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CARBOHYDRATE RESEARCH

Carbohydrate Research 313 (1998) 1–14

Synthesis and characterisation of muramic acid 1′,2-lactam-β-(1→4)-D-glucosamine derivatives related to repeating units of bacterial spore cortex

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Received 18 May 1998; accepted 11 September 1998

Abstract

A procedure for preparing allyl 4,6-*O*-benzylidene-*N*-phthaloyl-muramic acid methyl ester is described. Depending on the reaction conditions, a partial cleavage of the phthaloyl group and formation of the tricyclic allyl 4,6-*O*-benzylidene-muramic acid 1′,2-lactam also occurred; a successful route to the latter was via de-esterification (LiI) of the protected muramic acid ester group, dephthaloylation and cyclisation. This compound was characterised by X-ray structure analysis. Treatment of the protected muramic acid ester with tris(triphenylphosphine)rhodium(I) chloride produced the desired hemiacetal which was then transformed into the trichloroacetimidate donor and coupled with allyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside to provide allyl *O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside. De-esterification followed by removal of the two phthalimido groups and Ac_2O/Py treatment gave allyl *O*-(2-amino-4,6-*O*-benzylidene-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-1′,2-lactam)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside. The conformation analysis of allyl 4,6-*O*-benzylidene-muramic acid δ-lactam and propan-2-onyl glycoside of the protected muramic ester revealed in solid state for both compounds a chair conformation of glucopyranose and 1,3-dioxane rings with *trans* ring junction. The δ-lactam ring of the tricyclic muramyl lactam exhibits a conformation between *sofa* and *half-chair*.

Keywords: Muramic acid; muramic acid 1′,2-lactam derivatives; Bacterial spore cortex repeating units; NMR spectroscopy; X-ray structure; Conformational analysis

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0008-6215/98/$—see front matter
PII S0008-6215(98)00248-1

1. Introduction

It has been widely recognised that the bacterial spore peptidoglycan, so called spore cortex, has a composition different from that of the bacterial vegetative cell [1]. In the spore cortex, a substantial part of the muramic acid residues lack the peptide and *N*-acetyl substituents and instead form an intramolecularly cyclised δ-lactam structure [2,3]. Although the process of spore formation has been studied extensively in recent years by the molecular genetic approach [4], information on the chemistry of cortex and its fragments is still very scarce. We have reported [5,6] previously the synthesis, X-ray and conformational analysis of several muramic acid δ-lactams and their 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-substituted derivatives.

The aim of the present work was to prepare a disaccharide structurally and sequentially related to the cortex fragment, in which a muramic acid δ-lactam structure is β-glycosidically linked to the 4-position of the GlcNAc moiety.

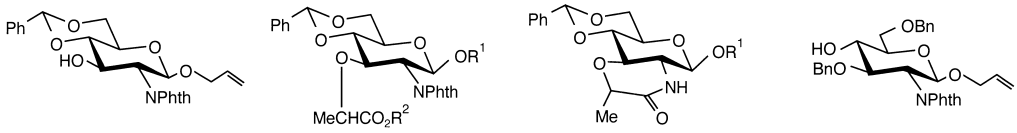
2. Results and discussion

Synthesis.—Attempts to convert a muramic acid δ-lactam derivative into an efficient glycosyl donor were not successful. Alternatively, we prepared 1-*O*-allyl-4,6-*O*-benzylidene-*N*-phthaloylmuramic acid methyl ester (**2**) as a suitably protected intermediate for glycosidation of the glucosamine unit.

As the starting compound for the synthesis of **2**, allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**1**) [7,8] was used. The phthalimido group was chosen for the protection of the amino function because of its activating and β-directing effects during glycosidation. It was found that in 1,4-dioxane using six equivalents of NaH, the introduction of the lactic acid residue at the C-3 of **1** is practically quantitative. During the reaction, a partial phthalimido-ring-opening had also occurred to give a mixture of 2-phthalimido- and 2-phthalamido-3-*O*-lactyl acid derivatives. Treatment of the crude product with Dowex H^+ resin and Ac_2O/Py converted the phthalamido acid substituent into the phthalimido ring without affecting the lactyl acid group. Esterification of the latter with diazomethane afforded pure **2** (73% calcd on **1**). The ^1H NMR data (δ 5.33, $J_{1,2}$ 8.3 Hz) were consistent with the β configuration, and the large $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ values (8.7–10.4 Hz) indicated the $^4C_1$ conformation of the pyranoid ring.

In an earlier experiment of this work two major products were isolated: the phthalimido ester **2** and the MurLactam derivative **3** (~30 and ~20%, respectively). In the latter case, apparently, the open-ring phthalamido derivative has undergone cleavage of the phthaloyl group to give an intermediary amino ester which cyclised to **3**. A characteristic feature of the NMR spectra of muramic acid 1′,2-lactam series is an upfield position of the signals for H-2 and for C-2 [5]. Indeed, in the spectra of **3** the H-2 signal at 3.42 ppm and the ^{13}C signal assigned to C-2 at 57.05 ppm were the most upfield resonances of the molecule. To confirm the tricyclic structure of **3** the X-ray crystallographic analysis was performed. The structure determined correlates to the spectroscopic data.

In order to obtain a higher yield of **3**, dephthaloylation of **2** was investigated. The reaction of **2** with ethanolic hydrazine hydrate was not a clean one; ^1H NMR spectra indicated that both, the lactyl methyl ester group and the allyl aglycon were affected by the reagent. Treatment of **2** with hydrazine acetate which was found [9] to be compatible with the methyl ester group resulted in cyclisation into the lactam ring together with reduction of the



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	R^1	R^2
2	$CH_2CH{=}CH_2$	Me
4	$CH_2CH{=}CH_2$	H
6	H	Me
7	CH_2COCH_3	Me
8	$C(=NH)CCl_3$	Me

	R^1
3	$CH_2CH{=}CH_2$
5	$CH_2CH_2CH_3$

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Scheme 1. Monosaccharide synthesis.

O-allyl double bond to give propyl MurLactam derivative **5** (69%) and the starting **2** (15%). Finally, **2** was de-esterified by using LiI in pyridine as the nucleophyle [10–12]; the liberated *N*-phtaloylmuramic acid derivative **4** was then directly treated with 1,2-diaminoethane [13] and Ac_2O/Py to give the desired lactam **3** (75%).

For the synthesis of the target disaccharide and conversion of **2** into the glycosyl donor, the de-*O*-allylation of **2** was required. Deprotection of **2** with $PdCl_2$ and CuCl [14] was accompanied by the Wacker oxidation process [15–17] involving hydration of the allylic double bond followed by oxidation to a methyl ketone. Thus, treatment of **2** afforded the reducing sugar **6** and the propan-2-onyl (acetonyl) β-glycoside **7** (37 and 40%, respectively). They were resolved by column chromatography; the structure of **7** was confirmed by X-ray structure analysis.

An efficient access to the reducing sugar **6** (73%) was by the selective removal of the allyl group in **2** using tris(triphenylphosphine)rhodium (I) chloride (Wilkinson catalyst) [18] and mercuric ion catalysed hydrolysis [13].

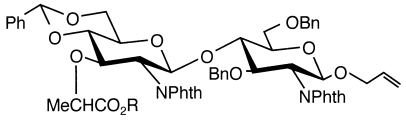
The hemiacetal **6** was transformed with trichloroacetonitrile [19] and diazabicyclo [5.4.0] undecene (DBU) as the base [20] into the trichloroacetimidate **8** (82%). Coupling of **8** with the allyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **9** [8,21,22] was performed in two ways. By using silver triflate as the catalyst [23] the disaccharide **10** (63% yield) was obtained along with unchanged acceptor **9** (21%) and the hemiacetal **6** (13%) formed by decomposition of **8**. Alternatively, the presence of catalytic amounts of silyl trifluoromethanesulphonate (0.3 equivalents) furnished the disaccharide **10** (66%). The β-D-disaccharide structure of **10** was confirmed by its ^{1}H NMR spectra which showed two doublets for anomeric protons at 5.47 and 5.02 ppm with *J* 8.71 and *J* 8.18 Hz and were assigned to the two glucopyranosyl residues of MurNPhth and GlcNPhth moiety, respectively.

Treatment of **10** with LiI, as described for **4** gave the disaccharide acid **11** (77%) which was then directly converted into the desired disaccharide **12** (76%) with 1,2-diaminoethane and Ac_2O/Py. The ^{1}H NMR spectra of **12** revealed the two anomeric protons at δ 4.96 ($J_{1,2}$ 7.9 Hz) and δ 4.48 ($J_{1',2'}$ 8.2 Hz); a high-field signal at δ 3.26 (H-2′) and one acetyl signal at δ 1.87 were consistent with the given structure.

