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METHANOLYSIS AND AMINOLYSIS OF *N*-ACETYLMURAMIC ACID LACTONES: EVIDENCE FOR THE RETENTION OF THE D-*gluco* CONFIGURATION

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ABSTRACT

Methanolysis of benzyl α-glycosides of *N*-acetylmuramic acid lactones with HO-6 free (**2**) and substituted (**4, 7, 10,** and **12**) is catalysed by small amounts of silica gel to give, exclusively, the corresponding methyl esters with HO-4 unsubstituted (**3, 5, 8, 11, 13**); opening of the lactone ring proceeds with retention of the D-*gluco* configuration and can be followed by ^{1}H-n.m.r. spectroscopy. Condensation of **2** with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**15**) gave the β-(1→6)-linked disaccharide lactone **16** which, on methanolysis, yielded the disaccharide methyl ester **17**, also obtained by condensation of **3** and **15**. In the presence of imidazole, the lactones **2** and **4** underwent aminolysis with amino acid and peptide esters as nucleophiles to give the *N*-acetylmuramoylamide derivatives **19–24**. The structures of methanolysis and aminolysis products were established by ^{1}H-n.m.r. spectroscopy and independent syntheses.

INTRODUCTION

2-Acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose (*N*-acetylmuramic acid) is a constituent of the repeating unit [β-GlcNAc-(1→4)-MurNAc-peptide] of bacterial cell-wall peptidoglycans. Since *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) is the minimal structure required for immunoadjuvant activities[1], many MDP derivatives and analogues have been synthesised[2]. The synthesis of the sugar–peptide bonds in each of these compounds was achieved by coupling a protected *N*-acetylmuramic acid derivative with suitably blocked peptide in the presence of a carbonyl activating and/or condensing agent.

N-Acetylmuramic acid and its derivatives with HO-4 unsubstituted easily undergo lactonisation[3]. Conventional acetylation of *N*-acetylmuramic acid results[4] exclusively in the formation of the δ-lactone involving HO-4 and the lactyl carboxyl group. Lactonisation of methyl and benzyl glycosides of *N*-acetylmuramic acid on

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heating, excessive drying, or treatment with dilute acetic acid at elevated temperature has been severally observed, and evidence for a δ-lactone has been provided[5–7]. However, these compounds were usually recognised as minor by-products, formed concomitantly with the desired *N*-acetylmuramic acid derivatives, and subjected, without isolation, to hydrolysis by treating a methanolic solution of the crude product with 2M sodium hydroxide at room temperature for several hours[3,5,8].

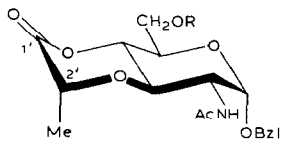
Work in this laboratory has demonstrated[9] that treatment of the *Brevibacterium divaricatum* mutant with penicillin causes immediate and massive secretion of non-cross-linked peptidoglycan fragments from which, on incubation with lysozyme, the repeating unit β-GlcNAc-(1→4)-MurNAc-pentapeptide was isolated and characterised[10]. This unit enhances[11] the immune response *in vivo* and interferes with the mitogenic action of some mitogens *in vitro*.

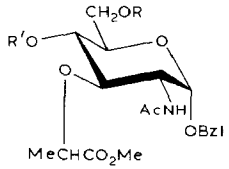
Thus, we became interested in the synthesis of compounds structurally related to peptidoglycan fragments and attention was turned to lactones of *N*-acetylmuramic acid as a starting material for syntheses in this field. We now report on the susceptibility of the lactone carbonyl group toward methanolysis and aminolysis and on the stereochemical outcome of these reactions.

RESULTS AND DISCUSSION

Treatment of the benzyl α-glycoside (**1**) of *N*-acetylmuramic acid[5,12] with dicyclohexylcarbodi-imide (DCC) and acetic anhydride–pyridine afforded the corresponding lactones with HO-6 free (**2**) and acetylated[6] (**4**), respectively, in high yields. When dissolved in dry methanol at room temperature, **2**, within a few hours, was converted completely into the methyl ester **3**[12] (77% isolated). Under similar conditions, the conversion of **4** into the 6-acetylated methyl ester **5** was complete after 24 h; warming (→45°) or the addition of a small proportion of silica gel substantially accelerated the reaction. Evidence for the structures of **3** and **5** was obtained by acetylation which gave benzyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside[12] (**6**) as the sole product. The identity of **6** was established by ^{1}H-n.m.r. spectroscopy and synthesis[5,12] involving debenzylidenation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside to give **3**, and then acetylation. The ^{1}H-n.m.r. spectra of **6** in $CDCl_3$ and pyridine-d_5 showed, *inter alia*, a deshielded doublet of doublets for H-4 at δ 5.10 and 5.45, with spacings of 9.0 and 10.0 Hz, and 8.9 and 9.8 Hz, respectively, thus indicating that opening of the lactone ring proceeded without epimerisation at C-4.

Evidence of a marked effect on the rate of methanolysis by the nature of the HO-6 substituent was obtained in experiments with *N*-acetylmuramic acid lactones **7**[5], **10**[6], and **12**, substituted at position 6 by trityl, tosyl, and pivaloyl groups, respectively; **12** was conveniently prepared from **1** and pivaloyl chloride–pyridine. The conversion of the above compounds in methanol into their respective methyl esters





2 R = H
4 R = Ac
7 R = Tr
10 R = Ts
12 R = Piv

3 R = R' = H
5 R = Ac, R' = H
6 R = R' = Ac
8 R = Tr, R' = H
9 R = Tr, R' = Ac
11 R = Ts, R' = H
13 R = Piv, R' = H
14 R = Piv, R' = Ac

was slow and gradually approached equilibrium. Monitoring of the reaction by t.l.c. and ^{1}H-n.m.r. spectroscopy (see below) revealed that, for 0.01–0.02M solutions in methanol at room temperature for 48 h, the efficiency of the conversions of **7**→**8** and **12**→**13** was 60 and 70%, respectively, whereas that of **10**→**11** in boiling methanol was only 25%. No other products could be detected during the reactions. The low reactivities of **7** and **12** may be ascribed primarily to steric factors associated with the bulky trityl and pivaloyl groups, respectively, whereas, for **10**, polar interactions with the electron-withdrawing tosyl group may be responsible for the enhanced stability of the lactone ring. 6-Tosylation of a glycopyranoside increases the stability of the glycosidic linkage towards hydrolysis[13].

When the above compounds were treated with methanol, in the presence of small amounts of silica gel, complete conversion into the corresponding methyl esters took place at room temperature within a few hours. The catalysis of S→O intramolecular[14] and O→O and S→O intermolecular[15,16] transesterification by silica gel has been observed and ascribed[17] to the binding properties of silica gel which polarises the ester (or thiol ester) carbonyl group. Evidence for the structures of **8**, **12**, and **13** was obtained from their physical and ^{1}H-n.m.r. data, and by conversion of **8** and **13** into the 4-acetates **9**[6] and **14**, respectively. Compounds **8** and **11** were also synthesised by tritylation and tosylation, respectively, of the methyl ester **3**[12].

