Carbohydrate Research, 241 (1993) 131–152
Elsevier Science Publishers B.V., Amsterdam

Synthesis and conformational analysis of muramic acid δ-lactam structures and their 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl) derivatives, characteristic of bacterial spore peptidoglycan

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(Received May 29th, 1992; accepted September 2nd, 1992)

ABSTRACT

1,6-Anhydro-4-*O*-benzyl-β-muramic acid 1′,2-lactam (**2**) was prepared by reduction of 1,6-anhydro-2-azido-4-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-1-methoxycarbonylethyl]-β-D-glucopyranose (**1**) followed by cyclisation. Debenzylation of **2** (→ **3**) and glycosylation of HO-4 with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride afforded 75% of a β-(1 → 4)-linked disaccharide derivative (**7**). Removal of the Phth group from **7**, then acetylation, and *O*-deacetylation yielded 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-amino-1,6-anhydro-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1′,2-lactam (**10**) Acetolysis of the 1,6-anhydro ring in the 4-acetate (**4**) of **3** and the 3′,4′,6′-triacetate (**9**) of **10**, with saponification of the products **5** and **11**, afforded 2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose 1′,2-lactam (**6**) and 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1′,2-lactam (**12**), respectively. The structure of **12** corresponds to that of the disaccharide unit characteristic of the glycan chains of bacterial spore peptidoglycan. ^{1}H NMR spectroscopy indicated that the β-D-glucopyranose ring in the 1,6-anhydro 1′,2-lactam derivatives adopts the $B_{O,3}$ conformation. On cleavage of the 1,6-anhydro ring by acetolysis, the D-glucopyranose ring adopts the $^4C_1$ conformation. X-ray analysis of **2**, **4**, and **5** confirmed the proposed structures. Molecular mechanics and molecular dynamics simulations were used to follow the transformation of the $B_{O,3}$ conformation of the D-glucopyranose ring via transition states to the $^4C_1$ form.

INTRODUCTION

Spore formation in bacteria serves as a strategy for survival; the multistage transformation process that occurs within the mother cell has been explored extensively by the molecular genetic approach[1,2]. Studies which involved the relationship between the dormancy and resistance properties of the bacterial spore and its structure have shown, inter alia, that the peptidoglycan of the spore has a

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composition different from that of the vegetative cell. Evidence has been presented[3,4] that a substantial part of the muramic acid residues in the glycan chains of the former lack the peptide and *N*-acetyl substituents and instead form a δ-lactam ring that involves the 2-amino and 3-*O*-lactyl carboxyl groups of the sugar residue. The muramic acid δ-lactam residue has not been detected in the cell walls of vegetative bacteria and is considered to be a unique spore component present in all spore peptidoglycans[1,2].

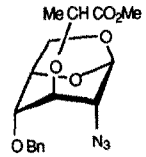
In contrast to the wealth of information on the chemistry of (1 → 4)-linked β-D-GlcNAc–MurNAc derivatives, little is known about the disaccharide units that contain the muramic acid lactam residue. The latter compound was obtained by conventional acetylation of muramic acid[5] and by *N*-deacetylation of the protected MurNAc, followed by condensation of the amino group liberated with the lactyl carboxyl[6]. In order to avoid the severe basic conditions required for *N*-deacetylation of 2-acetamido-2-deoxy sugars and ensure unambiguous glycosylation at HO-4, the known 1,6-anhydro-2-azidomuramic acid ester derivative[7] **1** was chosen as the starting material for the synthesis of muramic acid lactams.

RESULTS AND DISCUSSION

Synthesis.—Reduction of the azido group of 1,6-anhydro-2-azido-4-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-1-methoxycarbonylethyl]-β-D-glucopyranose[7] (**1**) with the $NiCl_2$–H_3BO_3–$NaBH_4$ system[8] gave the corresponding 2-amino-2-deoxy sugar which underwent, spontaneously (monitoring by TLC), intramolecular cyclisation with the ester group to give a δ-lactam ring. The product **2** was isolated (~ 80%) as a highly crystalline compound for which the ^1H NMR data (see Table I and the section on NMR spectroscopy) indicated that the β-D-glucopyranose ring had a flattened $B_{O,3}$ conformation. The structure of **2** was confirmed by X-ray analysis.

Catalytic hydrogenation of **2** furnished **3** with HO-4 unsubstituted, conventional acetylation of which afforded the 4-acetate **4**. The ^1H NMR spectra of **3** and **4** indicated the $B_{O,3}$ conformations in solution, and the same conformation for **4** in the crystal was shown by X-ray analysis. The 1,6-anhydro ring of **4** was opened by acid-catalysed acetolysis (10% trifluoroacetic acid in acetic anhydride) at room temperature to give a ~ 3:1 α,β-mixture of the known[5,6] 1,4,6-tri-*O*-acetylmuramic acid 1′,2-lactam (**5**). The ^1H NMR spectrum and X-ray analysis of **5** indicated the glucopyranose ring to be in the $^4C_1$ conformation in solution and in the solid state, respectively. *O*-Deacetylation (Zemplén) of **5** left the δ-lactam ring unaffected and gave the muramic acid lactam **6** as a ~ 1:1 α,β-mixture in practically quantitative yield.

Glycosylation of HO-4 of **3** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride in dichloromethane, using silver triflate as the catalyst, in the absence of a base[9], at ambient temperature, and in the presence of molecular sieves, gave the β-(1 → 4)-linked disaccharide derivative **7** (75% after column chromatography). The $B_{O,3}$ conformation of the β-D-glucopyranose ring in **7** was



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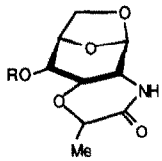
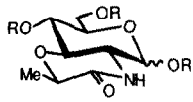
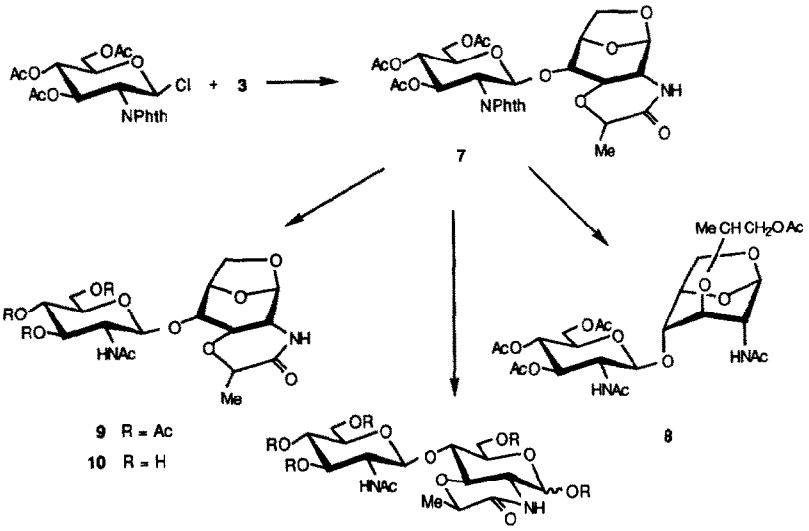


2 R = Bn
3 R = H
4 R = Ac



5 R = Ac
6 R = H



7

9 R = Ac
10 R = H

8

11 R = Ac
12 R = H

confirmed by NMR spectroscopy. Attempted removal of the phthalimido group in **7** by the reductive method[10,11] using $NaBH_4$–2-propanol–acetic acid caused only partial reduction and also opening of the δ-lactam ring to give, after *N*,*O*-acetylation, acetylated 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1,6-anhydro-2-deoxy-3-*O*-[(*R*)-1-hydroxyprop-2-yl]-β-D-glucopyranose (**8**) in which the β-D-glucopyranose ring had the conventional $^1C_4$ conformation. The phthalimido group in **7** was removed without affecting the δ-lactam ring, by sequential treatment with methanolic sodium methoxide, ethanolic hydrazine hydrate, and acetylation. The ^1H NMR spectrum of the resulting crystalline disaccharide derivative **9** was consistent with the $B_{O,3}$ conformation of the β-D-glucopyranose ring. Saponification of **9** afforded the disaccharide **10** with retention of the conformation.

Opening of the 1,6-anhydro ring in **9**, performed essentially as described for the synthesis of **5**, proceeded without cleavage of the glycosidic bond but at a considerably lower rate. The resulting acetylated (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose–muramic acid δ-lactam (**11**) was obtained as a ~3:1 α,β-mixture. Saponification of **11** afforded **12**. As expected, the ^{1}H NMR spectra of **11** and **12** were consistent with the $^4C_1$ conformation for each sugar moiety. Thus, the structure of **12** corresponds to that established[3,4] for the disaccharide unit that occurs in the glycan chains of the bacterial spore peptidoglycan.