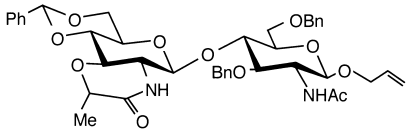
X-ray structure analysis of **3** *and* **7**.—The molecular structures of **3** and **7** with atom numbering are shown in Figs. 1 and 2, respectively. The molecule **3** is shown in ball and stick presentation [24] based on the isotropic refinement (limited number of reflections disabled an anisotropic refinement, see the Experimental section). It crystallizes with two independent molecules (**A** and **B**) in the asymmetric unit. The ORTEP drawing [25] for **7** is prepared with thermal ellipsoids at a 30% probability level. In both compounds D-enantiomers were selected according to the synthesis; the configuration at the anomeric C-1 in the crystal proved to be β.

Values of bond lengths and angles of both conformers of **3** (**A** and **B**) and **7** were not significantly different ($\leq 3\sigma$).

The molecular conformations of **3** and **7** are described by selected torsion angles (Table 1), Cremer–Pople [26] and asymmetry parameters [27] [28] (Table 2). In both conformers of **3** and **7** glucopyranose moieties adopt usual chair $^4C_1$ conformation (i.e. $^OC_3$ [29]). 1,3-Dioxane rings also adopt chair $^7C_5$ i.e. $^6C_{O4}$ conformation (Table 2). δ-lactam rings in both conformers of **3** adopt intermediate conformation between sofa E_3 and half-chair $^OH_3$; displacements from four-atom (C-2,N-2,C-33,C-31) best least-squares planes are for O-3, 0.16 Å (**A**), 0.26 Å (**B**) and for C-3, −0.53 Å (**A**), −0.50 Å (**B**). Ring junctions between glucopyranose and δ-lactam ring in 3 (molecules **A** and **B**) and between glucopyranose and 1,3-dioxane ring [both in **3** (molecules **A** and **B**) and in **7**] are *trans*.



	R
10	Me
11	H



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Scheme 2. Disaccharide synthesis.

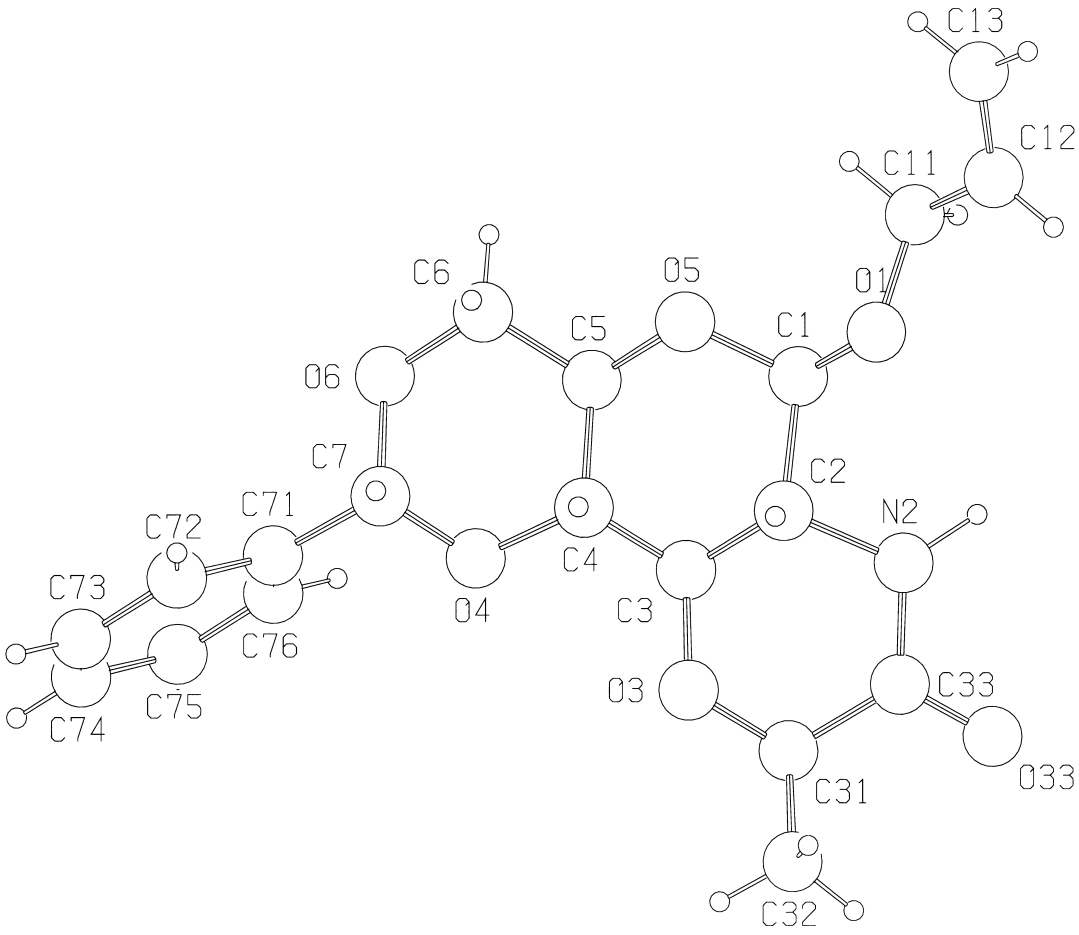

Fig. 1. Molecular structure of **3A** (ball and stick presentation [24]) with atom numbering.

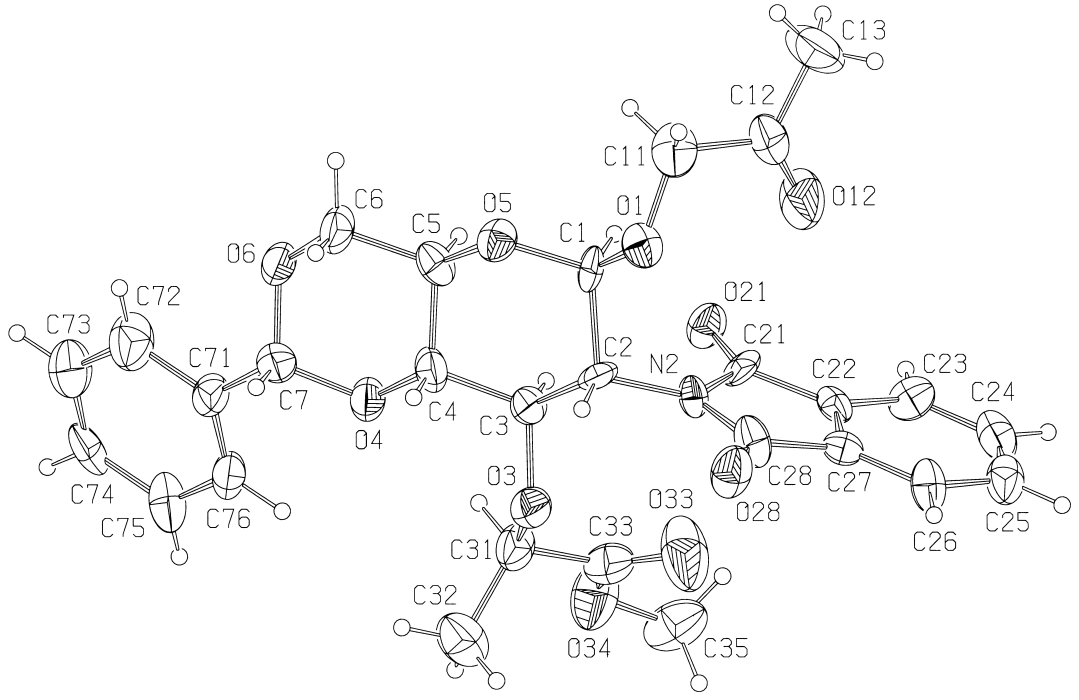

Fig. 2. Molecular structure of **7** (ORTEP drawing [25]) with atom numbering. The thermal ellipsoids are drawn at a 35% probability level.