The assignments in the 100-MHz ^{1}H-n.m.r. spectra (see Experimental) for solutions of each of the series of lactones in $CDCl_3$ were confirmed by spin-decoupling experiments. A general feature of the spectra is the strong deshielding of H-4 and the strong shielding of H-3; the value (3.6 Hz) of $J_{1,2}$ and the uniformly large magnitudes (9–10.5 Hz) of $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ indicate that the α-D-glucopyranose moiety adopts the $^4C_1$ conformation. The positions (δ 4.64–4.34) of the resonances for H-4, which are at lower field than those of all the other non-anomeric protons, are a consequence of attachment to the C adjacent to the partially positive oxygen atom of the lactone ring; this type of deshielding has been observed for sugar γ-lactones[18] and non-sugar lactones[19]. The upfield positions (δ

3.82–3.77) of the resonances for H-3 are due to shielding by the CH(Me) ether group. In all the spectra, the resonances of H-3 and H-5 were usually superimposed, at the highest field, except for **7** in which, because of the strong shielding effect of the trityl group[20], H-6,6′ were the most shielded. The H-2 resonances appeared consistently near δ 4.32. Relative to the signal positions for solutions in $CDCl_3$, those for solutions in pyridine-d_5 were ~0.50–0.3 p.p.m. downfield.

Opening of the lactone ring was reflected in ^{1}H-n.m.r. spectra of the methanolysis products **3, 5, 8, 11,** and **13** by the absence of the H-4 signal in the downfield region and, most evidently, by the well-separated signals for NH, H-1, and NAc, which were shifted downfield, and that for the lactyl methyl group, which was shifted upfield (Table I). Accordingly, methanolysis could be monitored on the basis of the relevant peak areas. The large (~1.9 p.p.m.) downfield shift of the NH resonance, accompanied by the shifts of the neighboring proton resonances, might reflect involvement of the NH proton in intramolecular hydrogen-bonds. In line with this view, there was slow (2–3 days) exchange of NH with D_2O and the finding that the position of the NH signal was essentially independent of temperature. Inspection of molecular models showed that it is possible for the acetamido proton to form a hydrogen bond with the oxygen of the lactyl carbonyl group. The ^{1}H-n.m.r. spectra of solutions of the 4-acetates **6, 9,** and **14**, in $CDCl_3$ and pyridine-d_5, allowed assignment of most of the signals for the ring protons; the H-4 signal always occurred to lower field of the resonances for the other non-anomeric protons, indicating that the *O*-acetyl group was located at position 4, and with vicinal coupling constants (9–10 Hz) fully consistent with the D-*gluco* configuration.

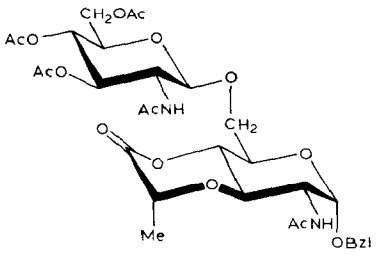
TABLE I

A COMPARISON OF ^{1}H-N M R PARAMETERS[a] FOR THE LACTONES **2, 4, 7, 10, 12,** AND **16** WITH THOSE OF THEIR METHANOLYSIS PRODUCTS **3, 5, 8, 11, 13,** AND **17**

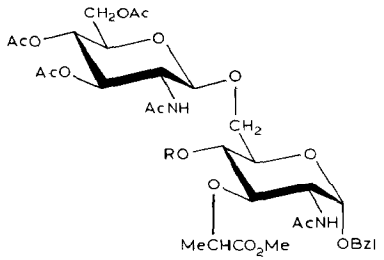
Compound	*H-1*		*AcN*[b]	*Me-lactyl*		*NH*	
	δ	$J_{1,2}$	δ	δ	$J_{Me,CH}$	δ	J
2	4.99d	3.66	1.98	1.48d	7.08	5.7d	9.3
3	5.45d	2.44	2 02	1.41d	7.08	7.6bs	
4	4.97d	3 66	1.99	1.49d	7.08	5 7d	9.3
5	5.34d	2.93	2.02	1.41d	6.84	7.5d	4.9
7	5.03d	3.66	1.99	1.45d	7.08	5.7d	9.5
8	5.31d	2.64	2.02	1.41d	7.08	[c]	
10	4 89d	3.66	1.96	1 45d	7.08	5.7d	9.2
11	5 23d	2.64	2.00	1.41d	7.08	[c]	
12	4.95d	3.66	1.99	1.49d	6.84	5.7d	9.3
13	5.33d	2.93	2.02	1.42d	7.08	7.5d	4.8
16[d]	4.96d	3.68	1.98	1.48d	6.97	5.61d	9.14
17	5.30d	2.00	2.02	1.42d	6.84	7.6bs	

[a]Recorded at 100 MHz for solutions in $CDCl_3$ (internal Me_4Si); *J* values given in Hz; multiplicities: d, doublet; bs, broad singlet. [b]Singlet. [c]Covered by signals of aromatic protons. [d]Data from 300-MHz spectrum.

To obtain some information on the stability of the δ-lactone ring under conditions commonly used in oligosaccharide synthesis, the lactone **2** with HO-6 unprotected was treated with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**15**) in 1,2-dichloroethane in the presence of toluene-*p*-sulphonic acid and molecular sieves at 90° to give, after column chromatography, 42% of the crystalline, fully protected (1→6)-β-D-disaccharide lactone **16**. If the reaction was carried out at lower temperature and/or in the presence of tetramethylurea, the yields of **16** dropped to <30%. When trimethylsilyl triflate[21] was used instead of toluene-*p*-sulphonic acid as the promoting agent, the reaction proceeded at lower (70°) temperature and better yields (55–59%) were obtained. The presence of tetramethylurea in the reaction mixture depressed the yield of **16**.



16



17 R = H
18 R = Ac

The 300-MHz ^{1}H-n.m.r. spectrum of a solution of **16** in $CDCl_3$ was largely first-order. The resonances of the anomeric protons were observed as one-proton doublets at δ 4.96 and 4.68 with coupling constants of 3.68 and 7.97 Hz, characteristic for H-1 of an α-D-muramic acid lactone (Table I) and a 2-acetamido-2-deoxy-β-D-glucopyranose residue, respectively. The chemical shifts and spacings of the signals assigned to the ring protons of the "aglycon" sugar component were fully consistent with the pattern observed in the spectra of monosaccharide lactones.

Methanolysis of **16** proceeded under very mild conditions, even in the absence of silica gel, to give, exclusively, the (1→6)-linked disaccharide methyl ester **17** with HO-4 free. The 100-MHz ^{1}H-n.m.r. spectrum of **17** was not amenable to analysis in the ring-proton region, but contained well-separated signals for NH, H-1, NAc, and lactyl methyl protons (Table I) at positions and with coupling constants closely similar to those observed for the monosaccharide analogues. Conventional acetylation of **17** afforded the 4-acetate **18**, the structure of which was indicated by its 300-MHz ^{1}H-n.m.r. spectrum; the signal assigned to H-4 of the "aglycon" sugar resonated as a doublet of doublets at low field (δ 5.05) with coupling constants ($J_{4,3}$ 8.58, $J_{4,5}$ 9.87 Hz) indicative of *trans*-diaxial arrangements of H-2,3,4,5.

