Carbohydrate Research, 112 (1983) 201–211
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESIS OF BENZYL 2-ACETAMIDO-3-*O*- AND -6-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-2-DEOXY-α-D-GALACTOPYRANOSIDE*

SAEED A. ABBAS, JOSEPH J. BARLOW AND KHUSHI L. MATTA**

Department of Gynecologic Oncology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14263 (U.S.A.)

(Received July 10th, 1982; accepted for publication, August 12th, 1982)

ABSTRACT

Glycosylation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide in dichloromethane, in the presence of silver trifluoromethanesulfonate, 2,4,6-trimethylpyridine, and molecular sieves, afforded benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranoside (**3**). Cleavage of the benzylidene group of **3** gave benzyl 2-acetamido-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranoside (**4**), which, on deacylation, followed by peracetylation, furnished the peracetylated disaccharide derivative (**7**). The structures of **3**, **4**, and **7** were established by ^{1}H-n.m.r. spectroscopy. *O*-Deacetylation of **7** afforded the title β-(1→3)-linked disaccharide (**8**). Compound **3** was also deacylated and then peracetylated, to give benzyl 2-acetamido-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside, which was *O*-deacetylated to give its 4,6-*O*-benzylidene derivative (**6**). For the synthesis of the β-(1→6)-linked disaccharide, the readily accessible benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside was condensed with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxozaline, and the product was isolated as its peracetylated derivative, which, on saponification, afforded the title β-(1→6)-linked disaccharide (**12**). The structures of compounds **6**, **8**, and **12** were established by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

The majority of glycoproteins isolated from mucous secretions consist of *O*-glycosylically linked carbohydrate chains that can be released by β-elimination in alkaline aqueous solution, with protection of the reducing groups, by sodium borohydride reduction[2,3]. Such oligosaccharides have been isolated, and characterized,

*Synthetic Studies in Carbohydrates, Part XXV. For Part XXIV, see ref. 1.
**To whom correspondence should be addressed.

from hog submaxillary-gland[4], human ovarian-cysts[5,6], and human[7], horse[8], and hog[9,10] gastric-mucins. The core structure of these complex oligosaccharides consists of either β-Gal-(1→3)-GalNAc or β-Gal-(1→3)-[β-GlcNAc-(1→6)]-GalNAc, attached to a peptide through an *O*-glycosylic linkage. Recently, oligosaccharides having a new type of core structure, namely, β-GlcNAc-(1→3)-GalNAc and β-GlcNAc-(1→3)-[β-GlcNAc-(1→6)]-GalNAc, further *O*-glycosylically linked to protein, have been identified in various mucus glycoproteins[11-13].

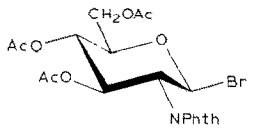
In our laboratory, we have initiated a program of chemical synthesis of oligosaccharides that occur as a part of mucinous glycoproteins. Our interest in these synthetic ventures has tremendously increased due to the fact that these synthetic compounds, having well defined structures, can be effectively utilized as acceptor-substrates in the search for glycosyltransferases involved in the biosynthesis of such glycoproteins[14]. Moreover, synthetic oligosaccharides can play an important role in specificity studies of glycosidases and lectins. We describe here the synthesis of the title disaccharides.

RESULTS AND DISCUSSION

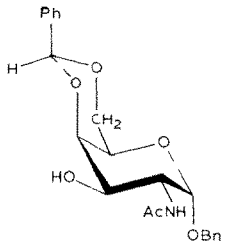
Two methods for the synthesis of 2-acetamido-2-deoxy-β-glycopyranosides of the D-*gluco* and D-*galacto* series, namely, the oxazoline and the phthalimide methods, have enjoyed wide application[15]. Although different laboratories prefer one method over the other, it appears from the current literature[15] that the two methods are complementary to one another. Thus, for glycosylation of primary hydroxyl groups, the oxazoline method gives excellent results, and, in most cases, the deblocking sequence involves only transesterification. Athough it has not been explicitly delineated, it would appear that it is a prerequisite to have benzyl groups[15], at least in the immediate vicinity of the secondary hydroxyl group to be glycosylated. In our experience, for example, HO-3 on a galactopyranoside ring was immune to glycosylation by the oxazoline method when O-2 and O-6 carried acetyl groups. However, glycosylation was readily accomplished[16] on replacing the acetyl groups with benzyl groups at the respective positions. The phthalimide method, on the other hand, has proved advantageous in numerous syntheses; however, the use of hydrazine at higher temperatures (to cleave the phthalimido group of the resulting oligosaccharide derivative) may not be entirely free from problems if that oligosaccharide is sensitive to alkali[15].

