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CHEMICAL SYNTHESIS OF THE DESIALYLATED HUMAN Cad-ANTIGENIC DETERMINANT*

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ABSTRACT

Benzyl 2-azido-2-deoxy-β-D-galactopyranoside was converted into benzyl 2-azido-4,6-*O*-benzyl-2-deoxy-β-D-galactopyranoside *via* benzylidenation, *p*-methoxybenzylation, acid hydrolysis, benzylation, and selective oxidation. Condensation of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose with benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside in the presence of trimethylsilyl triflate gave crystalline benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (76%), which was converted into benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-galactopyranoside and condensed with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide in the presence of silver silicate on alumina and molecular sieve 4 Å to give 61% of benzyl *O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside. Reduction with sodium borohydride followed by *N*-acetylation, *O*-deacetylation, and catalytic hydrogenolysis then gave *O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactopyranose, the desialylated human Cad-antigenic determinant.

INTRODUCTION

Cad is a rare human red-cell antigen inherited as an autosomal dominant character[1]. Preliminary investigations showed that the Cad determinant is a carbohydrate structure carried by glycophorin A and B, the main sialoglycoproteins of red-cell membranes[2]. Treatment of the Cad glycophorin A with alkaline boro-

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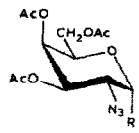
hydride released a reduced pentasaccharide[3] in which the sialic acid-rich tetrasaccharide described by Thomas and Winzler[4] is substituted by a 2-acetamido-2-deoxy-D-galactose residue, so that the structure of the Cad red-cell antigen is β-D-GalNAc-(1→4)-[α-NeuAc-(2→3)]-β-D-Gal-(1→3)-[α-NeuAc-(2→6)]-α-D-GalNAc. There is strong evidence[5] that blood-group Cad specificity is also carried by a novel ganglioside which is most probably derived from sialosylparagloboside, β-D-GalNAc-(1→4)-[α-NeuAc-(2→3)]-β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc-ceramide.

The Sd^a blood-group antigen[6] exhibits immune cross-reaction with the Cad-antigen, but has not yet been identified on red blood cells. A blood-group Sd^a active pentasaccharide has been isolated from the major human-urinary Tamm-Horsfall (T-H) glycoprotein and its structure is β-D-GalNAc-(1→4)-[α-NeuAc-(2→3)]-β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-D-Gal. Thus, the Sd^a antigen shares with the Cad-antigen the same trisaccharide unit at the non-reducing end. Whether the ganglioside isolated by Blanchard *et al.*[5] might be the carrier of the Sd^a antigen on human erythrocytes remains to be established.

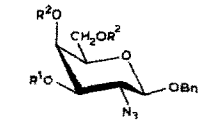
As part of a programme on the synthesis of the sub-units of the Cad-antigenic determinant carried by Glycophorin A and B, we now report the chemical synthesis of the asialylated trisaccharide β-D-GalNAc-(1→4)-β-D-Gal-(1→3)-D-GalNAc.

RESULTS AND DISCUSSION

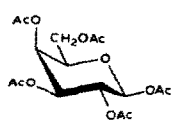
Benzyl 2-azido-2-deoxy-β-D-galactopyranoside (**3**) was prepared (78%) by the reaction of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide[7]



1 R = Br
2 R = ONO_2



3 $R^1 = R^2 = H$
4 $R^1 = H, R^2 = >CHPh$
5 $R^1 = MPM, R^2 = >CHPh$
6 $R^1 = MPM, R^2 = Bn$
7 $R^1 = H, R^2 = Bn$
MPM = p-MeO—$PhCH_2$



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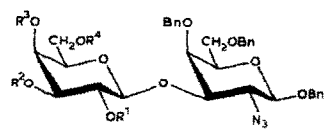
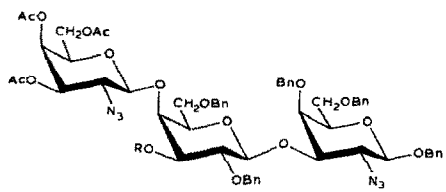
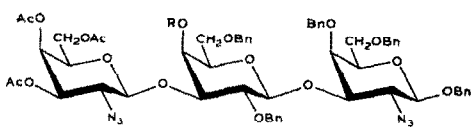


