ELSEVIER

Carbohydrate Research 259 (1994) 159–174

CARBOHYDRATE RESEARCH

Conformational analysis and computer modelling of muramic acid δ-lactam structures

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(Received June 22nd, 1993; accepted December 21st, 1993)

Abstract

Conformational analysis of 1,6-anhydromuramic acid δ-lactam, muramic acid δ-lactam, and 1,6-anhydromuramic acid was studied by X-ray structure analysis, molecular mechanics and dynamics calculations, and computer modelling (BIOSYM package). The X-ray structure of 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 (**1**) was determined. The crystals of **1** are monoclinic, space group $P2_1$, with the unit cell parameters: $a = 10.446(4)$, $b = 4.891(1)$, $c = 18.780(7)$ Å; $\beta = 94.33(2)°$; and $Z = 2$. The stability of the $B_{O,3}$ conformation of the β-D-glucopyranose ring involved in the tricyclic structures of **1** and 4-*O*-acetyl-2-amino-1,6-anhydro-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose 1′,2-lactam (**2**) was examined by computational chemistry methods. The influence of the 1,6-anhydro and δ-lactam rings on the conformation of the fused β-D-glucopyranose component was studied by computer simulations performed on **2**. New compounds (**3α,β** and **4**) were generated from **2** by opening of the 1,6-anhydro ring and cleavage of the δ-lactam ring, respectively. Conformational analysis of **3α,β** showed the minimum energy conformer of the D-glucopyranose ring to be $^4C_1$, whereas a distorted chair/sofa conformation $^1C_4/E_O$ was obtained for **4**.

1. Introduction

The conversion of a bacterial vegetative cell into the metabolically inactive spore cell has been the subject of intense research activity in molecular biology [1].

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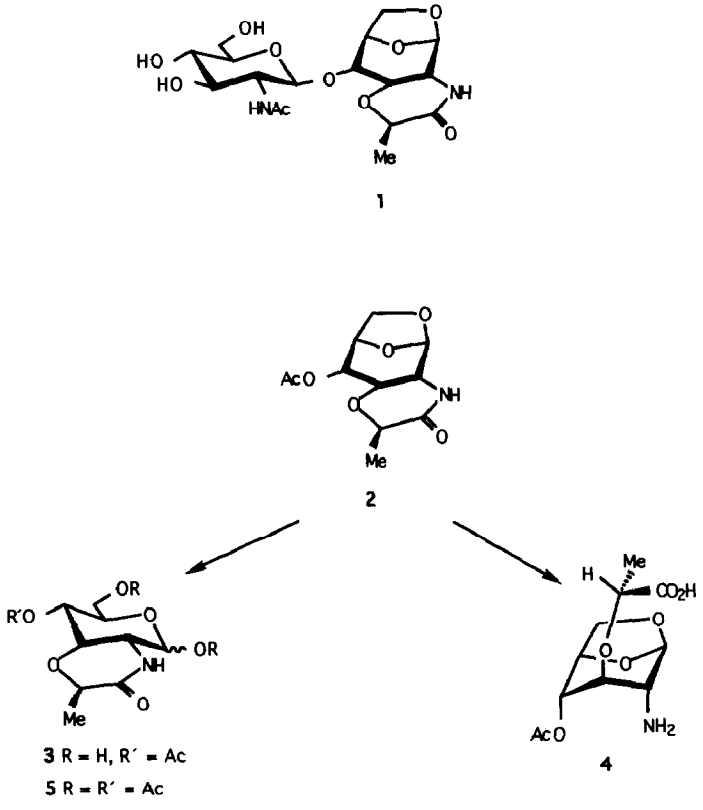
SSDI 0008-6215(94)00039-I

However, information on the biochemical and chemical changes by which the spore attains the resistant state is still very scarce. In contrast to the vegetative cell, the spore peptidoglycans contain a substantial amount of muramic acid residues that are intramolecularly cyclised into a bicyclic δ-lactam structure [2,3].

We have recently reported the synthesis and conformational analysis of several muramic acid δ-lactams and 1,6-anhydromuramic acid δ-lactams, as well as their 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-substituted derivatives [4]. ^{1}H NMR spectroscopy and X-ray analysis have revealed the β-D-glucopyranose ring in the tricyclic 1,6-anhydromuramic acid δ-lactam structure to be in the $B_{O,3}$ conformation in the crystalline state and in solution; opening of the 1,6-anhydro ring resulted in transformation to the more stable $^4C_1$ conformation. The conformational changes of the bicyclic 1,6-anhydro-β-D-glucopyranose structure upon conversion, by lactam formation, into the highly rigid tricyclic system have not been studied so far. The unique conformation of the [4.2.1.1] dodecane skeleton and the relatively high melting points (between 200 and 300°C) of this type of compound



Scheme 1. Chemical formulae of compounds studied in this paper.

make them a good choice for cooperative conformational analysis based on X-ray and computational chemistry methods. In addition, such studies might contribute to a better understanding of the enzymatic process operating in the vegetative bacterial cell, by which a lytic transglycosylase catalyses the conversion of the terminal *N*-acetylmuramic acid residue of the peptidoglycan into a 1,6-anhydro-*N*-acetylmuramoyl-peptide of low molecular weight [5].

