Carbohydrate Research, 122 (1983) 201–208
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

CHEMICAL SYNTHESIS OF THE HUMAN BLOOD-GROUP P_1-ANTIGENIC DETERMINANT*

PAUL-HENRI AMVAM ZOLLO, JEAN-CLAUDE JACQUINET, AND PIERRE SINAŸ**
Laboratoire de Biochimie Structurale, E.R.A. 739, U.E.R. de Sciences Fondamentales et Appliquées, 45046 Orléans-Cédex (France)

(Received March 18th, 1983; accepted for publication, April 11th, 1983)

ABSTRACT

Condensation of known benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride in dichloromethane in the presence of 2,4,6-trimethylpyridine, silver triflate, and molecular sieve 4A gave benzyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside. Catalytic hydrogenolysis gave crystalline *O*-α-D-galactopyranosyl-(1→4)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranose, the human blood-group P_1-antigenic determinant. A similar sequence of reactions was performed starting from allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside, in order to prepare a derivative of this determinant suitable for linkage to carrier molecules.

INTRODUCTION

The human blood-group P system, first[1] recognised in 1927, consists[2] of three antigens, P, P_1, and P^k. The P antigen is present on the erythrocytes of virtually all individuals, and 75% of the population also possess the P_1 antigen.

A trihexosylceramide was identified as the P^k antigen by hemagglutination inhibition[3] studies and by analysis of the glycosphingolipids from P^k erythrocytes[4]. Cox *et al.* and Garegg and Hultberg[5] reported syntheses of methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside, the carbohydrate moiety of this trihexosylceramide, which is considered to be the antigenic determinant of the P^k antigen. Globoside, the most abundant glycosphingolipid of normal erythrocytes, was identified in the same study as the P antigen. It contains the trihexosylceramide structure plus a terminal, non-reducing 2-amino-2-deoxy-β-D-galactosyl group. A chemical synthesis of the P-antigenic determinant has been re-

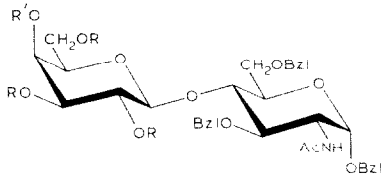
*Synthesis of Blood-Group Substances, Part 13. For Part 12, see *Carbohydr. Res.*, 92 (1981) 183–189.
**To whom enquiries should be sent.

0008-6215/83/$ 03.00

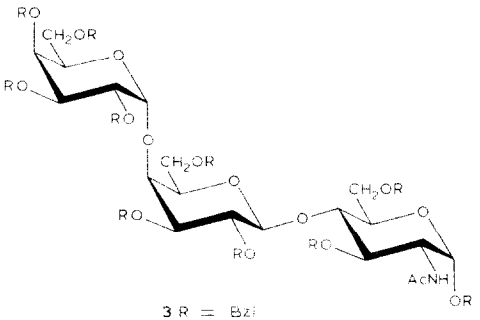
ported[6]. Finally, the structure of the blood-group P_1-glycosphingolipid of human erythrocytes has been established by Naiki *et al.*[7], and the trisaccharide α-Gal-(1→4)-β-Gal-(1→4)-GlcNAc (**4**), located at the non-reducing end of this glycosphingolipid, has been identified as the human blood-group P_1-antigenic determinant[8]. We now report the chemical synthesis of **4** and also a derivative suitable for subsequent elaboration into either an artificial P_1 antigen or a selective anti-P_1 immunoabsorbent.

RESULTS AND DISCUSSION

The synthesis of α-Gal-(1→4)-β-Gal-(1→4)-GlcNAc involved benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[9,10], which was condensed at 0° with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in dichloromethane, in the presence of 2,4,6-trimethylpyridine and silver triflate, to provide, after *O*-deacetylation, a quantitative yield of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside[11] (**1**). This reaction provides a fairly efficient and rather short route to derivatives of *N*-acetyl-lactosamine. The disaccharide derivative **1**, which had previously been prepared by lower-yielding procedures[11], was next converted into crystalline benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**2**) as previously described[11]. Attempts[11] to galactosylate the unsubstituted, axial hydroxyl group of **2**, using halide ion-catalysis[12] or the imidate procedure[13], failed. The expected protected-trisaccharide **3** was obtained (82%) by condensation of **2** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[14] at room temperature in dichloromethane in the presence of 2,4,6-trimethylpyridine, silver triflate, and molecular sieve 4A.