NMR spectroscopy.—The ^{1}H NMR spectra (Table I) of the compounds (**2–4**, **7**, **9**, and **10**) that contained a 1,6-anhydro-β-D-muramic acid 1′,2-lactam residue contained signals (dd or d) for H-2,3,4 with large (~9 Hz) $J_{2,3}$ and $J_{3,4}$ values and low (~0 Hz) $J_{1,2}$ and $J_{4,5}$ values. This finding is in contrast to the general pattern for most 1,6-anhydro-β-D-glucopyranose derivatives, the signals of H-2,3,4 of which are broad singlets, in agreement with their equatorial positions in a $^1C_4$ conformation of the glucopyranose ring[12,13]. Thus, the data obtained point to a *trans*-axial arrangement of H-2,3,4 and indicate the D-glucopyranose ring to be in the $B_{O,3}$ conformation. This conformation has been reported for 3-amino-1,6-anhydro-3-deoxy-β-D-glucopyranose[14] and the D-glucopyranose ring in acetylated 1,6-anhydro-3,2′ : 3′,4′-di-*O*-cyclohexylidene-β-lactose[15], postulated for the 2,4-ammonium derivatives of levoglucosan[16], and confirmed by X-ray analysis of 1,6-anhydro-2-chloro-2,4-dideoxy-4-*O*-(diphenylphosphoryl)-β-D-glucopyranose[17].

In the present example, steric hindrance and additional rigidity of the 1,6-anhydro-D-glucopyranose structure, imposed by the δ-lactam ring, seems to be essential for the $^1C_4 \rightarrow B_{O,3}$ transition. This inference was confirmed by converting the lactam derivative **7** into the lactam-free disaccharide derivative **8**. The ^{1}H NMR spectrum of **8** showed the signals for H-1 to H-5 of the 1,6-anhydro sugar residue as broad singlets with no measurable coupling constants. Furthermore, in the spectra of compounds belonging to the 1,6-anhydro-β-muramic acid 1′,2-lactam series, the signals for H-6*endo* are shifted substantially upfield as compared to those of 1,6-anhydro-β-D-glucopyranose derivatives in the $^1C_4$ conformation; a strong downfield shift (~0.6 ppm) of the H-6*endo* signal in the spectrum of **8** corroborates the assigned $^1C_4$ conformation.

The change of the $B_{O,3}$ to the $^4C_1$ conformation, by opening of the 1,6-anhydro ring in **4** and **9**, was reflected clearly by the magnitude of the *J* values and the chemical shifts of the ^{1}H and ^{13}C resonances for the resulting acetylated muramic acid lactam derivatives **5** and **11**, respectively. The ^{1}H NMR spectra of **5** and the *O*-deacetylated derivative **6** contained two, and those of **11** and **12** contained four, signals for anomeric protons, integration of which allowed evaluation of the α,β-ratio. Glycosylation at C-4 caused, in each series, deshielding of H-4 and shielding of H-3,5. As expected, the chemical shift data and *J* values for the 4-linked GlcNPhth and GlcNAc residues (in **7** and **9–12**, respectively) are typical for the β-D-*gluco* configuration in the $^4C_1$ conformation. The general pattern of the ^{1}H NMR spectra of muramic acid 1′,2-lactam derivatives (**5**, **6**, **11**, and **12**) is

similar to that exhibited by the α and β anomers of *N*-acetylmuramic acid derivatives. A distinct difference between the two groups of compounds is the upfield position of the H-2 signal for the muramic acid lactam structures, apparently due to the involvement of the 2-amino group in the lactam ring.

The ^{13}C NMR data (see Experimental) provided additional support for the structures assigned. A characteristic feature of the spectra of the 1,6-anhydro-β-D-muramic acid 1′,2-lactam series is a downfield shift of the signal for C-3 and an upfield shift of that for C-2, due to the etherification of HO-3 and substitution of HO-2 by NH_2, respectively. The site of benzyl and acetyl substitution in **2** and **4** was evident from the downfield shifts for the signal for C-4 (6.7 and 1.9 ppm) as compared to that of **3** with HO-4 unsubstituted and from the upfield shifts for the signals of C-3 (2.3 and 2.5 ppm) and C-5 (0.6 and 4.1 ppm), respectively. Furthermore, glycosylation of **3** resulted in a considerable downfield shift (6.0 and 6.5 ppm) of the C-4 signal in the disaccharide derivatives **7** and **9**, as compared to the value for the C-4 resonance of the parent $B_{O,3}$ conformer. Opening of the 1,6-anhydro ring and conversion into the $^4C_1$ conformation was reflected mostly in an upfield shift ($\sim$ 10 ppm) of the C-1 signal and a downfield ($\sim$ 6 ppm) of that of C-6. As with the ^1H NMR spectra, the general pattern of ^{13}C NMR spectra of muramic acid 1′,2-lactam series in the $^4C_1$ conformation is similar to that for MurNAc and its derivatives.

X-ray structure analysis of **2**, **4**, *and* **5**.—The structures of **2**, **4**, and **5** with atom numbering are shown in Figs. 1–3. The ORTEP plots[18] are drawn with thermal ellipsoids at 30% probability level for **2** and 50% for **4** and **5**. Selected bond lengths and angles for **4** are listed in Table II. Values of bond lengths and angles of 1,6-anhydro-4-*O*-benzyl-β-muramic acid 1′,2-lactam (**2**) and both conformers of its 4-acetate (**4**) were not significantly different ($\leq 3\sigma$). Thus, only the molecular geometry of **4** is listed. The dioxabicyclo[3.2.1]octane skeleton of 1,6-anhydro-β-D-glucopyranose has restricted flexibility and is strained, which affects particularly the bond angles C-4–C-5–C-6 and C-1–O-5–C-5 and those of the five-membered 1,6-anhydro ring (Table II). The bond angle at O-5 has been reduced to 100.2(4)° [average value for **2** and **4** (A, B)] as observed[19,20] for 1,6-anhydro-β-D-glucopyranose and its 3-amino derivative [102.0(2)°]. The variation in C–O bond lengths in the acetal moiety (anhydro ring) C-5–O-5–C-1–O-6–C-6 are within the range of 3σ. The shortening of two inner bonds observed in other analogous structures[21] was not detected in **2** and **4**. For the α anomer of **5** with no 1,6-anhydro ring, the values of bond lengths and angles are within ranges reported for other carbohydrates[22,23]. The differences in the lengths of the endocyclic and anomeric exocyclic C–O bonds are in the range of 3σ. Thus, in **5**α, with the C-1 substituent axial, the anomeric effect is not pronounced.

The conformations of **2**, **4** (conformers A and B), and **5** are described by selected torsion angles (Table III) and asymmetry parameters[24] (Table IV). The conformations of **2** and **4** are shown in a superposition diagram (Fig. 4). Both **2** and **4**, each of which has a 1,6-anhydroglucose residue, have distorted $B_{O,3}$ conforma-

TABLE I

300-MHz ^{1}H NMR data [a] for muramic acid 1′,2-lactam mono- (**2**–**6**) and di-saccharide (**7**–**12**) derivatives [b]

