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Aminolysis of *N*-Acetylmuramic Acid Lactones by Amino Acid and Peptide Esters — A Synthetic Route to *N*-Acetylmuramoyl-amide Derivatives

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Aminolysis of *N*-acetylmuramic acid (MurNAc) lactones, carrying different HO-6 substituents, with amino acid or peptide esters as nucleophiles was studied. The reaction of the protected (1 → 6) linked GlcNAc-MurNAc lactone I with *L*-alanine methyl ester, performed in dioxane in the presence of imidazole and tetra-*n*-hexylammonium benzoate (THAB), gives a moderate (42%) yield of the disaccharide-amide VI. This was converted into the 4-acetate VII, also prepared by coupling of the β-(1 → 6)-linked disaccharide acid V with Ala-OMe. Benzyl α-glycosides of HO-6 acetylated, tritylated and benzylated MurNAc lactones (IX, XI, XVIII) were used in aminolysis reactions with Ala-OMe and Ala-Gly-OBzl under various conditions. It is shown that the presence of imidazole and THAB, as well as the nature of the solvent, have a significant influence on the formation of the respective muramoylamides X, XII, XIII and XIX, resp.; conditions were elaborated to obtain these compounds in satisfactory (60—80%) yields. Additional evidence that the lactone ring-opening proceeds without epimerisation at C-4 is provided.

INTRODUCTION

Bacterial cell-wall peptidoglycan consists of polysaccharide chains joined to short peptides of characteristic amino acid composition¹. The basic structure of the glycan strands of the biopolymer is made up of alternating β-(1 → 4)-linked pyranosides of 2-acetamido-2-deoxy-*D*-glucose (GlcNAc) and 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-*D*-glucopyranose (*N*-acetylmuramic acid, MurNAc); the peptide units, attached through the amide bonds to the MurNAc carboxyl groups, are cross-linked in different directions². Peptidoglycan polymers have been known for a long time as effective stimulators of the immune response³. Since *N*-acetylmuramoyl-*L*-alanyl-*D*-isoglutamine (MDP) was shown⁴ to be the smallest structure possessing immunomodulating activities, many MDP derivatives and analogues have been synthesised with the objective of developing a new class of organic compounds usable for immunotherapeutic application³.

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Earlier work in this laboratory has shown⁵ that penicillin treatment of a *Brevibacterium divaricatum* mutant causes immediate and massive secretion of non-cross-linked peptidoglycan fragments into the culture medium. From the excreted linear polymer, the repeating unit [-GlcNAc- β -(1 \rightarrow 4)-MurNAc-pentapeptide] was isolated and characterised⁶⁻⁸. This unit enhances the immune response and interferes with some mitogens⁹⁻¹¹.

N-Acetylmuramic acid and its derivatives with HO-4 group unsubstituted easily undergo intramolecular esterification to give bicyclic lactones¹²⁻¹⁵. The latter compounds have often been recognised as byproducts in reactions involving MurNAc derivatives. However, in most cases, they were subjected, without isolation, to hydrolysis in alkaline methanolic solutions at elevated temperature. Recently, we have shown¹⁶ that methanolysis of glycosides of MurNAc lactones is catalysed, at room temperature, by small amounts of silica gel to give, exclusively, the corresponding HO-4 free methyl esters with retention of the *D*-*gluco* configuration. Analogously, methanolysis of the fully protected β -(1 \rightarrow 6)-linked disaccharide lactone I, prepared by condensation of the oxazoline derivative of 2-acetamido-2-deoxy-*D*-glucopyranose and the HO-6 free MurNAc lactone VIII, afforded the β -(1 \rightarrow 6)-disaccharide methyl ester II as the exclusive product.

The amide bond formation in the syntheses of different MDP-related compounds has been achieved^{4,17-20} by methods used in peptide chemistry, i. e. by coupling the sugar and peptide components in the presence of a carboxyl activating and/or condensing agent. Our preliminary experiments have shown¹⁶ that, in the presence of imidazole as catalyst, the MurNAc lactones undergo aminolysis with amino acid or peptide esters as nucleophiles to give the *N*-acetylmuramoylamide derivatives. In order to explore further the potential of this reaction as a synthetic route to compounds structurally related to peptidoglycan fragments, we have investigated the factors that influence the susceptibility of the MurNAc lactone carbonyl group toward aminolysis. Some new, suitably protected MurNAc derivatives were synthesised for this purpose.

RESULTS AND DISCUSSION

Selective hydrolysis of the methyl ester group of the HO-4 unsubstituted β -(1 \rightarrow 6)-linked disaccharide II, obtainable¹⁶ by either direct synthesis or by silica gel-catalysed methanolysis of the disaccharide lactone I, yielded the corresponding free acid III which was isolated and characterised. Conventional acetylation of III with acetic anhydride-pyridine led to cyclisation of the MurNAc moiety to give the disaccharide lactone I as the sole product. An attempt to couple the disaccharide acid III with *L*-alanine methyl ester, by using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide (DCC, HOSu) as the activating agents, failed because the majority of III lactonised during the activation step into I, thus providing a further example of the ease with which *N*-acetylmuramic acid derivatives undergo intramolecular esterification.