1 R = R' = H
2 R = Bzl, R' = H



3 R = Bzl
4 R = H

Athough **3** appeared homogeneous in t.l.c., it was an αβ-mixture (α:β ratio, 8:1), as suggested by the appearance of two signals for the acetamido group in the ^{1}H-n.m.r. spectrum (δ 1.62 for the α anomer, and 1.72 for the β anomer) and by a broadening of a signal in the C-1β region (δ 102.8 p.p.m.) of the ^{13}C-n.m.r. spec-

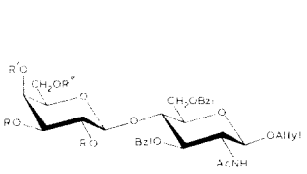
trum. Signals of the anomeric carbons appeared at 97.1 (C-1α), 100.5 (C''-1α), and 102.8 p.p.m. (C'-1β) for a solution in deuteriochloroform. The shifts of the signals of the anomeric carbons of methyl 2,3,4,6-tetra-*O*-benzyl-α- and -β-D-galactopyranosides are 98.8 and 105.0 p.p.m., respectively, and that for benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside is 97.2 p.p.m. The chemical synthesis of another protected trisaccharide of this type has been reported by Anderson *et al.*[15].

Hydrogenolysis of a solution of **3** in acetic acid in the presence of Pd/C gave *O*-α-D-galactopyranosyl-(1→4)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranose (**4**, 72% after crystallisation). No physical constants were reported when this trisaccharide was first obtained as a natural product after partial acid hydrolysis of a glycoprotein having blood-group P_1 specificity, which had been isolated from sheep hydatid-cyst fluid[8]. The trisaccharide **4** was a sensitive and selective inhibitor of the P_1–anti P_1 system using antisera either of animal (goat, No. 1110-20 Biotest) or human (absorbed Tahiri) origin[16]. The structural requirement of the human blood-group P_1-antigenic determinant, first proposed by Cory *et al.*[8], is thus confirmed.

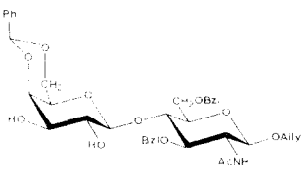
In order to use the immunological properties of the P_1-antigenic determinant, a derivative was required which would allow attachment to soluble proteins, to form immunogens, and to insoluble carriers for the preparation of immunoabsorbants. The allyl glycoside was proposed[17] as a suitable derivative for this general purpose, because such glycosides can be used to obtain a variety of spacer arms[18,19]. Hence, a synthesis of the derivative **11** was undertaken.

Condensation of allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside[9] with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl bromide in refluxing benzene, in the presence of mercuric cyanide and mercuric bromide, followed by *O*-deacetylation of the product, afforded 88% of the disaccharide-glycoside **5**. Reaction of **5** with benzaldehyde in the presence of zinc chloride gave 81% of the crystalline acetal **9**. Reaction of **9** with benzyl bromide in *N*,*N*-dimethylformamide in the presence of barium oxide and barium hydroxide octahydrate for 5 days at room temperature, with acid hydrolysis (aqueous 70% acetic acid, 100°, 20 min) of the product, gave crystalline diol **7**. Selective benzoylation of **7** with benzoyl cyanide[20] in dichloromethane–pyridine gave 89% of the crystalline 6'-benzoate **8**. Condensation of **8** at room temperature during 5 days with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[14] in dichloromethane, in the presence of 2,4,6-trimethylpyridine, silver triflate, and molecular sieve 4A, gave 78% of the derivative **10**. Although apparently homogeneous in t.l.c., **10** was an $\alpha\beta$-mixture (α:β-ratio, ~11:1), as indicated by the ^{1}H-n.m.r. signals for the acetamido group [δ 1.69 (α) and 1.79 (β), $CDCl_3$]. This inference was confirmed after *O*-debenzoylation using methanolic sodium methoxide; the trisaccharide derivative **11** (88%), $[\alpha]_D^{20}$ +30° (chloroform), could easily be separated, by chromatography on silica gel, from 8% of its β anomer **13**, $[\alpha]_D^{20}$ +16° (chloroform).

