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A CONVENIENT SYNTHETIC ROUTE TO THE DISACCHARIDE REPEATING-UNIT OF PEPTIDOGLYCAN

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ABSTRACT

Glycosylation of the readily accessible benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride (**2**), using the silver triflate method in the absence of a base, afforded 65–70% of the fully protected [β-D-GlcNPhth-(1→4)-MurNAc] methyl ester derivative **4**, the structure of which was ascertained on the basis of 500-MHz ^{1}H-n.m.r. data. 2,2′-Dideoxy-2,2′-diphthalimido-β,β-trehalose hexa-acetate was a by-product. Removal of the Phth group from **4**, followed by acetylation, yielded 90% of the acetylated 1,6-di-*O*-benzyl derivative **5**, which, on saponification and catalytic hydrogenation, afforded 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose. Similarly, **5** was converted into the acetylated methyl ester derivative, which, on selective removal of the methyl ester group, gave benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside. An alternative route for the preparation of **2** is described.

INTRODUCTION

Sharon *et al.*[1] have characterised 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*N*-acetylmuramic acid [**6**, β-GlcNAc-(1→4)-MurNAc], the repeating unit of the glycan chain of bacterial cell-wall peptidoglycan, as the fully acetylated methyl ester derivative **7**. Total syntheses of **7** have been reported[2,3]. New derivatives of **6** have been prepared and coupled to the requisite peptide component[4,5]; the disaccharide moiety was constructed by the introduction of the lactyl ether group at HO-3 of the reducing sugar residue of suitably blocked chitobiose derivatives[6]. Recently, an oxazoline-promoted β-(1→4) coupling of GlcNAc and MurNAc residues has been described[7] which required an ester bond between CO_2H of MurNAc and

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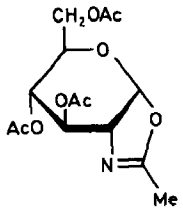
HO-6 of GlcNAc, in order to facilitate the glycosylation step (yield 20%). A short and efficient route for the direct glycosylation of the weakly reactive HO-4 of MurNAc has not been described so far.

We have reported[8] the synthesis of the 1,6-di-*O*-benzyl derivative (**3**) of *N*-acetylmuramic acid methyl ester (85–90% yield) in one step from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside[9,10] by modified reductive cleavage[11] of the acetal ring. We now report an efficient direct glycosylation of **3** to give the [β-GlcNPhth-(1→4)-MurNAc] derivative **4** and its conversion into the, hitherto synthetically unavailable, free disaccharide **6**. We also report an alternative preparation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride[12,13] (**2**).

RESULTS AND DISCUSSION

Although benzyl ether groups can enhance the reactivity of neighbouring hydroxyl groups in glycosylation reactions[14], the *N*-acetylmuramic acid derivative **3** did not react on treatment[15,16] with the oxazoline **1** in the presence of a catalyst (toluene-*p*-sulphonic acid, trimethylsilyl triflate), a base (tetramethylurea, *sym*-collidine), and molecular sieves. The reaction of **3** in the absence of base resulted in cyclisation to give the 1,6-di-*O*-benzyl-δ-lactone **9**[8] (53%) and the conversion of **1** into the methyl β-D-glycoside **11**[17] (22%).



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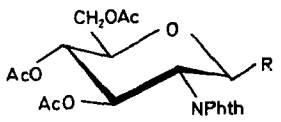
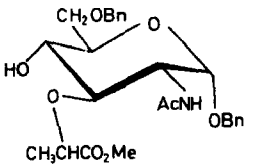
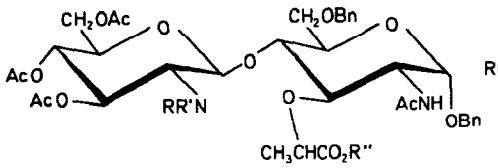


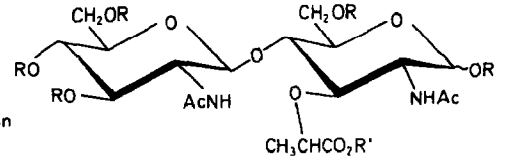
2 R = Cl
11 R = OMe
12 R = H, OH
13 R = OAc