Table 1
Selected torsion angles (°) for **3** (molecules **A** and **B**) and **7**

	3		7
	A	B	
Glucopyranose ring			
O-5–C-1–C-2–C-3	52(2)	67(3)	52(1)
C-1–C-2–C-3–C-4	−56(2)	−61(2)	−53(1)
C-2–C-3–C-4–C-5	59(3)	62(3)	59(1)
C-3–C-4–C-5–O-5	−65(3)	−65(3)	−65(1)
C-4–C-5–O-5–C-1	63(2)	62(2)	65(1)
C-5–O-5–C-1–C-2	−54(2)	−64(3)	−58(1)
δ-Lactam ring			
N-2–C-2–C-3–O-3	58(2)	58(2)	
C-2–C-3–O-3–C-31	−64(2)	−71(3)	
C-3–O-3–C-31–C-33	42(3)	51(3)	
O-3–C-31–C-33–N-2	−15(4)	−22(3)	
C-31–C-33–N-2–C-2	9(3)	15(3)	
C-33–N-2–C-2–C-3	−31(3)	−31(3)	
1,3-Dioxane ring			
C-4–O-4–C-7–O-6	70(3)	61(3)	61(1)
O-4–C-7–O-6–C-6	−69(3)	−64(3)	−65(1)
C-7–O-6–C-6–C-5	59(3)	57(3)	62(1)
O-6–C-6–C-5–C-4	−53(3)	−59(3)	−55(1)
C-6–C-5–C-4–O-4	50(3)	62(3)	56(1)
C-5–C-4–O-4–C-7	−60(2)	−56(2)	−58(1)

Crystal packing of **3** *and* **7**.—In the crystal structure of **3** an amide lactam nitrogen acts as a donor to two oxygen atoms O-3 and O-4 from molecules **A** and **B** forming a three-centre hydrogen bond [30,31] (Fig. 3 and Table 3). The basic motif includes two **A** and two **B** molecules (a sequence ...**ABAB**...) connected into a spiral along *b*. The crystal packing of **7** is realized via van der Waals interactions, only.

3. Experimental

General methods.—Melting points are uncorrected. Column chromatography was performed on silica gel (Merck 0.040–0.063 mm) and TLC on Silica Gel 60 with detection by charring with 10% H_2SO_4, chlorine–iodine reagent, or ninhydrin, if not stated otherwise. Optical rotations were determined with an Optical Activity LTD automatic AA-10 Polarimeter for solutions in $CHCl_3$. NMR spectra were recorded with a Varian Gemini 300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C for solutions in $CDCl_3$ (internal Me_4Si). Double resonance experiments were performed in order to assist in signal assignment.

*Allyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-2-phthalimido-*β*-*D*-glucopyranoside* (**2**).—To a solution of **1** (656 mg, 1.5 mmol) in dry 1,4-dioxane (8 mL) was added in portions NaH (50% in oil, 435 mg, 9 mmol), and the mixture was stirred under N_2 for 30 min at room temperature and for further 3 h at 85 °C. To the suspension cooled to 70 °C was then added over 1 h a solution of (*S*)-2-chloropropionic acid (310 mg, 2.8 mmol) in 1,4-dioxane (4 mL) and stirring at this temperature was continued for 18 h. Upon cooling, the mixture was diluted with dry THF (30 mL) and Dowex (H^+) resin was added under stirring at ~ 4 °C until all of the solid disappeared and a clear solution (pH ~4) was formed. The resin was removed by filtration, washed with THF, and the combined filtrate was concentrated and co-concentrated with toluene. The remaining white solid was treated at 0 °C with pyridine (5 mL) and Ac_2O (5 mL), the solution was left at room temperature for 24 h and then co-concentrated with toluene. The remaining material was extracted with chloroform (4×20 mL), the extracts were concentrated, and a solution of the residue in 1:1 $CHCl_3$–MeOH (4 mL) was treated at 0 °C with an ethereal diazomethane solution. After standing overnight in a refrigerator, excess of diazomethane was destroyed with a few drops of AcOH, and the solution was concentrated. Column chromatography (19:1 benzene–EtOAc) of the residue afforded **2**, isolated as a colourless syrup (573 mg, 73%), $[\alpha]_D$ +20° (*c* 1.2); R_f 0.45 (19:1 benzene–EtOAc), 0.65 (5:1 toluene–EtOAc); ^1H NMR ($CDCl_3$): δ 7.89–7.75 (m, 4 H, Phth), 7.48–7.37 (m, 5 H, Ph), 5.77–5.64 (m, 1 H, $CH_2CH=CH_2$), 5.59 (s, 1 H, PhC*H*), 5.33 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.14 and 5.04 (2 m, each 1 H, $CH_2CH=CH_2$), 4.43 (dd, 1 H, $J_{3,4}$ 8.7 Hz, H-3), 4.40 (dd, 1 H, $J_{6a,6b}$ 10.2 Hz, H-6a), 4.40 (q, $J_{CH,Me}$ 6.8 Hz, α-C*H* Lact), 4.29 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 4.27 and 4.03 (2 m, each 1 H, $CH_2CH=CH_2$), 3.85 (t, 1 H, $J_{6b,5}$ $J_{6b,6a}$ 10.2 Hz, H6-b), 3.76 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 3.66–3.58 (m, 1 H, $J_{5,6a}$ 4.9 Hz, H-5), 3.23 (s, 3 H, CO_2Me Lact), 1.28 (d, 3 H, $J_{Me,CH}$ 6.8 Hz, *Me* Lact). ^{13}C NMR ($CDCl_3$): δ 173.3 (*C*O Lact; 167.8 (*C*O Phth); 137.1–123.5 (aromatic *C* and $CH_2CH=CH_2$); 117.5 ($CH_2CH=CH_2$); 101.2 (PhCH); 97.9 (C-1); 83.1 (C-4); 75.5, 74.1, 70.0, 68.6, 65.6 (C-3, 5, 6, α-*C*H Lact and $CH_2CH=CH_2$); 55.3 (C-2); 51.3 (CO_2Me Lact); 18.7 (*Me*CH Lact). Anal. Calcd for $C_{28}H_{29}NO_9$: C, 64.24; H, 5.58; N, 2.67. Found: C, 64.54; H, 5.73; N, 2.95.

Table 2
Ring conformation analysis[a] for **3** (molecules **A** and **B**) and **7**

Glucopyranose ring

Comp.	Asymmetry parameter (°)	$w<\lvert$t.a.$\rvert>$ (°)	Q(Å)	Θ (°)	Φ (°)	Conform.
3A	$\Delta C_s(C-1)=\Delta C_s(C4)=5(2)$ $\Delta C_s(C-2)=\Delta C_s(C5)=4(2)$ $\Delta C_s(C-3)=\Delta C_s(O5)=9(2)$ $\Delta C_2(C-1-C-2)=\Delta C_2(C-4-C-5)=3(2)$ $\Delta C_2(C-2-C-3)=\Delta C_2(C-5-O-5)=9(3)$ $\Delta C_2(C-3-C-4)=\Delta C_2(O-5-C-1)=10(2)$	57.3(9)	0.58(2)	7(3)	281(21)	${}^{O}C_3={}^{4}C_1$
3B	$\Delta C_s(C-1)=\Delta C_s(C-4)=3(2)$ $\Delta C_s(C-2)=\Delta C_s(C-5)=4(2)$ $\Delta C_s(C-3)=\Delta C_s(O-5)=2(2)$ $\Delta C_2(C-1-C-2)=\Delta C_2(C-4-C-5)=2(3)$ $\Delta C_2(C-2-C-3)=\Delta C_2(C-5-O-5)=4(3)$ $\Delta C_2(C-3-C-4)=\Delta C_2(O-5-C-1)=5(3)$	63(1)	0.65(3)	4(2)	143(29)	${}^{O}C_3={}^{4}C_1$
7	$\Delta C_s(C-1-)=\Delta C_s(C-4)=9(1)$ $\Delta C_s(C-2)=\Delta C_s(C-5)=1(1)$ $\Delta C_s(C-3)=\Delta C_s(O-5)=9(1)$ $\Delta C_2(C-1-C-2)=\Delta C_2(C-4-C-5)=6(1)$ $\Delta C_2(C-2-C-3)=\Delta C_2(C-5-O-5)=7(1)$ $\Delta C_2(C-3-C-4)=\Delta C_2(O-5-C-1)=13(1)$	59.1(5)	0.60(1)	8(1)	269(8)	${}^{O}C_3={}^{4}C_1$