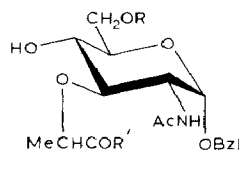
Additional evidence for the structure of **17** was obtained by condensation of

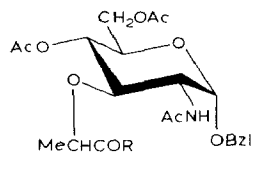
1-*O*-benzyl-*N*-acetylmuramic acid methyl ester (**3**) with the oxazoline **15**. If the coupling was catalysed by toluene-*p*-sulphonic acid, but without the addition of tetramethylurea, the product was the (1→6)-linked disaccharide lactone **16**, thus providing a further example of the ease with which *N*-acetylmuramic acid derivatives undergo lactonisation. The same reaction performed in the presence of the base gave 40% of the disaccharide methyl ester **17**. However, by using trimethylsilyl triflate as the promoting agent in the presence of tetramethylurea, the yield of **17** was 75%. Acetylation of **17** afforded the 4-acetate, the physical and spectroscopic data of which were indistinguishable from those of **18** formed by acetylation of the methanolysis product of **16**.

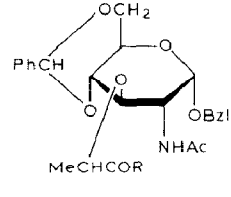
The susceptibility of *N*-acetylmuramic acid lactones to nucleophilic attack by the amino group of an amino acid ester was first examined by applying the conventional peptide-coupling active-ester procedure; under these conditions, the lactone carbonyl group did not react. Imidazole is an efficient catalyst in numerous reactions involving the formation of peptide[22,23] and ester bonds[24,25] (depsipeptides, glycosyl esters) with activated amino acids and alkyl esters as the acylating agents, probably because of participation of the intermediate *N*-acylimidazole in the transfer of the acyl group to the nucleophile.

When **2** and **4** were treated with glycine benzyl ester (2 equiv.) in 1,4-dioxane at room temperature in the presence of imidazole (5 equiv.), the crystalline *N*-acetylmuramoylamide derivatives **20** and **19** were obtained in yields of 70 and 76%, respectively. Similarly, by using the benzyl or methyl esters of L-alanine and L-alanylglycine as nucleophiles, **21–24** were formed in good yield. Acetylation of **24** afforded the 4-acetate **25**, the signals for the ring protons in the 300-MHz ^{1}H-n.m.r. spectrum ($CDCl_3$) of which could be assigned and indicated retention of the D-*gluco* configuration. In the spectrum of **25**, the signal for H-4 (δ 5.07, J 9.50 and 9.95 Hz) occurred at lower field, and that of H-3 (δ 3.67, J 9.50 and 10.10 Hz) at higher field, than all other ring-proton signals.

Compound **25** was also prepared by a different route, involving condensation of benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-







19 R = Ac, R′ = Gly–OBzl
20 R = H, R′ = Gly–OBzl
21 R = H, R′ = L-Ala–OBzl
22 R = Ac, R′ = L-Ala–OBzl
23 R = Ac, R′ = L-Ala–OMe
24 R = Ac, R′ = L-Ala–Gly–OMe

25

26

R = L-Ala–Gly–OMe

glucopyranoside with L-alanylglycine methyl ester to give **26**. Cleavage of the benzylidene group in **26**, followed by acetylation, then gave **25**.

EXPERIMENTAL

General methods. — Melting points were determined in capillaries and are uncorrected. Solvents were removed under reduced pressure at <45°. Methanol was distilled from Mg/I_2 onto 3A molecular sieve beads. Column chromatography was performed on Silica Gel (Merck, 0.025–0.2 mm), and t.l.c. on Silica Gel 60 (Merck); detection was effected by charring with sulphuric acid, or with the chlorine–iodine reagent for peptides. The solvents used were: *A*, chloroform–ethyl acetate–acetone (various proportions); *B*, chloroform–ethyl acetate (1:1); *C*, chloroform–ethyl acetate–light petroleum (1:1:1); *D*, chloroform–acetone (various proportions); *E*, chloroform–methanol (9:1). Optical rotations were determined for 1% solutions in chloroform, if not stated otherwise. I.r. spectra were recorded with a Perkin–Elmer Model 297 spectrometer. ^{1}H-N.m.r. spectra (100 MHz, internal Me_4Si) were recorded with Jeol JNM FX-100 and Bruker WH-300 spectrometers for solutions in $CDCl_3$, if not stated otherwise. Spin-decoupling experiments were used to confirm the proton assignments.

N-*Acetylmuramic acid lactones.* — Benzyl 2-acetamido-6-*O*-acetyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside 1′,4-lactone[6] (**4**), benzyl 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-6-*O*-trityl-α-D-glucopyranoside 1′,4-lactone[6] (**7**), and benzyl 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-6-*O*-tosyl-α-D-glucopyranoside 1′,4-lactone[5] (**10**) were prepared essentially by the literature procedures. Some modifications, leading to higher yields (75–85%) of pure products, were as follows. In the preparation of **4**, the reaction mixture was poured onto ice, extracted with chloroform, and worked-up in a conventional way (crystallisation from hot isopropyl ether–acetone + light petroleum). In the preparation of **7**, the tritylation procedure (90°) was extended to 9 h and elution of crude **7** from silica gel was carried out with solvent *B* (crystallisation as for **4**). Compound **10** crystallised from chloroform–light petroleum. Compound **4** had m.p. 170–171°, $[\alpha]_D$ +140°; lit.[6] m.p. 170–172°, $[\alpha]_D$ +144°. ^{1}H-N.m.r. data: δ 7.36 (Ph), 4.76, 4.50 (2 d, 2 H, J_{gem} 11.9 Hz, OCH_2Ph), 4.71 (q, *J* 7 Hz, MeC*H*), 4.39 (dd, $J_{4,3}$ 9.03, $J_{4,5}$ 10.01 Hz, H-4), 4.36 (dd, $J_{5,6}$ 4.15, $J_{6,6'}$ ~10 Hz, H-6), 4.31 (ddd, $J_{2,3}$ 10.0 Hz, H-2), 4.30 (dd, $J_{5,6'}$ 2.44 Hz, H-6′), 4.11–3.80 (m, 1 H, H-5), 3.82 (dd, H-3), and 2.11 (s, 3 H, AcO). Compound **7** had m.p. 218–220°, $[\alpha]_D$ +101°; lit.[6] m.p. 219–221°, $[\alpha]_D$ +103°. ^{1}H-N.m.r. data: δ 7.47–7.23 (m, 20 H, 4 Ph), 4.81, 4.54 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 4.65 (q, *J* 7 Hz, MeC*H*), 4.46 (dd, $J_{4,3}$ 9.03, $J_{4,5}$ 9.8 Hz, H-4), 4.34 (ddd, $J_{2,3}$ 10.0 Hz, H-2), 4.03–3.67 (m, H-5), 3.77 (dd, H-3), and 3.45–3.20 (m, 2 H, H-6,6′). Compound **10** had m.p. 186–187°, $[\alpha]_D$ +134°; lit.[5] m.p. 186–187°, $[\alpha]_D$ +130°. ^{1}H-N.m.r. data: δ 7.85–7.28 (m, 12 H, aromatic), 4.68, 4.46 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 4.61 (q, *J* 7 Hz, MeC*H*), 4.34–4.08 (m, 4 H, H-2,4,6,6′), 3.99–3.74 (m, H-5), and 3.76 (dd, $J_{3,4}$ 8.8, $J_{3,2}$ 10.2 Hz, H-3). Other signals for **4**, **7**, and **10** are given in Table I.