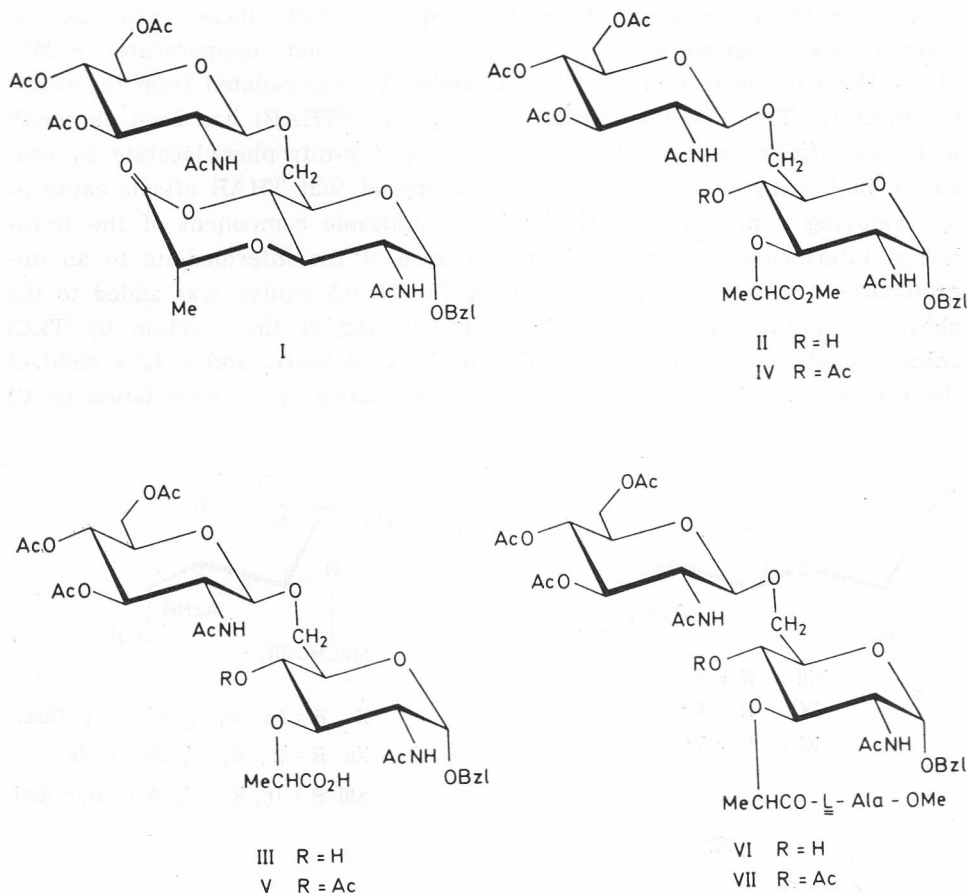
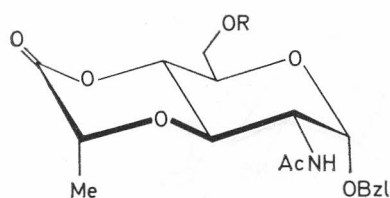


Chart 1

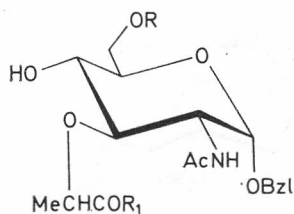
Methyl ester hydrolysis of the 4-*O*-acetyl derivative of II (IV¹⁶) led to the fully protected (1→6)-linked disaccharide acid V which, on condensation with *L*-alanine methyl ester in the presence of DCC and HOSu as activating agents, furnished the peracetylated 2-acetamido-2-deoxy-*D*-glucopyranosyl-β-(1→6)-*N*-acetylmuramoyl-*L*-alanine methyl ester derivative VII. The ¹H-NMR spectrum (CDCl₃) of VII was in agreement with the structure expected; the signals assigned to H-3', 4' and H-4 of the GlcNAc and MurNAc moiety, respectively, appeared at a lower field than all other ring proton signals and with coupling constants (~ 9 Hz) indicative of the *D*-gluco configuration of both monosaccharide units.

The alternative synthesis of VII via aminolysis of the disaccharide lactone I, was first performed by reacting I with *L*-alanine methyl ester in

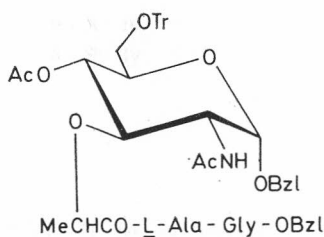
dioxane, in the presence of imidazole (5 equivs). Under these conditions, the reaction was sluggish and, after one week at room temperature, ~ 25% of the HO-4 unsubstituted disaccharide-amide VI was isolated from the reaction mixture. Tetra- *n*-hexylammonium benzoate (THAB) has been claimed²¹ to be an efficient catalyst in the aminolysis of *p*-nitrophenylacetate by imidazole in toluene at 25 °C; the authors proposed that THAB effects catalysis by removing a proton from the cationic imidazole component of the tetrahedral intermediate, thus permitting collapse of the intermediate to an unprotonated acylimidazole product. When THAB (0.5 equiv), was added to the above aminolysis mixture, a notable (monitoring of the reaction by TLC) enhancement of the rate of formation of VI took place, and a 42% yield of the product was obtained after silica gel chromatography. Acetylation of VI



- VIII R = H
IX R = Ac
XI R = Tr



- X R = Ac, R₁ = L-Ala-Gly-OBzl
XII R = Tr, R₁ = L-Ala-OMe
XIII R = Tr, R₁ = L-Ala-Gly-OBzl



XIV

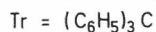


Chart 2

afforded the fully protected disaccharide-amide derivative with physical constants including ¹H-NMR data identical to those of VII prepared by the active ester method from the disaccharide acid V.

In order to investigate the conditions which would make the above reaction more effective for the synthesis of different muramoylamides, we turned to simpler members of the series, i. e. the benzyl α -glycosides of the HO-6 acetylated¹⁴ and -tritylated^{13,16} lactones IX and XI, respectively, and

studied their aminolysis by L-alanine methyl ester and L-alanyl-glycine benzyl ester under various conditions.