With these points in mind, we utilized the oxazoline method for the synthesis of the β-(1→6)-linked disaccharide, whereas, for the synthesis of its β-(1→3)-linked isomer, the phthalimide procedure was the method of choice.

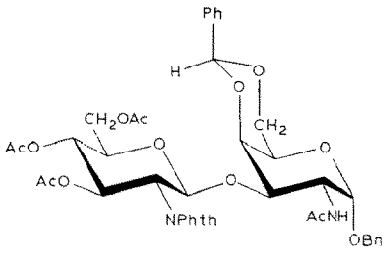
Condensation of tetra-*O*-acetyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl bromide (**1**) with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[17] in dichloromethane, in the presence of silver trifluoromethanesulfonate, 2,4,6-trimethylpyridine, and molecular sieves, for 4 h at room temperature afforded, after column chromatography, the fully protected disaccharide **3** in 73.5% yield. The ^{1}H-



1

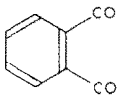


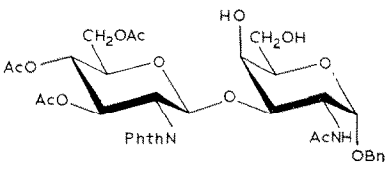
2



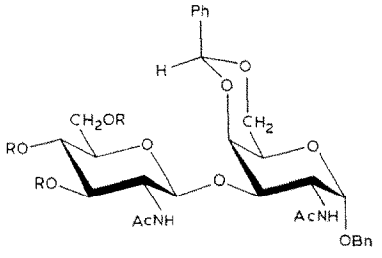
3

Phth =



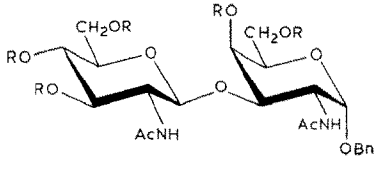


4



5 R = Ac

6 R = H



7 R = Ac

8 R = H

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a,b]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*CH_3CO*	*OCH_3 or $CH_2C_6H_5$*
c	101.56	55.41	74.21	70.44	76.74	60.88	—	—	—	—	—	—	22.96	50.02
d	97.63	54.02	70.58	70.49	72.42	60.62	—	—	—	—	—	—	22.43	53.56
e	96.08	49.59	67.19	67.55	71.34	60.53	—	—	—	—	—	—	22.49	67.99
2[f]	96.82	49.65	65.24	75.52	68.50	62.65	—	—	—	—	—	—	22.46	68.25
6[f]	96.73	48.07	75.05	73.86	68.55	62.63	101.62	55.96	74.60	70.66	76.78	61.30	22.47 22.86	68.55
8	95.94	48.11	76.41	67.12	71.23	60.73	101.50	55.85	74.85	70.18	76.59	60.73	22.49 22.87	67.91
12	96.04	49.55	67.00	67.47	69.34	68.56	101.49	55.08	74.06	70.53	76.77	60.92	22.51 22.93	68.28

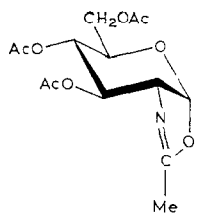
[a] In Me_2SO-d_6, with Me_4Si as the internal standard. [b] Carbonyl and aromatic carbon resonances are not shown. [c] Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside. [d] Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside. [e] Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside. [f] Benzylidene carbon resonances are at 99.49 and 99.60 p.p.m. for **2** and **6**, respectively.

n.m.r. spectrum of **3** was in agreement with the structure expected; H-1′ was observed as a doublet at 6.61, with spacings of 9 Hz, whereas H-1 resonated as a doublet at 5.00 (J 4 Hz), in accord with a β and an α configuration, respectively, of the two glycosidic linkages.