Benzyl 2-acetamido-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside 1',4-lactone (**2**). — A solution of dicyclohexylcarbodi-imide (DCC, 227 mg) in dry 1,4-dioxane (5 mL) was added with shaking to an ice-cooled solution of benzyl 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside[12] (**1**, 385 mg) in 1,4-dioxane (5 mL), and the mixture was left at room temperature overnight. *N,N'*-Dicyclohexylurea was removed, the filtrate was concentrated, and the solid residue was crystallised from acetone–light petroleum to give **2** (285 mg, 78%), m.p. 210–212°, $[\alpha]_D$ +155°; ν_{max}^{KBr} 3500–3200 (OH), 3315 (NH), 1760 (C=O lactone), 1655, 1555 (Amide I and II), and 740 cm^{-1} (phenyl). ^{1}H-N.m.r. data: δ 7.35 (s, Ph), 4.74, 4.49 (2 d, 2 H, J_{gem} 11.9 Hz, OC*H*$_2$Ph), 4.67 (q, *J* 7 Hz, MeC*H*), 4.34 (dd, $J_{4,3}$ 9.03, $J_{4,5}$ 10.5 Hz, H-4), 4.32 (ddd, $J_{2,3}$ 10.2 Hz, H-2), 4.0–3.7 (m, 4 H, H-3,5,6,6'), and 2.60 (bs, OH, exchangeable with D_2O) (other signals are given in Table I); (pyridine-d_5): δ 5.43 (d, $J_{1,2}$ 3.62 Hz, H-1), 4.97, 4.62 (2 d, 2 H, J_{gem} 11.7 Hz, OC*H*$_2$Ph), 4.94 (q, *J* 7 Hz, MeC*H*), 4.94 (t, $J_{4,3}$ 9.03, $J_{4,5}$ 10.5 Hz, H-4), 4.79 (ddd, $J_{2,3}$ 10.3 Hz, H-2), 2.11 (s, AcN), and 1.36 (d, *J* 7.08 Hz, *Me*CH).

Anal. Calc. for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.34; N, 3.84. Found: C, 58.92; H, 6.50; N, 3.86.

Benzyl 2-acetamido-2-deoxy-3-O-[(R)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**3**). — A solution of **2** (110 mg) in dry methanol (15 mL) was stored at room temperature; after 3 h, t.l.c. (solvent *A*, 3:1:4) indicated that the conversion of **2** (R_F ~0.5) into **3** (R_F ~0.4) was almost complete. After storage overnight, the solution was concentrated, and the residue was dried over sulphuric acid and crystallised from isopropyl ether–acetone–light petroleum to give **3** (92 mg, 77%), m.p. 130–132°, $[\alpha]_D$ +145° (lit.[12] m.p. 120–122°, $[\alpha]_D$ +137°); ν_{max}^{KBr} 3350–3250 (OH, NH), 1750 (C=O ester), 1650, 1555 (Amide I and II), and 730 cm^{-1} (phenyl). ^{1}H-N.m.r. data: δ 7.30 (s, Ph), 4.67, 4.45 (2 d, 2 H, J_{gem} 11.9 Hz, OCH$_2$Ph), 4.71 (q, *J* 7 Hz, MeC*H*), and 3.73 (s, CO_2Me) (other signals are given in Table I).

Anal. Calc. for $C_{19}H_{27}NO_8$: C, 57.42; H, 6.85; N, 3.52. Found: C, 57.62; H, 7.10; N, 3.50.

Conventional treatment of **3** (70 mg) with pyridine–acetic anhydride (1.5:1, 5 mL) gave a chromatographically (solvent *A*, 3:1:3) homogeneous solid that was crystallised from hot isopropyl ether + a few drops of acetone–light petroleum to afford benzyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**6**; 70 mg, 82.6%) as needles, m.p. 133–134°, $[\alpha]_D$ +119° (lit.[12] m.p. 128–129°, $[\alpha]_D$ +118°); ν_{max}^{KBr} 3325 (NH), 1743, 1738 (C=O ester), 1651, 1542 (Amide I and II), and 695 cm^{-1} (phenyl). ^{1}H-N.m.r. data: δ 7.70 (d, *J* ~4 Hz, NH), 7.33 (s, Ph), 5.43 (d, $J_{1,2}$ 2.70 Hz, H-1), 5.11 (dd, $J_{4,3}$ 9.0, $J_{4,5}$ 10.0 Hz, H-4), 4.68, 4.51 (2 d, 2 H, J_{gem} 12 Hz, OC*H*$_2$Ph), 4.24 (q, *J* 7 Hz, MeC*H*), 4.20 (dd, $J_{5,6}$ 4.4, $J_{6,6'}$ 12.4 Hz, H-6), 4.0 (dd, $J_{5,6'}$ 2.4 Hz, H-6'), 3.91–3.68 (m, 3 H, H-2,3,5), 3.77 (s, CO_2Me), 2.10, 2.09, 2.02 (3 s, 9 H, 2 AcO, AcN), and 1.36 (d, *J* 7.08 Hz, *Me*CH); (pyridine-d_5): δ 7.57–7.3 (m, Ph), 5.54 (d, $J_{1,2}$ 3.60 Hz, H-1), 5.47 (dd, $J_{4,3}$ 8.9, $J_{4,5}$ 9.8 Hz, H-4), 4.85, 4.57 (2 d, 2 H, J_{gem} 11.7 Hz, OC*H*$_2$Ph), 4.64 (dd, $J_{2,3}$ 10.7 Hz, H-2), 4.55 (q, *J* 7 Hz, MeC*H*), 4.50 (dd, $J_{5,6}$ 4.5, $J_{6,6'}$ 12 Hz, H-6),

4.29 (dd, $J_{5,6'}$ 2.4 Hz, H-6'), 4.30–4.11 (m, 1 H, H-5), 4.26 (t, H-3), 3.65 (s, CO_2Me), 2.14, 2.13, 2.06 (3 s, 9 H, 2 AcO, AcN), and 1.38 (d, *J* 7.08 Hz, *Me*CH).

Anal. Calc. for $C_{25}H_{31}NO_{10}$: C, 57.37; H, 6.49; N, 2.91. Found: C, 57.21; H, 6.60; N, 3.00.

Benzyl 2-acetamido-6-O-acetyl-2-deoxy-3-O-[(R)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**5**). — A solution of **4** (131 mg) in dry methanol (15 mL) was stored overnight at room temperature, shaken with silica gel (~500 mg) for ~1 h (monitoring of the reaction by t.l.c. in solvent *B*), filtered, and concentrated. The residue was crystallised from isopropyl ether–acetone–light petroleum to give **5** (111.4 mg, 79%), m.p. 122–124°, $[\alpha]_D$ +103°; ν_{max}^{KBr} 3440 (OH), 3340 (NH), 1760, 1730 (C=O ester), 1670, 1550 (Amide I and II), and 750 cm^{-1} (Ph). ^{1}H-N.m.r. data: δ 7.32 (s, Ph), 4.71 (q, *J* 7 Hz, MeC*H*), 4.67, 4.50 (2 d, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.60 (dd, $J_{5,6}$ 3.2, $J_{6,6'}$ 12 Hz, H-6), 3.96 (dd, $J_{5,6'}$ 1.95 Hz, H-6'), 3.75 (s, CO_2Me), 3.50 (t, $J_{3,2}$ 10.7, $J_{3,4}$ 9.0 Hz, H-3), 2.14, 2.02 (2 s, 6 H, AcO, AcN), 1.81 (bs, 1 H, OH, exchangeable with D_2O), and 1.41 (d, *J* 6.84 Hz, *Me*CH).