2. Results and discussion

General.—In the present study, the 4-*O*-glycosylated 1,6-anhydromuramic acid δ-lactam **1** has been the subject of conformational analysis based on X-ray data (Fig. 1) and computational chemistry. The previously synthesised compound **2**, which also involves the 1,6-anhydromuramic acid δ-lactam skeleton, of known molecular structure (from X-ray analysis), was subjected to computer-simulated ring opening of the 1,6-anhydro ring, resulting in an anomeric mixture of 4-*O*-acetyl-2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose 1′,2-lactam (**3α,β**). The conformational analysis by molecular mechanics and molecular dynamics simulations shows that the $^4C_1$ conformation of the D-glucopyranose ring is optimal for both the α and β anomer of **3** (Table 1). These results are in agreement with those for the synthetically prepared 1,4,6-tri-*O*-acetyl derivative **5** obtained as an anomeric mixture (^{1}H NMR); during crystallisation, the α anomer was deposited first, and its crystal structure was determined [4].

Since the 1,6-anhydro-β-D-glucopyranose derivatives selected from the Cambridge Structural Database [6] (version 5) revealed only a distorted chair/sofa conformation of the glucopyranose ring, we decided to design an appropriate model compound and to study its conformations. The computer-simulated cleavage of the δ-lactam ring performed on **2** resulted in 4-*O*-acetyl-2-amino-1,6-anhydro-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose (**4**) which was subjected to conformational analysis by computational chemistry methods (Table 1). The conformer of **4** of optimal energy exhibits a distorted conformation of the β-D-glucopyranose ring — between chair and sofa (Table 1). The computer modelling appears to be an advantageous method for studying the conformation of **4**. The high tendency for internal cyclisation of **4** would make the isolation of such a compound, as shown experimentally with the methyl ester of **4** [4], rather difficult. The present work indicates that compounds with the [4.2.1.1] dodecane skeleton have a strict preference for a particular ring conformation, giving them great rigidity.

X-ray structure analysis of **1**.—The structure with atom numbering is shown in Fig. 1. The ORTEP [7] plot is drawn with thermal ellipsoids at a 50% probability level. Selected bond lengths and angles are listed in Table 2. The geometry of the 1,6-anhydromuramic acid δ-lactam system reproduces the values already found in 1,6-anhydro-4-*O*-benzylmuramic acid 1′,2-lactam and the corresponding 4-*O*-acetyl analogue [4]. We shall discuss only the bond angles and lengths which depart from

the characteristic values defined by the atom type and hybridisation. 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 2). The bond angle at O-5 has been reduced to 100.3(3)°. The same effect was detected in the compounds containing the 1,6-anhydro-β-D-glucopyranose system [4,8,9]. The variations in C–O bond length in the anhydro ring are within the range of 3σ. The bond angle about the glycosidic linkage is 114.3(4)°; the C–O glycosidic bonds are asymmetrical, 1.437(4) and 1.386(5) Å, which is due to the exo-anomeric effect [10]. Bond lengths and angles of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue are in agreement with the values found in β-D-pyranosides of the $^4C_1$ conformation [11].

The conformation of **1** is described by selected torsion angles (Table 3) and asymmetry parameters [12] (Table 4). The 1,6-anhydro-β-D-glucopyranosyl residue has a somewhat distorted $B_{O,3}$ conformation of the β-D-glucopyranose ring (Tables 3 and 4). The 1,6-anhydro five-membered ring appears in an envelope conformer E_O. In the crystal structure of 4-*O*-acetyl-1,6-anhydromuramic acid 1',2-lactam (**2**), two crystallographically independent molecules were found, molecule A having a

Table 1
Conformational analysis of **2**, **5**, **3α**, **3β**, and **4** based on values of torsion angles (°) obtained from X-ray structure analysis and molecular dynamics simulations