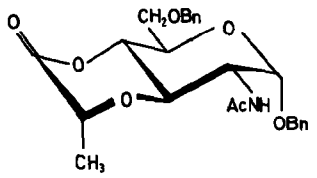
3



4 R,R' = Phth, R'' = Me
5 R,R' = H,Ac, R'' = Me
8 R,R' = H,Ac, R'' = H



6 R = H, R' = H
7 R = Ac, R' = Me



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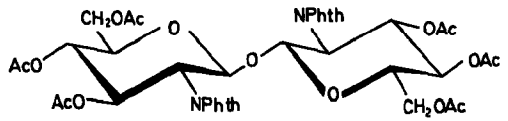

10

Attempted glycosylation of **3** by the silver triflate-promoted phthalimido method[13], using 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide, gave complex mixtures of products. When the less-reactive glycosyl chloride **2** was used in a poorly solvating solvent with silver triflate as the catalyst, a disaccharide derivative was formed but only under non-standard conditions. Glycosylation did not proceed below 0° and was almost totally inhibited in the presence of base (tetramethylurea or *sym*-collidine). A high yield (65–70%) of benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**) was obtained by the addition of **2** to **3** in dichloromethane in the presence of silver triflate (molar ratios, 3:1:4) and molecular sieves at room temperature. Strictly anhydrous conditions[18] and the use of concentrated solutions enhanced the efficiency of the reaction. The identity and homogeneity of **4**, isolated by column chromatography, was established by spectroscopic (see below) and analytical methods; the formation of the α-D-anomer of **4** was not detected (t.l.c., n.m.r. spectroscopy).

3,4,6,3′,4′,6′-Hexa-*O*-acetyl-2,2′-dideoxy-2,2′-diphthalimido-β,β-trehalose (**10**, up to 30%) was also formed in the reaction of **2** and **3**. The structure deduced from analytical and spectral data was confirmed by the synthesis of **10** from **2** and its hydrolysis product **12**, as well as from **2** alone, under conditions used for preparation of **4**. Iversen *et al.*[19] recorded the formation of a "trehalose"-type of impurity, arising from acetylated 2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide, in silver triflate–collidine-promoted glycosylations performed in the absence of molecular sieves.

A glycosylation method using triflic anhydride as catalyst, but not an organic base, was developed by Pavia *et al.*[20]. The driving force of the reaction is probably the trapping of liberated water as the insoluble hydroxonium triflate (H_3O^+ TfO^-) salt[21,22]. The conditions used for the synthesis of **4** suggest the operation of the same type of acid-catalysed mechanism involving ionisation of the glycosyl chloride **2** and liberation of the carbocation intermediate[13] which, without the intermediacy of an anomeric triflate, combines with the weakly nucleophilic HO-4 group of **3**. The phthalimido substituent ensures the formation of the 1,2-*trans* glycosidic linkage in **4** and **10**. Whilst this manuscript was in preparation, Paulsen *et al.*[23] reported an efficient, silver triflate-promoted glycosylation of 1,6-anhydro-β-muramic acid using 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide in the absence of a base.

The 500-MHz but not the 100-MHz ^{1}H-n.m.r. spectrum ($CDCl_3$) of **4** was amenable to first-order analysis. All of the ring proton signals were assigned and confirmed by the two-dimensional COSY spectrum, which identified all the connectivities. The large $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ values (9–10.5 Hz) for both sugar residues are indicative of the *trans*-diaxial arrangement of H-2,3,4,5 and $^4C_1$ conformations of the pyranoid rings.

Removal of the phthalimido group from **4** under conditions[19,24] (ethanolic hydrazine, reflux, 2 h) that did not affect a 1-(8-methoxycarbonyloctanoyl) group converted the 3-*O*-lactoyl group into its hydrazide derivative. However, on lowering the temperature of the reaction but not the concentration of the hydrazine, the selectivity for *N*-deprotection was improved. Thus, treatment of **4** with ethanolic hydrazine (molar ratio, 1:7) at 35° for ~40 h (monitoring by t.l.c.) gave, after acetylation and chromatography, 90% of benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**5**).

Saponification of the methyl ester group in **5** with 0.1 or 0.5M KOH in aqueous 1,4-dioxane yielded mixtures of **5**, the desired acid **8** (25–30%), and partially *O*-deacetylated derivatives of **8**. However, by using lithium iodide as the nucleophile[25,26], 86% of **8** was obtained from **5**. Alternatively, **5** was de-esterified with 0.5M KOH and the product was *O*-acetylated to give 82% of **8**.