Compound [c]	Chemical shifts (δ)										
	H-1	H-2	H-3	H-4	H-5	H-6*exo*	H-6*endo*	NH	CH	Me	Ac
2	5.26 s	3.24 d	3.67 dd	3.44 d	4.56 dd	3.81 dd	3.59 dd	7.6 bs	4.23 q	1.46 d	
3	5.32 s	3.20 d	3.50 dd	3.60 d	4.53 dd	3.88 dd	3.71 dd	7.8 s	4.31 q	1.47 d	
4	5.31 s	3.33 d	3.72 t	4.56 d	4.49 dd	3.91 dd	3.87 dd	7.5 s	4.32 q	1.48 d	2.182
5 α	6.23 d	3.79 dd	3.93 t	5.20 t	4.1–4.0 m	4.27 dd	4.10 dd	6.4 bs	4.26 q	1.47 d	2.180, 2.128
β	5.56 d	3.56 t	3.66 t		3.8–3.7 m			7.1 bs			2.093
6 [d] α	5.19 d	~3.3 [e]	3.77 t	3.57 t	3.8–3.7 m	3.74 dd	3.86 dd	[f]	4.24 q	1.44 d	
β	4.60 d	3.09 t	3.47 t	3.54 t	3.4–3.3 m	3.71 dd			4.22 q	1.43 d	
7	5.18 s	3.20 d	3.61 t	3.69 d	4.62 dd	3.82 dd	3.57 dd	6.9 bs	4.15 q	1.22 d	2.110, 2.040
	5.77 d	4.41 dd	5.70 t	5.19	3.89 ddd	4.31 dd	4.18 dd				1.859
8	5.24 bs	4.2 bs, m [g]	3.68 bs, m	3.49 bs	4.42 bd	3.67 dd	4.18 dd	6.7 d	4.3 m [g]	1.13	2.123, 2.094, 2.085
	4.48 d	4.1 m [g]	5.13 dd	5.07 dd	3.7–3.6 m	4.12 dd	4.29 dd	5.9 d			2.063, 2.048, 1.999
9 [h]	5.28 s	3.24 d	3.67 t	3.73 d	4.64 dd	3.90 dd	3.63 dd	[f]	4.32 q	1.45 d	2.087, 2.038
	5.08 d	3.79 ddd	5.34 dd	5.06 t	3.73 ddd	4.13 dd	4.26 dd				2.034, 1.931
10 [d]	5.28 s	3.22 d	3.61 dd	3.83 d	4.61 dd	3.86 dd	3.72 dd	[f]	4.31 q	1.40 d	
	4.70 d	3.63 ddd	3.50 dd	← 3.38–3.28 [e] →		3.68 dd	3.88 dd				1.989
10 [i]	5.37 s	3.26 d	3.61 dd	3.91 d	4.73 dd	3.86 dd	3.78 dd	[f]	4.32 q	1.39 d	
	4.83 d	3.66 dd	3.59 dd	← 3.4–3.3 [e] m →		3.68 dd	3.86 dd				1.966
11 α	6.15 d	3.65 dd	3.9 t [g]	4.31 t	3.9 m [g]	4.24 dd	4.11 dd	6.4 bs	4.36 q	1.45 d	2.147, 2.087 (×2),
	4.91 d	3.84 ddd	5.27 dd	5.09 dd	3.72 m	4.29 dd	4.11 dd	5.95 d			2.020 (×2), 1.935
β	5.51 d	3.42 bt	3.67 t	4.31 t	3.72 m	4.29 dd	4.11 dd	6.9 bs	4.36 q	1.45 d	
	4.95 d		5.30 dd	5.06 dd				5.94 d			
12 [d] α	5.17 d	3.39 dd	3.77 t	4.07 t	3.9–3.8 m	3.69 dd	3.81 dd	[f]	4.39 q	1.43 d	
	4.68 d	3.67 m	3.6 t	← 3.35–3.27 [e] →		3.71 dd	3.86 dd				2.006
β	4.59 d	3.11 bt	3.46 t	4.07 t	3.44 m	3.65 dd	3.85 dd		4.35 q	1.40 d	
	4.67										

Compound [c]	Coupling constants (Hz)								
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6exo}$	$J_{6exo,endo}$	$J_{6endo,5}$	$J_{NH,2}$	$J_{CH,Me}$
2	< 0.5	9.4	8.1	< 0.5	6.4	7.9	1.5	< 1	6.9
3	< 0.5	9.3	8.2	< 0.5	6.3	7.8	1.4	< 1	6.9
4	< 0.5	9.5	8.9	< 0.5	5.5	8.1	2.0	< 1	6.9
5 α	3.2	9.9	9.6	9.9	4.1	12.2	2.1	< 1	6.9
β	8.5	9.9	9.8		4.1			< 1	
6 [d] α	3.2	~ 9 [e]	8.9	8.5	5.4	12	2.2	[f]	6.9
β	8.0	9.3	8.9	9.2	5.4	12			6.9
7	< 0.5	9.1	8.5	< 0.5	6.7	8.0	1.3	< 1	6.9
	8.5	10.6	9.2	10.1	4.7	12.3	1.9		
8	~ 1	[j]	[j]	[j]	5.7	7.5	~ 1	10	6.1
	8.2	10.4	9.5	9.4	4.9	12.4	2.4	9	
9 [h]	< 1	9.3	9	< 0.5	6.6	8.0	1.6	[f]	6.9
	8.5	10.2	9.6	9.8	4.3	12.3	2.3		
10 [d]	< 0.5	9.7	8.0	< 0.5	6.6	8.1	1.9	[f]	6.9
	8.3	10.3	8.5	~ 9 [e]	5	12.2	1.9		
10 [i]	< 0.5	9.7	8.1	< 0.5	6.5	8.2	1.9	[f]	6.9
	8.2	10.2	8.6	~ 9	5	12.2	2.3		
11 α	3.0	9.8	9.3			12	2.1	~ 1	6.8
	8.5	10.2	9.4	9.8	4.7	12.4	2.1	8.7	
β	8.6	9.8	9					~ 1	6.8
	8.5		9.4	9.8			2	8.7	
12 [d] α	3.0	9.5	8.9	9.4	4.2	12	2.1	[f]	6.8
	8.5	10.2	8.6	~ 9	5.6	12.3	2.1		
β	8.1	9.5			4.2	12	2.1		6.8
	8.5								

[a] Other data: **2**, δ 7.4–7.3 (m, Ph), 4.82 and 4.68 (CH_2 Ph); **7**, δ 7.9 and 7.7 (2 m, 4 H, Phth); **8**, δ 3.92–3.84 (m, 2 H, CHCH_2O). [b] For convenience, the H-6a and H-6b of glucopyranose rings not involved in 1,6-anhydro linkage are listed under H-6*endo* (H-6a, smaller, $J_{6,5}$) and H-6*exo* (H-6b larger $J_{6,5}$ value), respectively. [c] Measured in $CDCl_3$ (internal Me_4Si), if not stated otherwise. [d] In MeOD. [e] Partly overlapped by the solvent signal. [f] H–D Exchange. [g] Overlapped signals. [h] In $CDCl_3$–MeOD (10:1). [i] In $(CD_3)_2CO$–D_2O (10:3). [j] Not recorded.

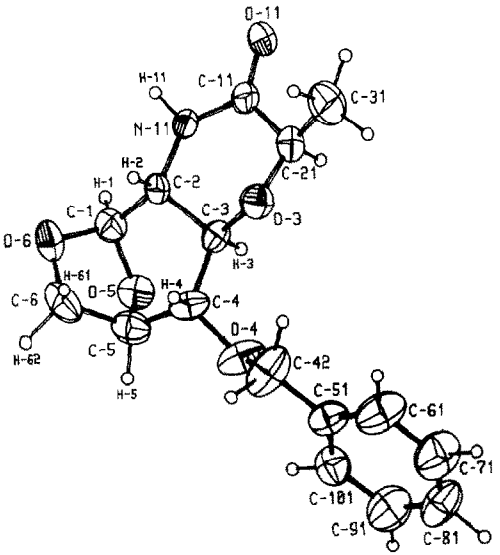


Fig. 1. Molecular structure (ORTEP drawing) of **2** with atom numbering. The thermal ellipsoids are at a 30% probability level.

tions of the β-D-glucopyranose ring. On opening the 1,6-anhydro ring, the resulting glucopyranose moiety adopts the $^4C_1$ conformation as detected in **5** (α anomer) (Table IV). The δ-lactam rings of **2** and **4A** have conformations between half-chair and sofa. The conformer **4B**, being slightly less distorted than **4A**, has a sofa conformation with C-3 displaced by 0.72(2) Å from the least-squares plane (O-3, N-11, C-2, C-11, C-21). The overall conformation of **2** is closer to that of **4A** and shows a somewhat higher distortion of ring moieties than **4B** (Fig. 4). In **5**, this ring residue has a half-chair conformation with C-3 and O-3 displaced out of the

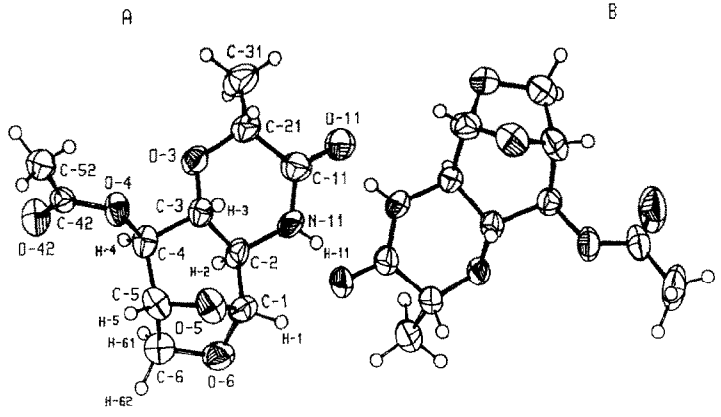


Fig. 2. Molecular structure of **4** with atom numbering for conformer A; the same applies to B. The thermal ellipsoids are at a 50% probability level.

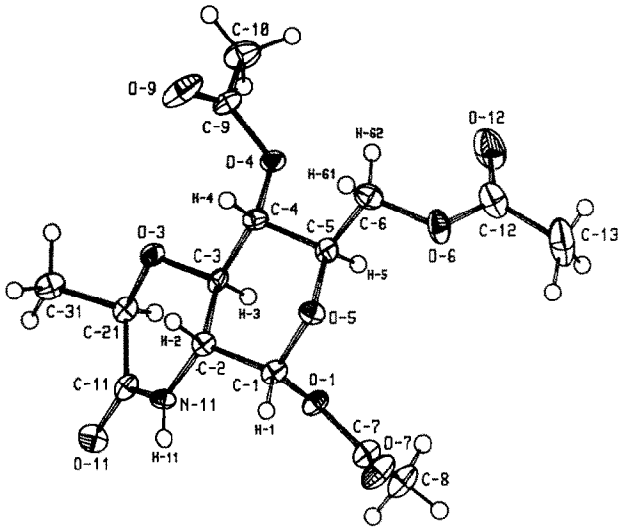


Fig. 3. Molecular structure of **5** with atom numbering. The thermal ellipsoids are at a 50% probability level.

four-atom plane [0.30(1) and 0.49(1) Å, respectively]. The slightly less distortion of the glucopyranose ring in **4B** enables a more regular shape of the 1,6-anhydro ring (envelope type) with puckering at O-5 (Table IV). However, in **2** and **4A**, the conformation is towards a twisted form with displacement of O-5 and C-1 atoms (Table IV).