	D-Glucopyranose moiety in muramic acid δ-lactam residue							1,6-Anhydro moiety		
	Bond sequences									
	C-1→	C-2→	C-3→	C-4→	C-5→	O-5→C-1	Conformer	O-5→	C-5→	C-6→
Compound **2**										
X-ray	0.9	−57.9	44.3	23.3	−82.0	67.0	$B_{O,3}$	40.3	−17.3	−12.9
MD	−14.0	−44.4	44.2	14.0	−72.5	72.7	$B_{O,3}$	46.8	−33.8	7.8
Compound **5**										
X-ray	55.8	−59.6	60.7	−59.2	58.8	−56.6	α-$^4C_1$			
MD	55.6	−56.0	54.2	−54.2	60.9	−60.9	α-$^4C_1$			
	36.0	−64.7	40.8	10.6	−42.7	17.3	$^2S_3$			
Compound **3α**										
MD	55.5	−56.5	55.1	−54.1	58.9	−59.3	α-$^4C_1$			
Compound **3β**										
MD	55.5	−56.3	55.1	−53.5	58.1	−59.1	β-$^4C_1$			
	29.3	−64.0	47.2	3.3	−41.3	23.4	$^2S_3/B_{O,3}$			
Compound **4**										
MD	−55.8	33.6	−33.7	55.1	−76.4	77.7	$^1C_4/E_O$	45.2	−33.0	8.3
	−23.5	−33.8	38.8	14.1	−71.5	77.6	$B_{O,3}$	46.9	−37.9	14.1
Ref. 9 [a]										
	−56.3	32.6	−33.4	55.1	−76.0	77.7	$^1C_4/E_O$	45.1	−33.7	9.9

[a] 3-Amino-1,6-anhydro-3-deoxy-β-D-glucopyranose selected from the Cambridge Structural Database.

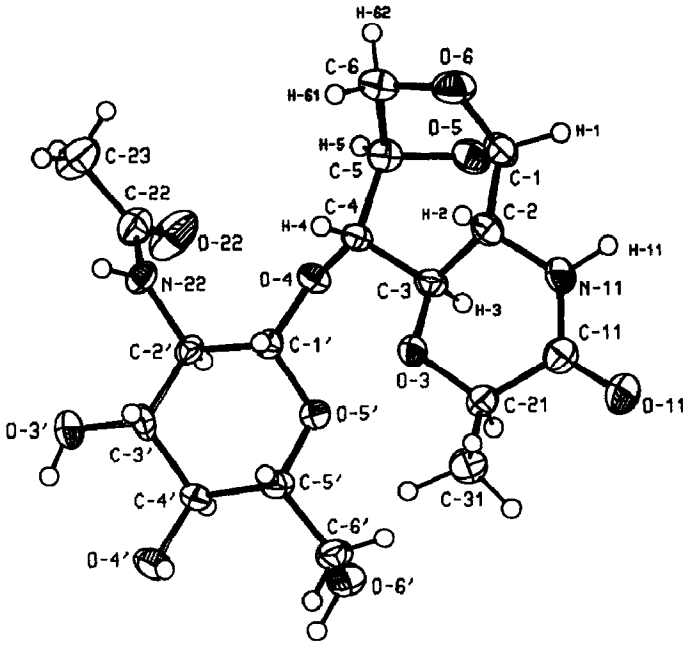


Fig. 1. Molecular structure of 1 (ORTEP drawing) with atom numbering. The thermal ellipsoides are drawn at a 50% probability level.

δ-Lactam moiety									
O-6 →	C-1 → O-5	Conformer	C-2 →	C-3 →	O-3 →	C-21 →	C-11 →	N-11 → C-2	Conformer
39.8	−50.7	$^{1}T_{O}$	56.0	−69.0	46.2	−15.6	7.2	−26.5	$^{O}H_{3}/E_{3}$
21.3	−43.5	$E_{O}/^{5}T_{O}$	60.0	−56.6	29.7	−4.5	10.2	−37.7	E_{3}
			54.3	−70.9	48.1	−12.8	−0.6	−19.2	$^{O}H_{3}$
			54.1	−56.8	34.5	−7.9	7.7	−30.8	E_{3}
			46.6	−55.1	37.9	−11.1	4.8	−22.6	$^{O}H_{3}/E_{3}$
			53.9	−56.6	34.0	−7.0	6.7	−30.1	E_{3}
			55.5	−56.7	33.4	−7.7	9.4	−33.2	E_{3}
			49.8	−55.7	36.9	−11.2	7.9	−27.3	E_{3}
19.7	−41.5	$E_{O}/^{5}T_{O}$							
15.1	−39.7	$^{5}T_{O}$							
18.4	−40.6	$^{5}T_{O}/E_{O}$							