Anal. Calc. for $C_{21}H_{29}NO_9$: C, 57.39; H, 6.65; N, 3.19. Found: C, 57.44; H, 6.68; N, 3.27.

Acetylation of **5** (50 mg) gave **6** (57 mg, 78%).

Benzyl 2-acetamido-2-deoxy-3-O-[(R)-1-(methoxycarbonyl)ethyl]-6-O-trityl-α-D-glucopyranoside (**8**). — (*a*) Treatment of **7**[6] (125 mg) in dry methanol (15 mL) with silica gel (~500 mg) for ~4 h (monitoring by t.l.c. in solvent *C*) gave **8** (110 mg, 84%), m.p. 170–172° (from isopropyl ether–light petroleum), $[\alpha]_D$ +79°. ^{1}H-N.m.r. data: δ 7.50–7.26 (m, 20 H, 4 Ph), 4.67 (q, *J* 7 Hz, MeC*H*), 4.64, 4.44 (2 d, 2 H, J_{gem} 12 Hz, OCH_2Ph), 3.8–3.5 (m, 4 H, H-2,3,4,5), 3.73 (s, CO_2Me), 3.39–3.26 (m, 2 H, H-6,6'), and 2.92 (bs, 1 H, OH, exchangeable with D_2O) (other signals are given in Table I).

Anal. Calc. for $C_{38}H_{41}NO_8$: C, 71.34; H, 6.46; N, 2.19. Found: C, 71.54; H, 6.61; N, 2.24.

Acetylation of **8** (80 mg) gave the 4-acetate **9** (55 mg, 64.5%), m.p. 165–166° (from isopropyl ether–light petroleum), $[\alpha]_D$ +101°, which tended[26] to form solvates. A sample crystallised from chloroform–light petroleum could not be freed from the solvent even after 5 days at 100°/0.01 mmHg; lit.[12] m.p. 149–151° (from ethyl acetate–light petroleum), $[\alpha]_D$ +101°. ^{1}H-N.m.r. data: δ 7.71 (d, *J* 5 Hz, NH), 7.5–7.2 (m, 20 H, 4 Ph), 5.48 (d, $J_{1,2}$ 2.69 Hz, H-1), 5.08 (dd, $J_{4,3}$ 9.03, $J_{4,5}$ 10.0 Hz, H-4), 4.86, 4.56 (2 d, 2 H, J_{gem} 11.9 Hz, OCH_2Ph), 4.16 (q, *J* 7 Hz, MeC*H*), 3.92 (ddd, H-2), 3.85–3.64 (m, 2 H, H-3,5), 3.74 (s, CO_2Me), 3.12–2.97 (m, 2 H, H-6,6'), 2.05, 1.75 (2 s, 6 H, AcN, AcO), and 1.37 (d, 3 H, *J* 7.08 Hz, *Me*CH); (pyridine-d_5): δ 5.63 (d, $J_{1,2}$ 3.42 Hz, H-1), 5.50 (dd, $J_{4,3}$ 9.0, $J_{4,5}$ 10.0 Hz, H-4), 5.02, 4.65 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 4.67 (ddd, $J_{2,3}$ 10.5 Hz, H-2), 4.50 (q, *J* 7 Hz, MeC*H*), 4.21 (dd, H-3), 4.23 (dq, $J_{5,6}$ 4.2, $J_{5,6'}$ 2.4 Hz, H-5), 3.61 (s, CO_2Me), 3.40–3.29 (m, 2 H, H-6,6'), 2.14, 1.88 (2 s, 6 H, AcN, AcO), and 1.34 (d, *J* 7.08 Hz, *Me*CH).

Anal. Calc. for $C_{40}H_{43}NO_9$: C, 70.47; H, 6.35; N, 2.05. Found: C, 70.22; H, 6.51; N, 2.16.

(*b*) A mixture of **3** (115 mg), pyridine (5 mL), and trityl chloride (168 mg) was stirred, with exclusion of moisture, for 6 h at 90° (monitoring by t.l.c. in solvent *C*). Chloroform was added to the reaction mixture, the solution was poured into ice–water, and the organic layer was worked-up in the conventional way. The product was eluted from silica gel with solvent *C* to give **8** (102 mg, 55%) and a mixture (25 mg) of **8** and **7**. After crystallisation from isopropyl ether–acetone–light petroleum, the product was indistinguishable from **8** prepared by route (*a*).

*Benzyl 2-acetamido-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-6-*O*-toluene-*p*-sulphonyl-α-*D*-glucopyranoside* (**11**). — (*a*) Methanolysis of **10**[5] (monitoring by t.l.c. in solvent *B*), as described for **8**, gave **11** (79 mg, 72%), m.p. 104–106° (from acetone–light petroleum), $[\alpha]_D$ +86°; ν_{max}^{KBr} 1735 (C=O ester), 1650, 1550 (Amide I and II), 1365, 1180 (sulphonyl), 670, and 830 cm^{-1} (aromatic). ^{1}H-N.m.r. data: δ 7.86–7.23 (m, 9 H, aryl), 4.58, 4.41 (2 d, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.64 (q, *J* 7 Hz, MeC*H*), 3.75 (s, CO_2Me), 3.34 (bs, 1 H, OH, exchangeable with D_2O), and 2.45 (s, MeC) (other signals are given in Table I).

Anal. Calc. for $C_{26}H_{33}NO_{10}S$: C, 56.51; H, 6.03; N, 2.54. Found: C, 56.75; H, 6.23; N, 2.82.

(*b*) Toluene-*p*-sulphonyl chloride (763 mg) was added with stirring to an ice-cold solution of **3** (159 mg) in pyridine (8 mL). The mixture was stored overnight at room temperature and then worked-up as described for **6**. Elution of the residue from silica gel with solvent *D* (11:5) and crystallisation of the product (199 mg) from acetone–light petroleum gave **11** (130 mg, 59%).

*Benzyl 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-6-*O*-pivaloyl-α-*D*-glucopyranoside 1',4-lactone* (**12**). — A solution of **1** (192 mg) in dry pyridine (2.5 mL) containing molecular sieves (~500 mg) was treated with pivaloyl chloride (302 mg) at room temperature for 3 h (monitoring by t.l.c. in solvent *B*), and then processed as described for **6**. The product was crystallised from isopropyl ether–acetone–light petroleum to give **12** (162 mg, 72%), m.p. 118–119°, $[\alpha]_D$ +130°. ^{1}H-N.m.r. data: δ 7.36 (s, Ph), 4.75, 4.49 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 4.71 (q, *J* 7 Hz, MeC*H*), 4.41 (dd, $J_{4,3}$ 8.8, $J_{4,5}$ 10.0 Hz, H-4), 4.32 (ddd, $J_{2,3}$ 10.25 Hz, H-2), 3.81 (dd, H-3), 1.98 (s, 3 H, AcN), 1.49 (d, *J* 6.84, *Me*CH), and 1.23 (s, 9 H, Me_3C) (other signals are given in Table I).