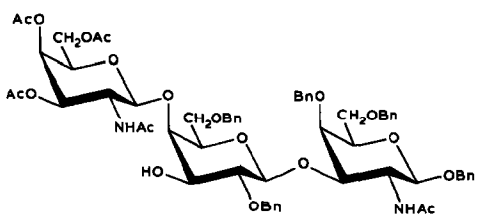
9 $R^1 = R^2 = R^3 = R^4 = Ac$
10 $R^1 = R^4 = H; R^2, R^3 = >CMe_2$
11 $R^1 = R^2 = H; R^3, R^4 = >CMe_2$
12 $R^1 = R^4 = Bn; R^2, R^3 = >CMe_2$
13 $R^1 = R^4 = Bn; R^2 = R^3 = H$



14 R = H
15 R = Ac



16 R = H
17 R = Ac



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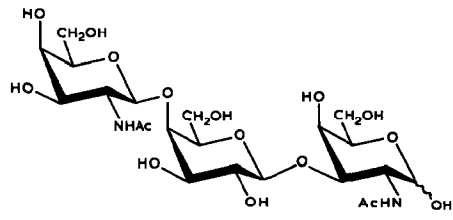

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(**1**) with benzyl alcohol in toluene in the presence of silver oxide and molecular sieve 4 Å, followed by saponification. Alternatively, treatment of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl nitrate[7] (**2**) with sodium benzyloxide[8] in toluene, followed by saponification, gave 81% of **3**. The physical properties of **3** reported by Paulsen *et al.*[8] {m.p. 119° (amorphous solid), $[\alpha]_D^{20}$ +13.3° (methanol)} are at variance with our data {m.p. 137–138° (from ethyl acetate–hexane), $[\alpha]_D$ +28° (methanol)}. Treatment[9] of **3** with 1,1-dimethoxytoluene gave 78% of **4**. Attempts to β-D-galactosylate HO-3 of **4** with 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose in the presence of trimethylsilyl triflate, a method which has been reported to require acid-stable protecting groups[10], gave poor results. A more stable type of protection of HO-4,6 was achieved by replacing the benzylidene group with two benzyl groups. Temporary protection of HO-3 was achieved by alkylation of **4** with *p*-methoxybenzyl bromide in *N,N*-dimethylformamide in the presence of sodium hydride to give >90% of **5**. Treatment of **5** with aqueous 60% acetic acid (10 min at 100°) followed by reaction with benzyl bromide–*N,N*-dimethylformamide–sodium hydride gave 72% of crystalline **6** and selective oxidative removal of the *p*-methoxybenzyl ether[11] gave 84% of crystalline benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**7**). The *p*-methoxybenzyl group is therefore a suitable protecting group in the preparation of such compounds as **7**.

Treatment[10] of **7** with 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose gave 76% of the crystalline disaccharide derivative **9**. The β configuration of the new interglycosidic linkage in **9** was apparent from the n.m.r. data (δ 4.70 $J_{1',2'}$ 8.5 Hz, H-1′; δ 102.1, C-1′). The chemical shift of the latter signal is close to that (101.5 p.p.m.) reported[12] for C-1 of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside, in contrast to that (96.5 p.p.m.) of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside. Saponification of **9** followed by treatment at room temperature with acetone–toluene-*p*-sulphonic acid gave 68% of the amorphous isopropylidene derivative **10** together with a small proportion of the 4,6-*O*-isopropylidene derivative **11**. The structures of **10** and **11** were based on ^{13}C-n.m.r. data[13] for isopropylidene acetals. Conventional benzylation of **10**, followed by treatment with aqueous 80% acetic acid at 100°, gave the diol **13** in excellent yield.

Condensation of **13** with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide in toluene from −20° to room temperature, in the presence of silver silicate on alumina and molecular sieve 4 Å, gave, after column chromatog-