Molecular mechanics and dynamics calculations on **2**.—Conformational analysis of the 1,6-anhydro-β-D-glucopyranose moiety of **2** performed by the computational chemistry approach is summarised in Table V. The results obtained by Straathof et al.[26] on 1,6-anhydro-β-D-glucopyranose (molecular mechanics approach, DELPHI[27,28]) have been used for comparison. The values of torsion angles of **2** in the $B_{O,3}$ conformation, determined by X-ray analysis, are in good agreement with those[26] for the minimum energy $B_{O,3}$ conformer described as the "global $B_{O,3}$ minimum". The results achieved by DISCOVER[29] and MMPMI[30] are consistent, although the optimised conformer of the latter is even closer to the conformer in the crystalline state. The 1,6-anhydro-β-D-glucopyranose moiety in **2** cannot form intramolecular hydrogen bonds. Energy optimisation procedures performed on the molecule "in vacuo" (gas phase) and in a water environment ended with the conformer close to that detected in the crystalline state. These findings support the fact that the 1,6-anhydro-β-D-glucopyranose skeleton has a restricted flexibility.xxx

In order to follow the conversion of the boat into the chair conformation, computer simulation was performed by opening of the 1,6-anhydro-β-D-glucopyranose ring; the energy optimised conformer was intermediate of the boat and twisted forms. In order to examine the transition barrier between twisted and expected chair conformations, molecular dynamics simulation over 5 ps ("in

TABLE II

Selected bond lengths (Å) and angles (°) for **4**

	A	B		A	B
C-1–C-2	1.541(7)	1.533(6)	O-5–C-1–C-2	109.6(4)	109.3(4)
C-2–C-3	1.507(6)	1.505(6)	O-6–C-1–C-2	109.8(4)	110.0(4)
C-3–C-4	1.515(5)	1.508(5)	O-5–C-1–O-6	104.6(3)	104.8(3)
C-4–C-5	1.544(6)	1.520(5)	C-1–C-2–C-3	108.7(4)	109.4(3)
C-5–C-6	1.523(6)	1.514(7)	N-11–C-2–C-3	107.8(3)	106.8(3)
C-5–O-5	1.429(5)	1.453(5)	N-11–C-2–C-1	114.1(4)	113.1(4)
C-6–O-6	1.451(6)	1.420(6)	C-2–C-3–C-4	108.4(3)	110.9(3)
C-1–O-6	1.390(5)	1.408(5)	O-3–C-3–C-2	109.6(4)	108.2(3)
C-1–O-5	1.410(6)	1.429(6)	O-3–C-3–C-4	111.6(3)	112.1(4)
C-2–N-11	1.465(5)	1.458(4)	C-3–C-4–C-5	107.3(4)	108.4(4)
N-11–C-11	1.333(6)	1.335(4)	O-4–C-4–C-3	107.2(3)	106.3(3)
C-11–O-11	1.247(5)	1.238(4)	O-4–C-4–C-5	110.9(3)	111.1(3)
C-11–C-21	1.522(7)	1.514(6)	C-4–C-5–C-6	115.1(4)	115.0(4)
C-21–C-31	1.498(7)	1.498(5)	O-6–C-6–C-5	103.0(3)	105.2(4)
C-21–O-3	1.428(5)	1.431(4)	O-5–C-5–C-4	107.7(2)	109.6(2)
C-3–O-3	1.419(5)	1.422(4)	O-5–C-5–C-6	103.2(4)	100.2(4)
C-4–O-4	1.441(5)	1.441(6)	C-1–O-5–C-5	99.9(3)	100.3(3)
O-4–C-42	1.342(5)	1.334(6)	C-1–O-6–C-6	104.7(3)	106.2(4)
C-42–O-42	1.188(4)	1.198(7)	C-3–O-3–C-21	111.3(3)	110.3(3)
C-42–C-52	1.494(7)	1.490(8)	C-2–N-11–C-11	123.3(4)	121.9(4)
			N-11–C-11–C-21	119.7(4)	119.8(3)
			O-11–C-11–C-21	119.5(4)	118.0(3)
			O-11–C-11–N-11	120.8(4)	122.2(4)
			O-3–C-21–C-11	112.3(4)	114.2(2)
			O-3–C-21–C-31	108.3(4)	107.2(3)
			C-11–C-21–C-31	110.7(4)	111.8(3)
			C-4–O-4–C-42	117.6(3)	116.8(4)
			O-4–C-42–O-42	124.2(4)	123.4(5)
			O-4–C-42–C-52	110.5(3)	110.7(5)
			O-42–C-42–C-52	125.3(4)	125.9(5)

vacuo") was applied. The energy-optimised molecule was a twisted conformation with a total energy 1.8 kcal/mol lower than that of the intermediate boat/twisted conformer. In the next step, the molecular-dynamics simulation resulted in a chair conformer. Energy optimisation of the chair conformer revealed a total energy difference of 23 kcal/mol compared to the boat/twisted conformer.

Crystal packing and hydrogen bonds.—The crystal packing of **2**, **4**, and **5** is dominated by hydrogen bonds (Table VI). The crystals belong to the $P2_1$ space group. Hydrogen bonds between lactam groups, N-11–H ··· O-11 (Table VI) connect molecules into an infinite chain running along the *c* axis in **2** and along the *a* axis in **5**. However, the crystal packing of **4** with two conformers exhibits different arrangement (Table VI) with 8% higher density; N-11–H ··· O-11 connects molecules A and B in dimers, only (packing diagrams of **2**, **4**, and **5** have been deposited).

TABLE III

Selected torsion angles (°) for **2**, **4**, and **5**

	2	**4**		**5**
		A	B	
Glucopyranose moiety				
O-5–C-1–C-2–C-3	−3.1(8)	0.9(5)	−8.9(5)	55.8(4)
C-1–C-2–C-3–C-4	−54.3(8)	−57.9(5)	−50.9(5)	−59.6(3)
C-2–C-3–C-4–C-5	43.9(8)	44.3(4)	46.0(5)	60.7(4)
C-3–C-4–C-5–O-5	20.9(8)	23.3(4)	17.0(5)	−59.2(4)
C-4–C-5–O-5–C-1	−79.0(7)	−82.0(4)	−76.7(4)	58.8(3)
C-5–O-5–C-1–C-2	67.2(7)	67.0(4)	70.9(4)	−56.6(4)
1,6-Anhydro moiety				
C-1–O-5–C-5–C-6	42.4(7)	40.3(4)	44.5(3)	
O-5–C-5–C-6–O-6	−19.7(8)	−17.3(4)	−27.8(4)	
C-5–C-6–O-6–C-1	−10.9(9)	−12.9(4)	−0.2(4)	
C-6–O-6–C-1–O-5	38.9(8)	39.8(4)	29.2(4)	
O-6–C-1–O-5–C-5	−52.5(7)	−50.7(4)	−47.0(4)	
δ-Lactam moiety				
N-11–C-2–C-3–O-3	58.1(7)	56.0(4)	63.0(4)	54.3(3)
C-2–C-3–O-3–C-21	−69.9(8)	−69.0(4)	−69.0(4)	−70.9(4)
C-3–O-3–C-21–C-11	47.8(8)	46.2(5)	39.7(5)	48.1(4)
O-3–C-21–C-11–N-11	−17.0(10)	−15.6(6)	−7.8(6)	−12.8(4)
C-21–C-11–N-11–C-2	9.4(11)	7.2(7)	5.2(7)	−0.6(6)
C-11–N-11–C-2–C-3	−29.1(9)	−26.5(6)	−31.6(6)	−19.2(5)

Concluding remarks.—The members of both tri- and bi-cyclic muramic acid δ-lactam series are crystalline compounds which possess high melting points, particularly those with the 4-*O*-glycosylated 1,6-anhydro structure (**9** and **10**).