Table 2
Bond lengths (Å) and bond angles (°) for **1**

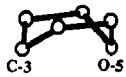
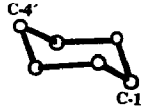
Bond length		Bond angle	
C-1–C-2	1.532(6)	O-5–C-1–C-2	109.6(3)
C-2–C-3	1.521(5)	O-6–C-1–C-2	109.8(4)
C-3–C-4	1.510(5)	O-5–C-1–O-6	105.4(3)
C-4–C-5	1.546(6)	C-1–C-2–C-3	109.4(3)
C-5–C-6	1.516(7)	N-11–C-2–C-3	106.3(3)
C-5–O-5	1.454(6)	N-11–C-2–C-1	113.9(4)
C-6–O-6	1.434(5)	C-2–C-3–C-4	111.5(3)
C-1–O-5	1.419(5)	O-3–C-3–C-2	106.3(3)
C-1–O-6	1.417(6)	O-3–C-3–C-4	112.7(4)
C-2–N-11	1.444(5)	C-3–C-4–C-5	107.7(4)
N-11–C-11	1.341(5)	O-4–C-4–C-3	112.5(3)
C-11–O-11	1.232(6)	O-4–C-4–C-5	106.8(3)
C-11–C-21	1.523(6)	C-4–C-5–C-6	114.7(5)
C-21–C-31	1.509(7)	O-6–C-6–C-5	103.7(4)
C-21–O-3	1.432(5)	O-5–C-5–C-4	110.1(3)
C-3–O-3	1.433(6)	O-5–C-5–C-6	100.7(3)
C-4–O-4	1.437(4)	C-1–O-5–C-5	100.3(3)
O-4–C-1′	1.386(5)	C-1–O-6–C-6	106.8(3)
C-1′–C-2′	1.530(5)	C-3–O-3–C-21	108.0(4)
C-2′–C-3′	1.523(6)	C-2–N-11–C-11	122.8(4)
C-3′–C-4′	1.523(6)	N-11–C-11–C-21	118.5(4)
C-4′–C-5′	1.522(6)	O-11–C-11–C-21	118.6(4)
C-5′–C-6′	1.510(6)	O-11–C-11–N-11	122.7(4)
C-5′–O-5′	1.443(6)	O-3–C-21–C-11	113.7(3)
C-1′–O-5′	1.427(5)	O-3–C-21–C-31	108.6(4)
C-6′–O-6′	1.417(7)	C-11–C-21–C-31	111.8(3)
N-22–C-2′	1.446(6)	C-4–O-4–C-1′	114.3(4)
N-22–C-22	1.333(7)	O-4–C-1′–C-2′	111.1(3)
C-22–C-23	1.489(7)	O-4–C-1′–O-5′	108.0(4)
C-22–O-22	1.237(8)	C-2′–C-1′–O-5′	108.0(3)
C-3′–O-3′	1.423(5)	C-1′–C-2′–C-3′	106.7(3)
C-4′–O-4′	1.423(6)	C-2′–C-3′–C-4′	109.4(4)
		C-3′–C-4′–C-5′	109.8(3)
		C-4′–C-5′–O-5′	108.4(3)
		C-1′–O-5′–C-5′	111.0(4)
		C-1′–C-2′–N-22	113.0(3)
		C-3′–C-2′–N-22	111.6(4)
		N-22–C-22–O-22	122.7(4)
		N-22–C-22–C-23	116.9(6)
		O-22–C-22–C-23	120.4(5)
		C-2′–C-3′–O-3′	108.5(3)
		O-3′–C-3′–C-4′	111.7(3)
		C-3′–C-4′–O-4′	109.6(4)
		O-4′–C-4′–C-5′	111.6(3)
		C-4′–C-5′–C-6′	114.1(3)
		O-5′–C-5′–C-6′	108.6(4)
		C-5′–C-6′–O-6′	113.1(4)

Table 3
Selected torsion angles values (°) for the ring residues of **1** obtained from X-ray structure analysis and molecular mechanics calculations (DISCOVER)

	D-Glucopyranose moiety in muramic acid δ-lactam residue							1,6-Anhydro moiety					
	Bond sequences												
	C-1→	C-2→	C-3→	C-4→	C-5→	O-5→C-1	Conformer	O-5→	C-5→	C-6→	O-6→	C-1→O-5	Conformer
X-ray	−12.4	−48.8	46.2	14.9	−75.5	73.0	$B_{O,3}$	45.9	−31.0	4.2	25.3	−45.1	E_O
MM	−14.3	−43.8	43.9	14.0	−72.5	72.7	$B_{O,3}$	47.2	−34.5	8.6	20.8	−43.5	$E_O/{}^5T_O$

	δ-Lactam moiety							D-Glucopyranose moiety in *N*-acetylglucosamine residue						
	Bond sequences													
	C-2→	C-3→	O-3→	C-21→	C-11→	N-11→C-2	Conformer	C-1′→	C-2′→	C-3′→	C-4′→	C-5′→	O-5′→C-1′	Conformer
X-ray	64.6	−73.5	46.1	−12.9	7.2	−32.2	E_3	63.5	−58.4	56.3	−57.1	64.1	−68.0	β-${}^4C_1$
MM	60.0	−57.9	31.7	−5.8	10.2	−36.7	E_3	50.6	−51.8	55.5	−56.9	60.5	−58.0	β-${}^4C_1$