raphy, 61% and 11% of the trisaccharide derivatives **14** and **16**, respectively. This remarkable (6:1) regioselectivity was discovered and developed in Paulsen's group[14]. The β configuration of the new interglycosidic linkage in **14** was clear from the $J_{1,2}$ value of 8 Hz for the H-1″ doublet and from the ^{13}C signal for C-1″ (102.3 p.p.m.). Furthermore, the attribution in the acetyl derivative **15** of a deshielded signal at δ 4.94 (dd, $J_{2',3'}$ 10.3, $J_{3',4'}$ 3.4 Hz) to H-3′ geminal to the newly formed acetoxy function clearly showed that the condensation had taken place at HO-4′. The β configuration of the new interglycosidic linkage in the minor trisaccharide derivative **16** was clear from the $[\alpha]_D$ value [−21° (chloroform)] which was close to that (−16°) of **14**. The derivative **16** was derived by glycosylation of HO-3′, since H-4′ (δ 5.43, $J_{3',4'}$ 3.1 Hz) in its acetyl derivative was deshielded. The azido groups of the trisaccharide derivative **14** were reduced with sodium borohydride in ethanol, in the presence of nickel dichloride hexahydrate and 2% of boric acid. Selective *N*-acetylation then gave the trisaccharide derivative **18**, which was *O*-deacetylated and then catalytically hydrogenolysed to give the amorphous title trisaccharide *O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactopyranose (**19**) after purification on Sephadex G-10. The high-field ^1H-n.m.r. spectrum of **19** was in agreement with the structure, and demonstrated the purity of the sample.

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler apparatus and are uncorrected. Optical rotations were measured at 20–22° with a Perkin–Elmer 241 polarimeter. ^1H-N.m.r. spectra were recorded with Varian CFT-20 (79.9 MHz) and Bruker AM-300 (300.133 MHz) instruments for ~10% solutions in $CDCl_3$ (internal Me_4Si), unless otherwise stated. ^{13}C-N.m.r. spectra were recorded with a Bruker AM-300 (75.5 MHz) spectrometer. I.r. spectra were recorded on neat liquids or Nujol mulls with a Pye-Unicam SP3-300 spectrometer. The purity of products was determined by t.l.c. on Silica Gel 60 F_{254} (Merck) with detection by charring with sulphuric acid. Column chromatography was performed on Silica Gel 60 (Merck, 63–200 μm) which was used without pretreatment. Elemental analyses were performed with a Carlo Elemental Analyzer Model 1106.

Benzyl 2-azido-2-deoxy-β-D-galactopyranoside (**3**). — (*a*) *From* **1**. A mixture of dry benzyl alcohol (750 mg), anhydrous silver oxide (1.46 g), 4 Å activated powdered molecular sieve (1.3 g), and dry toluene (10 mL) was stirred for 1 h at room temperature under dry argon. The mixture was cooled to 0° and a solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (**1**, 1.25 g) in dry toluene (10 mL) was added dropwise. The mixture was stirred for 2 h at 0° and then overnight at room temperature, diluted with dichloromethane (100 mL), filtered, and concentrated. A solution of the residue in anhydrous methanol (10 mL) was treated with freshly prepared methanolic 0.25M sodium methoxide (3 mL) overnight at room temperature, then neutralised with Amberlyst 15 (H^+) resin, filtered,

and concentrated. The residue crystallised from ethyl acetate–hexane to give **3** (740 mg, 78%), m.p. 137–138°, $[\alpha]_D$ +28° (*c* 1, methanol); ν_{max} 2120 cm^{-1} (N_3). ^{1}H-N.m.r. data [$(CD_3)_2SO$–D_2O, 80 MHz]: δ 7.45–7.28 (m, 5 H, Ph), 4.40 (d, 1 H, $J_{1,2}$ 8 Hz, H-1).

Anal. Calc. for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80; N, 14.33. Found: C, 52.69; H, 5.77; N, 14.17.

(*b*) *From* **2**. A solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl nitrate (**2**, 1.12 g) in dry toluene (10 mL) was added dropwise at 0° with stirring to sodium benzyloxide [from sodium (105 mg) and anhydrous benzyl alcohol (7 mL)]. The mixture was stirred for 30 min at room temperature, then diluted with methanol (20 mL), stirred with Amberlyst MB-3 (H^+) resin (10 mL), filtered, and concentrated. The residue was eluted from a column of silica gel (20 g) with ethyl acetate to give **3** (720 mg, 81%); lit.[8] m.p. 119° (amorphous solid), $[\alpha]_D$ +13.3° (*c* 0.72, methanol).

Benzyl 2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranoside (**4**). — A solution of **3** (3.60 g) in dry *N,N*-dimethylformamide (10 mL) was stirred for 1.5 h at 60° under reduced pressure (Büchi apparatus) in the presence of 1,1-dimethoxytoluene (1.9 mL) and toluene-*p*-sulphonic acid (20 mg). The mixture was then cooled, stirred with saturated aqueous $NaHCO_3$ (5 mL), and extracted with dichloromethane (2 × 75 mL). The combined extracts were dried ($MgSO_4$) and concentrated, and the residue (4.18 g) was crystallised from ethyl acetate to give **4** (3.64 g, 78%) as white needles, m.p. 143–144°, $[\alpha]_D$ −6° (*c* 1, chloroform). ^{1}H-N.m.r. data (80 MHz): δ 7.60–7.20 (m, 10 H, 2 Ph), 5.55 (s, 1 H, PhC*H*), 4.34 (d, 1 H, $J_{1,2}$ 8 Hz, H-1).