δ-lactam ring

Comp.	Asymmetry parameter (°) $\Delta C_s(C-3)$ (°) $=\Delta C_s(C-33)$(°)	 $\Delta C_2(C-3-O-3)$(°) $=\Delta C_2(N-2-C-33)$ (°)	$w\langle\lvert$t.a.$\rvert\rangle$ (°)	Q(Å)	Θ (°)	Φ (°)	Conform.
3A	8(2)	16(3)	45(1)	0.474(2)	42(3)	50(4)	$E_3\rightarrow{}^{O}H_3$
3B	14(2)	8(3)	44(1)	0.52(3)	40(3)	45(5)	${}^{O}H_3/E_3$
3A	$\Delta C_s(O-4)=\Delta C_s(C-6)=9(2)$ $\Delta C_s(C-4)=\Delta C_s(O-6)=13(2)$ $\Delta C_s(C-5)=\Delta C_s(C-7)=2(2)$ $\Delta C_2(O-4-C-4)=\Delta C_2(C-6-O-6)=18(3)$ $\Delta C_2(C-4-C-5)=\Delta C_2(O-6-C-7)=9(3)$ $\Delta C_2(C-5-C-6)=\Delta C_2(C-7-O-4)=9(3)$		60(1)	0.61(3)	11(3)	295(15)	${}^{6}C_{O4}={}^{7}C_5$
3B	$\Delta C_s(O-4)=\Delta C_s(C-6)=3(2)$ $\Delta C_s(C-4)=\Delta C_s(O-6)=5(2)$ $\Delta C_s(C-5)=\Delta C_s(C-7)=3(2)$ $\Delta C_2(O-4-C-4)=\Delta C_2(C-6-O-6)=4(3)$ $\Delta C_2(C-4-C-5)=\Delta C_2(O-6-C-7)=4(3)$ $\Delta C_2(C-5-C-6)=\Delta C_2(C-7-O-4)=7(3)$		59(1)	0.58(3)	0(3)	120(45)	${}^{6}C_{O4}={}^{7}C_5$
7	$\Delta C_s(O-4)=\Delta C_s(C-6)=7(1)$ $\Delta C_s(C-4)=\Delta C_s(O-6)=4(1)$ $\Delta C_s(C-5)=\Delta C_s(C-7)=3(1)$ $\Delta C_2(O-4-C-4)=\Delta C_2(C-6-O-6)=8(1)$ $\Delta C_2(C-4-C-5)=\Delta C_2(O-6-C-7)=2(1)$ $\Delta C_2(C-5-C-6)=\Delta C_2(C-7-O-4)=6(1)$		59.6(5)	0.59(1)	2(1)	251(30)	${}^{6}C_{O4}={}^{7}C_5$

[a] $w<\lvert$t.a.$\rvert>$ is weighted average of ring torsion angles absolute values: Q, Θ and Φ are Cremer–Pople parameters for atom sequences: O-5–C-1–C-2–C-3–C-4–C-5 (glucopyranose ring), O-3–C-3–C-2–N-2–C-33–C-31 (δ-lactam ring) and O-4–C-4–C-5–C-6–O-6–C-7 (1,3-dioxane ring); calculated by PLATON [24].

*Allyl 2-amino-4,6-*O*-benzylidene-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-*β*-*D*-glucopyranoside 1′,2-lactam (1-*O*-allyl-4,6-*O*-benzylidene-muramic acid 1′,2-lactam)* (**3**).—(**a**) The reaction of **1** (437 mg, 1 mmol) with (*S*)-2-chloropropionic acid (206 mg, 1.9 mmol) was performed as described above. After treatment with Dowex (H^+) resin and concentration of the filtrate, the residue was eluted from a column of silica gel with 20:2:1 $CHCl_3$–MeOH–AcOH. Fractions containing H_2SO_4 and peptide positive components ($R_f\approx0.8$–0.4) were combined, concentrated and treated with diazomethane as described above. TLC (19:1 benzene–EtOAc, and 2:1 toluene–EtOAc) of the crude product showed

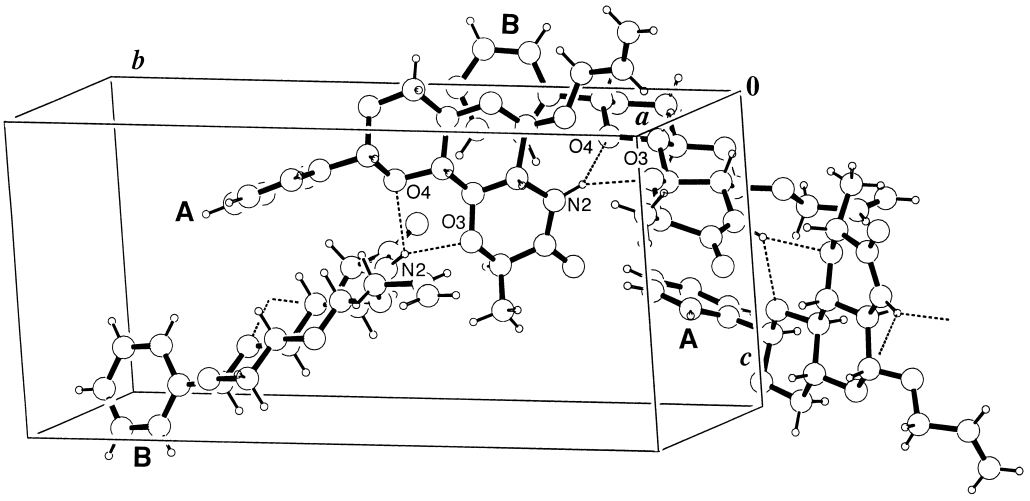

Fig. 3. Crystal packing of **3**.

Table 3
Hydrogen bond geometry in the crystal packing of **3**

	D···A (Å)	D-H (Å)	H···A (Å)	D-H···A (°)	Symmetry operation on *A*
N-2**A**–H···O-3**B**	3.03(3)	1.01(2)	2.14(2)	147(1)	x−1, y, z
N-2**A**–H···O-4**B**	3.33(3)	1.01(2)	2.54(2)	136(1)	x−1, y, z
N-2**B**–H···O-3**A**	3.09(3)	1.01(2)	2.20(2)	147(1)	−x+1, y+1/2−1, −z+1
N-2**B**–H···O-4**A**	3.24(2)	1.01(2)	2.49(2)	131(1)	−x+1, y+1/2−1, −z+1

Two molecules in the asymmetric unit: **A** and **B**.

two major components (R_f 0.5 and 0.05, and 0.8 and 0.4, respectively). Column chromatography in 5:1 toluene–EtOAc afforded the methyl ester **2** (168 mg, 32%) as a syrup; further elution with pure EtOAc yielded the lactam **3** (90 mg, 25%) as a white solid which was crystallised from EtOAc–light petroleum, mp 186–188° (softening at 183 °C), $[\alpha]_D$ −49° (*c* 0.9); ^{1}H NMR ($CDCl_3$): δ 7.50–7.36 (m, 5 H, Ph), 6.07 (bs, 1 H, disappeared on H–D exchange, NH), 5.95–5.84 (m, 1 H, $CH_2CH=CH_2$), 5.58 (s, 1 H, PhC*H*), 5.35–5.26 (m, 2 H, $CH_2CH=CH_2$), 4.48 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 4.39 and 4.10 (2 m, each 1 H, $CH_2CH=CH_2$), 4.36 (dd, 1H, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.31 (q, 1 H, $J_{CH,Me}$ 6.8 Hz, α-C*H* Lact), 3.87 (t, 1 H, $J_{6b,5}$ 10.3 Hz, H6-b), 3.82 (t, 1 H, $J_{4,5}$ 9.0 Hz, H-4), 3.77 (t, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 3.59–3.51 (m, 1 H, $J_{5,6a}$ 4.9 Hz, H-5), 3.42 (t, 1 H, $J_{2,3}$ 8.9 Hz, H-2), 1.51 (d, 3 H, $J_{Me,CH}$ 6.8 Hz, *Me* Lact). ^{13}C NMR ($CDCl_3$): δ 171.0 (*C*O Lactam); 136.6, 129.2, 128.2, 126.2 (aromatic *C*); 132.6 ($CH_2CH=CH_2$); 118.8 ($CH_2CH=CH_2$); 101.9 (Ph*C*H); 99.8 (C-1); 78.2 (C-4); 75.0, 74.6, 70.4, 68.4, 67.9 (C-3, 5, 6, α-*C*H Lact and $CH_2CH=CH_2$); 57.05 (C-2); 17.40 (*Me*CH Lact). Anal. Calcd for $C_{19}H_{23}NO_6$: C, 63.15; H, 6.41; N, 3.87. Found: C, 63.22; H, 6.52; N, 3.80.