Anal. Calc. for $C_{23}H_{31}NO_8$: C, 61.45; H, 6.95; N, 3.12. Found: C, 61.15; H, 7.13; N, 3.31.

*Benzyl 2-acetamido-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-6-*O*-pivaloyl-α-*D*-glucopyranoside* (**13**). — Methanolysis of **12** (166 mg) with monitoring by t.l.c. (solvent *D*, 3:1), as described for **8**, gave **13** (117 mg, 73.3%), m.p. 132–133° (from isopropyl ether–light petroleum), $[\alpha]_D$ +104°. ^{1}H-N.m.r. data: δ 7.3 (s, Ph), 4.71 (q, *J* 7 Hz, MeC*H*), 4.68, 4.49 (2 d, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.54 (q, $J_{5,6}$ 3.5, $J_{6,6'}$ 12.2 Hz, H-6), 4.02 (q, $J_{5,6'}$ 2.2 Hz, H-6'), 3.77–3.45 (m, 4 H, H-2,3,4,5), 3.75 (s, CO_2Me), and 1.23 (s, 9 H, Me_3C) (other signals are given in Table I).

Anal. Calc. for $C_{24}H_{35}NO_9$: C, 59.86; H, 7.33; N, 2.91. Found: C, 59.59; H, 7.46; N, 2.67.

Acetylation of **13** (96 mg) gave the 4-acetate **14** (73 mg, 70%), m.p. 106–108° (from isopropyl ether–light petroleum), $[\alpha]_D$ +122°. ^{1}H-N.m.r. data: δ 7.7 (bs, NH), 7.32 (s, Ph), 5.41 (d, $J_{1,2}$ 2.44 Hz, H-1), 5.10 (dd, $J_{4,3}$ 9.03, $J_{4,5}$ 10.0 Hz, H-4), 4.68, 4.49 (2 d, 2 H, J_{gem} 11.98 Hz, OCH_2Ph), 4.24 (q, J 7 Hz, MeC*H*), 4.12–4.00 (m, 2 H, $J_{5,6}$ 3.7, $J_{5,6'}$ 2.2, $J_{6,6'}$ 11 Hz, H-6,6'), 3.86 (ddd, $J_{2,3}$ 10.2 Hz, H-2), 3.77 (s, CO_2Me), 3.90–3.66 (m, H-3,5), 2.10, 2.03 (2 s, 6 H, AcO, AcN), 1.37 (d, J 7.08 Hz, *Me*CH), and 1.22 (s, 9 H, Me_3C); (pyridine-d_5): δ 5.50 (d, $J_{1,2}$ 3.42 Hz, H-1), 5.47 (dd, $J_{4,3}$ 8.9, $J_{4,5}$ 9.8 Hz, H-4), 4.82, 4.57 (2 d, 2 H, J_{gem} 12.2 Hz, OCH_2Ph), 4.48 (q, J 7 Hz, MeC*H*), 4.34 (dd, $J_{5,6}$ 4.88, $J_{6,6'}$ ~10 Hz, H-6), 3.63 (s, CO_2Me), 2.08, 2.05 (2s, 6 H, AcO, AcN), 1.38 (d, J 7.08 Hz, *Me*CH), and 1.24 (s, 9 H, Me_3C).

Anal. Calc. for $C_{26}H_{37}NO_{10}$: C, 59.64; H, 7.12; N, 2.68. Found: C, 59.80; H, 7.07; N, 2.62.

*Benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-gluco-pyranosyl)-3-*O*-[(R)-1-carboxyethyl]-2-deoxy-α-*D*-glucopyranoside 1',4-lactone* (**16**). — (*a*) A solution of **2** (111.6 mg) and 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**15**, 205 mg) in dry 1,2-dichloroethane (7 mL) containing trimethylsilyl triflate[27] (35.2 mg) and molecular sieve 4A (~300 mg) was stirred under N_2 (anhydrous conditions) for 9 h at 70°. After cooling and the addition of a few drops of pyridine, the mixture was concentrated and the residue was eluted from a column of silica gel (50 g) with solvent *A* (3:1:3) to give **16** (124 mg, 58.6%). Crystallisation from acetone–chloroform (4:1) and light petroleum gave **16**, m.p. 282–284° (dec.), $[\alpha]_D$ +73°. ^{1}H-N.m.r. data (300 MHz): δ 7.37–7.27 (m, 5 H, Ph), 5.64 (d, 1 H, $J_{NH,2'}$ 8.59, NH), 5.61 (d, 1 H, $J_{NH,2}$ 9.14 Hz, NH), 5.24 (dd, 1 H, $J_{3',4'}$ 9.32, $J_{3',2'}$ 10.36 Hz, H-3'), 5.08 (t, 1 H, $J_{4',5'}$ 9.70 Hz, H-4'), 4.96 (d, 1 H, $J_{1,2}$ 3.68 Hz, H-1), 4.72, 4.48 (2 d, 2 H, J_{gem} 11.72 Hz, OCH_2Ph), 4.71 (q, 1 H, J 7 Hz, MeC*H*), 4.68 (d, 1 H, $J_{1',2'}$ 7.97 Hz, H-1'), 4.39 (t, 1 H, $J_{4,3}$ 9.44, $J_{4,5}$ 9.60 Hz, H-4), 4.34 (ddd, 1 H, $J_{2,3}$ 10.01 Hz, H-2), 4.25 (dd, 1 H, $J_{5',6'a}$ 4.66, $J_{6'a,6'b}$ 12.3 Hz, H-6'a), 4.15 (dd, 1 H, $J_{5',6'b}$ 2.40 Hz, H-6'b), 4.13 (ddd, H-2'), 3.94 (dd, 1 H, $J_{5,6a}$ 4.49, $J_{6a,6b}$ 10.2 Hz, H-6a), 4.0–3.93 (m, 1 H, H-5'), 3.83 (dd, $J_{3,4}$ 9.40, $J_{3,2}$ 9.99 Hz, H-3), 3.79–3.69 (m, 2 H, H-5,6b), 2.088, 2.034, 2.031, 1.977, 1.887 (5 s, 15 H, 3 AcO, 2 AcN), and 1.48 (d, 3 H, J 6.97 Hz, *Me*CH).

Anal. Calc. for $C_{32}H_{42}N_2O_{15}$: C, 55.32; H, 6.09; N, 4.03. Found: C, 55.13; H, 5.96; N, 4.18.

(*b*) A mixture of **2** (182.7 mg), **15** (300 mg), toluene-*p*-sulphonic acid (17 mg), and molecular sieves (~400 mg) in 1,2-dichloroethane (9 mL) was stirred under anhydrous conditions for 8 h at 90°. The mixture was processed as in (*a*) to give **16** (142 mg, 42%).

(*c*) When **3** (129 mg) was treated with **15** (300 mg), as in (*b*), elution of the residue from silica gel with solvent *A* (3:1:3) gave a ~6:1 mixture (79 mg, 35%) of **16** and **17**, as indicated by ^{1}H-n.m.r. spectroscopy. Crystallisation (2 ×) from acetone–chloroform–light petroleum gave **16**.

*Benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-gluco-*

pyranosyl)-2-deoxy-3-O-[(R)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**17**). (*a*) A suspension of **16** (102 mg) in dry methanol was stirred at 40° for 1 h. The clear solution was stored overnight at room temperature and then concentrated, and the residue was crystallised from acetone–light petroleum to give **17** (85 mg, 79.7%), m.p. 222–224°, $[\alpha]_D$ +87°. ^{1}H-N.m.r. data: δ 7.60 (bs, 1 H, NH of MurNAc), 7.31 (s, Ph), 6.51 (d, $J_{NH,2'}$ 8.3 Hz, NH), 5.37 (dd, 1 H, $J_{3',4'}$ 9.3, $J_{3',2'}$ 10.2 Hz, H-3'), 5.30 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.05 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1'), 5.03 (t, $J_{4',5'}$ 9.8 Hz, H-4'), 4.76 (q, *J* 7 Hz, MeC*H*), 4.66, 4.45 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 3.75 (s, CO_2Me), 2.094, 2.030 (6 H), 2.015, 1.964 (4 s, 15 H, 3 AcO, 2 AcN), and 1.42 (d, *J* 6.84 Hz, *Me*CH); [$(CD_3)_2$SO]: δ 2.02, 1.97, 1.91, 1.83 1.74 (5 s, each 3 H, 3 AcO, 2 AcN), and 1.30 (d, *J* 6.85 Hz, *Me*CH).

Anal. Calc. for $C_{33}H_{46}N_2O_{16}$: C, 54.54; H, 6.38; N, 3.86. Found: C, 54.42; H, 6.33; N, 4.01.

Conventional acetylation of **17** (74 mg) with acetic anhydride–pyridine (1:2, 3 mL) and chromatography of the product on silica gel (solvent *A*, 3:1:3) gave the 4-acetate **18** (70 mg, 89%), m.p. 260–262° (acetone–light petroleum), $[\alpha]_D$ +64°. ^{1}H-N.m.r. data (300 MHz): δ 7.67 (d, 1 H, $J_{NH,2}$ 2.46 Hz, NH), 7.34 (m, 5 H, Ph), 5.89 (d, $J_{NH,2'}$ 8.66 Hz, NH), 5.41 (d, 1 H, $J_{1,2}$ 2.59 Hz, H-1), 5.18 (t, $J_{3',4'}$ 9.4, $J_{3',2'}$ 10.0 Hz, H-3'), 5.10 (t, $J_{4',5'}$ 9.50 Hz, H-4'), 5.05 (dd, $J_{4,3}$ 8.68, $J_{4,5}$ 9.97 Hz, H-4), 4.65, 4.48 (2 d, 2 H, J_{gem} 11.9 Hz, OCH_2Ph), 4.46 (d, $J_{1',2'}$ 8.40 Hz, H-1'), 4.26 (dd, $J_{5',6'a}$ 4.65, $J_{6'a,6'b}$ 12.2 Hz, H-6'a), 4.23 (q, *J* 7 Hz, MeC*H*), 4.13 (dd, $J_{5',6'b}$ 2.42 Hz, H-6'b), 4.05 (ddd, H-2'), 3.96 (q, 1 H, $J_{5,6b}$ 2.00, $J_{6a,6b}$ 10.91 Hz, H-6b), 3.86–3.77 (m, 3 H, H-2,3,5), 3.76 (s, CO_2Me), 3.69–3.64 (m, 1 H, H-5'), 3.33 (q, 1 H, $J_{5,6a}$ 4.95, $J_{6a,6b}$ 10.92 Hz, H-6a), 2.132, 2.094, 2.033, 2.026, 2.024, 1.912 (6 s, 18 H, 4 AcO, 2 AcN), and 1.35 (d, 3 H, *J* 7.04 Hz, *Me*CH); [$(CD_3)_2$SO]: δ 2.087, 2.013, 1.967, 1.907, 1.806, and 1.730 (6 s, each 3 H, 4 AcO, 2 AcN).

Anal. Calc. for $C_{35}H_{48}N_2O_{17}$: C, 54.68; H, 6.29; N, 3.64. Found: C, 54.55; H, 6.11; N, 3.49.

(*b*) A mixture of **3** (132.5 mg), **15** (250 mg), trimethylsilyl triflate (25 mg), tetramethylurea (0.1 mL), and molecular sieves 4A (~300 mg) in 1,2-dichloroethane (7 mL) was stirred under N_2 (anhydrous conditions) for 7 h at 70° and then concentrated, and the residue was eluted (2 ×) from silica gel with ethyl acetate–acetone (4:1) to give **17** (181 mg, 75%).

Acetylation of the above sample (70 mg) gave crystals (55 mg, 75%) indistinguishable (m.p., ^{1}H-n.m.r.) from **18** obtained *via* (*a*).

(*c*) The reaction of **3** (129 mg) and **15** (214 mg) in 1,2-dichloroethane (8 mL), performed in the presence of toluene-*p*-sulphonic acid (15 mg) and tetramethylurea at 88° for 9 h, gave, after chromatography on silica gel, **17** (93 mg, 40%).

N-[*2-O-(Benzyl 2-acetamido-6-O-acetyl-2,3-dideoxy-α-D-glucopyranoside-3-yl-(R)-lactoyl]-glycine benzyl ester* (**19**). — To a cooled suspension of glycine benzyl ester toluene-*p*-sulphonate (109 mg) in dry 1,4-dioxane (10 mL) was added *N*-methylmorpholine (0.036 mL) followed, after stirring for ~30 min, by **4** (66 mg) and imidazole (51 mg). The mixture was kept at room temperature for 3 days with

monitoring by t.l.c. (solvent *E*, 6:1), and then concentrated. The residue was eluted from a column of silica gel with solvent *E* (9:1); fractions containing **19** were collected and concentrated, and the residue was crystallised from chloroform–light petroleum to give **19** (71 mg, 76%), m.p. 154–155°, $[\alpha]_D$ +105° (methanol). ^{1}H-N.m.r. data: δ 7.32 (10 H, 2 Ph), 6.48 (d, *J* 8 Hz, NH), 5.10 (s, 2 H, CO_2CH_2Ph), 4.86 (d, $J_{1,2}$ 3.3 Hz, H-1), 2.05, 1.81 (2 s, 6 H, AcO, AcN), and 1.40 (d, *J* 7 Hz, *Me*CH).

Anal. Calc. for $C_{29}H_{36}N_2O_{10}$: C, 60.83; H, 6.34; N, 4.89. Found: C, 60.93; H, 6.43; N, 5.03.

N-[2-O-*(Benzyl 2-acetamido-2,3-dideoxy-α-*D-*glucopyranoside-3-yl)-(*R*)-lactoyl*]-*glycine benzyl ester* (**20**). — Treatment of **2** (118 mg) with glycine benzyl ester (liberated from 218 mg of the TsOH salt) and imidazole, as described above, gave **20** (120 mg, 70%), m.p. 180–181° (chloroform–light petroleum), $[\alpha]_D$ +132° (methanol).

Anal. Calc. for $C_{27}H_{34}N_2O_9$: C, 61.12; H, 6.46; N, 5.28. Found: C, 60.96; H, 6.63; N, 5.51.