Anal. Calc. for $C_{20}H_{21}N_3O_5$: C, 62.65; H, 5.52; N, 10.96. Found: C, 62.75; H, 5.44; N, 11.05.

Benzyl 2-azido-4,6-O-benzylidene-2-deoxy-3-O-p-methoxybenzyl-β-D-galactopyranoside (**5**). — A solution of **4** (1.77 g) in dry *N,N*-dimethylformamide (10 mL) was added dropwise at 0° with stirring to a stirred suspension of sodium hydride (165 mg) in dry *N,N*-dimethylformamide (10 mL). The mixture was stirred for 1.5 h at 0° and then for 1.5 h at room temperature, cooled to 0°, and treated with freshly prepared *p*-methoxybenzyl bromide[15] (1.21 g). The mixture was stirred for 4 h at room temperature, methanol (2 mL) was added, and stirring was continued for 15 min at 0°. The mixture was concentrated and a solution of the residue in dichloromethane (150 mL) was washed with ice–water, dried ($MgSO_4$), and concentrated. The residue crystallised from methanol to give **5** (1.92 g, 82%), m.p. 152–153°, $[\alpha]_D$ +4° (*c* 0.8, chloroform); ν_{max} 2120 cm^{-1} (N_3). ^{1}H-N.m.r. data (80 MHz): δ 7.60–7.20 and 6.81 (m and d, 14 H, aromatic protons), 5.42 (s, 1 H, PhC*H*), 4.28 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.73 (s, 3 H, OMe).

Anal. Calc. for $C_{28}H_{29}N_3O_6$: C, 66.78; H, 5.81; N, 8.35. Found: C, 66.88; H, 5.69; N, 8.35.

When the crude residue was eluted from a column of silica gel (hexane–ethyl acetate, 7:3), the yield of **5** increased to 92%.

*Benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-p-methoxybenzyl-β-*D*-galactopyranoside* (**6**). — Compound **5** (1.46 g) was heated for 10 min at 100° under stirring with aqueous 60% acetic acid (25 mL). The mixture was cooled and then concentrated, and toluene (3 × 20 mL) was evaporated from the residue, a solution of which in dry *N,N*-dimethylformamide (30 mL) was then stirred for 1 h at 0° in the presence of sodium hydride (480 mg). Freshly distilled benzyl bromide (0.9 mL) was then added, the mixture was stirred for 2 h at room temperature, methanol (3 mL) was added, and stirring was continued for 15 min at 0°. The mixture was concentrated, and the residue was treated with ice–water (150 mL) and extracted with dichloromethane (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was eluted from a column of silica gel (100 g) with 3:1 hexane–ethyl acetate to give **6** (1.25 g, 72%), m.p. 54–55° (from methanol), $[\alpha]_D$ −37.5° (*c* 1, chloroform); ν_{max} 2120 cm^{-1} (N_3). ^{1}H-N.m.r. data (80 MHz): δ 7.42–7.23 and 6.85 (m and d, 19 H, aromatic protons), 4.26 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.79 (s, 3 H, MeO).

Anal. Calc. for $C_{35}H_{37}N_3O_6$: C, 70.57; H, 6.26; N, 7.05. Found: C, 70.68; H, 6.16; N, 6.95.

*Benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-β-*D*-galactopyranoside* (**7**). — A solution of **6** (1.33 g) in dichloromethane (30 mL) and water (1.5 mL) was stirred for 3 h at room temperature in the presence of 2,3-dichloro-5,6-dicyanobenzoquinone (0.66 g). The mixture was then diluted with dichloromethane (100 mL), washed with water (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL), and water (20 mL), dried ($MgSO_4$), and concentrated. The residue crystallised from methanol to give **7** (0.88 g, 84%), m.p. 123–123.5°, $[\alpha]_D$ −14° (*c* 0.6, chloroform); ν_{max} 2120 cm^{-1} (N_3). N.m.r. data: ^{1}H (80 MHz), δ 7.45–7.20 (m, 15 H, 3 Ph), 4.26 (d, 1 H, $J_{1,2}$ 8 Hz, H-1); ^{13}C ($CDCl_3$), δ 100.5 (C-1).