(**b**) A solution of **2** (200 mg, 0.38 mmol) in dry pyridine (6 mL) containing granulated 4 Å molecular sieves (0.4 g) was stirred for 1 h at room temperature under N_2. Anhydrous LiI (820 mg, 6.1 mmol) was added and the mixture was stirred at 105 °C for 24 h under N_2. TLC (9:1 $CHCl_3$–MeOH), detection: $Ce(SO_4)_2$ showed a complete conversion of **2** (R_f 0.9) into a new product (R_f 0.35). After cooling, the mixture was co-concentrated with toluene (3×), the residue was taken in $CHCl_3$–water (2:1, 40 mL), and the aqueous layer was acidified with 2.5 M HCl to pH 3. The product was extracted with $CHCl_3$ (4×), the combined extracts washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (9:1 $CHCl_3$–MeOH) of the residue afforded the acid **4** as a syrup (149 mg, 77%) that was used directly in the next step; ^{1}H NMR ($CDCl_3$) δ 7.8–7.5 (m, 4 H, Phth), 7.5–7.3 (m, 5 H, Ph), 5.76–5.63 (m, 1 H, $CH_2CH=CH_2$), 5.60 (s, 1 H, PhC*H*), 5.36 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.16–5.03 (m, 2 H, $CH_2CH=CH_2$), 4.42 (dd, $J_{6a,5}$ 5.1, $J_{6a,6b}$ 10.3 Hz, H-6a) 4.41 (dd, $J_{3,4}$ 8.6 Hz, H-3), 4.32 (q, $J_{CH,Me}$ 6.9 Hz, α-C*H* Lact), 4.29 (dd, $J_{2,3}$ 9.8 Hz, H-2), 4.27 and 4.05 (2 m, each 1 H, $CH_2CH=CH_2$), 3.85 (t, 1 H, $J_{6b,5}$ $J_{6b,6a}$ 10.25 Hz, H-6b), 3.77 (t, 1 H, $J_{4,3}$ 8.7, $J_{4,5}$

9.5 Hz, H-4), 3.69–3.60 (m, 1H, H-5), 1.21 (d, 3H, $J_{Me,CH}$ 6.8 Hz, *Me* Lact).

A solution of **4** (130 mg, 0.25 mmol) in *n*-butanol (14 mL) was treated with 1,2-diaminoethane (1 mL, 15 mmol) for 20 h at 90 °C under N_2. After cooling and co-concentration with toluene (4×), the residue was treated at 4 °C with 1:1 Ac_2O–pyridine (5 mL), and the solution was left overnight at room temperature. Concentration and co-distillation with toluene left a solid that was extracted with 1:1 toluene–EtOAc (4×10 mL). The extracts were concentrated, and the residue was fractionated on a silica gel column with 1:1 toluene–EtOAc to give the lactam **3** (69 mg, 75%) as a crystalline solid which physical and NMR data were indistinguishable from those of **3** obtained under (**a**).

Propyl 2-amino-4,6-O-*benzylidene-3*-O-*[(*R*)-1-carboxyethyl]-2-deoxy*-β-D-*glucopyranoside 1′,2-lactam* (**5**).—A solution of **2** (94 mg, 0.18 mmol) and hydrazine acetate (714 mg, 7.8 mmol) in dry MeOH (20 mL) was refluxed under stirring for 36 h. The solution was concentrated, the residue partioned between water and $CHCl_3$, the organic layer was washed with water, dried and concentrated. Column chromatography (3:2 toluene–EtOAc) of the residue gave, first, the unreacted **2** (R_f 0.8, 14 mg, 15%) and then the propyl lactam **5** (R_f 0.25, 45 mg, 69%). Crystallisation of **5** from 1:10 EtOAc–light petroleum gave material with mp 172–174 °C, $[\alpha]_D$ −45° (*c* 1), ^{1}H NMR ($CDCl_3$): δ 7.5–7.3 (m, 5 H, Ph), 6.02 (bs, 1 H, NH), 5.58 (s, 1 H, PhC*H*), 4.43 (d, $J_{1,2}$ 8.14 Hz, H-1), 4.35 (dd, $J_{6a,5}$ 5.0, $J_{6a,6b}$ 10.3 Hz, H-6a), 4.31 (q, $J_{CH,Me}$ 6.7 Hz, α-C*H* Lact), 3.87 (t, $J_{6b,5}$ 10.3 Hz, H-6b), 3.85 (t, $J_{4,3}$ 9.2 Hz, H-4), 3.84–3.65 (m, 1H, 1/2 $OCH_2CH_2CH_3$), 3.77 (t, $J_{3,2}$ 9.5 Hz, H-3), 3.59–3.51 (m, 1H, $J_{5,4}$ 9.9 Hz, H-5) 3.51–3.44 (m, 1H, 1/2 $OCH_2CH_2CH_3$), 3.39 (t, 1 H, H-2), 1.70–1.59 (m, 2 H, $OCH_2CH_2CH_3$), 1.51 (d, 3 H, $J_{Me,CH}$ 6.7 Hz, *Me* Lact), 0.95 (t, 3 H, *J* 7.4 Hz, $OCH_2CH_2CH_3$). Anal. Calcd for $C_{19}H_{25}NO_6$: C, 62.80; H, 6.93; N, 3.85. Found: C, 63.00; H, 7.19; N, 4.02.

4,6-O-*Benzylidene-2-deoxy-3*-O-*[(*R*)-1-(methoxycarbonyl)ethyl]-2-phthalimido*-β-D-*glucopyranose* (**6**).—Procedure (**a**) (with Wilkinson catalyst): A solution of **2** (200 mg, 0.38 mmol), tris-(triphenylphosphine)rhodium (I) chloride (92.5 mg, 0.1 mmol) and 1,8-diazabicyclo [2.2.2] octane (Dabco, 18 mg, 0.16 mmol) in 10:5:1.25 ethanol–benzene–water (16.5 mL) was refluxed for 30 h (monitoring by TLC in 19:1 benzene–EtOAc). The solvent was removed, the residue was dissolved in acetone (5 mL) containing mercuric oxide (2 mg, 0.009 mmol) and to this mercuric chloride (515 mg, 1.9 mmol) was added in 9:1 acetone-H_2O (10 mL) and the mixture was stirred for 2 h at room temperature. Following solvent evaporation, the residue was extracted with $CHCl_3$ and the combined extracts were washed with 30% aq KI followed by water and dried (Na_2SO_4). Evaporation left a syrup which was purified by column chromatography with 10:1 benzene–acetone to give **6** (136 mg, 74%) as a shiny foam. Crystallisation from EtOAc–petroleum ether afforded crystals mp 170–172 °C, $[\alpha]_D$ +31° (*c* 1), ^{1}H NMR ($CDCl_3$ and $CDCl_3$–D_2O): δ 7.89–7.73 (m, 4 H, Phth), 7.47–7.37 (m, 5 H, Ph), 5.60 (s, 1 H, PhC*H*), 5.53 (t, 1 H, $J_{1,2}$∼$J_{1,OH}$ 8.4 Hz; changes to doublet on H–D exchange, H-1), 4.50 (dd, 1 H, $J_{3,4}$ 8.6 Hz, H-3), 4.40 (dd, 1 H, $J_{6a,6b}$ 10.1 Hz, H-6a), 4.40 (q, 1H, $J_{CH,Me}$ 6.8 Hz, α-C*H* Lact), 4.19 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 3.83 (t, $J_{6b,5}$ 10.1 Hz, H-6b), 3.77 (t, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 3.72–3.64 (m, 1 H, $J_{5,6a}$ 4.7 Hz, H-5), 3.24 (s, 3 H, CO_2Me Lact), 1.29 (d, 3 H, $J_{Me,CH}$ 6.8 Hz, *Me* Lact). Anal. Calcd for $C_{25}H_{25}NO_9$: C, 62.10; H, 5.21; N, 2.90. Found: C, 62.25; H, 5.30, N, 3.0.