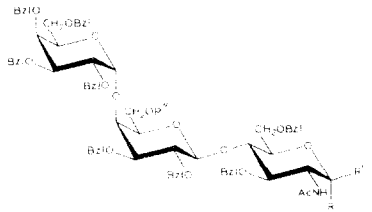
O-Deallylation of **11** with potassium *tert*-butoxide in dimethyl sulfoxide, fol-



5 R = R′ = R″ = H
6 R = R′ = R″ = Ac
7 R = Bz, R′ = R″ = H
8 R = Bz, R′ = H, R″ = H

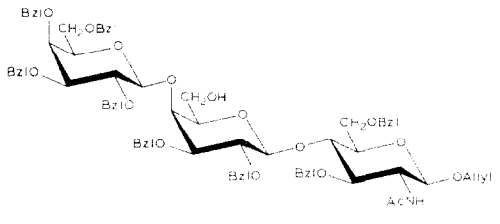


9

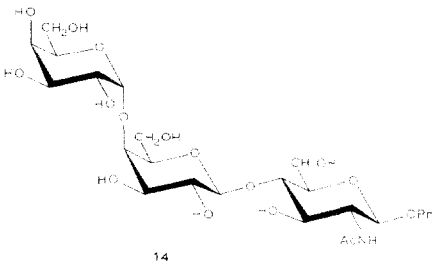


10 R = H, R′ = OAllyl, R″ = Bz
11 R = R″ = H, R′ = OAllyl
12 R = OH, R′ = R″ = H

lowed by acidic treatment and catalytic hydrogenolysis (10% Pd/C) in acetic acid, gave **4**. Catalytic hydrogenolysis (10% Pd/C) of **11** in acetic acid gave propyl 2-acetamido-2-deoxy-4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**14**)*, which had selective inhibitory properties comparable with those of **4** and thus is a good candidate for the preparation of artificial P_1 antigens and anti-P_1 immunoabsorbents.



13



14

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 22–24° with a Perkin–Elmer model 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Perkin–Elmer R-32 instrument. ^{13}C-N.m.r. spectra were recorded at 15.08 MHz with a Bruker instrument. C.i.-m.s. (ammonia as reagent gas) was performed on an R-10-10 Nermag spectrometer. Purity of products was determined by t.l.c. on silica gel 60 F 154 (Merck) with detection by charring with sulphuric acid. Column chromatography was performed on silica gel 60 (Merck, 0.063–0.200 mm), which was used without pre-treatment. Elemental analyses were obtained from the Service Central de Micro-Analyse du Centre National de la Recherche Scientifique.

*Note added in proof: after submission of this manuscript, a synthesis of compound **14** was reported [M. A. Nashed and L. Anderson, *Carbohydr. Res.*, 114 (1983) 43–52].

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-β-*D*-galactopyranosyl-α-*D*-glucopyranoside* (**1**). — A solution of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (2 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (3.34 g) in dichloromethane (40 mL) containing 2,4,6-trimethylpyridine (2 mL) and freshly prepared silver triflate (2.08 g) was stirred at 0° in the dark for 1 day. The mixture was diluted with dichloromethane, filtered, washed successively with water, aqueous 10% sulphuric acid, saturated aqueous sodium hydrogencarbonate, and saturated aqueous sodium chloride, dried (Na_2SO_4), and concentrated. The residue was *O*-deacetylated (methanolic sodium methoxide) to give a quantitative yield (2.64 g) of **1**, m.p. 154–155° (from methanol–water), $[\alpha]_D$ +86° (*c* 1, methanol); lit.[11] m.p. 154–155°, $[\alpha]_D$ +86° (*c* 1, methanol).

Compound **1** was then used for the synthesis of **2**.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-α-*D*-glucopyranoside* (**3**). — A solution of **2** (400 mg) and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (725 mg) in dichloromethane (5 mL) containing 2,4,6-trimethylpyridine (0.1 mL) and activated, powdered molecular sieve 4A (200 mg) was stirred for 0.5 h in an atmosphere of dry nitrogen. Freshly prepared silver triflate (334 mg) was then added quickly, and the mixture was stirred for 5 days in the dark, diluted with dichloromethane, filtered, washed successively with water, aqueous 10% sulphuric acid, saturated aqueous sodium hydrogencarbonate, saturated aqueous sodium chloride, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from silica gel (15 g) with ethyl acetate–hexane (1:1), to give amorphous **3** (513 mg, 82%), $[\alpha]_D$ +59° (*c* 1.5, chloroform). N.m.r. data: ^1H, 6.25–7.12 (m, 50 H, 10 Ph), 1.72 (β) and 1.62 (α) (2 s, 2 NAc, α:β-ratio, 8:1); ^{13}C, 97.1 (C-1α), 100.5 (C″-1α), and 102.8 p.p.m. (C′-1β, broadened by C″-1β). Mass spectrum: *m/z* 1447 (M + 1) and 1464 (strong, M + 18).