Table 4
Ring conformation analysis based on asymmetry parameters [a] values for **1**, using X-ray data and molecular mechanics results (DISCOVER)

Ring residue	Asymmetry parameter	X-ray			MM	
D-Glucopyranose moiety in muramic acid δ-lactam residue	ΔC_s(O-5)	2.5	$B_{O,3}$		0.2	$B_{O,3}$
	ΔC_s(C-1–C-2)	26.9			28.8	
1,6-Anhydro moiety	ΔC_s(O-5)	4.1	E_O		10.0	E_O
	ΔC_2(O-6)	17.9			10.7	$\frac{}{^5T_O}$
δ-Lactam moiety	ΔC_s(C-3)	10.1	E_3		4.2	E_3
D-Glucopyranose moiety in *N*-acetylglucosamine residue	ΔC_s(C-1′)	4.2	β-$^4C_1$		6.6	β-$^4C_1$
	ΔC_s(C-2′)	8.4			6.9	
	ΔC_s(C-3′)	4.5			4.5	
	ΔC_2(C-1′–C-2′)	8.7			5.6	
	ΔC_2(C-2′–C-3′)	9.2			3.6	
	ΔC_2(C-3′–C-4′)	1.0			7.9	

[a] The asymmetry parameters according to ref. 12.

twist and molecule B an envelope conformation of the 1,6-anhydro ring. The δ-lactam ring in **1** appears in a sofa conformation, puckered at C-3. In analogous monosaccharide compounds, we detected a sofa and a conformer intermediate between half-chair and sofa [4]. The β-D-glucopyranose ring of the *N*-acetylglucosaminyl residue exhibits a chair $^4C_1$ conformation (Tables 3 and 4). The conformation about the glycosidic bond is defined by O-5′–C-1′–O-4–C-4 of −99.1(4)° and C-1′–O-4–C-4–C-3 of 76.3(5)°. The same type of conformation has been observed in the crystal structure of methyl 4-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (methyl β-cellobioside) (−88.9°, 80.3°) [13].

Crystal packing and hydrogen bonds of **1**.—The crystal packing is dominated by hydrogen bonds (Table 5, Fig. 2). The hydroxyl protons and the N–H group of the *N*-acetylglucosamine moiety together with N–H of the δ-lactam ring act as donors

Table 5
Hydrogen bond geometry for **1**

	D–H···*A*(Å)	*D*–H(Å)	H···*A*(Å)	*D*–H···*A*(°)	Symmetry operations on *A*
N-11–H···O-3′	2.979(6)	1.01(6)	2.44(7)	113(5)	$x+1, y-1, z$
N-22–H···O-22	3.035(7)	1.01(6)	2.06(6)	162(4)	$x, y+1, z$
O-4′–H···O-6′	2.651(6)	0.97(9)	1.70(9)	164(8)	$x, y+1, z$
O-6′–H···O-4′	2.691(4)	0.98(6)	1.71(5)	175(5)	$-x, y+1/2-1, -z+1$
O-3′–H···O-11	2.769(6)	0.98(7)	1.91(7)	146(5)	$x-1, y+1, z$
O-3′–H···O-4′	2.861(4)	0.98(7)	2.35(6)	112(5)	x, y, z