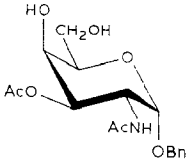
Cleavage of the benzylidene group of **3** in 60% aqueous acetic acid at 85° furnished **4**, which was purified in a column of silica gel. Deacylation of **4** with 85% hydrazine hydrate in ethanol, and peracetylation according to the procedure of Garegg and Norberg[18], afforded, after column-chromatographic purification, the peracetylated, crystalline disaccharide **7**, the ^{1}H-n.m.r. spectrum of which contained signals in support of its overall structure (see Experimental section). *O*-Deacetylation of **7** with methanolic sodium methoxide gave the title disaccharide **8**. The ^{13}C-n.m.r. spectrum of **8** was consistent with the structure proposed (see Table I).

Although the deblocking sequence described was adopted in order to expose HO-6 for further glycosylation with bromide **1** (to give a related trisaccharide, the synthesis of which has been achieved[19]), it was also possible to deacylate compound **3** and then acetylate the resulting intermediate to furnish **5**, exactly as described for **4** (to give **7**). *O*-Deacetylation of **5** afforded the benzylidenated disaccharide **6**, whose identity was evidenced by its ^{13}C-n.m.r. spectrum (see Table I).

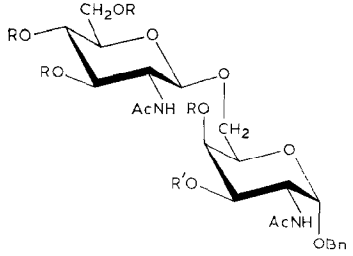
On condensation of the oxazoline **9** with **10** in 1,2-dichloroethane in the presence of *p*-toluenesulfonic acid, examination of the crude product by thin-layer chromatography (t.l.c.) with 14:14:1 benzene–ether–methanol revealed the presence of a major product, marginally slower-migrating than **10**, and some faster-migrating contaminants (presumably due to decomposition of **9**). After customary processing, the crude product was acetylated with 1:2 acetic anhydride–pyridine to give, after



9



10



11 R = R′ = Ac

12 R = R′ = H

column chromatography, crystalline **11** in 68.5% yield. *O*-Deacetylation of **11** with methanolic sodium methoxide gave **12**. The overall structure of **11** was evidenced by its ^{1}H-n.m.r. spectrum (see Experimental section), and that of **12** was supported by its ^{13}C-n.m.r. spectrum (see Table I).

Comments on the ^{13}C-n.m.r. assignments

A reasonable degree of uniformity in assigning the ^{13}C-n m.r. resonances of a series of compounds can only be attained when the spectra are recorded under similar conditions[20]. This principle is herein adhered to, all of the spectra being recorded for samples in Me_2SO-d_6, with Me_4Si as the internal standard. The spectrum of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, which has previously been reported[21,22] for samples in D_2O, is also recorded under the same conditions. The assignments of the resonances for the carbon atoms of the latter compound follow the same pattern as those reported[21,22], the only difference being the reversal of the resonances of C-2 and OCH_3 given in ref. 21.

The assignments of the ^{13}C signals of the disaccharides **6**, **8**, and **12** were made by comparison of their spectra with those of benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (or its 4,6-*O*-benzylidene derivative **2**), and of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (see Table I).

In the ^{13}C-n.m.r. spectrum of disaccharide **6**, the resonances for C-6, C-5, and C-1, respectively, showed little change in comparison to their counterparts in the spectrum of **2**. However, the resonances for C-2 and C-4 were shifted upfield by 1.58 and 1.66 p.p.m., respectively, as would be expected because of substitution at O-3. The resonance for C-3 exhibited a considerable downfield-shift (9.81 p.p.m.) compared to that of C-3 in the spectrum of **2**, clearly indicating the site of glycosylation to be O-3.

Whereas, in the ^{13}C-n.m.r. spectrum of the debenzylidenated disaccharide **8**, the resonance of C-2 remained close to that in the spectrum of **6**, the expected, downfield shifts for C-3 and C-5, and upfield shifts for C-4 and C-6, were observed as a consequence of removing the substituents at O-4 and O-6. Interestingly, the C-1 resonance in the spectrum of **8** suffered downfield shifts of 0.79 and 0.14 p.p.m., by comparison to that of **6**, and to that of the parent glycoside (*i.e.*, benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside).