Typical results of the reaction of the lactone IX with L-alanyl-glycine benzyl ester are summarised in Table I. From the data presented, it is evident that the presence of THAB, as well as the nature of the solvent have significant effects on the extent of the formation of the muramoyldipeptide ester X; treatment of IX with 1.5 equiv of the dipeptide ester, 5 equivs of imidazole and 1 equiv of THAB in dry tetrahydrofuran (Expt. No 5) resulted in the clean formation of X in 72% yield. The fact that in the presence of THAB, but without imidazole, the reaction failed completely, agrees well with the proposed²¹ catalytic role of the former in the aminolysis of esters by imidazole in aprotic solvents. In the present case, apparently, the formed N-acylimidazole participates²²⁻²⁴ in the transfer of the muramoyl acyl group to the nucleophile.

TABLE I

Formation of N-Acetylmuramoyldipeptide Ester Derivative X in the Reaction of HO-6 Acetylated MurNAc Lactone IX with L-Ala-Gly-OBzl Under Various Conditions

Expt. No.	Reaction Conditions ^a				Yield of X ^b %
	Molar ratio to IX		Solvent	Time days	
	Imidazole	THAB			
1	2	--	dioxane	2	15
2	2	0.5	dioxane	6	37
3	5	0.5	dioxane	7	44
4	5	1.0	dioxane	3	57
5	5	1.0	THF	3	72

^a Each experiment was performed with 0.25 mmol (102 mg) of IX and 0.37 mmol (1.5 molar excess) of H-L-Ala-Gly-OBzl, liberated from the TFA salt, in a total of 12 ml of the solvent indicated at room temp.

^b Refers to the isolated, chromatographically homogeneous product, except in Expt. No. 1.

Evidence of a marked effect on the extent of aminolysis of the nature of the substituent was obtained in experiments with the HO-6 tritylated lactone XI. While the 6-O-acetyl lactone IX underwent aminolysis with L-alanine methyl ester in dioxane at room temperature in the presence of imidazole (5 equivs) to give a 52% yield of the corresponding muramoylamide¹⁶, the lactone XI was practically inert under these conditions, and only trace

amounts ($\sim 10\%$) of the expected product XII were revealed by TLC. The difference in reactivity between the two lactones can be ascribed mainly to steric factors; the access of the nucleophile's amino group to the lactone carbonyl of XI should be much more sterically hindered due to the presence of the bulky trityl group. However, addition of either THAB or tetra-*n*-butylammonium benzoate (TBAB) accelerated the reaction to give XII in high (76—79%) yields; no significant difference between the efficiency of the two tetraalkylammonium benzoate salts was observed. It is interesting that aminolysis of XI by L-alanyl-glycine benzyl ester was greatly influenced by the solvent used; under otherwise identical experimental conditions, the reactions performed in dioxane and tetrahydrofuran afforded the muramoyl-dipeptide ester XIII in yields of 41 and 60%, respectively.

Acetylation of XIII afforded the 4-acetate derivative XIV; its $^1\text{H-NMR}$ spectrum (Py-d_5) revealed the H-4 proton at position (δ 5.20) and with vicinal coupling constants ($J_{4,3}$ 9.03, $J_{4,5}$ 9.52 Hz) fully consistent with the *D-glucosyl* configuration, thus providing evidence that under the conditions elaborated the lactone ring-opening proceeds without epimerisation at C-4.

In order to prepare a HO-6 benzylated and HO-4 unsubstituted *N*-acetylmuramic acid derivative, suitable for glycosylation at O-4, we undertook the reductive ring opening of the 4,6-*O*-benzylidene acetal of benzyl α -glycoside of *N*-acetylmuramic acid methyl ester²⁵ (XV) with sodium cyanoborohydride-hydrogen chloride. This reaction, introduced in carbohydrate chemistry by Garegg^{26,27} proceeds with high regioselectivity to give the benzyl group at O-6 and is compatible with the presence of ester and acetamido groups in the molecule. However, a direct application of the procedure to XV resulted in a very low yield of the desired HO-6 benzylated derivative XVI and a high recovery of the starting XV. Nevertheless, when the reaction was performed with a large excess (12 equivs) of sodium cyanoborohydride, which was added gradually to the reaction mixture over a prolonged (~ 24 h) time, XVI was obtained in excellent ($> 85\%$) yields. $^1\text{H-NMR}$ data of the product and its acetylated derivative XVII provided evidence for the expected structure: the H-4 signal in the spectrum (CDCl_3) of XVII resonated at the lowest field of other non-anomeric protons, thus indicating that the *O*-acetyl group was located at position 4.

Selective hydrolysis of the methyl ester group of XVI afforded a free acid which was directly subjected to DCC condensation to give the HO-6 benzylated lactone XVIII (72% overall yield). The same compound could be obtained, in a considerably lower (38%) yield, by treating the HO-6 unsubstituted lactone VIII with benzyl bromide in the presence of silver carbonate. Dissolution of XVIII in methanol, in the presence of a catalytic amount of silica gel, led to opening of the lactone ring to give a single product, indi-

stinguishable from XVI obtained by regioselective ring opening of XV; acetylation of the methanolysis product afforded XVII.

Aminolysis of the lactone XVIII, having a rather bulky substituent at 0-6, by L-alanine methyl ester proceeded smoothly, when performed in tetrahydrofuran and in the presence of imidazole and THAB; the HO-6 benzylated muramoylamide derivative XIX was isolated in a 75% yield. With dioxane as solvent, the reaction proceeded at a slower rate, whereas in the absence of THAB, only a very low amount (~ 15%) of the expected product was formed within several days. The structure of XIX was confirmed by conversion into the 4-O-acetyl derivative XX whose ¹H-NMR spectrum (CDCl₃) showed signals in support of the structures assigned.