Catalytic hydrogenation of **5** followed by acetylation afforded the known[1–3] fully acetylated [β-GlcNAc-(1→4)-MurNAc] methyl ester **7** that was mainly the α anomer. Pure **7α** was isdolated by chromatography and its structure was confirmed by the ^{1}H- and ^{13}C-n.m.r. data. *O*-Deacetylation and de-esterification of **5** with 0.5M KOH, followed by catalytic hydrogenolysis of the 1-*O*-benzyl group, gave 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose (**6**). The ^{13}C-n.m.r. data for **6** were consistent with the values reported[27] for the disaccharide obtained from the natural peptidoglycan monomer[28] by hydrolysis with *N*-acetylmuramoyl-L-alanine amidase[29]; the signal (δ 76.2) assigned to the glycosidically linked C-4 of the MurNAc moiety was shifted downfield (~7 p.p.m.) compared to that of unsubstituted MurNAc[27,30].

The 500-MHz ^{1}H-n.m.r. spectrum of **6** (D_2O) contained signals (δ 5.12 and 4.46, $J_{1,2}$ 2.81 and 8.26 Hz, ratio 3:1) for H-1α and H-1β of the MurNAc residue, the H-1′ signal of the β-GlcNAc moiety appeared as two doublets (δ 4.327 and 4.319, $J_{1',2'}$ 8.47 Hz, ratio 3:1), and the lactyl CH proton as two quartets (δ 4.49 and 4.38) of relative intensities corresponding to the anomeric ratio. The 2D COSY spectrum of **6** permitted assignment of the signals of the ring protons (see Experimental); the relative proportions of the doubled signals exhibited by the MurNAc residue reflected the anomeric ratio. A downfield shift for the signal of H-4 and an upfield shift of that for H-3 of the MurNAc residue, as compared to GlcNAc, are consistent with the glycosylation site and HO-3 substitution; the *J* values for the reducing residue are similar to those of the β-GlcNAc moiety and resemble those of the corresponding residue in chitobiose[31].

An alternative route to the glycosyl chloride **2** involves introduction of the phthalyl group by the Nefkens method[32]. Thus, treatment of 2-amino-2-deoxy-D-glucose hydrochloride with *N*-ethoxycarbonylphthalimide in aqueous sodium carbonate at room temperature gave 2-deoxy-2-phthalimido-D-glucopyranose[33] (**12**), which was not isolated but treated with acetyl chloride–aluminium chloride[13,34] to give 35% of **2**.

EXPERIMENTAL

General. — Melting points were determined in capillaries and are uncorrected. Solvents were removed under reduced pressure at <45°. Column chromatography was performed on Silica Gel (Merck 0.040–0.063 mm) and t.l.c. on Silica Gel 60 (Merck) with detection by charring with sulphuric acid. Optical rotations were determined at room temperature for 0.9–1.1% solutions. I.r. spectra were recorded with a Perkin–Elmer Model 297 spectrometer and mass spectra with a Varian CH7 spectrometer operating at 70 eV. N.m.r. spectra (internal Me_4Si) were recorded with a Jeol FX 90 F.t. spectrometer operating at 100 (^1H) and 22.5 MHz (^{13}C), if not stated otherwise. The 500-MHz ^1H-n.m.r. spectra were recorded with a Bruker AM-500 spectrometer operating in the F.t. mode and equipped with a Bruker Aspect 3000 computer. $^1H,^1H$-COSY spectra were obtained using the Jeener sequence with a 45° detection pulse and digital resolution in each dimension of 2–3.5 Hz/point.

*Benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-[(*R*-1-(methoxycarbonyl)ethyl]-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**4**). — A two-armed reaction vessel[18] was used. Silver triflate (411 mg, 1.6 mmol), in the flat-bottomed arm, was dried at 90°/0.05 Torr for 7 h; **3** (195 mg, 0.4 mmol), powdered 4-Å molecular sieves (500 mg), and a stirring bar were then added, the glycosyl chloride **2** (550 mg, 1.2 mmol) was placed in the conical arm, and drying at room temperature (liquid nitrogen trap) was continued for 5 h. After the admission of nitrogen, the ground-glass stopper was replaced by a rubber septum, dry dichloromethane (2 × 2 mL) was introduced with a syringe into both arms, and, with vigorous stirring, one-third of the halide solution was added, at room temperature, into the flat-bottomed arm. Addition of the halide was repeated after 2 and 4 h, and the mixture was stirred vigorously for a further 18 h. The suspension was diluted with chloroform (2 × 20 mL) and centrifuged, and the combined supernatant solutions were washed with aqueous sodium hydrogencarbonate and water, dried, and concentrated. Column chromatography (8:1:4 ethyl ether–2-propanol–light petroleum) of the residue gave, first, a mixture (R_F 0.50–0.45) of **12, 13,** and **3**, then **4** (R_F 0.39; 254 mg, 70%), and finally **10** (R_F 0.29, 116 mg, 22% based on **2**). Crystallisation of **4** from ether–light petroleum gave needles, m.p. 145–146°, $[\alpha]_D$ +38° (chloroform); ν_{max}^{KBr} 1760, 1739 (C=O), 1720 (C=O Phth), 1675, 1660 (Amide I and II), 750, 730 cm^{-1} (aromatic). N.m.r. data: (^1H, 500 MHz, $CDCl_3$), δ 7.95–7.7 (m, 5 H, Phth + NH MurNAc), 5.76 (dd, $J_{3',4'}$ 9.0 Hz, H-3'),

5.39 (d, $J_{1,2}$ 3.4 Hz, H-1), 5.37 (d, $J_{1',2'}$ 8.5 Hz, H-1'), 5.12 (dd, $J_{4',5'}$ 10.5 Hz, H-4'), 4.66 (q, $J_{CH,Me}$ 7.0 Hz, lactyl CH), 4.65 and 4.47 (2 d, J_{gem} 11.6 and 12.1 Hz, 2 OCH_2Ph), 4.29 (dd, $J_{6'a,6'b}$ 12.5 Hz, H-6'a), 4.23 (t, $J_{4,5}$ 9.1 Hz, H-4), 4.19 (dd, $J_{2',3'}$ 11.0 Hz, H-2'), 3.91 (dd, $J_{5',6'b}$ 2.2 Hz, H-6'b), 3.78 (s, 3 H, CO_2Me), 3.77 (ddd, $J_{2,3}$ 10.9 Hz, H-2), 3.60 (dd, $J_{3,4}$ 9.1 Hz, H-3), 3.44 (dq, $J_{5,6a}$ 2.7 Hz, H-5), 3.43 (dd, $J_{6a,6b}$ 11.6 Hz, H-6a), 3.41 (dd, $J_{5,6b}$ 2.2 Hz, H-6b), 3.31 (dq, $J_{5',6'a}$ 3.7 Hz, H-5'), 2.01, 2.00, 1.99, 1.85 (4 s, NAc, 3 OAc), 1.51 (d, *J* 7 Hz, CH*Me*); ^{13}C, δ 176.5 (CO_2Me), 170.7, 170.4, 170.0, 169.5 (NAc, 3 OAc), 168.0, 167.2 (Phth CO), 96.58, 96.31 (C-1,1'), 75.4, 75.2, 75.1 (C-3,4, lactyl *C*H), 72.7, 71.6, 70.6, 70.3, 70.2 (C-5,3',5', 2 CH_2Ph), 68.6, 68.0 (C-6,4'), 61.2 (C-6'), 55.2, 54.4 (C-2,2'), 52.2 (CO_2CH_3), 23.0, 20.5, 20.4 (NAc, 3 OAc), 18.5 (lactyl CH_3).

Anal. Calc. for $C_{46}H_{52}N_2O_{17}$: C, 61.06; H, 5.79; N, 3.10. Found: C, 60.99; H, 5.67; N, 3.08.

3,4,6,3',4',6'-Hexa-O-acetyl-2,2'-dideoxy-2,2'-diphthalimido-β,β-trehalose (**10**). — (*a*) Crystallisation of **10** from ethyl ether–light petroleum gave material with m.p. 268–269°, $[\alpha]_D$ −37° (chloroform); ν_{max}^{KBr} 1750 (C=O Ac), 1720 cm^{-1} (C=O Phth). N.m.r. data ($CDCl_3$): ^1H, δ 7.9–7.7 (m, 8 H, 2 Phth), 5.75 (dd, 2 H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.3 Hz, H-3,3'), 5.55 (d, 2 H, $J_{1,2}$ 8 Hz, H-1,1'), 4.94 (t, 2 H, $J_{4,5}$ 9.8 Hz, H-4,4'), 4.19 (dd, 2 H, H-2,2'), 4.02–3.64 (m, 6 H, H-5,5',6,6'), 1.97, 1.95, 1.83 (18 H, 6 OAc); ^{13}C, δ 97.16 (C-1,1'), 71.7 (C-5,5'), 70.3 (C-3,3'), 68.5 (C-4,4'), 61.8 (C-6,6'), 54.0 (C-2,2').