Procedure (**b**) *(with $PdCl_2$-CuCl)*. To a solution of **2** (143 mg, 0.27 mmol) was added $PdCl_2$ (50 mg, 0.28 mmol) and CuCl (28 mg, 0.28 mmol), and through the stirred suspension oxygen was bubbled at room temperature for 4 h.; TLC (1:1 ethyl acetate–petroleum ether) then showed complete disappearance of the starting material (R_f 0.8) and the presence of two overlapping spots (R_f ∼0.3). The mixture was diluted with 1:1 ether–EtOAc, filtered through Celite, and the filtrate concentrated to a residue which was extracted with chloroform. The combined extracts were washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (10:1 benzene–acetone) of the residue separated the majority of the two products. Crystallisation of the slower moving product from EtOAc–petroleum ether afforded **6** (49 mg, 37%) which mp, $[\alpha]_D$ and NMR data were indistinguishable from those of **6** obtained under procedure (**a**). Found: C, 62.19; H, 5.31; N, 2.65. Crystallisation of the faster moving product (EtOAc–petroleum ether) gave propan-2-onyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-2-phthalimido-β-D-glucopyranoside (**7**) (59 mg, 40%); mp 146–147 °C; $[\alpha]_D$ −1° (*c* 1.5); ^{1}H NMR ($CDCl_3$): δ 7.90–7.74 (m, 4 H, Phth), 7.47–7.26 (m, 5 H, Ph), 5.60 (s, 1 H, PhC*H*), 5.28 (d, $J_{1,2}$ 8.4 Hz, H-1), 4.50

(dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.40 (q, 1 H, $J_{CH,Me}$ 6.8 Hz, α-C*H* Lact), 4.38 (dd, 1 H, $J_{6a,6b}$ 10.4 Hz, H-6a), 4.33 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 4.18 (2 d, 2 H, ABq, *J* 17.1 Hz, OCH_2COCH_3), 3.82 (t, 1 H, $J_{6b,5}$ 10.2 Hz, H-6b), 3.76 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 3.65–3.57 (m, 1 H, $J_{5,6a}$ 4.8 Hz, H-5), 3.24 (s, 3H, CO_2Me Lact), 2.02 (s, 3 H, OCH_2COCH_3), 1.29 (d, 3 H, $J_{Me,CH}$ 6.8 Hz, *Me* Lact). Anal. Calcd for $C_{28}H_{29}NO_{10}$: C, 62.33; H, 5.42; N, 2.60. Found: C, 62.48; H, 5.49; N, 2.50.

4,6-O-*Benzylidene-2-deoxy-3*-O-*[(*R*)-1-(methoxycarbonyl)ethyl]-2-phthalimido*-β-D-*glucopyranosyl trichloroacetimidate* (**8**).—To a stirred solution of hemiacetal **6** (160 mg, 0.33 mmol) in dichloromethane (5 mL) were added trichloroacetonitrile (460 μL, 4.6 mmol) and DBU (42 μL, 0.28 mmol) at 0 °C under N_2. The mixture was stirred for 2 h at room temperature when TLC (1:1 EtOAc–petroleum ether) indicated the disappearance of **6** (R_f 0.35) and presence of a new compound (R_f 0.80). Column cromatography (2:1 petroleum ether–EtOAc) of the partially concentrated solution afforded **8** as an amorphous mass (170.8 mg, 82%), ^{1}H NMR ($CDCl_3$): δ 8.62 (s, 1 H, NH), 7.89–7.72 (m, 4 H, Phth), 7.48–7.38 (m, 5 H, Ph), 6.59 (d, $J_{1,2}$ 8.71 Hz, H-1), 5.62 (s, 1 H, PhC*H*), 4.57–4.38 (m, 4 H, H-3, -6a, -2, α-C*H* Lact), 3.89–3.30 (m, 3 H, H-6b, -4, -5), 3.25 (s, 3 H, CO_2Me Lact), 1.30 (d, 3 H, $J_{Me,CH}$ 6.7 Hz, *Me* Lact). Anal. Calcd for $C_{27}H_{25}Cl_3N_2O_9$: C, 51.65; H, 4.01; N, 4.46. Found: C, 51.40; H, 4.26; N, 4.76.

Allyl O-*(4,6*-O-*benzylidene-2-deoxy-3*-O-*[(*R*)-1-(methoxycarbonyl)ethyl]-2-phthalimido*-β-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**10**).—(**a**) A mixture of imidate **8** (163 mg, 0.26 mmol), allyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside [8, 21,22] **9** (106 mg, 0.2 mmol) and AgOTf (67 mg, 0.26 mmol) was dried in high vacuo; the flask was then opened to N_2, dry CH_2Cl_2 (5 mL) was injected through a septum, and the reaction was stirred at room temperature in the dark for 48 h. The mixture was diluted with CH_2Cl_2 filtered through Celite, and the combined filtrate and washings were concentrated. Column chromatography (5:1 toluene–EtOAc) of the residue afforded **10** (125 mg, 63%) as a colourless syrup, TLC (5:1 toluene–EtOAc): R_f 0.60; recoveries of the acceptor **9** and the hemiacetal **6** (eluted with 1:1 toluene–EtOAc) were 21 and 13% respectively. Compound **10** had $[\alpha]_D$ +21 (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.96–7.66 (m, 8 H, 2×Phth), 7.43–6.90 (m, 15 H, 3×Ph), 5.68–5.54 (m, 1 H, CH_2–C*H*=CH_2), 5.48 (s, 1 H, PhC*H*), 5.47 (d, 1 H, $J_{1,2}$ 8.71 Hz, H-1′), 5.04–4.92 (2m, each 1 H, CH_2–CH=CH_2), 5.02 (d, 1 H, $J_{1,2}$ 8.18 Hz, H-1), 4.81 and 4.50 (2 d, ABq, 2H, *J* 12.35 Hz, PhCH_2), 4.55 and 4.48 (2d, ABq, 2H, *J* 11.93 Hz, PhCH_2), 4.43 (dd, 1H, $J_{3,4}$ 8.7 Hz, $J_{3,2}$ 10.39 Hz, H-3′), 4.39 (q, $J_{CH,Me}$ 7.0 Hz, α-C*H* Lact), 4.27 (dd, 1H, $J_{3,4}$ 8.4 Hz, $J_{3,2}$ 10.39 Hz, H-3), 4.24 (dd, 1 H, H-2), 4.22 (dd, 1 H, $J_{6a,5}$ 5 Hz, $J_{6a,6b}$ 10.4 Hz, H-6′a), 4.20 and 3.89 (2m, each 1 H, CH_2–CH=CH_2), 4.19 (t, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 4.16 (dd, 1 H, H-2′), 3.67 (t, 1 H, $J_{4,5}$ 9.3 Hz, H-4′), 3.53 (t, 1 H, $J_{6b,5}$ 10.4 Hz, H-6′b), 3.58–3.36 (m, 4 H, H-6a, H-6′b, H-5, H-5′), 3.25 (s, 3 H, CO_2Me Lact), 1.27 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, *Me* Lact). Calcd for $C_{56}H_{54}N_2O_{15}$: C, 67.59; H, 5.47; N, 2.81. Found: C, 67.76; H, 5.58; N, 2.85.

(**b**) To a stirred mixture of **9** (106 mg, 0.2 mmol) and powdered molecular sieves (200 mg) in dry CH_2Cl_2 (4 mL) cooled to 0 °C, was added, under N_2, a 0.1 M solution of Me_3SiOTf in dry CH_2Cl_2 (600 μL) followed by a solution of **8** (163 mg, 0.26 mmol) in CH_2Cl_2 (4 mL), and stirring was continued at room temperature overnight. The mixture was neutralised with Et_3N and further processed as described above; column chromatography (twice) gave pure **10** (131 mg, 66%) indistinguishable (R_f, ^{1}H NMR) from that obtained under (**a**).

Allyl O-*(2-amino-4,6*-O-*benzylidene-3*-O-*[(*R*)-1-carboxyethyl]-2-deoxy*-β-D-*glucopyranosyl 1′,2′-lactam)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-β-D-*glucopyranoside* (**12**).—The disaccharide **10** (150 mg, 0.15 mmol) was treated with anhydrous LiI (450 mg, 3.4 mmol) in dry pyridine (6 mL) containing granular molecular sieves at 107 °C for 20 h under N_2 as described for **4**. Evaporation and co-evaporation in vacuo, dissolution of the residue in 2:1 $CHCl_3$–water, acidification of the aqueous layer with 0.1 M HCl to pH 3, extraction of the liberated acid with $CHCl_3$ (4×), followed by drying (Na_2SO_4) and removal of the solvent, left a syrup that was purified by column chromatography (6:1 toluene–2-propanol), yielding the acid **11** (114 mg, 77%), TLC (6:1 toluene–2-propanol): R_f 0.40 as an amorphous mass that was used directly in the next step. Compound **11**, ^{1}H NMR ($CDCl_3$): δ 7.9 and 7.7 (2m, 8 H, 2×Phth), 7.4–6.9 (m, 15 H, 3×Ph), 5.62–5.54 (m, 1 H, CH_2–C*H*=CH_2), 5.49 (s, 1 H, PhC*H*), 5.48 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1′), 5.03, 5.00, 4.96, 4.93 (4m, 2 H, CH_2–CH=CH_2), 5.01 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.81 and 4.49 (2d, ABq, *J* 12.6 Hz, PhCH_2O), 4.53 and 4.47 (2d, ABq, 2 H, *J* 12.0 Hz,

PhCH_2), 4.41 (t, 1 H, $J_{3,4}$ 8.9 Hz, $J_{3,2}$ 10 Hz, H-3′), 4.39 (q, 1 H, $J_{CH,Me}$ 6.9 Hz, α-C*H* Lact), 4.3–4.0 (m, 6 H, H-2, 3, 4 H-2′, 6′a, 1/2 CH_2–CH = CH_2), 3.92–3.86 (m, 1 H, 1/2 CH_2-CH = CH_2), 3.66 (t, 1 H, $J_{4,5}$ 9 Hz, H-4′), 3.53 (t, 1 H, $J_{6b,5} = J_{6b,6a}$ 10.2 Hz, H-6′b), 3.56–3.30 (m, 4 H, H-5, 6a, 6b, H-5′), 1.27 (d, 3H, $J_{Me,CH}$ 6.9 Hz, *Me* Lact).