TABLE IV

Ring conformation analysis using X-ray data for **2**, **4**, and **5**

Compound	Ring residue	Conformation	Asymmetry parameters (°)	Mean torsion angle (°)
D-Glucopyrano				
2	β-D	distorted boat, $B_{O,3}$	ΔC_s (C-3) = 13.7, ΔC_s (C-1–C-2) = 26.4	44.7
4 A *	β-D	distorted boat, $B_{O,3}$	ΔC_s (C-3) = 18.4, ΔC_s(C-1–C-2) = 26.9	46.0
4 B *	β-D	distorted boat, $B_{O,3}$	ΔC_s (C-3) = 6.6, ΔC_s (C-1–C-2) = 26.4	45.1
5	α-D	chair, $^4C_1$	ΔC_s (C-2) = 3.2, ΔC_2 (C-2–C-3) = 3.9	58.4
δ-Lactam				
2		half-chair/sofa	ΔC_s (C-3) = 13.5, ΔC_2 (O-3–C-3) = 11.2	38.5
4 A *		half-chair/sofa	ΔC_s (C-3) = 13.9, ΔC_2 (O-3–C-3) = 10.9	36.9
4 B *		sofa	ΔC_s (C-3) = 6.9, ΔC_2 (O-3–C-3) = 22.3	36.3
5		half-chair	ΔC_s (C-3) = 20.7, ΔC_2 (O-3–C-3) = 6.3	34.3
1,6-Anhydro				
2		twisted	ΔC_s (O-5) = 15.3, ΔC_2 (C-6) = 6.7	32.9
4 A *		twisted	ΔC_s (O-5) = 17.4, ΔC_2 (C-6) = 3.1	31.9
4 B *		envelope	ΔC_s (O-5) = 2.0, ΔC_2 (C-6) = 22.3	29.6

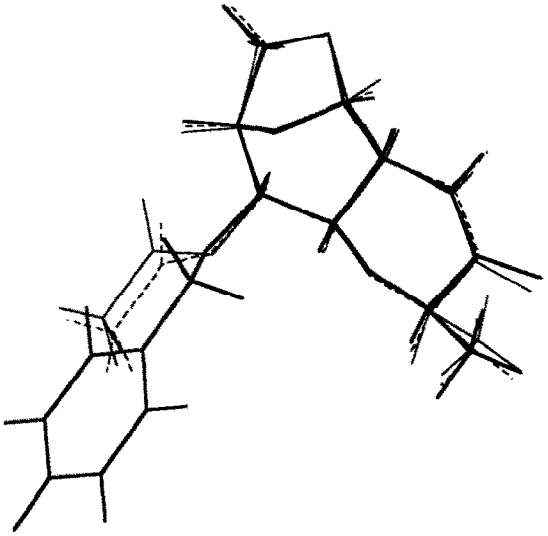


Fig. 4. Overlap diagram of X-ray conformations for **2** (heavy line), **4A** (dashed line), and **4B** (thin line).

According to the NMR data, no conformational change could be detected for the solutions of the tricyclic series (Table VII); the NMR spectrum of a solution of **10** in CD_3OD-D_2O did not change at 50°C. Molecular dynamics simulation did not reveal a direct transition from the $B_{O,3}$ to the $^4C_1$ conformation, but an intermediate twisted conformation. 1,6-Anhydromuramic acid residues have been isolated from peptidoglycan of vegetative bacterial cells[31–33]; however, the existence of 1,6-anhydromuramic acid lactam units in bacterial spores is not known.

TABLE V

Comparative analysis of the 1,6-anhydro-β-D-glucopyranose moiety conformation based on X-ray and computational chemistry methods for **2** in the $B_{O,3}$ conformation and 1,6-anhydro-β-D-glucopyranose[26]

Torsion angle (°)	X-ray	Compound **2** DISCOVER[14]		MMPMI[15]	Ref. 26
		a [a]	b [a]	a	a
O-5–C-1–C-2–C-3	−3.1	−16	−14	−15	−4.4
C-1–C-2–C-3–C-4	−54.3	−43	−43	−52	−48.7
C-2–C-3–C-4–C-5	43.9	43	42	44	40.2
C-3–C-4–C-5–O-5	20.9	14	16	19	20.7
C-4–C-5–O-5–C-1	−79.0	−72	−72	−78	−76.1
C-5–O-5–C-1–C-2	67.2	72	71	69	66.9
C-1–O-5–C-5–C-6	42.4	47	47	43	43.6
O-5–C-5–C-6–O-6	−19.7	−31	−29	−23	−20.1
C-5–C-6–O-6–C-1	−10.9	2	0	−7	−10.3
C-6–O-6–C-1–O-5	38.9	27	29	34	37.2
O-6–C-1–O-5–C-5	−52.5	−45	−48	−49	−51.2

[a] Energy optimisation performed in a, the gas phase; and b, a water environment.

TABLE VI

Hydrogen-bond geometry for **2**, **4**, and **5**

Compound		D–H···A (Å)	D–H (Å)	H···A (Å)	D–H···A (°)	Symmetry operations on A
2	N-11–H···O-11	2.783(10)	0.97(6)	1.86(6)	157(5)	$-x$, $y+1/2-1$, $-z+1$
4	N-11–H(A)···O-11(B)	2.832(5)	1.01(4)	1.83(4)	172(3)	$-x$, $y+1/2-1$, $-z+1$
	N-11–H(B)···O-11(A)	2.840(5)	1.01(4)	1.84(4)	170(3)	$-x$, $y+1/2$, $-z+1$
5	N-11–H···O-11	2.791(5)	1.01(4)	1.81(4)	161(3)	$-x+1$, $y+1/2$, $-z$

The opening of the 1,6-anhydro ring in **4** and **7** yielded **5** and **11**, respectively which exhibited the more-stable $^4C_1$ conformation of the D-glucopyranose ring. In these compounds, H-6,6 were not sterically blocked and, apparently, the C-6 rotamer populations in the solid state and in the solution were different (Table VII).

TABLE VII

Values of torsion angles (°) of protons in the D-glucopyrano ring for compounds **2**, **4**, and **5** from X-ray data (a) compared to those derived [a] from experimental J values (b)

Angle	**2**		**4**		**5**	
	a [b]	b [b]	a	b	a	b
H-1–C-1–C-2–H-2	116	~100	122, 113 [c]	~100	60	~50 or ~130
H-2–C-2–C-3–H-3	178	~180	174, −176	~180	178	~180
H-3–C-3–C-4–H-4	169	~160	164, 170	~170	−180	~180
H-4–C-4–C-5–H-5	−98	~100	−99, −106	~100	−172	~180
H-5–C-5–C-6–H-6*endo*	108	~110	109, 92	~115		
H-5–C-5–C-6–H-6*exo*	−14	~30	−12, −26	~30 or ~140		
H-5–C-5–C-6–H-6a					−170	~50 or ~120
H-5–C-5–C-6–H-6b					71	~40 or ~130

[a] According to the Karplus curve and Hall[25]. [b] See Table V. [c] Compound **4** has two conformers (A and B) in the crystalline state.

EXPERIMENTAL

General.—Melting points were determined in capillaries and are uncorrected. Solvents were removed under reduced pressure at < 45°C. Column chromatography was performed on silica gel (Merck, 0.040–0.063 mm) and TLC on Silica Gel 60 with detection by ninhydrin, the chlorine–iodine reagent, or charring with H_2SO_4. The solvents used were *A*, $CHCl_3$–MeOH; *B*, benzene–acetone; *C*, $CHCl_3$–EtOAc; *D*, $CHCl_3$–acetone–MeOH; *E*, EtOAc–EtOH–water; in proportions given in the text. Optical rotations were determined with an Optical Activity LTD automatic AA-10 Polarimeter for 1% solutions unless stated otherwise. 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), unless stated otherwise. Double resonance experiments were performed in order to assist in signal assignment.

1,6-Anhydro-2-azido-4-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-β-D-glucopyranose (**1**) was prepared from 1,6-anhydro-2-azido-4-*O*-benzyl-2-deoxy-β-D-glucopyranose and (*S*)-2-chloropropionic acid, followed by esterification with diazomethane, as described by Paulsen et al.[7].

2-Amino-1,6-anhydro-4-O-benzyl-3-O-[(R)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1',2-lactam (1,6-anhydro-4-O-benzylmuramic acid 1',2-lactam) (**2**).—To a stirred solution of **1** (608 mg, 1.67 mmol) in ethanolic $NiCl_2$ (4% $NiCl_2 \cdot 6H_2O$, 2% H_3BO_3) (15 mL) was added dropwise at 5°C a suspension of $NaBH_4$ (~ 500 mg) in EtOH (~ 5 mL) until the black colour of the mixture persisted and TLC (solvent *A*, 9:1) indicated that **1** had disappeared. Stirring was continued at room temperature for 2 h, the mixture was concentrated, and toluene was evaporated (3 ×) from the residue, which was extracted with $CHCl_3$ (3 × 100 mL). The extracts were combined and concentrated to leave an oil which gradually solidified; TLC monitoring (solvent *B*, 5:2) indicated (~ 2 days) an almost complete conversion of the primary ninhydrin-positive (R_f ~ 0.2) into the ninhydrin-negative and peptide-positive (R_f ~ 0.3) product **2** (477 mg, 85%). Column chromatography (solvent *B*, 5:2) afforded **2**; mp 162–164°C from $CHCl_3$–light petroleum); $[\alpha]_D$ +18.5° ($CHCl_3$). ^{13}C NMR data: δ 172.1 (CO), 101.8 (C-1), 81.3 (C-4), 77.4 (C-3), 76.3 (C-5), 75.5 (α-C Lact), 72.5 (CH_2Ph), 67.2 (C-6), 58.1 (C-2), and 18.0 (Me). Anal. Calcd for $C_{16}H_{19}NO_5$ (305.34): C, 62.94; H, 6.27; N, 4.59. Found: C, 63.02; H, 6.03; N, 4.47.