Anal. Calc. for $C_{40}H_{40}N_2O_{19}$: C, 56.34; H, 4.73; N, 3.29. Found: C, 56.58; H, 4.99; N, 3.14.

(*b*) To a solution of **12** (87 mg) and **2** (90 mg) in dichloromethane (7 mL) at room temperature was added silver triflate (52 mg). The solution was stirred overnight, then diluted with chloroform, and processed as for **4**, to yield **10** (49 mg, 28%) identical with the product obtained in (*a*).

(*c*) A mixture of **2** (180 mg), silver triflate (102 mg), and dichloromethane (7 mL) was stirred at room temperature for 18 h. Processing, as described above, yielded **10** (60 mg, 35%).

*Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)-6-O-benzyl-2-deoxy-3-O-[(R)-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**5**). — To a solution of **4** (160 mg, 0.18 mmol) in aqueous 96% EtOH (8 mL) was added hydrazine hydrate (60 μL), and the solution was kept at 35° for 43 h. The reaction was monitored by t.l.c. (9:1 chloroform–methanol) and terminated by addition of a few drops of acetic acid. After evaporation of the solvent, the dried residue was treated with acetic anhydride–pyridine (1:1, 3 mL) overnight. Conventional work-up and column chromatography (2:1:3 ethyl acetate–2-propanol–light petroleum) of the product gave **5** (132 mg, 91%). Recrystallisation from ethyl acetate–light petroleum afforded needles, m.p. 208–209°, $[\alpha]_D$ +61° (methanol); ν_{max}^{KBr} 3320 (NH), 1745 (C=O), 1660, 1540 cm^{-1} (Amide I and II). N.m.r. data ($CDCl_3$): ^1H, δ 7.95 (d, NH MurNAc), 7.47–7.29 (m, 2 Ph), 5.39 (d, $J_{1,2}$ 2.9 Hz, H-1), 5.01 (dd, *J* 9 Hz, H-3'), 4.97 (d, $J_{1',2'}$ 8.2 Hz, H-1'), 4.56 and 4.48 (2 dd, J_{gem}

12 and 11.9 Hz, 2 OCH_2Ph), 3.72 (s, CO_2Me), 2.02, 1.98 (15 H, 2 NAc, 3 OAc), 1.37 (d, *J* 7 Hz, *Me*CH); ^{13}C, δ 100.28 (C-1'), 96.67 (C-1), 78.0, 75.1, 75.0 (C-3,4, lactyl *C*H), 74.0, 73.2, 71.7, 70.5, 70.3 (C-5,3',5', 2 CH_2Ph), 68.4, 67.8 (C-6,4'), 61.6 (C-6'), 54.5, 54.3 (C-2,2'), 52.1 (CO_2CH_3), 18.5 (lactyl CH_3).

Anal. Calc. for $C_{40}H_{52}N_2O_{16}$: C, 58.82; H, 6.42; N, 3.43. Found: C, 58.73; H, 6.13; N, 3.31.