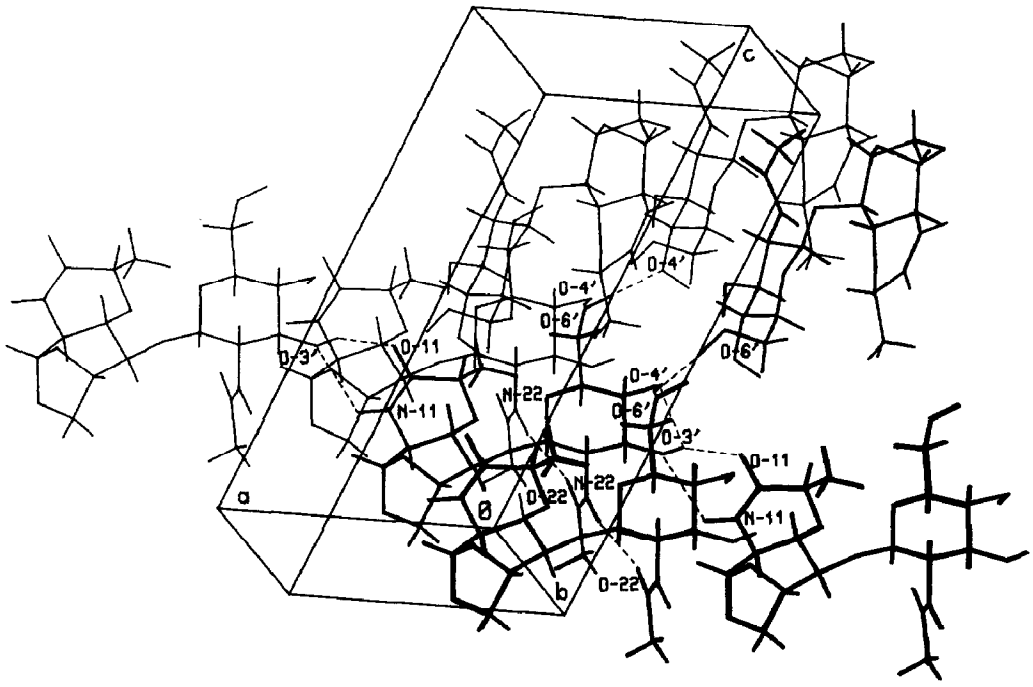


Fig. 2. Crystal structure with the hydrogen bond network for 1.

(five donor sites) to five acceptor oxygens. The three-centered (bifurcated) bond involves O-3′–H′ (of *N*-acetylglucosamine) in intramolecular (to O-4′) and intermolecular hydrogen bonds (to O-11 of the δ-lactam residue). The hydrogen bonds N-11–H · · · O-3′ and O-3′–H · · · O-11 connect molecules into the plane (110), whereas N-22–H · · · O-22 and O-4′–H · · · O-6′ join molecules in direction [010]. A three-dimensional network is completed by O-6′–H · · · O-4′ along *c*.

Molecular mechanics and dynamics study of **1**.—A comparative conformational analysis of 1,6-anhydromuramic acid δ-lactam and *N*-acetylglucosaminyl residues based on X-ray analysis and molecular mechanics (DISCOVER [14]) calculations is summarised in Tables 3 and 4. In order to explore the conformational stability of the molecule, molecular dynamics simulations (in vacuo, DISCOVER [14]) at high temperatures (1200 K) were carried out. The results obtained do not reveal any conformational changes of the ring residues. They are in agreement with the values obtained by the molecular mechanics geometry optimisation listed in Tables 3 and 4. A bacterial spore represents a dormant stage of life which excludes the presence of water and therefore molecular dynamics simulations of the muramic acid δ-lactam structure in water have not been considered to be of biological interest. The relatively flexible part of the molecule appears to be about the β-(1 → 4) glycosidic linkage. The systematic rotation by 10° increments about C-1′–O-4 (Φ) and O-4–C-4 (Ψ) was applied in order to select the optimal conformer(s) by the method of "prudent ascent" [15] using the MM3(92) program [16,17]. A conforma-

