosphate (Alfa), were purchased from Aldrich. Reagents and dried solvents were added via oven-dried syringes through septa. All reactions were monitored by TLC on Silica Gel 60 F-254 plates (E. Merck) with detection by spraying with 50% H_2SO_4 solution. Flash column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). Uncorrected melting points were determined on a Büchi apparatus. Optical rotations were determined on a Perkin–Elmer 241 polarimeter in a 1-dm cell at 20°C. ^1H NMR spectra were recorded with a Bruker AM-500 instrument. Chemical shifts are referenced to HDO (δ 4.55) for compound **8** in 0.04 M D_2O solution at 303 K. All evaporations were carried out under reduced pressure at 40°C.

*Allyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-β-*D*-galactopyranoside* (**2**).—Compound **1** [7] (600 mg, 1.40 mmol) was conventionally acetylated to afford, after flash chromatography (1:1 hexane–EtOAc), **2** (640 mg, 97%); mp 129–130°C (from hexane); $[\alpha]_D$ −13.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.84 (m, 1 H, -C*H*=), 5.57 (d, 1 H, $J_{2,NH}$ 8.5 Hz, N*H*), 5.33 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.28 (dd, 1 H, $J_{2,3}$ 11.0 Hz, H-3), 5.24, 5.16 (2 m, 2 H, =CH_2), 4.73 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.31 (m, 1 H, OCH_aH–), 4.13 (dd, 1 H, $J_{6a,6b}$ 11.0, $J_{5,6a}$ 7.0 Hz, H-6a), 4.06 (m, 1 H, OCH_bH–), 4.04 (dd, 1 H, $J_{5,6b}$ 7.0 Hz, H-6b), 3.94 (dd, 1 H, H-5), 3.91 (ddd, 1 H, H-2), 1.93, 1.92 (2 s, 6 H, 2 Ac), 1.22 and 1.14 (2 s, 18 H, 2 *tert*-BuCO). Anal. Calcd for $C_{23}H_{37}O_9N$: C, 58.58; H, 7.91; N, 2.97. Found: C, 58.32; H, 8.02; N, 3.07.

*1-Propenyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-β-*D-galactopyranoside (**3**).—To a solution of **2** (620 mg, 1.32 mmol) in dry THF (40 mL), a catalytic amount of (cycloocta-1,5-diene)bis(methyldiphenylphosphine)iridium hexafluorophosphate was added. The solution was degassed and left for ca. 1 min under H_2 until the orange colour turned yellow. The solution was degassed again and left under N_2 for 1 h (TLC 1:1 hexane–EtOAc). After evaporation of the solvent the residue was dissolved in CH_2Cl_2, washed with a saturated aq solution of $NaHCO_3$ and with water, and dried under reduced pressure. Flash chromatography (2:3 hexane–EtOAc) of the crude product afforded the propenyl derivative **3** (572 mg, 92%); mp 149–150°C (from hexane); $[\alpha]_D$ + 3.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.17 (m, 1 H, OC*H*=), 5.39–5.32 (m, 3 H, H-3, H-4 and N*H*), 5.11 (m, 1 *H*, =C*H*–), 4.96 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.15 (dd, 1 H, $J_{6a,6b}$ 11.0, $J_{5,6a}$ 7.0 Hz, H-6a), 4.06 (dd, 1 H, $J_{5,6b}$ 7.0 Hz, H-6b), 4.01 (dd, 1 H, H-5), 3.92 (ddd, 1 H, $J_{2,3}$ 11.0, $J_{2,NH}$ 8.5 Hz, H-2), 1.95, 1.93 (2 s, 6 H, 2 Ac), 1.51 (m, 3 H, =CH-CH_3), 1.23 and 1.15 (2 s, 18 H, 2 *tert*-BuCO). Anal. Calcd for $C_{23}H_{37}O_9N$: C, 58.58; H, 7.91; N, 2.97. Found: C, 58.61; H, 7.85; N, 2.90.