N-[2-O-*(Benzyl 2-acetamido-2,3-dideoxy-α-*D-*glucopyranoside-3-yl)-(*R*)-lactoyl*]-L-*alanine benzyl ester* (**21**). — A mixture of **2** (110 mg), L-alanine benzyl ester (liberated from 211 mg of the TsOH salt), and imidazole (102 mg) in 1,4-dioxane (12 mL) was kept at room temperature for 48 h, and then concentrated. A solution of the residue in chloroform was washed with M hydrochloric acid, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. The residue was crystallised from acetone–light petroleum to give **21** (83 mg, 50%), m.p. 162–163°, $[\alpha]_D$ +109° (acetone). ^{1}H-N.m.r. data (Me_2CO-d_6): δ 7.35 (10 H, 2 Ph), 5.14 (s, 2 H, CO_2CH_2Ph), 4.93 (d, $J_{1,2}$ 3.42 Hz, H-1), 1.89 (s, AcN), 1.42, and 1.33 (2 d, 6 H, *J* 7 Hz, 2 MeC*H*).

Anal. Calc. for $C_{28}H_{36}N_2O_9$: C, 61.75; H, 6.66; N, 5.15. Found: C, 61.98; H, 6.83; N, 5.08.

N-[2-O-*(Benzyl 2-acetamido-6-*O*-acetyl-2,3-dideoxy-α-*D-*glucopyranoside-3-yl)-(*R*)-lactoyl*]-L-*alanine benzyl ester* (**22**). — Treatment of **4** (122 mg), as described for **21**, gave **22** (116 mg, 66%), m.p. 161–162° (from acetone–light petroleum), $[\alpha]_D$ +90°; +105° (acetone).

Anal. Calc. for $C_{30}H_{38}N_2O_{10}$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.60; H, 6.54; N, 4.57.

N-[2-O-*(Benzyl 2-acetamido-6-*O*-acetyl-2,3-dideoxy-α-*D-*glucopyranoside-3-yl)-(*R*)-lactoyl*]-L-*alanine methyl ester* (**23**). — Treatment of **4** (82 mg) with L-alanine methyl ester (liberated from 67 mg of the HCl salt) and imidazole (68 mg), as described for **19**, gave, after chromatography and crystallisation, **23** (53 mg, 52%), m.p. 168–169°, $[\alpha]_D$ +92° (*c* 0.5).

Anal. Calc. for $C_{24}H_{34}N_2O_{10}$: C, 56.46; H, 6.71; N, 5.49. Found: C, 56.21; H, 6.73; N, 5.29.

N-[2-O-*(Benzyl 2-acetamido-6-*O*-acetyl-2,3-dideoxy-α-*D-*glucopyranoside-3-yl)-(*R*)-lactoyl*]-L-*alanylglycine methyl ester* (**24**). — To a solution of dry L-

alanylglycine methyl ester trfluoroacetate [prepared from Boc-Ala-Gly-OMe (260 mg)] in 1,4-dioxane (8 mL) was added *N*-methylmorpholine (0.11 mL) followed by **4** (204 mg) and imidazole (150 mg), and the reaction was continued as for **19** to give **24** (145 mg, 52%), m.p. 202–203° (from chloroform–light petroleum). ^{1}H-N.m.r. data: δ 7.34 (s, Ph), 7.11 (d, J 6.8 Hz, NH), 6.31 (d, J 8.7 Hz, NH), 4.93 (d, $J_{1,2}$ 3.5 Hz, H-1), 3.96 (d, 2 H, collapsed to s on exchange with D_2O, NHCH_2CO), 3.72 (s, CO_2Me), 2.13, 1.92 (2 s, 6 H, AcO, AcN), 1.41, and 1.43 (2 d, 6 H, J 7 Hz, 2 *Me*CH).

Anal. Calc. for $C_{26}H_{37}N_3O_{11}$: C, 55.02; H, 6.57; N, 7.40. Found: C, 55.24; H, 6.45; N, 7.47.

Conventional acetylation of **24** (60 mg) and chromatography (solvent *D*, 1:2) of the product on a column of silica gel gave the 4-acetate **25** (46 mg, 72%), m.p. 192–193° (from acetone–light petroleum), $[\alpha]_D$ +67°. ^{1}H-N.m.r. data (300 MHz): δ 7.44–7.31 (m, 5 H, Ph), 6.93 (d, 1 H, J 6.99 Hz, NH of MurNAc), 6.81 (m, 1 H, NH of Gly), 5.92 (d, 1 H, J 9.50 Hz, NH of Ala), 5.07 (t, 1 H, $J_{4,3}$ 9.50, $J_{4,5}$ 9.95 Hz, H-4), 4.89 (d, 1 H, $J_{1,2}$ 3.63 Hz, H-1), 4.71, 4.51 (2 d, 2 H, J_{gem} 11.67 Hz, OCH_2Ph), 4.38 (m, $J_{2,3}$ 10.27 Hz, H-2), 4.37 (q, 1 H, J 7 Hz, MeC*H* of Ala), 4.22 (dd, 1 H, $J_{6,5}$ 4.50, $J_{6,6'}$ 12.3 Hz, H-6), 4.05 (dd, $J_{6',5}$ 2.35 Hz, H-6'), 4.02 (q, 1 H, J 7 Hz, MeC*H* of MurNAc), 4.00 (d, 2 H, J 5.30 Hz, CH_2 of Gly), 3.92 (m, 1 H, H-5), 3.73 (s, CO_2Me), 3.67 (t, 1 H, H-3), 2.12, 2.08, 1.90 (3 s, each 3 H, 2 AcO, AcN), 1.46 (d, 3 H, J 7.1 Hz, *Me*CH of Ala), and 1.34 (d, 3 H, J 6.7 Hz, *Me*CH of MurNAc).

Anal. Calc. for $C_{28}H_{39}N_3O_{12}$: C, 55.16; H, 6.45; N, 6.89. Found: C, 55.35; H, 6.46; N, 7.10.

N-[*2-O-(Benzyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-*D*-glucopyranoside-3-yl)-(*R*)-lactoyl*]-L-*alanylglycine methyl ester* (**26**). — Benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside[12] (471 mg) was treated at 0° with ethyl chloroformate (0.1 mL) and *N*-methylmorpholine (0.11 mL) in dry tetrahydrofuran (10 mL), and to the mixed anhydride was added L-alanylglycine methyl ester trifluoroacetate [prepared from Boc-Ala-Gly-OMe (260 mg)]. The mixture was stored at room temperature for 20 h, filtered, and eluted from a column of silica gel with solvent *E* (6:1) to give **26** (330 mg, 54%), m.p. 264–266° (from chloroform–light petroleum), $[\alpha]_D$ +104° (*N*,*N*-dimethylformamide).

Anal. Calc. for $C_{31}H_{39}N_3O_{10}$: C, 60.67; H, 6.41; N, 6.85. Found: C, 60.47; H, 6.69; N, 6.89.

A suspension of **26** (195 mg) in aqueous 60% acetic acid (10 mL) was stirred at 90° for 45 min, and then concentrated, and the residue was conventionally acetylated. The crude product was eluted from a column of silica gel with solvent *D* (1:2) to give **25** (107 mg, 63%).

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