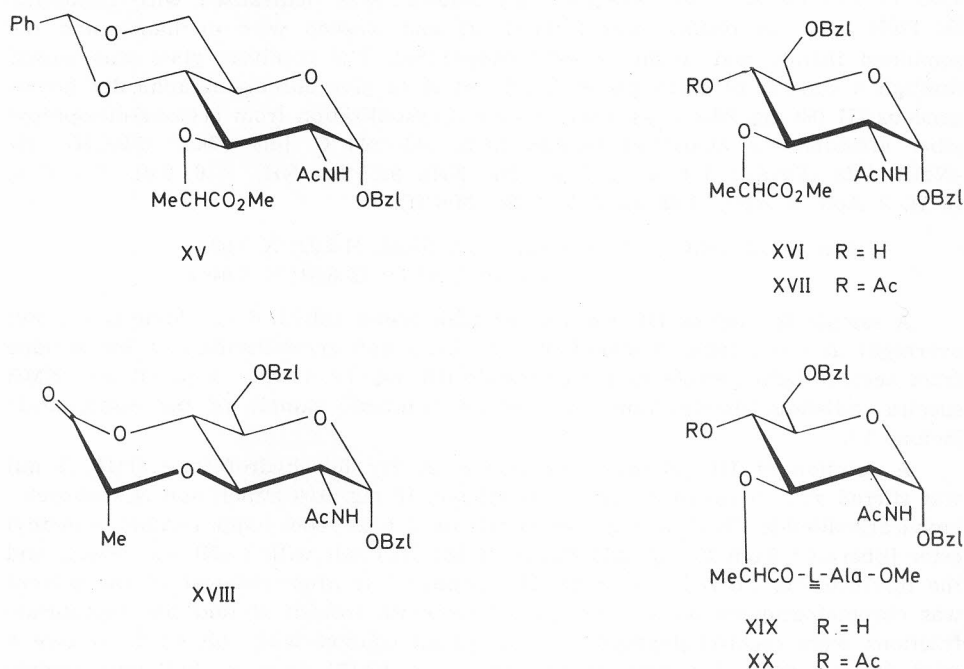


Chart 3

To conclude, the results obtained with different lactones of *N*-acetylmuramic acid derivatives suggest that the aminolysis approach is a promising synthetic route to several peptidoglycan-related structures.

EXPERIMENTAL

General

Melting points were determined in capillaries and are not corrected. Solvents were removed under reduced pressure at < 45 °C. Column chromatography was performed on Silica Gel (Merck, 0.02–0.2 mm) and TLC 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 ethyl acetate-ethanol-water (5:2:1); B chloroform-ethyl acetate-acetone (3:1:3); C ethyl acetate-acetone (4:1); D chloroform-acetone (proportions are given in the text); E chloroform-ethyl acetate (1:1); F chloroform-acetone-light petroleum (3:2:2). Optical rotations were determined for 1% solutions in chloroform, if not stated otherwise. ¹H-NMR spectra (100 MHz, internal Me₄Si) were recorded with a Jeol JNM FX-100 spectrometer for solutions in CDCl₃, if not stated otherwise.

Benzyl 2-acetamido-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (III)

To a stirred solution of the HO-4 unsubstituted disaccharide methyl ester II¹⁶ (109.5 mg, 0.15 mmol) in 1,4-dioxane (5 ml) 0.1 M aq. KOH (1.98 ml) was added under cooling (ice-water bath); the progress of the reaction was monitored by TLC in solvent A. After ~70 min, the solution was neutralised with Amberlite IR 120B (H⁺), the residue was filtered off and washed with methanol, and the combined filtrate and washings were evaporated. The resulting glass was passed through a column of silica gel with solvent A to give chromatographically homogeneous III (90 mg, 83.8%) as a white solid. Crystallisation from acetone-diisopropyl ether afforded the analytical sample, m.p. 242–244 °C, [α]_D + 58.2° (MeOH). ¹H-NMR data (Py-*d*₅): δ 9.44 (d, *J* 8.5 Hz, NH), 9.27 (bs NH), 2.16, 2.04, 2.01 (3 s, 15 H, 2 AcN, 3 AcO), 1.68 (d, *J* 7.08 Hz, MeCH).

Anal. C₃₂H₄₄N₂O₁₆ (712.69) calc'd.: C 53.92; H 6.22; N 3.93%
found: C 53.75; H 6.54; N 4.04%

A sample (25 mg) of III was treated with acetic anhydride-pyridine (1:1, 2 ml) overnight at room temp. Conventional work-up and crystallisation of the residue from acetone-light petroleum gave crystals (18 mg, 74%) with m.p., IR and NMR spectra indistinguishable from those of an authentic sample of the disaccharide lactone I¹⁶.

A solution of III (40 mg, 0.056 mmol) in dry tetrahydrofuran (THF, 3 ml) was stirred with *N*-hydroxysuccinimide (HOSu, 10 mg, 0.09 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC, 18 mg, 0.09 mmol) for 5 h at room temp. *L*-Alanine methyl ester [liberated from 15 mg (0.11 mmol) of the HCl salt with Et₃N] was added, and the mixture was stirred overnight. The residue left after removal of the solvent was chromatographed on a silica gel column with solvent B, and the appropriate fractions were rechromatographed on a second column with solvent C to give a solid (25 mg, 64%), indistinguishable (m.p., IR, NMR) from an authentic sample of the lactone I.