In the ^{13}C-n.m.r. spectrum of **12**, the resonances for C-1 (96.04 p.p.m.) and C-2 (49.55 p.p.m.) remained close to those (96.08 and 49.49 p.p.m., respectively) for the parent glycoside. The resonances for C-3 and C-4 showed upfield shifts of 0.19 and 0.08 p.p.m., respectively, whereas that of C-5 was shifted upfield by 2 p.p.m. The latter, noticeable upfield-shift for C-5, coupled with an appreciable downfield-shift (8.03 p.p.m.) for C-6, by comparison to that of the parent glycoside, is a clear indication that O-6 was the site of the glycosylation.

The resonances for C-1′ in the ^{13}C-n.m.r. spectrum of **6** (101.74 p.p.m.), **8** (101.50 p.p.m.), and **12** (101.49 p.p.m.) were all in the range expected for the β configuration at the respective, interglycosidic linkage.

Finally, it may be pertinent to comment on the assignment of the ^{13}C resonances for benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside. With the exception of the resonances for C-3 and C-4, the ^{13}C resonances for this glycoside follow the same pattern as those reported for 2-acetamido-2-deoxy-α-D-galactopyranose, 2-amino-2-deoxy-α-D-galactopyranose hydrochloride[21] and methyl 2-acetamido-2-deoxy-α-D-galactopyranoside[24]. However, in those reports, the assignments of the resonances for C-3 and C-4 were either reversed[21,23], compared to our entries in Table I, or left without definitive assignment[24]. We were inclined to adopt the entries in Table I by comparison of the signals for C-3 and C-4 of the benzyl glycoside with their counterparts in its benzylidenated derivative **2**, and in the disaccharides **6** and **8**; because of substitution, the downfield and upfield shifts expected are observed. This is particularly apparent on comparing the signal for C-4 in the spectrum of the benzyl glycoside with that of the disaccharide **8**, in which the signal for C-4 is shifted upfield by 0.43 p.p.m. Should the resonances for C-3 and C-4 in the benzyl glycoside be reversed, the signals for C-4 in both compounds would remain very close to each other, contrary to the upfield shift invariably observed on substituting at a β carbon atom[25]. The present assignment for C-3 and C-4 agree with those reported for somewhat similar glycosides[26] and with those for the α-GalNAc unit in the Forssman hapten[27].

EXPERIMENTAL

General methods. — These were the same as those described in ref. 20, except that the following solvent systems (v/v) were used for chromatography: *A*, 3:1 ethyl acetate–hexane; *B*, 2:1 ethyl acetate–hexane; *C*, 1:1 chloroform–acetone; *D*, 2:1 chloroform–acetone; and *E*, 4:1 chloroform–acetone.

*Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-galactopyranoside* (**3**). — A mixture of **2** (2.4 g), silver trifluoromethanesulfonate (2.2 g), 2,4,6-trimethylpyridine (0.9 g), and molecular sieves type 4A (6 g) in dichloromethane (90 mL), protected from light and moisture, was stirred for 1 h at room temperature in an atmosphere of nitrogen. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**1**) in dichloromethane (60 mL) was added dropwise, with stirring, during 1 h, and stirring was continued for a total of 4 h. T.l.c. (solvent *A*) then revealed the presence of a major product, faster-migrating than **2**; a trace of **2**, as well as some faster-migrating products (presumably, due to decomposition of **1**), were also revealed by t.l.c. The mixture was diluted with an equal volume of dichloromethane, and the solids were filtered off, and washed with dichloromethane. The filtrate and washings were combined, successively washed with ice-cold water, cold 3% aqueous hydrochloric acid, cold saturated sodium hydrogencarbonate, and water, dried, and concentrated to a small volume. The concentrate was applied to a column of silica gel, and eluted with solvent *B*, followed by solvent *A*. Earlier fractions contained the faster-migrating contaminants. On evaporation, fractions corresponding to the major