Anal. Calc. for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found: C, 68.24; H, 6.27; N, 8.69.

*Benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**9**). — A solution of **7** (670 mg) and commercial 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**8**, 720 mg) in anhydrous dichloromethane (10 mL) was stirred for 1 h at room temperature under dry argon in the presence of 4 Å activated powdered molecular sieve (2.5 g). Trimethylsilyl trifluoromethanesulphonate (0.3 mL) was added, and the mixture was stirred for 3 h, then diluted with dichloromethane (50 mL), washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (200 g) with 19:1 dichloromethane–acetone to give **9** (870 mg, 76%), which crystallised from methanol; m.p. 98–100°, $[\alpha]_D$ −31° (*c* 1, chloroform); ν_{max} 2120 cm^{-1} (N_3). N.m.r. data: ^{1}H (80 MHz), δ 7.40–7.12 (m, 15 H, 3 Ph), 4.70 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 4.23 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 2.10, 2.07, 1.97, and 1.93 (4 s, 12 H, 4 Ac); ^{13}C ($CDCl_3$), δ 100.3 (C-1), 102.1 (C-1').

Anal. Calc. for $C_{41}H_{47}N_3O_{14}$: C, 61.11; H, 5.88; N, 5.21. Found: C, 61.03; H, 5.86; N, 5.16.

Benzyl 2-azido-4,6-di-O-benzyl-2-deoxy-3-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-galactopyranoside (**10**). — A solution of **9** (2.07 g) in dry methanol (20 mL) was treated with freshly prepared methanolic 0.25M sodium methoxide (3 mL), left overnight at room temperature, then neutralised with Amberlyst 15 (H^+) resin, filtered, and concentrated. Toluene (3 × 50 mL) was evaporated from the residue, a solution of which in dry acetone (50 mL) was then stirred for 7 h at room temperature in the presence of toluene-*p*-sulphonic acid (20 mg). The mixture was neutralised with triethylamine (0.5 mL) and concentrated, and the residue was eluted from a column of silica gel (120 g) with 3:2 ethyl acetate–hexane to give, first, amorphous **10** (1.18 g, 68%), $[\alpha]_D$ −4° (*c* 1, chloroform); ν_{max} 2120 cm^{-1} (N_3). N.m.r. data: ^1H (80 MHz), δ 7.40–7.10 (m, 15 H, 3 Ph), 4.36 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 1.43 and 1.30 (2 s, 6 H, Me_2C); ^{13}C ($CDCl_3$), δ 111.6 (CMe_2), 105.6 (C-1′), 102.0 (C-1), 29.5 and 27.7 (CMe_2).

Anal. Calc. for $C_{36}H_{43}N_3O_{10}$: C, 63.80; H, 6.40; N, 6.20. Found: C, 63.61; N, 6.56; N, 6.22.

Eluted second was benzyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-(4,6-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-galactopyranoside (**11**; 320 mg, 18%), $[\alpha]_D$ −40.5° (*c* 0.9, chloroform); ν_{max} 2120 cm^{-1} (N_3). N.m.r. data: ^1H (80 MHz), δ 7.50–7.20 (m, 15 H, 3 Ph), 1.50 and 1.45 (2 s, 6 H, Me_2C); ^{13}C ($CDCl_3$), δ 104.6 (C-1′), 100.4 (C-1), 98.7 (CMe_2), 29.0 and 18.4 (CMe_2).

Anal. Calc. for $C_{36}H_{43}N_3O_{10}$: C, 63.80; H, 6.40; N, 6.20. Found: C, 63.77; H, 6.38; N, 6.26.

Benzyl 2-azido-4,6-O-benzyl-2-deoxy-3-O-(2,6-di-O-benzyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-galactopyranoside (**12**). — A solution of **10** (860 mg) in dry *N,N*-dimethylformamide (10 mL) was stirred for 1 h at 0° in the presence of sodium hydride (250 mg). Freshly distilled benzyl bromide (0.5 mL) was then added, the mixture was stirred for 5 h at room temperature, methanol (3 mL) was added, and stirring was continued for 15 min at room temperature. The mixture was concentrated, and the residue was treated with ice–water (100 mL) and extracted with dichloromethane (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was eluted from a column of silica gel (120 g) with 3:1 hexane–ethyl acetate to give amorphous **12** (1.05 g, 96%), $[\alpha]_D$ +9.5° (*c* 1.4, chloroform); ν_{max} 2120 cm^{-1} (N_3). N.m.r. data: ^1H (80 MHz), δ 7.40–7.20 (m, 25 H, 5 Ph), 4.32 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 1.31 (s, 6 H, Me_2C).