Benzyl-2-acetamido-4-O-acetyl-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (V)

A solution of the fully protected disaccharide methyl ester IV¹⁶ (120 mg, 0.156 mmol) in dioxane was treated with 0.1 M KOH (2.42 ml) under cooling for ~60 min and further processed as described for III to give the crude acid V (112 mg, 95%) as a white solid that was thoroughly dried and directly used in the next step. ¹H-NMR data (Py-*d*₅): δ 9.43 (bs, NH), 9.25 (d, *J* 8.8 Hz, NH), 2.15, 2.14, 2.06, 2.01 (4 s, 18 H, 2 AcN, 4 AcO), 1.63 (d, *J* 7.08 Hz, MeCH); DMSO-*d*₆: δ 7.33 (5 H, Ph), 2.014, 2.010, 1.967, 1.904, 1.811, 1.727 (6 s, 18 H, 2 AcN, 4 AcO), 1.48 (d, *J* 7 Hz, MeCH).

N-{2-O-[Benzyl 2-acetamido-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-4-O-acetyl-2,3-dideoxy- α -D-glucopyranoside-3-yl]-*(R)*-lactoyl}-L-alanine methyl ester (VII)

(a) *By condensation.* — To a stirred solution of V (55 mg, 0.073 mmol) in dry THF (4 ml) HOSu (17.5 mg, 0.15 mmol) and DCC (31 mg, 0.15 mmol) were added at room temp. and, after 5 h, L-alanine methyl ester [liberated from 21 mg (0.15 mmol) of the HCl salt with Et₃N] in THF (1 ml). The mixture was stirred for additional 24 h, the solvent was evaporated, and the residue was chromatographed on a silica gel column with solvent B. Concentration of the appropriate fractions gave crude VII (37 mg, 60%) which was crystallised from acetone-light petroleum to give the pure product (23 mg, 37.5% calc'd. on IV), m. p. 272–274 °C. ¹H-NMR data: δ 7.35 (s, Ph), 6.90 (d, *J* 6.6 Hz, NH), 6.06 (d, *J* 8.55 Hz, NH), 5.85 (d, *J* 9.28 Hz, NH), 5.14, 5.07, 5.04 (3 overlapped t, 3 H, *J* ~9 Hz, H-3',4',4), 4.93 (d, *J*_{1,2} 3.66 Hz, H-1), 3.70 (s, CO₂Me), 2.117, 2.087, 2.027, 2.018, 1.972, 1.871 (6 s, 18 H, 2 AcN, 4 AcO), 1.44 (d, *J* 7.3 Hz, MeCH), 1.32 (d, *J* 6.8 Hz, MeCH).

Anal. C₃₈H₅₃N₃O₁₈ (839.83) calc'd.: C 54.34; H 6.36; N 5.00%
found: C 54.58; H 6.51; N 5.15%

(b) *By aminolysis.* — To the disaccharide lactone I (69.5 mg, 0.1 mmol) in 1,4-dioxane (10 ml) were added imidazole (34 mg, 0.5 mmole), L-alanine methyl ester [liberated from the HCl salt (35 mg, 0.25 mmol) with *N*-methylmorpholine] and tetra-*n*-hexylammonium benzoate (THAB, 24 mg, 0.05 mmol, prepared according lit.²¹⁻²⁸ and dried at 80 °C/0.01 mm for 9h). The reaction mixture was kept under anhydrous conditions at room temp., with occasional stirring, for one week. After removal of the solvent, the residue was chromatographed on silica gel with solvent D (1:3) to give homogenous VI (32 mg, 40%) which crystallised from diisopropyl ether-acetone-light petroleum, m. p. 234–236 °.

Treatment of the above compound with Ac₂O-Py (1:1, 2 ml) at room temp. overnight, followed by conventional work-up, left a residue which was crystallised from acetone-light petroleum to give crystals (24.8 mg, 74%), m. p. 272–274 °C which was indistinguishable (mixed m. p., ¹H-NMR spectrum) from VII obtained by route (a). (Found: C 54.24; H 6.60; N 5.10%).

N-[2-O-(Benzyl 2-acetamido-6-O-acetyl-2,3-dideoxy- α -D-glucopyranoside-3-yl)-*(R)*-lactoyl]-L-alanyl-glycine benzyl ester (X)

A solution of the 6-O-acetyl-lactone IX^{14,16} (102 mg, 0.25 mmol) and imidazole (85 mg, 1.25 mmol) in dry THF (10 ml) was stirred at room temp. for 30 min; a solution of dry L-alanyl glycine benzyl ester trifluoroacetate [prepared from Boc-Ala-Gly-OBzl (124 mg, 0.37 mmol) in THF (1 ml) was treated with *N*-methylmorpholine (41 μ l) and then added to the reaction mixture, followed by THAB (119 mg, 0.25 mmol) in THF (1 ml). The reaction was kept at room temp., with occasional stirring, for 3 days (monitoring of the reaction by TLC in solvent D) and then concentrated. The residue was passed through a column of silica gel with solvent D (1:2.5) to give chromatographically homogeneous X (116 mg, 72%, *R_F* 0.69). Crystallisation from diisopropylether-acetone-light petroleum deposited crystals, m. p. 189–190°, [α]_D + 60°. ¹H-NMR data: δ 7.33 (10 H, 2 Ph), 7.10 (d, *J* 7.9 Hz, NH of MurNAc), 6.91 (bs, NH of Gly), 6.30 (d, *J* 8.5 Hz, NH of Ala), 5.14 (s, 2 H, CO₂CH₂Ph), 4.92 (d, *J*_{1,2} 3.51 Hz, H-1), 3.99 (d, 2 H, *J* 5.57 Hz, collapsed to s on deuteration, CH₂ of Gly), 2.11 (s, 4 H, AcO + HO-4; after deuteration: 3 H), 1.91 (s, AcN), 1.41 and 1.39 (2 d, *J* 7.32 and 6.44 Hz, 2 MeCH).