product yielded a foam, which was dissolved in dichloromethane. Precipitation by the addition of ether–hexane afforded **3** (3.6 g, 73.5%), a white powder: $[\alpha]_D$ +88.6° (*c* 0.82, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.90–7.26 (m, 14 H, aromatic), 5.68 (dd, 1 H, *J* 9 and 10 Hz, H-2′), 5.61 (d, 1 H, *J* 9 Hz, H-1′), 5.52 (s, 1 H, PhCH), 5.34 (d, 1 H, *J* 9 Hz, NH), 5.18 (t, 1 H, *J* 10 Hz, H-3′), 5.00 (d, 1 H, *J* 4 Hz, H-1), and 2.02, 1.82, and 1.44 (s, 12 H, 3 OAc and NAc).

Anal. Calc. for $C_{42}H_{44}N_2O_{15}$: C, 61.75; H, 5.44; N, 3.43. Found: C, 61.97; H, 5.74; N, 3.41.

*Benzyl 2-acetamido-2-deoxy-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-galactopyranoside* (**4**). — Compound **3** (3 g) in 60% aqueous acetic acid (60 mL) was stirred for 1.5 h at 85°. T.l.c. (solvent *C*) then revealed the presence of a slower-migrating, major product. Traces of a slower- and a faster-migrating contaminant were also revealed by t.l.c. The acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several portions of toluene, and the residue was purified in a column of silica gel by elution with solvent *C*. The fractions corresponding to the debenzylidenated disaccharide were evaporated to dryness, and the residue was dissolved in a small volume of dichloromethane. Addition of ether caused the crystallization of **4** (2.4 g, 89.9%), a fine white powder, m.p. 118–121°, $[\alpha]_D$ +110.4° (*c* 1.2, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 8.00–7.25 (m, 9 H, aromatic), 5.73 (dd, 1 H, *J* 10 and 8 Hz, H-2′), 5.53 (d, 1 H, *J* 8 Hz, H-1′), 5.32 (d, 1 H, *J* 9 Hz, exchangeable by D_2O, NH), 5.15 (t, 1 H, *J* 9 Hz, H-3′), 4.86 (d, 1 H, *J* 4 Hz, H-1), 2.88 and 2.70 (broad s, 2 H, exchangeable by D_2O, 2 OH), and 2.10, 2.04, 1.84, and 1.28 (s, 12 H, 3 OAc and NAc).

Anal. Calc. for $C_{35}H_{40}N_2O_{15}$: C, 57.68; H, 5.54; N, 3.84. Found: C, 57.39; H, 5.43; N, 3.74.

*Benzyl 2-acetamido-3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)-4,6-di-O-acetyl-2-deoxy-α-*D*-galactopyranoside* (**7**). — Compound **4** (0.5 g) was heated for 15 min at 70° in a mixture of ethanol (10 mL) and 85% hydrazine hydrate (5 mL). The mixture was evaporated, and several portions of ethanol were added to, and evaporated from, the residue, which was then taken up in 1:2 (v/v) acetic anhydride–pyridine (16 mL), and the mixture heated for 20 min at 90°, cooled, concentrated, and purified by column chromatography on silica gel, using solvent *D* and then solvent *C* as the eluants, to afford, after recrystallization from chloroform–ether–hexane, **7** (0.38 g, 76%); m.p. 193–195°, $[\alpha]_D$ +95.3° (*c* 0.53, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.36 (s, 5 H, aromatic), 2.20–1.80 (cluster of singlets, 21 H, 5 OAc and 2 NAc), and 6.15–3.40 (unresolved signals, 18 H).

Anal. Calc. for $C_{33}H_{44}N_2O_{16}$: C, 54.68; H, 6.13; N, 3.87. Found: C, 54.36; H, 5.99; N, 3.81.

*Benzyl 2-acetamido-3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)-4,6-O-benzylidene-2-deoxy-α-*D*-galactopyranoside* (**5**). — Compound **3** (0.5 g) was deacylated, and then acetylated, exactly as described for **4** (to give **7**). The crude product was purified in a column of silica gel (solvent *D*) to give, after

recrystallization from chloroform–ether, **5** (0.34 g, 76.4%); m.p. 246–248°, $[\alpha]_D$ +116.42° (*c* 1.23, chloroform).