Anal. Calc. for $C_{90}H_{95}NO_{16}$: C, 74.71; H, 6.61; N, 0.96. Found: C, 74.57; H, 6.68; N, 0.95.

O-α-*D*-*Galactopyranosyl-(1→4)-*O*-β-*D*-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α-*D*-glucopyranose* (**4**). — A solution of **3** (400 mg) in acetic acid (5 mL) was hydrogenolysed in the presence of 10% Pd/C (300 mg) for 2 days, filtered, and concentrated. Crystallisation of the residue gave **4** (109 mg, 72%), m.p. 179–180° (de.; from methanol–acetone), $[\alpha]_D$ +68→ +72° (*c* 0.36; methanol–water, 9:1; 18 h). ^1H-N.m.r. data (D_2O, external Me_4Si): 5.68 (d, 1 H, $J_{1,2}$ 2 Hz, H-1α), 5.42 (d, 1 H, $J_{1'',2''}$ 3 Hz, H″-1α), 5.00 (d, 1 H, $J_{1',2'}$ 9 Hz, H′-1β), and 2.52 (s, 3 H, Ac).

Anal. Calc. for $C_{20}H_{35}NO_{16} \cdot 2\ H_2O$: C, 41.33; H, 6.76; N, 2.40. Found: C, 41.33; H, 6.31; N, 2.41.

*Allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**6**). — A suspension of allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (1.764 g) in benzene (20 mL) containing mercuric cyanide (2.016 g) and mercuric bromide (144 mg) was heated at

90° in an atmosphere of dry nitrogen. A freshly prepared solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (3.29 g) in benzene (10 mL) was then added dropwise during 1 h and the mixture was boiled under reflux overnight. The cooled mixture was filtered, washed successively with aqueous 10% potassium iodide, aqueous 10% sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was *O*-deacetylated (methanolic sodium methoxide), and eluted from silica gel (100 g) with chloroform–methanol (22:3) to give the disaccharide derivative **5** (532 mg, 88%) as a hygroscopic powder, $[\alpha]_D$ +14° (*c* 0.25, chloroform). Acetylation (acetic anhydride–pyridine) of a portion (100 mg) gave syrupy **6** (119 mg, 94%), $[\alpha]_D$ −21° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.33 (m, 10 H, 2 Ph), 6.02 (d, 1 H, *J* 9 Hz, NH), and 2.10–1.93 (5 s, 15 H, 5 Ac).

Anal. Calc. for $C_{30}H_{49}NO_{15}$: C, 60.70; H, 6.40; N, 1.81. Found: C, 61.08; H, 6.48; N, 1.80.

*Allyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-2-deoxy-β-*D*-glucopyranoside* (**9**). — A suspension of **5** (1.050 g) in benzaldehyde (15 mL) containing anhydrous, powdered zinc chloride (1 g) was shaken overnight at room temperature and then diluted with di-isopropyl ether (100 mL). The precipitate was collected and crystallised twice from ethyl acetate to give **9** (975 mg, 81%), m.p. 203–204°, $[\alpha]_D$ −35.5° (*c* 1, methanol).

Anal. Calc. for $C_{38}H_{45}NO_{11}$: C, 65.12; H, 6.61; N, 2.00; O, 26.25 Found: C, 65.23; H, 6.64; N, 2.04; O, 25.82.

*Allyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3-di-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**7**). — A solution of **9** (346 mg) in *N*,*N*-dimethylformamide (15 mL) was stirred at room temperature in the presence of freshly distilled benzyl bromide (0.3 mL), barium oxide (1.36 g), and barium hydroxide octahydrate (0.35 g). More benzyl bromide (0.3 mL) was added after 2 and 4 days. After 5 days, the large excess of benzyl bromide was destroyed with methanol (5 mL), and the mixture was diluted with chloroform, washed successively with ice-cold 50% aqueous acetic acid, water, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. A solution of the residue in aqueous 70% acetic acid (20 mL) was kept for 20 min at 100°, cooled, and concentrated, and the residue was eluted from silica gel (25 g) with chloroform–methanol (19:1) to give **7** (174 mg, 70%), m.p. 167–168° (from ethyl acetate), $[\alpha]_D$ +16° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.30–7.20 (m, 20 H, 4 Ph), 6.15 (d, 1 H, *J* 8 Hz, NH), 2.92 (s, 1 H, OH), and 1.80 (s, 3 H, Ac).