Anal. C₃₂H₄₁N₃O₁₁ (643.67) calc'd.: C 59.71; H 6.42; N 6.53%
found: C 59.52; H 6.28; N 6.62%

N-[2-*O*-(Benzyl 2-acetamido-2,3-dideoxy-6-*O*-trityl- α -D-glucopyranoside-3-yl)-(R)-lactoyl]-L-alanine methyl ester (XII)

To a solution of 6-*O*-trityl-lactone XI^{14,16} (61 mg, 0.1 mmol) in dioxane (7 ml) were added L-alanine methyl ester [liberated from HCl salt (28 mg, 0.2 mmol) with *N*-methylmorpholine], imidazole (34 mg, 0.5 mmol) and tetra-*n*-butylammonium benzoate²⁸ (TBAB, 182 mg, 0.5 mmol) in a total of 8 ml of dioxane. After 5 days at room temperature, the solvent was evaporated, and the residue passed through a silica gel column with solvent *E* to give chromatographically pure XII (56 mg, 79%) which crystallised from acetone-light petroleum, m. p. 217–218 °C, $[\alpha]_D + 74^\circ$. ¹H-NMR data: δ 7.54–7.25 (m, 20 H, 4 Ph), 6.92 (d, *J* 7.2 Hz, NH of Ala), 6.18 (d, *J* 9 Hz, NH of MurNAc), 4.93 (d, *J*_{1,2} 3.42 Hz, H-1), 3.69 (s, CO₂Me), 3.41–2.33 (m, 2 H, H-6,6'), 1.92 (s, 4 H, AcN + HO-4; after deuteration: 3 H), 1.43 and 1.39 (2 d, *J* 7.08 and 6.84 Hz, 2 MeCH).

Anal. C₄₁H₄₆N₂O₉ (710.79) calc'd.: C 69.28; H 6.52; N 3.94%
found: C 69.38; H 6.79; N 4.15%

N-[2-*O*-(Benzyl 2-acetamido-2,3-dideoxy-6-*O*-trityl- α -D-glucopyranoside-3-yl)-(R)-lactoyl]-L-alanyl-glycine benzyl ester (XIII)

6-*O*-Trityl-lactone XI (182 mg, 0.3 mmol) was treated with L-alanyl-glycine benzyl ester [prepared from Boc-Ala-Gly-OBzl (151 mg, 0.45 mmol) via trifluoroacetate salt] in the presence of imidazole (102 mg, 1.5 mmol) and THAB (143 mg, 0.3 mmol) in dry THF (total 15 ml), exactly as described for X. After 3 days, (monitoring of the reaction in solvent *D* by TLC), the solvent was removed, the residue passed through silica gel with solvent *E*, and the chromatographically homogeneous XIII (152 mg, 60%) crystallised from acetone-light petroleum, m. p. 178–179 °C, $[\alpha]_D + 50^\circ$. ¹H-NMR data: δ 7.49–7.23 (m, 25 H, 5 Ph), 7.03–6.85 (m, 2 H, NH of Gly and MurNAc), 6.16 (d, *J* 7.9 Hz, NH of Ala), 5.11 (s, 2 H, CO₂CH₂Ph), 4.92 (d, *J*_{1,2} 3.22 Hz, H-1), 3.97 (d, 2 H, *J* 5.27 Hz, CH₂ of Gly) 3.42–3.31 (m, 2 H, H-6,6'), 1.92 (s, 4 H, AcN + HO-4), 1.42 and 1.37 (2 d, *J* 7.03 and 6.44 Hz, 2 MeCH).

Anal. C₄₉H₅₃N₃O₁₀ (843.94) calc'd.: C 69.73; H 6.33; N 4.98%
found: C 69.76; H 6.39; N 5.20%

N-[2-*O*-(Benzyl 2-acetamido-4-*O*-acetyl-2,3-dideoxy-6-*O*-trityl- α -D-glucopyranoside-3-yl)-(R)-lactoyl]-L-alanyl-glycine benzyl ester (XIV)

Treatment of XIII (118 mg, 0.14 mmol) with Ac₂O-Py (1:2, 3 ml) for ~20 hrs, followed by conventional work-up and drying of the CHCl₃ extract over anhydrous Na₂SO₄, gave, after removal of the solvent, a glassy mass; trituration with light petroleum yielded XIV (97 mg, 78.2%) as an amorphous powder, m. p. 128–130 °C (softening at 80 °C), $[\alpha]_D + 60^\circ$. ¹H-NMR data: δ 7.5–7.2 (m, 25 H, 5 Ph), 7.0–6.8 (m, 2 H, 2 NH), 5.97 (d, *J* 9.5 Hz, NH), 5.13 (s, CO₂CH₂Ph), 4.97 (d, *J*_{1,2} 2.64 Hz, H-1), 4.85 and 4.54 (2 d, 2 H, *J*_{gem} 11.4 Hz, OCH₂Ph), 4.03 (d, *J* 5.27 Hz, CH₂ of Gly), 3.18–3.05 (m, 2 H H-6,6'), 1.92, 1.75 (2 s, AcO, AcN), 1.45 and 1.27 (2 d, *J* 7.08 and 6.69 Hz, 2 MeCH); (Py-d₅): δ 8.4–8.0 (m, 3 H, 3 NH), 7.7–7.2 (m, 5 Ph), 5.32 (d, *J*_{1,2} 3.66 Hz, H-1), 5.16 (s, CO₂CH₂Ph), 5.20 (dd, *J*_{4,3} 9.03, *J*_{4,5} 9.52 Hz, H-4), 4.24 (d, *J* 5.37 Hz, CH₂ of Gly), 3.41–3.35 (m, 2 H, H-6,6'), 2.04, 1.75 (2 s, AcO, AcN), 1.60 and 1.39 (2 d, *J* 7.08 and 6.84 Hz, 2 MeCH).