A stirred solution of **11** (97 mg, 0.1 mmol) in *n*-butanol (15 mL) was treated with ethylenediamine (1 mL) at 100 °C for 20 h under N_2. After evaporation and co-evaporation with toluene (4×), the residue was dissolved in 1:1 Ac_2O–pyridine at 4 °C and left at room temperature overnight. Excess Ac_2O was destroyed by 96% ethanol, and the solution concentrated to a syrup which was purified by column chromatography with 10:10:1 toluene–EtOAc–MeOH to give **12** (R_f 0.45, 57 mg, 76%) as a solid, mp 240–245 °C (decomp., softening at 190 °C), $[\alpha]_D$ +13° (*c* 0.9); ^{1}H NMR ($CDCl_3$): δ 7.47–7.30 (m, 15 H, 3×Ph), 6.45 (bs, 1 H, NH′), 5.94–5.81 (m, 1 H, CH_2–C*H* = CH_2), 5.61 (d, 1 H, $J_{NH,2}$ 7.4 Hz, NH), 5.46 (s, 1 H, PhC*H*), 5.29–5.17 (m, 2 H, CH_2–CH = CH_2), 4.96 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.84, 4.77, 4.59, 4.55 (4d, 4 H, ABq, $J \sim 12$ Hz, 2×PhCH_2), 4.48 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1′), 4.34–4.18 (m, 3H, H6′a, α-C*H* Lact,

Table 4
Crystal data and summary of experimental details for **3** and **7**

Compound	**3**	**7**
Molecular formula	$C_{19}H_{23}NO_6$	$C_{28}H_{29}NO_{10}$
M_r	361.39	539.54
Data collection		
Difractometer	Enraf–Nonius CAD4*F*, rot.anode	Enraf–Nonius CAD4
Radiation, λ(Å)	MoK_α, 0.71073	Cu$K\alpha$, 1.54184
T/(K)	154	295(3)
No. of reflections for cell determination	25	15
θ Range for cell determination (°)		11–21
θ Range for intensity measurements (°)	2.03–24.97	2.38–74.33
hkl Range	−11,11; −23,0; −11,0	0,9; −15,0; 0,24
Scan	ω/2Θ	ω/2Θ
Δω		0.73 + 0.31tnΘ
No. of measured reflections	3552	2133
No. of symm. independent reflections	3336	2109
No. of symm. ind. refl. with $F_o > 4\sigma(F_o)$	1289	665
Crystal data		
Solvent	EtOAc–petroleum ether	EtOAc–petroleum ether
Crystal size (mm)		0.072×0.036×0.324
Crystal colour	Colourless	Colourless
a (Å)	9.5278	8.435(1)
b (Å)	20.0326	14.044(2)
c (Å)	9.7031	22.649(2)
β (°)	97.058	90.00
V (Å^3)	1837.96	2682.8(6)
Crystal class	Monoclinic	Orthorhombic
Space group	$P2_1$	$P2_12_12_1$
Z	4	4
D_x (g cm^{-3})	1.3060	1.3356(3)
μ (cm^{-1})	0.9 (MoK_α)	8.2 (CuK_α)
F (000)	768	1136
Solution and refinement		
Programs used	HELENA,SHELXS86,SHELXL93	HELENA,SHELXS86,SHELXL93
No. of parameters	184	331
Minimisation function	$\Sigma w(\lvert F_o^2\rvert - \lvert F_c^2\rvert)^2$ $w = 1.0$	$\Sigma w(\lvert F_o^2\rvert - \lvert F_c^2\rvert)^2$ $w = q/[\sigma^2(F_o^2) + (aP)^2 + bP]$ $q = 1.0$, $a = 0.0622$, $b = 0.02$ $p = [\max(F_o^2) + 2F_c^2]/3$
$R(F)$za$F_o > 4\sigma(F_o)$	0.1703 (R_1)	0.0529 (R_1)
$R(F)$ all reflections	0.3412 (R_1)	0.3412 (R_1)
$wR(F^2)$ all reflections	0.4471 (wR_2)	0.1526 (wR_2)
S	1.635 all reflections	0.797 all reflections
$(\Delta/\sigma)_{max}$	—	< 0.389
$\Delta\rho_{max}$ (eÅ^{-3}), $\Delta\rho_{min}(eA^{-3})$	1.01, −0.82	0.21, −0.23

1/2 OCH_2–$CH=CH_2$), 4.12–4.06 (m, 2 H, H-6a, 1/2 OCH_2–$CH=CH_2$), 4.02 (t, 1 H, $J_{4,3}$ 8.7, $J_{4,5}$ 9.5 Hz, H-4), 3.82–3.51 (m, 6 H, H-2, 3, 6b, H-4′, 5′, 6′b), 3.46 (t, $J_{3,2}$ 9.5, $J_{3,4}$ 9.2 Hz, H-3′), 3.26 (t, 1 H, H-2′), 3.20 (m, 1 H, H-5), 1.87 (s, 3 H, NAc), 1.49 (d, 3 H, $J_{Me,CH}$ 6.9 Hz, *Me* Lact). Anal. Calcd for $C_{41}H_{48}N_2O_{11}$: C, 66.11; H, 6.49; N, 3.76. Found: C, 66.21; H, 6.28; N, 3.56.

Table 5
Final atomic coordinates and isotropic thermal parameters of non-hydrogen atoms for **3** (molecules **A** and **B**)