Anal. Calc. for $C_{45}H_{53}NO_{11}$: C, 68.95; H, 6.81; N, 1.78. Found: C, 69.13; H, 6.81; N, 1.78.

*Allyl 2-acetamido-4-*O*-(6-*O*-benzoyl-2,3-di-*O*-benzyl-β-*D*-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**8**). — A solution of **7** (2 g) in dichloromethane (40 mL) and pyridine (10 mL) was stirred for 5 h at room temperature in the presence of benzoyl cyanide (700 mg). After destruction of the excess of benzoyl cyanide with methanol (20 mL), the mixture was concentrated and the residue was eluted from silica gel (100 g) with ethyl acetate–hexane (2:1), to give

8 (2 g, 88%), m.p. 114–115° (from ethyl acetate–hexane), $[\alpha]_D$ +14° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.97–7.25 (m, 25 H, 5 Ph), 5.86 (d, 1 H, *J* 9 Hz, NH), 2.46 (s, 1 H, OH), and 1.80 (s, 3 H, Ac).

Anal. Calc. for $C_{52}H_{57}NO_{12}$: C, 70.33; H, 6.47; N, 1.57. Found: C, 70.10; H, 6.70; N, 1.39.

*Allyl 2-acetamido-4-*O*-[6-*O*-benzoyl-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**10**). — A solution of **8** (150 mg) and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (283 mg) in dichloromethane (2 mL) containing 2,4,6-trimethylpyridine (0.1 mL) and activated, powdered molecular sieve 4A (100 mg) was stirred for 0.5 h in an atmosphere of dry nitrogen. Freshly prepared silver triflate (130 mg) was added quickly, the mixture was stirred for 5 days in the dark, diluted with dichloromethane, filtered, washed successively with water, aqueous 10% sulphuric acid, saturated aqueous sodium hydrogencarbonate, saturated aqueous sodium chloride, and water, dried (Na_2SO_4), and concentrated. Elution of the residue from silica gel (15 g) with ethyl acetate–hexane (1:1) gave crude, amorphous **10** (186 mg, 78%), $[\alpha]_D$ +27° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 7.90 (dd, 2 H, Bz), 7.15–7.25 (m, 45 H, 9 Ph), and 1.70 (s, 3 H, Ac). Compound **10** (830 mg) was *O*-debenzoylated (methanolic sodium methoxide) and the product was eluted from silica gel (50 g) with ethyl acetate–hexane (2:1) to give, first, the β isomer **13** (60 mg, 8%), $[\alpha]_D$ +16° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 7.25–7.15 (m, 40 H, 8 Ph) and 1.80 (s, 3 H, Ac).

Anal. Calc. for $C_{79}H_{87}NO_{16}$: C, 72.62; H, 6.71; N, 1.07. Found: C, 72.38; H, 6.54; N, 1.06.

Further elution gave syrupy **11** (676 mg, 88%), $[\alpha]_D$ +30° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.25–7.20 (m, 40 H, 8 Ph) and 1.70 (s, 3 H, Ac).

Anal. Calc. for $C_{79}H_{87}NO_{16}$: C, 72.62; H, 6.71; N, 1.07. Found: C, 72.24; H, 6.68; N, 1.25.

*2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-*D*-glucopyranose* (**12**). — A solution of **11** (500 mg) in dimethyl sulfoxide (5 mL) containing potassium *tert*-butoxide (150 mg) was heated for 1 h at 100° in an atmosphere of dry nitrogen. After the usual work-up, the residue was dissolved in acetone–water (9:1, 10 mL), M hydrochloric acid (2.5 mL) was added, the mixture was boiled under reflux for 2 h, cooled, and diluted with water, and the acetone was distilled off. The aqueous mixture was then extracted with chloroform, and the extract was washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4), and concentrated. The residue was eluted from silica gel (30 g) with ethyl acetate–acetone (4:1), to give amorphous **12** (338 mg, 70%), $[\alpha]_D$ +47° (*c* 1.3, chloroform). ^{1}H-N.m.r. data: δ 7.30–7.20 (m, 40 H, 8 Ph), 5.70 (d, 1 H, *J* 8 Hz, NH), and 1.72 (s, 3 H, Ac).