Anal. Calc. for $C_{36}H_{44}N_2O_{14}$: C, 59.32; H, 6.10; N, 3.84. Found: C, 59.43; H, 6.15; N, 4.05.

*Benzyl 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-α-*D*-galactopyranoside* (**6**). — Compound **5** (0.15 g) was *O*-deacetylated in 0.1M methanolic sodium methoxide (6 mL) for 5 h at room temperature. After de-ionization with Amberlite IR-120 (H^+) cation-exchange resin, the methanol was evaporated, and the residue was recrystallized from water containing a little ethanol, to afford **6** (0.1 g, 83.3%); m.p. 250° (dec.), $[\alpha]_D$ +35.6° (*c* 0.55, methanol); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{30}H_{38}N_2O_{11} \cdot H_2O$: C, 58.10; H, 6.51; N, 4.52. Found: C, 58.28; H, 6.18; N, 4.66.

*Benzyl 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-α-*D*-galactopyranoside* (**8**). — Compound **7** (0.25 g) was taken up in 0.1M methanolic sodium methoxide (10 mL), and stirred at room temperature. The suspended **7** rapidly dissolved, and, in 20 min, crystallization ensued. The mixture was kept for 2 h at room temperature, refrigerated for 2 h, the base neutralized by the addition of a few drops of glacial acetic acid, and the crystalline material filtered off, and thoroughly washed with cold methanol. Recrystallization from aqueous alcohol furnished **8** (0.16 g, 88.9%); m.p. 300° (dec.), $[\alpha]_D$ +131.4° (*c* 0.41, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{23}H_{34}N_2O_{11} \cdot 0.5\ H_2O$: C, 52.76; H, 6.75; N, 5.35. Found: C, 52.62; H, 6.68; N, 5.04.

*Benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3,4-di-*O*-acetyl-2-deoxy-α-*D*-galactopyranoside* (**11**). — A mixture of benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**10**; 0.71 g), oxazoline **9** (1.0 g), and *p*-toluenesulfonic acid (11.4 mg), in 1,2-dichloroethane (20 mL), protected from moisture, was heated for 2 days at 70° in an atmosphere of nitrogen, an additional amount of **9** (0.5 g in 5 mL of 1,2-dichloroethane), and *p*-toluenesulfonic acid (7.5 mg, in 5 mL of 1,2-dichloroethane) being added after 16 h. The mixture was cooled, the acid neutralized by the addition of a few drops of pyridine, and evaporated to dryness. Examination of the crude product by t.l.c. with 14:14:1 benzene–ether–methanol revealed the presence of a major product, marginally slower-migrating than **10**; some faster-migrating contaminants (presumably due to decomposition of **9**) were also revealed by t.l.c. The dried residue was dissolved in pyridine (20 mL) and acetic anhydride (10 mL), and the solution was kept overnight at room temperature. The acetic anhydride and pyridine were evaporated under diminished pressure, the last traces being removed by co-evaporation with several portions of toluene. The solid residue (2.2 g) was dissolved in solvent *E* (6 mL), and applied to a column of silica gel. Elution with solvent *E* removed the faster-migrating contaminants. Continued elution of the column with solvent *C*, and evaporation of the fraction corresponding to the major product, afforded, after recrystallization from chloroform–

ether, **11** (1.0 g, 68.5%); m.p. 212–214°, $[\alpha]_D$ +56.9° (*c* 0.85, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.40 (s, 5 H, aromatic), 2.20–1.80 (cluster of singlets, 21 H, 5 OAc and 2 NAc), and 5.80–3.45 (unresolved signals, 18 H).

Anal. Calc. for $C_{33}H_{44}N_2O_{16}$: C, 54.68; H, 6.13; N, 3.87. Found: C, 54.44; H, 6.35; N, 3.77.

*Benzyl 2-acetamido-6-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-α-*D*-galactopyranoside* (**12**). — Compound **11** (0.2 g) was *O*-deacetylated in 0.1M methanolic sodium methoxide, as described for **7** (to give **8**). Recrystallization of the product from aqueous alcohol afforded **12** (0.12 g, 85.7%): m.p. 290° (dec.), $[\alpha]_D$ +109.5° (*c* 0.52, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{23}H_{34}N_2O_{11}$: C, 53.68; H, 6.67; N, 5.45. Found: C, 53.69; H, 6.55; N, 5.44.