2-Amino-1,6-anhydro-3-O-[(R)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1',2-lactam (**3**).—A mixture of **2** (462 mg, 1.51 mmol) and 10% Pd–C (417 mg) in EtOH (15 mL) was shaken under H_2 at 1 atm and room temperature for 24 h (monitoring by TLC in solvent *A*, 9:1). The suspension was centrifuged, the catalyst was washed with MeOH–$CHCl_3$, the combined filtrate and washings were concentrated, and the residue was dried (P_2O_5) to give **3** (305 mg, 94%). Crystallisation from $CHCl_3$–MeOH (9:1)–light petroleum gave **3**; mp 189–190°C (darkening at 170°C); $[\alpha]_D$ −24° ($CHCl_3$–MeOH, 9:1). ^{13}C NMR data ($CDCl_3$–MeOD, 10:1): δ

172.8 (CO), 101.9 (C-1), 79.7 (C-3), 77.0 (C-5), 75.8 (α-C Lact), 74.5 (C-4), 67.6 (C-6), 58.0 (C-2), and 18.1 (Me). Anal. Calcd. for $C_9H_{13}NO_5$ (215.21): C, 50.23; H, 6.09; N, 6.51. Found: C, 50.13; H, 6.25; N, 6.43.

Conventional treatment of **3** (215 mg, 1 mmol) with pyridine–acetic anhydride (1:1, 5 mL) overnight at room temperature and crystallisation of the product from $CHCl_3$–light petroleum afforded the 4-acetate **4** (217 mg, 84%); mp 186–188°C; $[\alpha]_D$ −49.5° ($CHCl_3$). ^{13}C NMR data: δ 172.2, 170.0 (CO), 101.8 (C-1), 77.2 (C-4), 76.5 (C-3), 75.8 (α-C Lact), 72.9 (C-5), 67.2 (C-6), 58.0 (C-2), 21.0 (Me Ac), 17.9 (Me Lact); (C_6D_6): δ 171.7, 170.3 (CO), 102.1 (C-1), 77.4 (C-4), 76.8 (C-3), 75.8 (α-C-Lact), 73.2 (C-5), 67.0 (C-6), 58.1 (C-2), 20.3 (Me Ac), and 18.1 (Me Lact). Anal. Calcd for $C_{11}H_{15}NO_6$: C, 51.36; H, 5.88; N, 5.45. Found: C, 51.46; H, 5.72; N, 5.22.

*1,4,6-Tri-*O*-acetyl-2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-*D*-glucopyranose 1',2-lactam* (**5**).—Trifluoroacetic acid (1 mL) was added to a solution of **4** (129 mg, 0.5 mmol) in acetic anhydride (9 mL) with stirring. The solution was left at room temperature for 2 days (monitoring by TLC in solvent *C*, 1:1), then diluted with toluene (10 mL), and concentrated, and toluene was evaporated (2×) from the residue. Column chromatography (solvent *C*, 2:5) then gave **5** (142 mg, 79%) as a ~4:1 α,β-mixture. After two crystallisations (EtOAc–light petroleum), the α,β-ratio was ~8:1 and the product had mp 202–205°C (softening at 198°C); $[\alpha]_D$ +101° ($CHCl_3$); lit.[5] for the pure α anomer, mp 200–205°C; $[\alpha]_D$ +118° ($CHCl_3$). ^{13}C NMR data (**5**α): δ 171.1, 170.7. 169.6, 169.4 (CO), 89.5 (C-1), 75.1 (α-C Lact), 72.8 (C-3), 71.2 (C-4), 66.9 (C-5), 61.6 (C-6), 55.0 (C-2), 21.0, 20.8, 20.7 (Me Ac), and 17.7 (Me Lact). Anal. Calcd for $C_{15}H_{21}NO_9$: C, 50.13; H, 5.89; N, 3.90. Found: C, 50.18; H, 6.06; N, 3.89.

*2-Amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-*D*-glucopyranose 1',2-lactam (muramic acid 1',2-lactam)* (**6**).—To a solution of **5** (82 mg, 0.23 mmol) in dry MeOH (4 mL) was added methanolic 0.1 M NaOMe (1 mL). The solution was stirred at room temperature for ~2 h (monitoring by TLC in solvent *A*, 9:1), neutralised with Amberlite IR-120 (H^+) resin, the resin was collected and washed with MeOH, and the combined filtrate and washings were concentrated to give **6** as a glass that, after drying over P_2O_5, turned into a white solid (α,β-ratio ~1.2:1). A solution of **6** in EtOAc–MeOH (9:1) was diluted with di-isopropyl ether, to give **6** as a white hygroscopic powder; mp 170–180°C; $[\alpha]_D$ +47° (*c* 1.8, MeOH); lit.[6] syrup, $[\alpha]_D$ +35° (MeOH). ^{13}C NMR data (CD_3OD): δ 96.2 (C-1β), 92.1 (C-1α), 79.8 (C-3β), 79.1 (C-3α), 76.4 (C-5β), 75.8 and 75.7 (C-α Lact), 74.3 (C-5α), 69.1 (C-4), 62.5 (C-6), 58.7 (C-2β), 57.1 (C-2α), 18.2 and 18.1 (Me Lact). Anal. Calcd for $C_9H_{15}NO_6$: C, 46.35; H, 6.48; N, 6.01. Found: C, 46.61; H, 6.29; N, 5.79.

*2-Amino-1,6-anhydro-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-β-*D*-glucopyranose 1',2-lactam* (**7**).—A mixture of **3** (215 mg, 1 mmol), powdered activated 4A molecular sieves (1.2 g), and silver triflate (780 mg, 3 mmol) in dry CH_2Cl_2 (5 mL) was stirred at room temperature under N_2 for 10 min. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-

phthalimido-β-D-glucopyranosyl chloride (918 mg, 2 mmol) in dry CH_2Cl_2 (5 mL) was then added portionwise with a syringe during 1.5 h, and stirring was continued for 16 h. The mixture was diluted with $CHCl_3$ and centrifuged, and the supernatant solution was washed with aq $NaHCO_3$ and water, dried, and concentrated. Column chromatography (solvent *B*, 5:3) of the residue gave **7** (527 mg, 83%) which, after recrystallisation (yield 75%) from EtOAc–light petroleum, had mp 162–164°C; $[\alpha]_D$ +4.2° ($CHCl_3$). ^{13}C NMR data: δ 171.8 (CO Lact), 170.6, 170.3, 170.1 (CO Ac), 169.4 (CO Phth), 101.8 (C-1), 95.9 (C-1′), 80.5 (C-4), 77.2 (C-3), 75.4 (α-C Lact), 73.4, 72.4, 70.8, and 68.7 (C-5,5′,3′,4′), 67.0 (C-6), 62.0 (C-6′), 57.3 (C-2), 54.0 (C-2′), 20.7, 20.6, and 20.4 (Me Ac), and 17.6 (Me Lact). *Anal.* Calcd for $C_{29}H_{32}N_2O_{14}$: C, 55.06; H, 5.10; N, 4.43. Found: C, 55.24; H, 5.00; N, 4.69%.