Anal. C₅₁H₅₅N₃O₁₁ (885.97) calc'd.: C 69.14; H 6.26; N 4.74%
found: C 69.29; H 6.50; N 4.80%

Benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-[(R)-1-(methoxycarbonyl)ethyl]- α -D-glucopyranoside (XVI)

To a stirred solution of the 4,6-*O*-benzylidene acetal XV (971 mg, 2 mmol) and sodium cyanoborohydride (376 mg, 6 mmol) in dry THF (30 ml) containing 3A molecular sieves (~3 g) hydrogen chloride in dry diethyl ether was added at room temp. until the solution was acidic (pH paper, ~15 min). More cyanoborohydride (3 × 376 mg) followed by HCl/Et₂O were added after 2, 4 and 18 hrs; after a

total reaction time of ~30 h, TLC in solvent *F* indicated complete reaction. The mixture was poured into ice-water, the product was extracted with chloroform, and the combined extracts were washed successively with water, saturated sodium hydrogen carbonate solution and water, and kept overnight over sodium sulphate + ~0.5 g of silica gel. After removal of the solvent, the residue was purified by flash chromatography on a column of silica gel (50 g) with solvent *F* to give XVI (873 mg, 90%) as a glassy oil that crystallised on standing. Crystallisation from diisopropyl ether-acetone-light petroleum afforded pure XVI, m.p. 116–117 °C, $[\alpha]_D + 128^\circ$. $^1\text{H-NMR}$ data: δ 7.51 (bs, NH), 7.31, 7.26 (10 H, 2 Ph), 5.33 (d, $J_{1,2}$ 2.7 Hz, H-1), 3.73 (s, CO_2Me), 2.01 (s, AcN), 1.41 (d, J 7.08 Hz, MeCH).

Anal. $\text{C}_{26}\text{H}_{33}\text{NO}_8$ (487.55) calc'd.: C 64.05; H 6.82; N 2.87%
found: C 64.21; H 6.91; N 2.86%

Benzyl 2-acetamido-4-O-acetyl-6-O-benzyl-2-deoxy-3-O-[(R)-1-(methoxy-carbonyl)ethyl- α -D-glucopyranoside (XVII)

A sample (93 mg, 0.18 mmol) of XVI was treated with $\text{Ac}_2\text{O-Py}$ (1:2, 6 ml) at room temp. overnight, and after conventional work-up, the residue was chromatographed on a silica gel column with solvent *E* to give the 4-acetate XVII (73 mg, 78%) as a chromatographically homogeneous solid. Crystallisation from diisopropyl ether-acetone-light petroleum afforded the analytical sample, m.p. 151–152°, $[\alpha]_D + 147^\circ$. $^1\text{H-NMR}$ data: δ 7.72 (bs, 1H, NH), 7.32 (10 H, 2 Ph), 5.43 (d, $J_{1,2}$ 2.68 Hz, H-1), 5.10 (dd, $J_{4,3}$ 8.79, $J_{4,5}$ 10.3 Hz, H-4), 4.71, 4.50 (2 d, J_{gem} 11.9 Hz, OCH_2Ph), 4.51 (2 H, 6-O- CH_2Ph), 4.23 (q, J 7 Hz, MeCH), 3.76 (s, CO_2Me), 3.50–3.45 (m, 2 H, H-6,6'), 2.02 1.95 (2 s, AcO, AcN), 1.35 (d, J 7.08 Hz, MeCH).

Anal. $\text{C}_{28}\text{H}_{35}\text{NO}_9$ (529.59) calc'd.: C 63.50; H 6.66; N 2.65%
found: C 63.59; H 6.48; N 2.83%

Benzyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-[(R)-1-carboxyethyl]- α -D-glucopyranoside 4,1'-lactone (XVIII)

(a) *From XVI.* — To a stirred solution of XVI (244 mg, 0.5 mmol) in dry dioxane (7 ml) 0.1 M aq. KOH (9 ml) was added at room temp., and after ~45 min (monitoring of the reaction by TLC in solvent *A*) Amberlite IR-120B (H^+); the mixture was further processed as described for III to give the free acid as glass (239 mg) which was dried over P_2O_5 and used for the next step without purification.

A solution of the above acid in dry dioxane (5 ml) was treated with DCC (100 mg, 0.53 mmol) in dioxane (2 ml) for 20 hrs; dicyclohexylurea was filtered off, the filtrate concentrated, and the residue was passed through silica gel with solvent *D* (4:1) to give the lactone XVIII as a viscous oil. Crystallisation from diisopropyl ether-acetone-light petroleum afforded the product (154.4 mg, 72%) as fluffy crystals, m.p. 160–161 °C, $[\alpha]_D + 144^\circ$. $^1\text{H-NMR}$ data: δ 7.33 (10 H, 2 Ph), 5.63 (d, J 9.5 Hz, NH), 5.00 (d, $J_{1,2}$ 3.66 Hz, H-1), 4.67 (q, J 7 Hz, MeCH), 4.75, 4.49 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 4.61 (2 H, 6-O- CH_2Ph), 4.48 (t, J 9.3 Hz, H-4), 4.33 (ddd, $J_{2,3}$ 10.2 Hz, H-2), 3.82 (dd, $J_{3,2}$ 10.2, $J_{3,4}$ 9.2 Hz, H-3), 3.99–3.71 (m, 3 H, H-5, 6,6'), 1.97 (s, AcN), 1.47 (d, J 7.08 Hz, MeCH).