*2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-1,6-di-*O*-acetyl-2-deoxy-3-*O*-[(R)-1-(methoxycarbonyl)ethyl]-*D*-glucopyranose* (**7**). — A solution of **5** (110 mg, 0.15 mmol) in ethanol–acetic acid–water (6:1.5:1.5, 9 mL) was hydrogenated (10% Pd/C, 60 mg) at ambient temperature and pressure overnight, then centrifuged, and concentrated. The residue was treated with acetic anhydride–pyridine overnight. Column chromatography (ethyl acetate–acetone, 4:1) of the product gave **7** (70 mg, 73%) as a solid highly enriched in α anomer. Crystallisation from acetone–isopropyl ether–light petroleum afforded pure **7α**, m.p. 234–236°, $[\alpha]_D$ +38° (chloroform); lit.[1] m.p. 235–236°, $[\alpha]_D$ +40°. N.m.r. data ($CDCl_3$): ^1H, δ 7.92 (d, NH MurNAc), 6.41 (d, $J_{1,2}$ 2.9 Hz, H-1), 6.1 (d, *J* 8 Hz, NH GlcNAc), 5.15 (d, $J_{1',2'}$ 8.5 Hz, H-1'), 3.77 (s, CO_2Me), 2.14, 2.11, 2.07, 2.02, 2.00, 1.97 (21 H, 2 NAc, 5 OAc), 1.39 (d, *J* 7.0 Hz, *Me*CH); ^{13}C, δ 101.81 (C-1'), 90.07 (C-1), 78.2, 75.2, 74.5 (C-3,4, lactyl CH), 73.1, 72.2, 71.5 (C-5,3',5'), 68.3 (C-4'), 61.8 (C-6,6'), 54.2, 53.1 (C-2,2'), 52.3 (CO_2CH_3) 18.6 (lactyl CH_3).

Anal. Calc. for $C_{30}H_{44}N_2O_{18}$: C, 50.50; H, 6.15; N, 3.89. Found: C, 50.02; H, 6.41; N, 4.02.

*Benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-6-*O*-benzyl-3-*O*-[(R)-1-carboxyethyl]-2-deoxy-α-*D*-glucopyranoside* (**8**). — (*a*) A mixture of **5** (250 mg), anhydrous LiI (270 mg), and dry pyridine (6 mL) was stirred at 100° for 20 h under N_2 with the exclusion of moisture. Pyridine was removed *in vacuo*, the residue was taken in water–chloroform, the aqueous layer was acidified with 6M HCl to pH ~2, and the product was extracted with chloroform. Flash column chromatography (toluene–2-propanol–methanol, 4:1:2) of the product gave **8** (211 mg, 86%). Crystallisation from acetone–isopropyl ether gave material with m.p. 146–148°, $[\alpha]_D$ +54° (chloroform); ν_{max}^{KBr} 3260, 3060 (broad, NH, OH), 1750 (C=O), 1650, 1550 cm^{-1} (Amide I and II). N.m.r. data: ^1H ($CDCl_3$), δ 7.33, 7.29 (2 Ph), 5.30 (dd, $J_{3',4'}$ 9.3 Hz, H-3'), 5.19 (d, $J_{1,2}$ 2.3 Hz, H-1), 1.98, 1.94, 1.88 (15 H, 2 NAc, 3 OAc), 1.45 (d, *J* 7.1 Hz, *Me*CH); ^{13}C (CD_3OD), δ 100.28 (C-1'), 97.1 (C-1).

Anal. Calc. for $C_{39}H_{50}N_2O_{16}$: C, 58.35; H, 6.28; N, 3.49. Found: C, 58.49; H, 6.12; N, 3.38.

(*b*) A solution of **5** (82 mg) in 1,4-dioxane (4 mL) was stirred with 0.5M KOH (2 mL) at room temperature for 2 days [monitoring by t.l.c. ($CHCl_3$–MeOH, 4:1)]. After neutralisation [Amberlite IR-120 (H^+) resin] and removal of the solvent, the residue was acetylated with acetic anhydride–pyridine. Column chromatography of the product as in (*a*) gave **8** (66 mg, 82%).