*2-Methyl-(3-*O*-acetyl-1,2-dideoxy-4,6-di-*O*-pivaloyl-α-*D*-galactopyrano)-[2,1-*d*]-2-oxazoline* (**4**).—Compound **3** (556 mg, 1.18 mmol) was dissolved in dry THF (16 mL) under N_2; I_2 (594 mg, 2.34 mmol) and 5.2 mL of a 10% (v/v) solution of DBU (3.34 mmol) in THF were added. After 15 min at room temperature (TLC 85:15 toluene–MeOH), the mixture was diluted with $CHCl_3$ and washed with 5% $K_2S_2O_3$ solution, water, dried (Na_2SO_4) and the solvent evaporated. Flash chromatography (85:15 toluene–MeOH) of the residue afforded **4** (264 mg, 54%); mp 112–113°C (from hexane); $[\alpha]_D$ + 84.1° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.94 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1), 5.46 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 3.0 Hz, H-4), 4.88 (dd, 1 H, $J_{2,3}$ 7.0 Hz, H-3), 4.26 (ddd, 1 H, $J_{5,6a}$ 7.0, $J_{5,6b}$ 7.0 Hz, H-5), 4.18 (dd, 1 H, $J_{6a,6b}$ 11.0 Hz, H-6a), 4.02 (dd, 1 H, H-6b), 3.95 (ddq, 1 H, J_{2,CH_3} 1.5 Hz, H-2), 2.02 (d, 3 H, -CH_3), 2.01 (s, 3 H, Ac), 1.22 and 1.16 (2 s, 18 H, 2 *tert*-BuCO). Anal. Calcd for $C_{20}H_{31}O_8N$: C, 58.10; H, 7.56; N, 3.39. Found: C, 58.81; H, 7.49; N, 3.23.

*Allyl 3-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-β-*D*-galactopyranosyl)-2,4,6-tri-*O*-benzyl-α-*D*-galactopyranoside* (**6**).—To a mixture of **5** [10] (294 mg, 0.60 mmol) in dry CH_2Cl_2 (6 mL) and powdered molecular sieves 4 Å, 5.2 mL of a 2.4% (v/v) solution of Me_3SiOTf (0.64 mmol) in dry CH_2Cl_2, and a solution of **4** (248 mg, 0.60 mmol) in dry CH_2Cl_2 (6 mL) were added. After 18 h at room temperature (TLC 1:1 toluene–EtOAc), the reaction was filtered and the filtrate was washed with a saturated aq $NaHCO_3$ solution, H_2O, dried (Na_2SO_4) and the solvent evaporated. Flash chromatography (1:1 toluene–EtOAc) of the residue afforded **6** (430 mg, 79%); mp 182–183°C (from hexane–CH_2Cl_2); $[\alpha]_D$ + 5.4° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.37–7.20 (m, 15 H, Ph-*H*), 5.87 (m, 1 H,-C*H*=), 5.32 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.25, 5.15 (2 m, 2 H, =CH_2), 5.11 (d, 1 H, $J_{NH,2'}$ 10.0 Hz, N*H*), 4.98 (dd, 1 H, $J_{3',2'}$ 11.0 Hz, H-3'), 4.96–4.34 (m, 6 H, Ph-CH_2 –), 4.81 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 4.79 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.32 (ddd, 1 H, H-2'), 4.16–3.91 (m, 8 H, H-2, H-3, H-4, H-5', H-6'a, H6'b and OCH_2 –), 3.89 (dd, 1 H, $J_{5,6a}$ 6.5 Hz, $J_{5,6b}$ 6.5 Hz, H-5), 3.48 (dd, 1 H, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.34 (dd, 1 H, H-6b), 1.93, 1.69 (2 s, 6 H, 2 Ac), 1.21 and 1.14 (2 s, 18 H, 2*tert*-BuCO). Anal. Calcd for $C_{50}H_{65}O_{14}N$: C, 66.43; H, 7.25; N, 1.55. Found: C, 66.19; H, 7.05; N, 1.42.