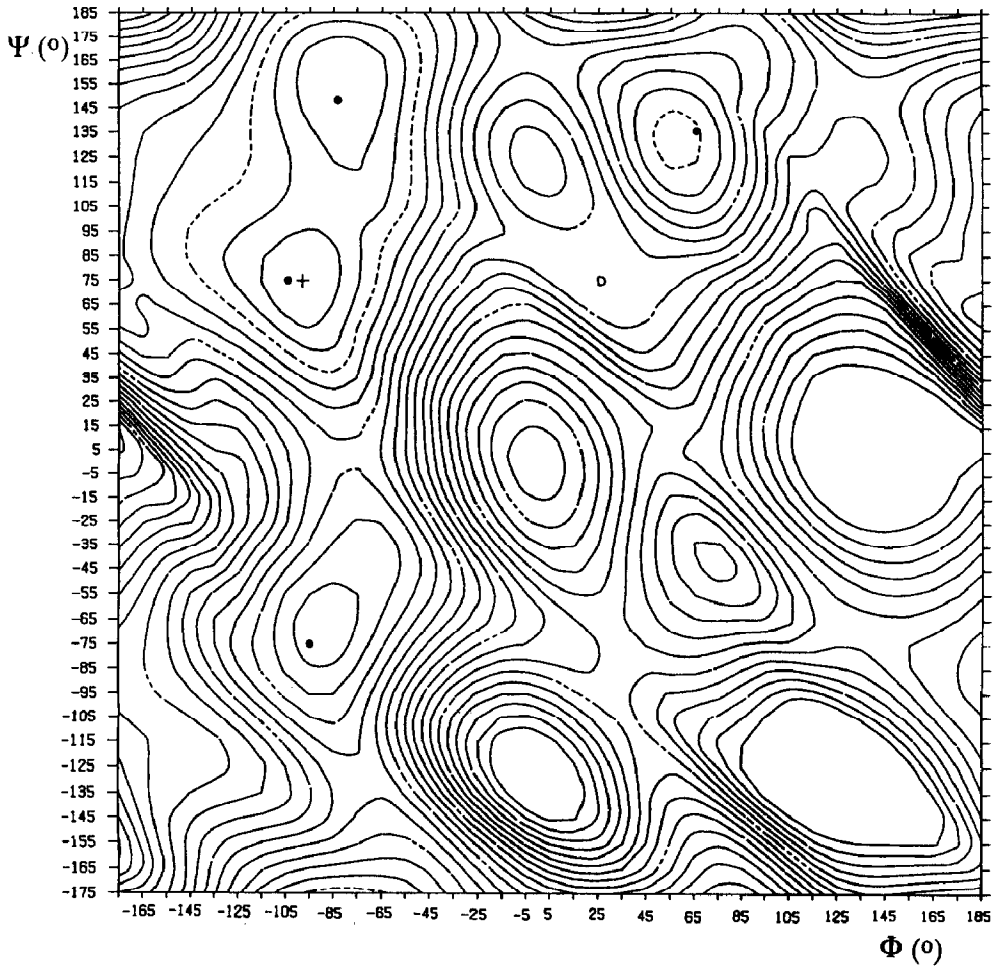


Fig. 3. Two-dimensional energy surface (kcal/mol) with a separation of energy levels of one kcal/mol as a function of two torsion angles Φ and Ψ (simultaneous rotation in 10-degree steps) for 1. Detected energy minima (obtained by molecular mechanics) correspond to four conformers indicated by dots. The X-ray conformer is marked by the + sign.

tional energy map as a function of these two angles (Fig. 3) has revealed four energy optimal conformers; two of them (−105°, 75° and −85°, 145°) represent a wide global minimum. The conformation determined by X-ray (−99.1°, 76.3°) is very close to the global minimum conformer (−105°, 75°). However, the energy difference among the four optimal conformers does not exceed 4 kcal/mol. Molecular dynamics simulations (DISCOVER [14]) at 1200 K detected also three groups of conformations about the glycosidic linkage. By the optimisation procedure, they led to the conformers (defined by Φ and Ψ, respectively): (−94°, 69°), (−76°, 154°), and (50°, 125°). The first two will be the preponderant conformations separated by a very small barrier, giving the linkage a rather flexible character. The

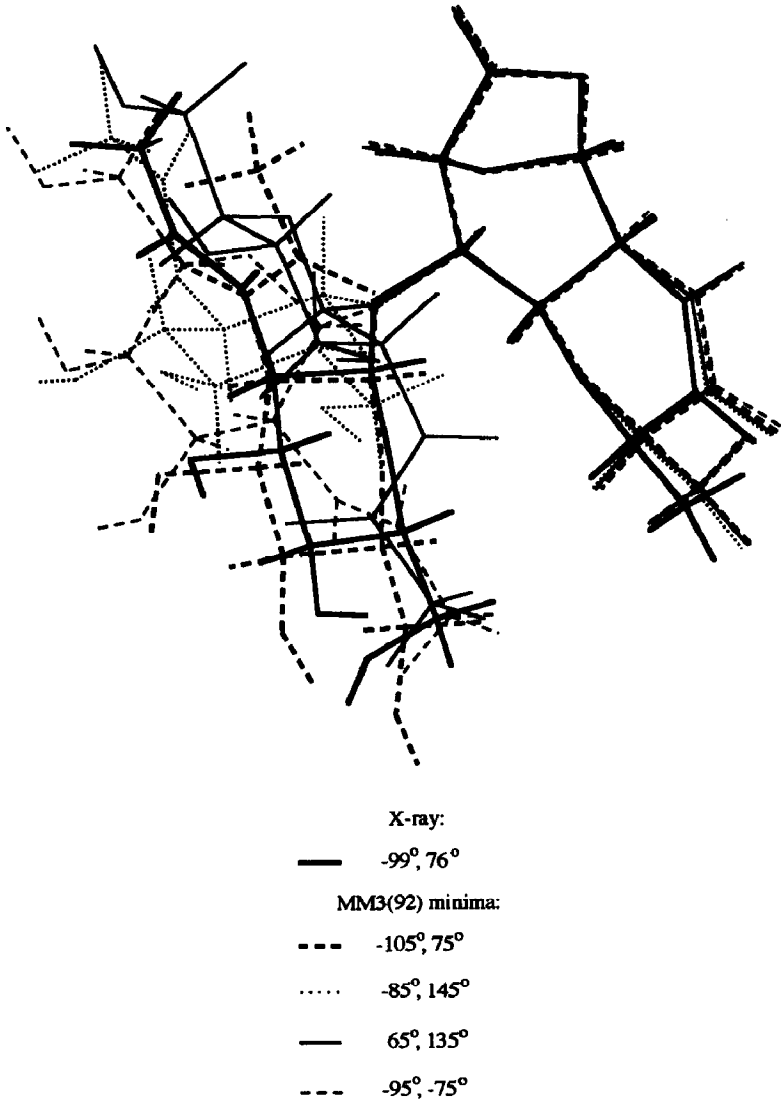


Fig. 4. Superposition of conformers (based on the least-squares fit of the D-glucopyranose ring) obtained by X-ray structure analysis and molecular mechanics study for 1.

values agree very well with those obtained by molecular mechanics study by the method of "prudent ascent". The overlap of energy minima conformers found by the method of "prudent ascent" and the conformer found by X-ray analysis is shown in Fig. 4.