Atom	*x*	*y*	*z*	U_{iso} (Å^2)
Molecule **A**				
O-1	0.0745(16)	0.2711(11)	0.0854(16)	0.023(4)
O-3	0.2154(16)	0.4098(12)	0.4628(16)	0.022(4)
O-4	0.2773(17)	0.5135(11)	0.2659(16)	0.025(4)
O-5	0.1505(15)	0.37483(0)	0.0340(15)	0.017(4)
O-6	0.2767(18)	0.5461(13)	0.0353(18)	0.033(5)
O-33	−0.0292(19)	0.2910(13)	0.5719(19)	0.040(5)
N-2	0.0295(19)	0.3044(13)	0.3554(19)	0.021(5)
C-1	0.060(3)	0.3363(15)	0.118(2)	0.021(6)
C-2	0.129(2)	0.3450(15)	0.273(2)	0.019(6)
C-3	0.156(2)	0.4121(14)	0.321(2)	0.009(5)
C-4	0.243(2)	0.4430(14)	0.230(2)	0.015(5)
C-5	0.168(3)	0.4420(14)	0.081(2)	0.020(6)
C-6	0.246(3)	0.4796(16)	−0.018(3)	0.033(7)
C-7	0.364(3)	0.5395(16)	0.177(3)	0.032(7)
C-11	−0.005(3)	0.2524(16)	−0.044(3)	0.032(7)
C-12	0.033(3)	0.1805(17)	−0.067(3)	0.038(8)
C-13	0.077(3)	0.159(2)	−0.190(3)	0.061(10)
C-31	0.121(2)	0.3826(14)	0.550(2)	0.013(5)
C-32	0.207(3)	0.3689(17)	0.682(3)	0.040(8)
C-33	0.040(3)	0.3228(15)	0.495(3)	0.023(6)
C-71	0.3943(16)	0.6123(10)	0.2227(15)	0.014(5)
C-72	0.5329(14)	0.6352(11)	0.2339(17)	0.032(7)
C-73	0.5654(13)	0.6991(11)	0.2838(18)	0.044(8)
C-74	0.4592(17)	0.7401(10)	0.3226(17)	0.024(6)
C-75	0.3206(15)	0.7172(11)	0.3114(17)	0.045(8)
C-76	0.2881(12)	0.6533(11)	0.2615(17)	0.030(7)
Molecule **B**				
O-1	0.5464(17)	0.0202(12)	0.3752(17)	0.030(5)
O-3	0.9178(16)	0.1640(12)	0.2988(15)	0.021(4)
O-4	0.7105(17)	0.2641(12)	0.1969(17)	0.024(4)
O-5	0.4861(16)	0.1194(12)	0.2604(16)	0.022(4)
O-6	0.4702(16)	0.2886(12)	0.1273(16)	0.022(4)
O-33	1.0476(18)	0.0394(12)	0.5488(17)	0.032(5)
N-2	0.8241(19)	0.0546(13)	0.4386(19)	0.015(5)
C-1	0.582(2)	0.0895(14)	0.368(2)	0.011(5)
C-2	0.727(2)	0.0880(15)	0.330(3)	0.022(6)
C-3	0.780(2)	0.1636(15)	0.326(2)	0.015(5)
C-4	0.671(2)	0.1930(14)	0.211(2)	0.009(5)
C-5	0.531(2)	0.1891(15)	0.249(3)	0.019(6)
C-6	0.423(3)	0.2195(15)	0.138(3)	0.027(7)
C-7	0.601(2)	0.2905(16)	0.097(3)	0.026(6)
C-11	0.420(3)	0.0049(16)	0.447(3)	0.037(8)
C-12	0.411(3)	−0.0726(16)	0.457(3)	0.029(7)
C-13	0.295(3)	−0.0990(18)	0.435(3)	0.040(8)
C-31	1.007(3)	0.1410(16)	0.412(3)	0.027(7)
C-32	1.162(2)	0.1379(15)	0.374(2)	0.022(6)
C-33	0.958(2)	0.0705(15)	0.470(2)	0.017(6)
C-71	0.6468(16)	0.3668(10)	0.0794(16)	0.012(5)
C-72	0.7126(17)	0.4017(11)	0.1932(12)	0.035(7)
C-73	0.7522(16)	0.4678(11)	0.1786(13)	0.026(6)
C-74	0.7261(18)	0.4991(10)	0.0501(17)	0.037(8)
C-75	0.6603(18)	0.4642(12)	−0.0637(12)	0.037(7)
C-76	0.6206(17)	0.3981(11)	−0.0491(13)	0.044(8)

X-ray structure determination of **3** *and* **7**. The crystals for X-ray analysis were grown from EtOAc–petroleum ether (b.p. 50–60 °C) for both **3** and **7** at 4 °C for several weeks. Numerous attempts to get good quality crystals of **3** were not successful. Only very tiny plates were obtained. The crystal data and summary of experimental details are listed in Table 4. The X-ray intensity data were collected with an Enraf–Nonius CAD4*F* diffractometer using graphite-monochromatised Mo*Kα* radiation and rotating anode for **3** and Enraf–Nonius CAD4 diffractometer using graphite-monochromatised Cu*Kα* radiation for **7** [32]. There were no significant variations in intensity for the standard reflections. The data were corrected for Lorentz and polarisation effects using program HELENA [33]. The structures were solved by SHELXS-86 [34] and refined by full-matrix least-squares minimisation by SHELXL-93 [35] using F^2 values. Due to poor ratio of reflections per variable, **3** was refined isotropicaly, only. Hydrogen atoms were generated on the stereochemical grounds and refined riding on their respective carbon atoms. The O–H and N–H bond distances were normalised to the values obtained by neutron diffraction (O–H 0.983 Å and N–H 1.009 Å). Details of the refinement procedure are given in the Table 4. During the structure determinations, the D enantiomers were selected according to the assignment *R* at C-5; chirality on C-31 (*δ*-lactam residue) proved to be *R*. The molecular geometries were calculated by program PLATON [24] and ORTEP

Table 6
Final atomic coordinates and equivalent isotropic thermal parameters of non-hydrogen atoms for **7**

Atom	*x*	*y*	*z*	U_{eq} (Å^2)[a]
O-1	0.4469(9)	0.3595(6)	0.2535(4)	0.060(4)
O-3	0.5678(11)	0.3616(6)	0.0496(3)	0.068(4)
O-4	0.4097(10)	0.5411(6)	0.0423(3)	0.064(4)
O-5	0.3478(9)	0.4768(5)	0.1968(3)	0.058(3)
O-6	0.2540(10)	0.6578(6)	0.0870(3)	0.065(4)
O-12	0.7357(12)	0.3558(9)	0.3051(4)	0.113(5)
O-21	0.8558(10)	0.4335(6)	0.1728(3)	0.062(3)
O-28	0.5624(12)	0.1602(6)	0.1744(4)	0.078(4)
O-33	0.8832(15)	0.3130(10)	0.0508(4)	0.151(7)
O-34	0.9319(14)	0.4083(8)	−0.0259(5)	0.117(6)
N-2	0.6766(12)	0.3108(7)	0.1672(3)	0.046(4)
C-1	0.4869(13)	0.4203(9)	0.2075(5)	0.048(5)
C-2	0.5355(14)	0.3622(9)	0.1525(5)	0.054(6)
C-3	0.5494(16)	0.4262(9)	0.0989(5)	0.056(5)
C-4	0.3937(15)	0.4832(10)	0.0931(5)	0.066(6)
C-5	0.3709(15)	0.5406(9)	0.1487(5)	0.056(5)
C-6	0.2279(17)	0.6036(9)	0.1390(5)	0.077(7)
C-7	0.2697(18)	0.5992(9)	0.0371(5)	0.069(6)
C-11	0.4605(16)	0.3962(11)	0.3121(5)	0.081(7)
C-12	0.624(2)	0.3788(11)	0.3362(6)	0.076(7)
C-13	0.6390(17)	0.3967(11)	0.3995(5)	0.121(9)
C-21	0.8280(17)	0.3517(9)	0.1771(5)	0.053(5)
C-22	0.9290(11)	0.2720(5)	0.1909(3)	0.047(5)
C-23	1.0899(11)	0.2695(6)	0.2042(3)	0.065(6)
C-24	1.1619(8)	0.1837(9)	0.2188(3)	0.080(7)
C-25	1.0732(12)	0.1003(6)	0.2201(3)	0.072(6)
C-26	0.9123(12)	0.1028(5)	0.2068(3)	0.061(6)
C-27	0.8403(7)	0.1886(7)	0.1922(3)	0.047(5)
C-28	0.6743(18)	0.2129(10)	0.1784(6)	0.068(6)
C-31	0.669(2)	0.4015(10)	0.0038(6)	0.068(6)
C-32	0.6078(17)	0.3665(11)	−0.0551(6)	0.109(8)
C-33	0.835(3)	0.3667(13)	0.0150(7)	0.086(8)
C-35	1.0936(17)	0.3776(9)	−0.0268(6)	0.106(8)
C-71	0.2898(12)	0.6599(7)	−0.0168(3)	0.065(6)
C-72	0.2099(11)	0.7463(9)	−0.0194(4)	0.097(8)
C-73	0.2283(12)	0.8052(6)	−0.0683(5)	0.105(8)
C-74	0.3266(13)	0.7776(7)	−0.1145(4)	0.076(7)
C-75	0.4066(10)	0.6912(9)	−0.1118(3)	0.084(7)
C-76	0.3881(10)	0.6323(5)	−0.0629(5)	0.066(6)

[a] $U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a_i} \cdot \mathbf{a_j}$

plot for **7** was prepared by program ORTEP [25]. Ball and stick molecular structure presentation and packing diagrams for **3** were prepared by program PLUTON [24]. The final atomic coordinates and equivalent isotropic thermal parameters for **3** and **7** are listed in the Tables 5 and 6, respectively. Calculations were performed on Silicon Graphics INDIGO2 workstation of the Laboratory for chemical and biological crystallography, Department of Physical Chemistry, Ruđer Bošković Institute, Zagreb, Croatia.

4. Supplementary material

Tables of atomic coordinates, thermal parameters, bond lengths, and bond angles for **3** and **7** have been deposited with the Cambridge Crystallographic Data Centre. These tables may be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

Acknowledgements

This work was supported by Ministry of Science and Technology of Republic of Croatia grants 00980704 and 00980608. The authors thank Professor Dr. Jan Kroon, Head, Department of Crystal and Structural Chemistry, Bijvoet Centre for Biomolecular Research, Utrecht University, for enabling the use of high-intensity X-ray beam suitable for data collection and his valuable comments.

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