TABLE VIII

Crystal data and summary of experimental details for compounds **2**, **4**, and **5**

	2	**4**	**5**
Molecular formula	$C_{16}H_{19}NO_5$	$C_{11}H_{15}NO_6$	$C_{15}H_{21}NO_9$
M_r	305.33	257.24	359.33
Crystal size (mm)	0.60×0.30×0.10	0.45×0.30×0.10	0.45×0.20×0.10
a (Å)	5.394(1)	10.091(10)	11.332(4)
b (Å)	7.552(1)	5.199(2)	7.546(5)
c (Å)	19.204(1)	23.123(35)	11.383(7)
β (°)	94.96(1)	93.93(5)	110.32(2)
V (Å^3)	779.4(2)	1210(2)	912.8(9)
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1$	$P2_1$	$P2_1$
D_x (g cm^{-3})	1.301	1.412	1.307
Z	2	4	2
μ (Mo*Kα*) (cm^{-1})	0.9	1.1	1.0
F (000)	324	544	380
T (K)	295	295	105
No. of reflections used for cell	25	25	25
Parameters and θ range (°)	5–18	8–17	7–17
θ Range for intensity measurement	5–11	2–25	2–25
hkl range	(0.6; 0.9; −22.22)	(−1.12; −1.6; −27.27)	(−1.13; −1.8; −13.13)
Scan	ω	ω/2Θ	ω/2Θ
Δω	1+0.35 tan θ	0.8+0.35 tan θ	0.8+0.35 tan θ
No. of measured reflections	1307	2980	2304
No. of symmetry-independent reflections	930 $I > 2\sigma(I)$	1666 $I > 2\sigma(I)$	1580 $I > 2\sigma(I)$
No. of variables	211	409	309
R	0.062	0.035	0.033
R_w, $w^{-1} = k(\sigma F_o^2 + gF)$	0.061	0.037	0.033
Final shift/error	0.062 (C42, *y*)	0.582 (O4B, *y*)	0.050 (C8, *x*)
Residual electron density $(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (eÅ^{-3})	0.19, −0.19	0.18, −0.17	0.19, −0.20

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-[(R)-1-acetoxyprop-2-yl]-1,6-anhydro-2-deoxy-β-D-glucopyranose (**8**).—To a solution of **7** (65 mg, 0.1 mmol) in 2-propanol–water (6:1, 2 mL) was added portionwise[10] $NaBH_4$ (50 mg, 1.3 mmol) during 4 h at room temperature. After 8 h, the pH of the mixture was adjusted (glacial acetic acid) to 5, and the mixture was heated at 80°C for 5 h and concentrated. Conventional acetylation of the residue and column chromatography in solvent *D* (14:2:1) of the product afforded **8** (46 mg, 81.5%); mp 214–216°C (from $CHCl_3$–light petroleum); $[\alpha]_D$ −114° ($CHCl_3$). Anal. Calcd for $C_{27}H_{40}N_2O_{15}$: C, 51.26; H, 6.37; N, 4.43. Found: C, 51.52; H, 6.52; N, 4.50.

4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-amino-1,6-anhydro-3-O-[(R)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1',2-lactam (**9**).—To a stirred solution of **7** (158 mg, 0.25 mmol) in dry MeOH (9 mL) was added methanolic 0.1 M NaOMe (0.75 mL). The solution was stirred at room temperature for 15 min and then neutralised with Amberlite IR-120 (H^+) resin, the resin was colleced and washed with MeOH, and the combined filtrate and washings were concentrated. To a solution of the residue in EtOH (10 mL) was added ethanolic 5% hydrazine hydrate (2 mL), and the solution was boiled under reflux for 2 h (monitoring by TLC in solvent *E*, 5:2:1). The solvent was evaporated, and

TABLE IX

Final atomic coordinates and equivalent isotropic thermal parameters for **2**

Atom	*x*	*y*	*z*	U_{eq} (Å^2) [a]
O-3	0.3769(9)	0.8394(10)	0.3436(2)	0.0760(19)
O-4	0.2453(9)	0.7847(13)	0.1899(2)	0.101(2)
O-5	−0.1245(8)	0.5131(0)	0.2577(2)	0.0771(19)
O-6	0.0682(13)	0.2888(11)	0.3115(3)	0.104(3)
O-11	0.0971(10)	0.8652(10)	0.5050(3)	0.091(2)
N-11	0.0712(11)	0.6557(11)	0.4234(3)	0.064(2)
C-1	−0.0405(14)	0.4508(14)	0.3243(4)	0.075(3)
C-2	0.1465(13)	0.5774(12)	0.3604(3)	0.058(3)
C-3	0.1932(13)	0.7275(12)	0.3106(3)	0.056(2)
C-4	0.2695(13)	0.6531(13)	0.2428(3)	0.072(3)
C-5	0.0979(15)	0.4985(13)	0.2229(4)	0.081(3)
C-6	0.1907(18)	0.3202(14)	0.2499(5)	0.102(4)
C-11	0.1423(14)	0.8149(13)	0.4468(4)	0.068(3)
C-21	0.2795(15)	0.9319(13)	0.3992(4)	0.075(3)
C-31	0.4900(16)	1.0309(15)	0.4387(4)	0.112(4)
C-42	0.4671(16)	0.8552(15)	0.1752(5)	0.122(4)
C-51	0.4217(10)	1.0104(12)	0.1267(3)	0.082(3)
C-61	0.5942(10)	1.1484(12)	0.1302(3)	0.123(5)
C-71	0.5570(10)	1.2941(12)	0.0859(3)	0.138(6)
C-81	0.3472(10)	1.3017(12)	0.0381(3)	0.116(5)
C-91	0.1747(10)	1.1637(12)	0.0346(3)	0.123(5)
C-101	0.2120(10)	1.0180(12)	0.0790(3)	0.100(4)

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

toluene was evaporated (3 ×) from the residue which was acetylated conventionally with pyridine–acetic anhydride (2:1, 6 mL) overnight at room temperature. Column chromatography (solvent *A*, 10:1) of the product gave **9** (92 mg, 69%), a solution of which in EtOAc–MeOH (20:1) was diluted with light petroleum to give **9**; mp 283–285°C; $[\alpha]_D$ −14° ($CHCl_3$–MeOH, 9:1). ^{13}C NMR data ($CDCl_3$–MeOD, 10:1): δ 101.8 (C-1), 99.1 (C-1′), 81.1 (C-4), 77.0 (C-3), 75.6 (α-C Lact), 74.8 (C-5), 72.6, 72.2, and 69.1 (C-5′,3′,4′), 66.9 (C-6), 62.6 (C-6′), 57.7 (C-2), 54.8 (C-2′), and 18.0 (Me Lact). Anal. Calcd for $C_{23}H_{32}N_2O_{13}$: C, 50.73; H, 5.92; N, 5.14. Found: C, 50.52; H, 6.20; N, 4.99.

4-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-2-amino-1,6-anhydro-3-O-[(R)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1′,2-lactam (**10**).—To a solution of **9** (60 mg, 0.11 mmol) in dry MeOH (5 mL) was added methanolic 0.1 M NaOMe (0.5 mL). The solution was stirred for 2 h at room temperature (monitoring by TLC in solvent *E*, 5:2:1), neutralised with Amberlite IR-120 (H^+) resin, and processed as described for **6**, to give, after drying over P_2O_5, **10** (42 mg, ~ 100%) which, after crystallisation from MeOH–di-isopropyl ether, had mp 242–244°C; $[\alpha]_D$ −28° (MeOH–water, 20:1). Anal. Calcd for $C_{17}H_{26}N_2O_{10}$: C, 48.80; H, 6.26; N, 6.69. Found: C, 48.33; H, 6.00; N, 6.46.

4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6-di-O-acetyl-2-amino-3-O-[(R)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1′,2-lactam (**11**).—To **9** (87 mg, 0.16 mmol) was added, with shaking, a cold 10% solution of trifluoroacetic acid in acetic anhydride (5 mL). The mixture was kept at room temperature for 3 days (monitoring by TLC in solvent *A*, 10:1), then processed, as described for **5**. Column chromatography (solvent *A*, 9:1) of the product gave **11** (80 mg, 80%) as a ~ 3:1 α,β-mixture. Crystallisation from $CHCl_3$–light petroleum afforded material with mp 244–248°C; $[\alpha]_D$ +61° ($CHCl_3$). ^{13}C NMR data: δ 101.0 (C-1′), 91.3 (C-1β), 89.3 (C-1α), 77.8, 75.4, and 74.8 (C-3, α-C Lact, C-4), 72.3, 72.0, and 71.4 (C-3,5′,5), 68.4 (C-4′), 62.3 and 62.1 (C-6,6′), 55.1 and 54.7 (C-2,2′), and 17.8 (Me Lact). Anal. Calcd for $C_{27}H_{38}N_2O_{16}$: C, 50.15; H, 5.92; N, 4.33. Found: C, 50.26; H, 5.85; N, 4.48.

4-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-2-amino-3-O-[(R)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1′,2 lactam (**12**).—Saponification of a solution of **11** (65 mg, 0.1 mmol) in MeOH (5 mL) with 0.1 M NaOMe–MeOH (0.5 mL), as described for **6** [monitoring by TLC in solvents *A* (9:1) and *E* (5:2:1)], gave **12** (44 mg, ~ 100%), with an α,β-ratio of ~ 1:1). Crystallisation from MeOH–di-isopropyl ether gave **12** as a white powder; mp 206–208°C; $[\alpha]_D$ +67° (MeOH). Anal. Calcd for $C_{17}H_{28}N_2O_{11}$: C, 46.78; H, 6.47; N, 6.42. Found: C, 46.65; H, 6.70; N, 6.30.

X-ray structure analysis of **2**, **4**, *and* **5**.—Crystals suitable for X-ray analysis were grown from $CHCl_3$–light petroleum (**2** and **4**) and EtOAc–light petroleum (**5**) at room temperature during 3–6 days. At an early stage of cyrstallisation from the α,β-mixture of **5**, the α anomer was picked out for X-ray analysis. The crystal data and a summary of the experimental details for **2**, **4**, and **5** are listed in Table VIII.