ACKNOWLEDGMENTS

We are grateful to Mr. Conrad F. Piskorz for his valuable technical assistance, and to Mrs. Onda D. Simmons for recording the n.m.r. spectra. We also thank Denise Kendall for kindly typing the manuscript. The n.m.r. studies were supported by National Cancer Institute Core Grant CA-16056. This investigation was supported by Grant No. IRO1-GM-24392-03, awarded by the National Institutes of Health.

REFERENCES

1 S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 100 (1982) c33–c34.
2 R. N. IYER AND D. M. CARLSON, *Arch. Biochem. Biophys.*, 142 (1971) 101–105.
3 B. ANDERSON, L. ROVIS, AND E. A. KABAT, *Arch. Biochem Biophys.*, 148 (1972) 304–314.
4 D. M. CARLSON, *J. Biol. Chem.*, 243 (1968) 616–626.
5 L. ROVIS, B. ANDERSON, E. A. KABAT, F. GRUEZO, AND J. LIAO, *Biochemistry*, 12 (1973) 5340–5354.
6 F. MAISONROUGE-MCAULIFFE AND E. A. KABAT, *Arch. Biochem. Biophys.*, 75 (1976) 90–113.
7 M. D. G. OATES, A. C. ROSBOTTOM, AND J. SCHRAGER, *Carbohydr. Res.*, 34 (1974) 115–137.
8 W. NEWMAN AND E. A. KABAT, *Arch. Biochem. Biophys.*, 172 (1976) 535–550.
9 B. L. SLOMIANY AND K. MEYER, *J. Biol. Chem.*, 247 (1972) 5062–5070.
10 V. A. DEREVITSKAYA, N. P. ARBATSKY, AND N. K. KOCHETKOV, *Eur. J. Biochem.*, 86 (1978) 423–437.
11 G. LAMBLIN, M. LHERMITTE, A. BOERSMA, AND P. ROUSSEL, *J. Biol. Chem.*, 255 (1980) 4595–4598.
12 A. SLOMIANY AND B. L. SLOMIANY, *J. Biol. Chem.*, 253 (1978) 7301–7306.
13 E. F. HOUNSELL, M. FUKUDA, M. E. POWELL, T. FEIZI, AND S. HAKOMORI, *Biochem. Biophys. Res. Commun.*, 92 (1980) 1143–1150.
14 D. WILLIAMS, G. LONGMORE, K. L. MATTA, AND H. SCHACHTER, *J. Biol. Chem.*, 255 (1980) 11,253–11,261.
15 H. PAULSEN, *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 155–173.
16 S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, unpublished results.
17 H. M. FLOWERS AND D. SHAPIRO, *J. Org. Chem.*, 30 (1965) 2041–2043.
18 P. J. GAREGG AND T. NORBERG, *Acta Chem. Scand.*, 36 (1982) 65–66.
19 S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, in press.
20 S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 106 (1982) 59–69.
21 N. YAMAOKA, T. USUI, H. SUGIYAMA, AND S. SITO, *Chem. Pharm. Bull.*, 22 (1974) 2196–2200.

22 S. J. PERKINS, L. N. JOHNSON, D. C. PHILIPS, AND R. A. DWEK, *Carbohydr. Res.*, 59 (1977) 12–34.
23 D. R. BUNDLE, H. J. JENNINGS, AND I. C. P. SMITH, *Can. J. Chem.*, 51 (1973) 3812–3819.
24 K. BLUMBERG AND C. A. BUSH, *Anal. Biochem.*, 119 (1982) 397–406.
25 D. E. DORMAN AND J. D. ROBERTS, *J. Am. Chem. Soc.*, 93 (1971) 4463–4472.
26 B. FERRARI AND A. A. PAVIA, *Bioorg. Chem.*, 11 (1982) 85–95.
27 T. A. W. KOERNER, JR., L. W. CARY, S.-C. LI, AND Y.-T. LI, *Biochem. J.*, 195 (1981) 529–533.