Anal. $\text{C}_{25}\text{H}_{29}\text{NO}_7$ (455.51) calc'd.: C 65.92 H 6.42; N 3.08%
found: C 66.09; H 6.29; N 3.14%

(b) *From VIII.* — A suspension of the HO-6 free lactone VIII (73 mg, 0.2 mmol), freshly prepared silver carbonate (150 mg), benzyl bromide (120 mg, 0.7 mmol) and molecular sieves (~300 mg) in dry benzene (12 ml) was stirred at room temp. for 48 hrs. The precipitate was centrifuged off, washed with benzene, and the combined supernatant and washings were concentrated. The residue was passed through silica gel with solvent *D* (4:1) to give chromatographically homogeneous XVIII (35 mg, 38%); crystallisation from diisopropyl ether-acetone-light petroleum afforded crystals indistinguishable (m.p., NMR) from those of XVIII prepared by route (a). (Found: C 65.65; H 6.65; N 3.27%).

Treatment of a sample (30 mg, 0.066 mmol) of XVIII in dry methanol (8 ml) with silica gel (200 mg) for 2 hrs at room temp. led to complete conversion into the methyl ester XVI (monitoring by TLC in solvent D, 4:1); removal of the solvent and acetylation of the residue (Ac₂O—Py 1:2, 3 ml), followed by silica gel chromatography of the product, gave the 4-acetate XVII (25 mg) in 78% yield.

N-[2-O-(Benzyl 2-acetamido-6-O-benzyl-2,3-dideoxy- α -D-glucopyranoside-3-yl)-(R)lactoyl]-L-alanine methyl ester (XIX)

6-O-Benzyl lactone XVIII (91.7 mg, 0.2 mmol) was treated with L-alanine methyl ester [prepared from Boc-Ala-OMe (83 mg, 0.4 mmol) via trifluoroacetate salt] in the presence of imidazole (68 mg, 1 mmol) and THAB (21.3 mg, 0.04 mmole) in dry THF (total 10 ml) exactly as described for X. After 5 days (monitoring of the reaction by TLC in solvent B), the solvent was evaporated and the residue was chromatographed over a silica gel column with solvent B to give homogenous XIX (84 mg, 75.2%) which crystallised from hot diisopropyl ether + some drops of acetone: m.p. 125–126° (softening at 122°C), $[\alpha]_D + 90^\circ$. ¹H-NMR data: δ 7.34, 7.33 (10 H, 2 Ph), 6.97 (d, *J* 8.79 Hz, NH), 6.10 (d, *J* 8.8 Hz, NH), 4.92 (d, *J*_{1,2} 3.66 Hz, H-1), 4.72, 4.45 (2 d, 2 H, *J*_{gem} 11.7 Hz, O—CH₂Ph), 4.60 (2 H, 6-O—CH₂Ph), 3.70 (s, CO₂Me), 1.90 (s, AcN), 1.41, 1.40 (2 d, *J* 6.84 and 7.08 Hz, 2 MeCH).

Anal. C₂₉H₃₈N₂O₉ (558.61) calc'd.: C 62.35; H 6.86; N 5.01%
found: C 62.22; H 6.82; N 4.98%

N-[2-O-(Benzyl 2-acetamido-4-O-acetyl-6-O-benzyl-2,3-dideoxy- α -D-glucopyranoside-3-yl)-(R)-lactoyl]-L-alanine methyl ester (XX)

A sample (77 mg, 0.14 mmol) of XIX was treated with Ac₂O—Py (2:1, 3 ml) at room temp. overnight. After work-up, the residue was crystallised from diisopropyl ether-acetone to give the 4-acetate XX (66 mg, 80%) as shiny needles; m.p. 146–148°C (softening at 138°C), $[\alpha]_D + 95^\circ$. ¹H-NMR data: δ 7.33 (10 H, 2 Ph), 6.88 (d, *J* 6.5 Hz, NH), 5.88 (d, *J* 10 Hz, NH), 5.05 (dd, *J*_{4,3} 9.7, *J*_{4,5} 10.0 Hz, H-4), 4.88 (d, *J*_{1,2} 3.52 Hz, H-1), 4.53 (2 H, 6-O—CH₂Ph), 3.70 (s, CO₂Me), 3.54–3.45 (m, 2 H, H-6,6'), 1.971, 1.876 (2 s, AcO, AcN), 1.44 and 1.31 (2 d, *J* 7.32 and 6.74 Hz, 2 MeCH).

Anal. C₃₁H₄₀N₂O₁₀ (600.67) calc'd.: C 61.99; H 6.71; N 4.66%
found: C 62.20; H 6.96; N 4.56%

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SAŽETAK

Aminoliza laktona *N*-acetilmuraminske kiseline s esterima aminokiselina i peptida. Sintetski put do derivata *N*-acetilmuramoilamida

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Ispitivana je reakcija aminolize HO-6-supstituiranih laktona *N*-acetilmuraminske kiseline (MurNAc) s esterima aminokiselina i peptida kao nukleofilnom komponentom. Zaštićeni, (1 → 6) povezani disaharid GlcNAc-MurNAc laktan I reagirao je s *L*-alanin-metilesterom dajući odgovarajući disaharidamid VI čija je struktura potvrđena i alternativnom sintezom. Reakcije benzilglikozida HO-6 acetiliranog, benziliranog, odnosno tritiliranog MurNAc laktana (IX, XI, XVIII) s Ala-OME i Ala-Gly-OME ispitivane su pod različitim uvjetima; utvrđeno je da prisutnost imidazola i tetraheksilamonijbenzoata, kao i priroda otapala, bitno utječu na iskorištenja u kojima nastaju odgovarajući muramoilamidi (X, XII, XIII, XIX). Pruženi su dokazi da otvaranje laktonskog prstena teče bez epimerizacije na položaju C-4, tj. uz zadržavanje *D*-gluko-konfiguracije.