TABLE X

Final atomic coordinates and equivalent isotropic thermal parameters for **4**–molecules A and B

Atom	Molecule A				Molecule B			
	x	*y*	*z*	U_{eq} (Å^2) [a]	*x*	*y*	*z*	U_{eq} (Å^2) [a]
O-3	0.8785(3)	0.8738(6)	0.187(1)	0.0502(9)	0.1904(3)	0.0843(6)	0.2914(1)	0.0471(9)
O-4	0.9173(3)	1.2873(5)	0.1014(1)	0.0485(9)	0.0320(3)	0.2377(7)	0.3873(1)	0.0580(10)
O-5	0.6035(3)	1.2217(6)	0.0881(1)	0.0552(10)	0.3119(3)	0.5343(6)	0.4214(1)	0.0574(13)
O-6	0.5309(3)	0.8511(7)	0.0507(1)	0.0651(13)	0.4696(3)	0.2401(0)	0.4449(1)	0.0663(13)
O-11	0.6575(3)	0.6700(8)	0.2932(1)	0.0845(17)	0.4003(3)	0.4127(7)	0.1958(1)	0.0589(10)
O-42	1.0563(3)	1.1255(6)	0.0394(1)	0.0559(13)	−0.0132(4)	−0.0570(8)	0.4532(1)	0.0904(16)
N-11	0.6153(4)	0.7638(9)	0.1990(1)	0.0571(13)	0.4260(4)	0.3592(8)	0.2926(1)	0.0484(11)
C-1	0.5534(5)	0.9794(10)	0.1032(2)	0.0546(18)	0.4240(5)	0.4071(11)	0.3999(2)	0.0579(18)
C-2	0.6590(4)	0.8347(9)	0.1420(2)	0.0424(15)	0.3790(4)	0.2518(9)	0.3457(1)	0.0414(15)
C-3	0.7798(4)	1.0038(9)	0.1515(1)	0.0411(14)	0.2296(5)	0.2502(9)	0.3384(1)	0.0423(15)
C-4	0.8290(5)	1.0708(8)	0.0929(2)	0.0419(14)	0.1707(5)	0.1698(9)	0.3938(1)	0.0415(15)
C-5	0.7063(5)	1.1424(8)	0.0525(1)	0.0465(16)	0.2417(5)	0.3157(9)	0.4438(1)	0.0526(18)
C-6	0.6431(5)	0.9198(9)	0.0178(2)	0.0507(16)	0.3564(6)	0.1731(11)	0.4749(2)	0.060(2)
C-11	0.6969(5)	0.7531(10)	0.2468(2)	0.0587(18)	0.3594(4)	0.3267(9)	0.2412(1)	0.0454(15)
C-21	0.8395(5)	0.8468(9)	0.2450(2)	0.0496(16)	0.2255(4)	0.1943(9)	0.2378(1)	0.0426(15)
C-31	0.9338(5)	0.6646(11)	0.2767(2)	0.0782(19)	0.2179(4)	−0.0164(9)	0.1935(1)	0.0577(16)
C-42	1.0288(4)	1.2871(8)	0.0729(1)	0.0402(13)	−0.0496(6)	0.1091(12)	0.4199(2)	0.071(2)
C-52	1.1108(6)	1.5194(10)	0.0885(2)	0.0513(19)	−0.1884(5)	0.2048(16)	0.4097(2)	0.108(3)

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

TABLE XI

Final atomic coordinates and equivalent isotropic thermal parameters for **5**

Atom	*x*	*y*	*z*	U_{eq} (Å^2) [a]
O-1	0.1836(2)	0.0634(4)	0.0288(2)	0.0200(6)
O-3	0.4572(2)	−0.2448(4)	0.2845(2)	0.0188(6)
O-4	0.2529(2)	−0.2691(4)	0.3734(2)	0.0203(7)
O-5	0.2061(2)	0.1720(0)	0.2291(2)	0.0189(7)
O-6	−0.0112(2)	0.1405(5)	0.2977(2)	0.0287(8)
O-7	0.0863(2)	0.3219(5)	−0.0420(2)	0.0336(8)
O-9	0.4174(2)	−0.2652(5)	0.5546(2)	0.0422(9)
O-11	0.5488(2)	−0.1776(4)	0.0147(2)	0.0235(7)
O-12	−0.1138(2)	−0.1030(5)	0.3230(3)	0.0469(11)
N-11	0.4450(3)	0.0009(5)	0.1043(3)	0.0178(9)
C-1	0.2655(3)	0.1507(6)	0.1395(3)	0.0186(10)
C-2	0.3821(3)	0.0377(6)	0.1930(3)	0.0163(10)
C-3	0.3481(3)	−0.1386(6)	0.2375(3)	0.0167(9)
C-4	0.2908(3)	−0.1021(6)	0.3363(3)	0.0185(10)
C-5	0.1725(3)	0.0097(6)	0.2760(3)	0.0189(10)
C-6	0.1080(3)	0.0587(7)	0.3661(3)	0.0260(11)
C-7	0.0959(3)	0.1666(7)	−0.0565(3)	0.0265(11)
C-8	0.0205(4)	0.0561(7)	−0.1650(4)	0.0333(14)
C-9	0.3317(3)	−0.3442(6)	0.4796(3)	0.0247(10)
C-10	0.2953(4)	−0.5321(6)	0.4893(4)	0.0332(14)
C-11	0.4984(3)	−0.1543(5)	0.0944(3)	0.0193(10)
C-12	−0.1140(3)	0.0417(7)	0.2784(4)	0.0346(14)
C-13	−0.2297(4)	0.1337(8)	0.1938(6)	0.0522(18)
C-21	0.4949(3)	−0.3037(6)	0.1823(3)	0.0191(10)
C-31	0.6214(3)	−0.3940(6)	0.2389(3)	0.0255(11)

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

The X-ray intensity data were collected with an Enraf–Nonius CAD-4F diffractometer with graphite-monochromatised Mo$K\alpha$ radiation. There were no significant variations in intensity for the standard reflections. The data were corrected for Lorentz and polarisation effects, using the Enraf–Nonius SDP/VAX package[34]. Structures were solved by SHELX86[35]. Refinement was by full-matrix least-squares minimising $\Sigma w(|F_o| - |F_c|)^2$ with the SHELX77[36] system of programs using F values. For the structures solved in the polar space group $P2_1$, the origin was fixed with the y coordinate of O-5. The H atom coordinates were determined from successive difference Fourier syntheses. For structure **2**, the H atoms attached to the benzene ring and to the C-31 methyl group (attached to the δ-lactam ring) were calculated on stereochemical grounds and refined riding on their respective C atoms. This procedure was applied to **4**, to the C-31 methyl groups of molecules A and B, and the terminal methyl group C-52 (of acetyl residue) of molecule A. In the structures **4** and **5**, the N-11–H bond distances were normalised to the values obtained by neutron diffraction (N–H, 1.009 Å). Atomic scattering factors were those included in SHELX77[36]. Details of the refinement procedures are given in Table VIII. During the structure determinations of **2**, **4**, and **5**, D enantiomers were

selected according to the assignment *R* at C-5; chirality on C-21 (δ-lactam residue) proved to be *R*. The molecular geometry was calculated by program package EUCLID[37]. Drawings were prepared by the program PLUTON incorporated in EUCLID and ORTEP II[18]. The final atomic coordinates and equivalent isotropic thermal parameters are listed in Tables IX–XI *. Calculations were performed on Micro-VAX II and IRIS-4D25G computers of the X-ray Laboratory, Rudjer Bošković Institute (Zagreb, Croatia).

Molecular mechanics and dynamics calculations.—The consistent valence force-field approach of Lifson and Warschel[38] and Hagler et al.[39,40], incorporated in the program DISCOVER version 2.7.0 (Biosym, 1991)[29], was used. Steepest descent and conjugate gradients and, in some calculations, modified Newton–Raphson algorithms were used. An alternative approach, the MMPMI program[30] was applied also. Energy optimisation was performed for molecules in the gas phase and a water environment with the initial atomic coordinates obtained by X-ray diffraction analysis (for **2**, Table IX). A lone-pair correction (for oxygen) was applied. These calculations were performed on the integral molecule of **2**. In order to follow the glucopyranose ring conversion from boat ($B_{O,3}$) into a chair ($^4C_1$) conformation on opening of the 1,6-anhydro ring, molecular dynamics (DISCOVER) was applied.

ACKNOWLEDGMENTS

This work was supported partially by Ministry of Science, Technology and Informatics grants Nos. 1-07-192 and 1-07-179. We thank Mrs. B. Metelko and Mr. Z. Marinić for recording the NMR spectra and Mrs. D. Orlić for technical assistance.

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