Anal. Calc. for $C_{50}H_{55}N_3O_{10}$: C, 69.99; H, 6.46; N, 4.90. Found: C, 70.15; H, 6.36; N, 4.84.

Benzyl 2-azido-4,6-di-O-benzyl-2-deoxy-3-O-(2,6-di-O-benzyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**13**). — Compound **12** (510 mg) was heated for 25 min at 100° under stirring with aqueous 80% acetic acid (30 mL). The mixture was cooled and concentrated, and toluene (2 × 20 mL) was evaporated from the residue which was then eluted from a column of silica gel (60 g) with 1:1 ethyl acetate–hexane to give syrupy **13** (450 mg, 91%), $[\alpha]_D$ +3° (*c* 1.1, chloroform); ν_{max} 2120 cm^{-1} (N_3). N.m.r. data: ^1H (300 MHz), δ 7.41–7.18 (m, 25 H, 5 Ph), 4.63

(d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1'), 4.33 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.96 (m, 2 H, H-4,4'), 3.89 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2); ^{13}C ($CDCl_3$), δ 104.5 (C-1'), 100.8 (C-1).

Anal. Calc. for $C_{47}H_{51}N_3O_{10}$: C, 69.02; H, 6.28; N, 5.14. Found: C, 69.10; H, 6.12; N, 5.34.

Benzyl O-*(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-*O*-(2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-(1→3)-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-*D*-galactopyranoside* (**14**). — A solution of **13** (130 mg) in anhydrous toluene (1 mL) was stirred for 1 h at room temperature under dry argon in the presence of silver silicate on alumina (122 mg) and 4 Å activated powdered molecular sieve (150 mg), and then cooled to −20°. A solution of **1** (65 mg) in anhydrous toluene (1 mL) was added, and the mixture was stirred for 6 h in the dark at −20° and then overnight at room temperature, diluted with dichloromethane (30 mL), filtered, washed with water (2 × 10 mL), dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel (40 g) with acetone–dichloromethane (30:1→2:1) to give, first, the amorphous trisaccharide derivative **16** (19 mg, 11%), then **13** (22 mg, 17%), and finally amorphous **14** (111 mg, 61%), $[\alpha]_D$ −16° (*c* 1.4, chloroform); ν_{max} 2100 cm^{-1} (N_3). N.m.r. data: ^1H (300 MHz), δ 7.24–7.47 (m, 25 H, Ph), 5.30 (dd, 1 H, $J_{3'',4''}$ 3.7, $J_{4'',5''}$ 0.4 Hz, H-4''), 4.76 (dd, 1 H, $J_{2'',3''}$ 12.6, $J_{3'',4''}$ 3.7 Hz, H-3''), 4.74 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.33 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.12 (dd, 1 H, $J_{3',4'}$ 3, $J_{4',5'}$ 0.4 Hz, H-4'), 4.10 (d, 1 H, $J_{1'',2''}$ 8 Hz, H-1''), 3.99 (dd, 1 H, $J_{5'',6''a}$ 6.1, $J_{6''a,6''b}$ 11.2 Hz, H-6''a), 3.92 (dd, 1 H, $J_{3,4}$ 2.55, $J_{4,5}$ 0.4 Hz, H-4), 3.81 (ddd, 1 H, $J_{4'',5''}$ 0.4, $J_{5'',6''a}$ 6.1, $J_{5'',6''b}$ 6.1 Hz, H-5''), 3.61 (dd, 1 H, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 9.2 Hz, H-6a), 3.52 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 2.55 Hz, H-3), 3.46 (ddd, 1 H, $J_{4,5}$ 0.4, $J_{5,6a}$ 5.5, $J_{5,6b}$ 5.5 Hz, H-5), 3.37 (dd, 1 H, $J_{5,6b}$ 5.5, $J_{6a,6b}$ 9.2 Hz, H-6b), 2.15, 2.01, and 2.00 (3 s, 9 H, 3 Ac); ^{13}C ($CDCl_3$), δ 104.9 (C-1'), 102.3 (C-1''), 101.0 (C-1).