Anal. Calc. for $C_{76}H_{83}NO_{16}$: C, 72.07; H, 6.60; N, 1.10. Found: C, 72.02; H, 6.76; N, 1.06.

Hydrogenolysis (10% Pd/C) of **12** gave **4** described above.

Propyl 2-acetamido-2-deoxy-4-O-(4-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**14**). — A solution of **11** (200 mg) in acetic acid (3 mL) was hydrogenated in the presence of 10% Pd/C (150 mg) for 2 days, filtered, and concentrated, to give **14** (70 mg, 78%), $[\alpha]_D$ +32° (*c* 1.3; methanol–water, 9:1).

Anal. Calc. for $C_{23}H_{41}NO_{16} \cdot 2\ H_2O$: C, 45.61; H, 7.15; N, 2.31. Found: C, 45.10; H, 7.21; N, 2.05.

ACKNOWLEDGMENTS

We thank Dr. G. Lukacs for ^{13}C-n.m.r. experiments, Nermag for the m.s. work, Dr. C. Muller (C.N.T.S.) for inhibition studies, and the Centre National de la Recherche Scientifique (E.R.A. 739) and the Institut National de la Santé et de la Recherche Médicale (C.R.L. no. 79 1129) for financial support.

REFERENCES

1 K. LANDSTEINER AND P. LEVINE, *Proc. Soc. Exp. Biol. Med.*, 24 (1927) 941–942.
2 R. R. RACE AND R. SANGER, *Blood Groups in Man*, 6th edn., Blackwell, Oxford, 1975, pp. 131–177.
3 M. NAIKI AND D. M. MARCUS, *Biochem. Biophys. Res. Commun.*, 60 (1974) 1105–1111.
4 M. NAIKI AND S. K. KUNDER, *Proc. Natl. Acad. Sci. U.S.A.*, 73 (1976) 3263–3267.
5 D. D. COX, E. K. METZNER, AND E. J. REIST, *Carbohydr. Res.*, 63 (1978) 139–147; P. J. GAREGG AND H. HULTBERG, *ibid.*, 110 (1982) 261–266.
6 H. PAULSEN AND A. BÜNSCH, *Carbohydr. Res.*, 101 (1982) 21–30.
7 M. NAIKI, J. FONG, R. LEDEEN, AND D. M. MARCUS, *Biochemistry*, 14 (1975) 4831–4837.
8 H. T. CORY, A. D. YATES, A. S. R. DONALD, W. M. WATKINS, AND W. T. J. MORGAN, *Biochem. Biophys. Res. Commun.*, 61 (1974) 1289–1295.
9 J.-M. PETIT, J.-C. JACQUINET, AND P. SINAY, *Carbohydr. Res.*, 82 (1980) 130–134.
10 J.-C. JACQUINET, J.-M. PETIT, AND P. SINAY, *Carbohydr. Res.*, 38 (1974) 305–311.
11 J.-C. JACQUINET, D. DUCHET, M.-L. MILAT, AND P. SINAY, *J. Chem. Soc., Perkin Trans. 1*, (1981) 326–330 and references therein.
12 R. U. LEMIEUX, K. B. HENDRIKS, R. V. STICK, AND K. JAMES, *J. Am. Chem. Soc.*, 97 (1975) 4056–4062.
13 M.-L. MILAT, P.-H. AMVAM ZOLLO, AND P. SINAY, *Carbohydr. Res.*, 100 (1982) 263–271.
14 T. IVERSEN AND D. R. BUNDLE, *Carbohydr. Res.*, 103 (1982) 29–40.
15 M. A. NASHED, M. S. CHOWDHARY, C. W. SLIFE, AND L. ANDERSON, *Abstr. Pap. Int. Symp. Carbohydr. Chem., Xth, Sydney*, (1980) M7.
16 C. HUREL AND A. MULLER, unpublished data.
17 J.-C. JACQUINET AND P. SINAY, *Tetrahedron*, 35 (1979) 365–371.
18 J.-C. JACQUINET AND P. SINAY, *Carbohydr. Res.*, in press.
19 R. ROY AND H. J. JENNINGS, *Carbohydr. Res.*, 112 (1983) 63–72.
20 S. A. ABBAS AND A. H. HAINES, *Carbohydr. Res.*, 39 (1975) 358–363.