*2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-3-*O*-[(R)-1-car-*

boxyethyl]-*2-deoxy*-D-*glucopyranose* (**6**). — Saponification of **5** (60 mg) in 1,4-dioxane (3 mL) with 0.5M KOH (1.5 mL) was performed as described above. After neutralisation and concentration, a solution of the residue in EtOH–AcOH–H_2O (6:1.5:1.5, 9 mL) was hydrogenated (10% Pd/C, 80 mg) overnight. The product was eluted from a column of Sephadex G-10 with water and isolated as a hygroscopic solid that was dried (P_2O_5), then dissolved in methanol (0.5 mL), and precipitated with ether (20 mL) to yield **6** (34 mg, 90%). The analytical sample, dried at 60°/0.05 Torr for 30 h, had m.p. 166–168° (softening at 164°), $[\alpha]_D$ +4° (water). N.m.r. data: ^1H (500 MHz, D_2O): δ 5.12 (d, $J_{1,2}$ 2.81 Hz, H-1α), 4.49 (q, J 7.08 Hz, lactyl C*H* of α form), 4.45 (d, $J_{1,2}$ 8.26 Hz, H-1β), 4.38 (q, J 7.04 Hz, lactyl C*H* of β form), 4.33 (d, $J_{1',2'}$ 8.47 Hz, H-1′ of α form), 4.32 (d, $J_{1',2'}$ 8.47 Hz, H-1′ of β form), 3.75 (2 d, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ 12.6 Hz, H-6′a), 3.73 (2 d, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 12.6 Hz, H-6aβ), 3.63 (m, $J_{5,6a}$ 2.2 Hz, H-5α), 3.58 (2 d, $J_{2,3}$ 10.6 Hz, H-2α), 3.58 (2 d, $J_{5',6'b}$ 4.7 Hz, H-6′b), 3.55 (2 d, $J_{6a,6b}$ 12.6 Hz, H-6α), 3.54 (2 d, $J_{2',3'}$ 10.6 Hz, H-2′), 3.54 (dd, $J_{4,5}$ 10.3 Hz, H-4α), 3.51 (2 d, $J_{5,6b}$ 4.9 Hz, H-6bα), 3.48 (2 d, $J_{2,3}$ 10.6 Hz, H-2β), 3.35 (dd, $J_{3',4'}$ 9.9 Hz, H-3′), 3.35 (m, $J_{5,6a}$ 2.3 Hz, H-5β), 3.23 (m, $J_{5',6'a}$ 2.0 Hz, H-5′), 3.22 (t, $J_{3,4}$ 9.9 Hz, H-3α), 3.20 (t, $J_{4',5'}$ 10.0 Hz, H-4′), 1.83, 1.82 (2 NAc), 1.26 (d, J 7.14 Hz, *Me*CH); ^{13}C, δ 100.23 (C-1′), 89.73 (C-1), 78.9, 75.6, 75.3 (C-3,5′, lactyl *C*H), 76.2 (C-4), 73.6 (C-3′), 71.3, 70.5 (C-4′,5), 61.4, 59.8 (C-6,6′), 56.2, 54.4 (C-2,2′), 22.2 (2 NAc), 18.3 (lactyl CH_3).

Anal. Calc. for $C_{19}H_{32}N_2O_{13} \cdot 1.5\ H_2O$: C, 43.59; H, 6.74; N, 5.35; H_2O, 5.16. Found: C, 43.38; H, 6.77; N, 5.61; H_2O 5.21.

3,4,6-Tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl chloride* (**2**). — To a solution of 2-amino-2-deoxy-D-glucose hydrochloride (2.15 g, 10 mmol) and sodium carbonate (0.6 g, 6 mmol) in water (30 mL) was added powdered *N*-ethoxycarbonylphthalimide (2.1 g, 10 mmol). The mixture was then stirred at room temperature overnight, filtered, and concentrated. The residue was dried (P_2O_5) to constant weight and then stirred with acetyl chloride (20 mL) for 3 h at room temperature; dry aluminium trichloride (1.5 g) was then added and stirring was continued for 18 h. The mixture was diluted with chloroform and poured into ice–water, the aqueous layer was extracted with chloroform, and the combined extracts were washed with aqueous sodium hydrogencarbonate and water, dried, and concentrated. Flash column chromatography (1:1:1, ethyl acetate–ethyl ether–light petroleum) of the residue gave a semi-crystalline mixture (R_F 0.84) of **2** and its 1-acetate derivative **13**[12]. Trituration with ether (15 mL) left **2** (1.6 g, 35.5% calc. on $GlcNH_2 \cdot HCl$) as platelets, m.p. 146–147°, $[\alpha]_D$ +58° (chloroform); lit.[12] m.p. 149°, $[\alpha]_D$ +61.7°. N.m.r. ($CDCl_3$): ^1H, δ 6.2 (d, $J_{1,2}$ 9.3 Hz, H-1), 5.8 (dd, $J_{3,4}$ 9.3, $J_{3,2}$ 10.5 Hz, H-3), 5.24 (t, $J_{4,5}$ 10.5 Hz, H-4), 4.5 (dd, $J_{2,1}$ 9.3 Hz, H-2), 4.3–3.8 (m, H-5,6a,6b), 2.14, 2.04, 1.87 (3 OAc); ^{13}C, δ 85.61 (C-1), 75.7 (C-5), 70.7 (C-3), 68.3 (C-4), 61.7 (C-6), 57.6 (C-2).

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