Anal. Calc. for $C_{59}H_{66}N_6O_{17}$: C, 62.64; H, 5.88; N, 7.43. Found: C, 62.49; H, 5.83; N, 7.38.

Benzyl O-*(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-*O*-(3-*O*-acetyl-2,6-di-*O*-benzyl-β-*D*-galactopyranosyl-(1→3)-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-*D*-galactopyranoside* (**15**). — Treatment of **14** (32 mg) in dry pyridine (3 mL) with acetic anhydride (1 mL) gave, after the usual work-up and preparative t.l.c. [Silica Gel 60 F_{254} (Merck) using dichloromethane–acetone (19:1)], amorphous **15** (27 mg, 89%), $[\alpha]_D$ −22° (*c* 0.6, chloroform). ^1H-N.m.r. data (300 MHz): δ 7.50–7.20 (m, 25 H, 5 Ph), 5.29 (dd, 1 H, $J_{3'',4''}$ 3.2, $J_{4'',5''}$ 0.4 Hz, H-4''), 4.94 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3',4'}$ 3.4 Hz, H-3'), 4.74 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 4.64 (dd, 1 H, $J_{2'',3''}$ 11.1, $J_{3'',4}$ 3.2 Hz, H-3''), 4.22 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.20 (d, 1 H, $J_{1'',2''}$ 7.6 Hz, H-1''), 4.18 (dd, 1 H, $J_{3',4'}$ 3.4, $J_{4',5'}$ 0.4 Hz, H-4'), 4.12 (dd, $J_{5'',6''a}$ 6.9, $J_{6''a,6''b}$ 11.1 Hz, H-6a''), 3.96 (dd, 1 H, $J_{1',2'}$ 7.6, $J_{2',3'}$ 10.3 Hz, H-2'), 3.95 (dd, 1 H, $J_{5'',6''b}$ 6.4, $J_{6''a,6''b}$ 11.1 Hz, H-6''b), 3.91 (dd, 1 H, $J_{3,4}$ 3, $J_{4,5}$ 0.4 Hz, H-4), 3.88 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 10.3 Hz, H-2), 3.77 (ddd, 1 H, $J_{4'',5''}$ 0.4, $J_{5'',6''a}$ 6.9, $J_{5'',6''b}$ 6.4 Hz, H-5''), 3.64 (dd, 1 H, $J_{1'',2''}$ 7.9, $J_{2'',3''}$ 11.1 Hz, H-2''), 3.61 (dd, 1 H, $J_{5,6a}$ 6, $J_{6a,6b}$ 9.4 Hz, H-6a), 3.53 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3 Hz, H-3), 3.43 (ddd, 1 H, $J_{4,5}$ 0.4, $J_{5,6a}$ 6, $J_{5,6b}$ 5.6 Hz, H-5), 3.36 (dd, 1 H, $J_{5,6b}$ 5.6, $J_{6a,6b}$ 9.4 Hz, H-6b), 2.14, 2.07, and 2.02 (3 s, 12 H, 4 Ac).

Anal. Calc. for $C_{61}H_{68}N_6O_{18}$: C, 62.45; H, 5.84; N, 7.16. Found: C, 62.33; H, 5.95; N, 7.02.

Benzyl *O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-(1→3)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**16**) had $[\alpha]_D$ −21° (*c* 0.7, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.43–7.25 (m, 25 H, 5 Ph), 5.31 (dd, 1 H, $J_{3'',4''}$ 3.2, $J_{4'',5''}$ 0.4 Hz, H-4''), 4.74 (dd, 1 H, $J_{2'',3''}$ 11.8, $J_{3'',4''}$ 3.2 Hz, H-3''), 3.95 (dd, 1 H, $J_{2,3}$ 11.5, $J_{3,4}$ 0.4 Hz, H-3), 2.50 (s, 1 H, OH), 2.16, 2.07, and 1.99 (3 s, 9 H, 3 Ac).

Anal. Calc. for $C_{59}H_{66}N_6O_{17}$: C, 62.64; H, 5.88; N, 7.43. Found: C, 62.73; H, 5.96; N, 7.31.