*Allyl 3-O-(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-2,4,6-tri-O-benzyl-α-*D*-galactopyranoside (*7*)*.—To a solution of **6** (416 mg, 0.46 mmol) in MeOH (8 mL), 2.6 mL of 1.3 M MeONa in MeOH were added. After 3 h at reflux (TLC 95:5 EtOAc–MeOH) the reaction mixture was neutralized with Dowex-50 × 8 (H^+), filtered and the solvent evaporated. Flash chromatography (95:5 EtOAc–MeOH) of the residue afforded **7** (294 mg, 92%); mp 87–88°C (from hexane–EtOAc; $[\alpha]_D$ + 25.5° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.37–7.20 (m, 15 H, Ph–H), 6.16 (d, 1 H, $J_{NH,2'}$ 3.0 Hz, N*H*), 5.88 (m, 1 H, -C*H*=), 5.27, 5.17 (2 m, 2 H, =CH_2), 4.98 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.92–4.34 (m, 6 H, Ph–CH_2–), 4.54 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 4.13 (m, 1 H, OCH_aH–), 4.11 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 4.08 (dd, 1 H, $J_{3,4}$ 2.0 Hz, H-3), 4.04 (br d, 1 H, H-4), 3.99 (dd, 1 H, $J_{5',6'a}$ 5.5, $J_{6'a,6'b}$ 11.5 Hz, H-6'a), 3.95 (dd, 1 H, $J_{5,6a}$ 6.0, $J_{5,6b}$ 6.0 Hz, H-5), 3.92 (m, 1 H, OCH_bH–), 3.90 (dd, 1 H, $J_{5',6'b}$ 4.5 Hz, H-6'b), 3.89 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 3.72 (ddd, 1 H, $J_{2',3'}$ 10.0 Hz, H-2'), 3.56 (dd, 1 H, H-3'), 3.56 (dd, 1 H, H-5'), 3.50 (dd, 1 H, $J_{6a,6b}$ 9.5 Hz, H-6a), 3.41 (dd, 1 H, H-6b) and 1.59 (s, 3 H, Ac). Anal. Calcd for $C_{38}H_{47}O_{11}N$: C, 65.79; H, 6.83; N, 2.02. Found: C, 65.27; H, 6.97; N, 2.26.

*Propyl 3-O-(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-α-*D*-galactopyranoside (***8***)*. —To a solution of **7** (278 mg, 0.40 mmol) in MeOH (20 mL), 140 mg of 10% Pd/C were added and the mixture was stirred for 4 h under H_2 atmosphere (TLC 3:3:1 EtOAc–*i*PrOH–H_2O). The mixture was filtered on Celite and the solvent evaporated. The residue was dissolved in water and then lyophilized to give **8** (138 mg, 81%); mp 215–218°C (dec) (from EtOAc); $[\alpha]_D$ + 89.2° (*c* 0.9, MeOH); ^1H NMR (D_2O): δ 4.73 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.46 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 4.01 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 3.79–3.71 (m, 4 H, H-5, H-4', H-2' and H-3), 3.69 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 3.61 (dd, 1 H,$J_{5',6'a}$ 7.5, $J_{6'a,6'b}$ 11.5 Hz, H-6'a), 3.57 (dd, 1 H, $J_{5',6'b}$ 4.5 Hz, H-6'b), 3.56 (dd, 1 H,$J_{2',3'}$ 10.0, $J_{3',4'}$ 3.5 Hz, H-3'), 3.54 (d, 2 H, $J_{5,6}$ 6.5 Hz, H_2-6), 3.51–3.45 (m, 2 H, OCH_aH and H-5'), 3.31 (m, 1 H, OCH_bH–) 1.86 (s, 3 H, Ac), 1.45 (m, 2 H, –CH_2–) and 0.74 (t, 3 H, –CH_3). Anal. Calcd for $C_{17}H_{31}O_{11}N$: C, 48.00; H, 7.34; N, 3.29. Found: C, 48.39; H, 7.90; N, 3.66.

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