Benzyl O-*(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-(1→3)-*O*-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside* (**17**). — Acetylation of **16** (23 mg) gave **17** (17 mg), $[\alpha]_D$ −44° (*c* 0.8, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.42–7.25 (m, 25 H, 5 Ph), 5.43 (d, 1 H, $J_{3',4'}$ 3.1 Hz, H-4'), 5.24 (dd, 1 H, $J_{3'',4''}$ 3.1, $J_{4'',5''}$ 0.4 Hz, H-4''), 4.63 (dd, 1 H, $J_{2'',3''}$ 10.3, $J_{3'',4''}$ 3.1 Hz, H-3''), 4.00 (dd, 1 H, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.1 Hz, H-3'), 3.95 (dd, 1 H, $J_{3,4}$ 3, $J_{4,5}$ 0.4 Hz, H-4), 2.17, 2.09, 2.05, and 2.00 (4 s, 12 H, 4 Ac).

The pure derivative was only prepared for recording the n.m.r. data and elemental analysis was not performed.

Benzyl O-*(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside* (**18**). — A solution of sodium borohydride in ethanol (10 mg/mL) was added dropwise at room temperature to a solution of **14** (21.6 mg) in tetrahydrofuran (1 mL) and ethanolic 4% nickel dichloride hexahydrate (2 mL) containing 2% of boric acid, until the green solution turned to persistent black. Acetic anhydride (1 mL) was added, and the mixture was stirred for 1 h at room temperature and then concentrated. The residue was eluted from a column of silica gel (20 g) with 9:1 dichloromethane–methanol to give **18** (14 mg, 63%), m.p. 103–104° (from ethyl acetate–hexane), $[\alpha]_D$ −33° (*c* 0.3, chloroform); ν_{max} 1750 (OAc), 1650 (Amide I), 1550 cm^{-1} (Amide II). ^{1}H-N.m.r. data (300 MHz): δ 7.50–7.28 (m, 25 H, 5 Ph), 5.59 (d, 1 H, *J* 7.8 Hz, NH), 5.31 (dd, 1 H, $J_{3'',4''}$ 2.9, $J_{4'',5''}$ 0.6 Hz, H-4''), 5.26 (d, 1 H, *J* 7 Hz, NH), 5.03 (dd, 1 H, $J_{2'',3''}$ 10.8, $J_{3'',4''}$ 2.9 Hz, H-3''), 2.14, 1.99, and 1.97 (3 s, 9 H, 3 OAc), 1.75 and 1.61 (2 s, 6 H, 2 NAc).

Anal. Calc. for $C_{63}H_{74}N_2O_{19}\cdot 1.25\ H_2O$: C, 63.81; H, 6.49; N, 2.36. Found: C, 63.48; H, 6.17; N, 2.57.

O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactopyranose (**19**). — A solution of **18** (20 mg) in 5:1:1 methanol–water–triethylamine (14 mL) was left overnight at room temperature and then concentrated. The residue was eluted from a column of silica gel (20 g) with 4:1 dichloromethane–methanol to give an amorphous product (12.4 mg, 70%), $[\alpha]_D$ −22° (*c* 0.95, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.42–7.25

(m, 25 H, 5 Ph), 5.36 (d, 1 H, *J* 7.2 Hz, NH), 4.98 (d, 1 H, *J* 8.4 Hz, NH), 1.98 and 1.66 (2 s, 6 H, 2 NAc).

A solution of this product (29 mg) in 4:1 methanol–water (5 mL) was hydrogenated in the presence of 10% Pd/C (20 mg) for 3 h, then filtered, and concentrated. The residue was eluted from a column (20 × 900 mm) of Sephadex G-10 with water. The appropriate fractions were combined and lyophilised to give amorphous **19** (14 mg, 90%), $[\alpha]_D$ +14° (*c* 0.35, water). ^{1}H-N.m.r. data [300 MHz; D_2O; internal sodium 3-(trimethylsilyl)propionate]: δ 5.21 (d, $J_{1,2}$ 3.8 Hz, H-1α), 4.69 and 4.62 (2 d, $J_{1'',2''}$ 8.4 Hz, H-1''$\alpha\beta$), 4.64 (d, $J_{1,2}$ 8.3 Hz, H-1β), 4.50 and 4.44 (2 d, $J_{1',2'}$ 7.9 Hz, H-1'$\alpha\beta$), 4.31 (dd, $J_{1,2}$ 3.8, $J_{2,3}$ 11 Hz, H-2α), 3.95 (dd, $J_{1,2}$ 8.3, $J_{2,3}$ 11 Hz, H-2β), 2.06 and 2.03 (2 s, 6 H, 2 NAc).

In order to save this material for biological studies, no destructive elemental analysis has been made. The high-field ^{1}H-n.m.r. spectrum demonstrates the purity of this compound.

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