Molecular mechanics and dynamics study on **2**, **5**, *and computer-designed structures* **3α**, **3β**, *and* **4**.—The results of conformational analysis based on torsional angles obtained from X-ray analysis and molecular dynamics simulation are listed in Table 1. The subjects of analysis are the β-D-glucopyranose, 1,6-anhydro, and

δ-lactam rings in the synthesised compounds **2** and **5** as well as in the computer-designed **3α,β** and **4**. For comparison, an example of a 1,6-anhydro-β-D-glucopyranose derivative from the Cambridge Structural Database [6] was selected.

Molecular mechanics was used to optimise the geometry of both conformers of **2** in the crystal lattice environments (DISCOVER [14]). The results obtained revealed the distorted $B_{O,3}$ conformation of the D-glucopyranose ring to be again more distorted for A than for B. However, the optimisation of both conformers in vacuo ended with a single conformer ($B_{O,3}$). Molecular dynamics simulations at 1200 K in vacuo (DISCOVER [14]) were initiated with the atomic coordinates of conformer A (Table 1). During the simulations, the $B_{O,3}$ conformation of the β-D-glucopyranose ring was maintained. However, the 1,6-anhydro ring has been changed from the twist (^1T_O) to the envelope/twist ($E_O/{}^5T_O$) conformation and the δ-lactam ring has exhibited transition from an intermediate half-chair/sofa ($^OH_3/E_3$) to sofa (E_3) conformation (Table 1).

The computer-simulated opening of the 1,6-anhydro ring in **2** has been accomplished in two ways: cleavage of the C-6–O-6 bond and accommodation of the HO group at C-1 in the equatorial position and cleavage of the C-1–O-6 bond and

Table 6
Crystal data and summary of experimental details for **1**

Molecular formula	$C_{17}H_{26}N_2O_{10}$
M_r	418.4
Crystal size (mm)	0.072×0.036×0.18
a(Å)	10.446(4)
b(Å)	4.891(1)
c(Å)	18.780(7)
β(°)	94.33(2)
V(Å^3)	956.76(9)
Crystal system	monoclinic
Space group	$P2_1$
D_x (gcm^{-3})	1.4523(1)
Z	2
μ (Cu$K\alpha$) (cm^{-1})	9.8
F (000)	444
T (K)	295(3)
No. of reflections used for cell parameters and θ range (°)	25 10–45
θ range for intensity measurement (°)	3–70
hkl range	0,12; 0,5; −22,22
Scan	ω/θ
$\Delta\omega$	0.80+0.15×tan θ
No. of measured reflections	2569
No. of symmetry independent reflections	1546, $I > 2\sigma(I)$
No. of variables	365
R	0.035
R_w, $w^{-1} = k(\sigma^2 F_o + gF_o^2)$	0.040
Final shift/error	−0.106(C4, y)
Residual electron density $(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$(eÅ^{-3})	0.16, −0.19

accommodation of the HO group at C-1 in the axial position of the boat conformation, giving the new compounds **3α** and **3β**, respectively. A molecular mechanics calculation (DISCOVER [14]) was used to optimise the geometry of **3α** in vacuo. The conformation of the β-D-glucopyranose ring changed from $B_{O,3}$ to $^4C_1$. The torsion angle C-5–O-5–C-1–O-1 of 64.8° corresponds to an axially positioned anomeric bond, typical of the α anomer. The δ-lactam ring appeared in a sofa (E_3) conformation. Molecular dynamics simulations at 1200 K in vacuo followed by an optimisation revealed the chair $^4C_1$ conformation of the glucopyranose ring (Table 1).

The 4-*O*-acetylated derivative (**5**) of **3α** was previously synthesised and characterised by X-ray structure analysis [4]. Its α-D-glucopyranose ring exhibits a $^4C_1$ conformation, whereas the δ-lactam ring is in a half-chair conformation. Molecular mechanics calculations performed in vacuo and for the crystalline lattice environment (DISCOVER [14]) revealed, as the optimal energy conformer, only the $^4C_1$ chair. Molecular dynamics simulations at 1200 K in vacuo and the optimisation of

Table 7
Final coordinates and equivalent isotropic thermal parameters of the nonhydrogen atoms for **1**

Atom	x	y	z	U_{eq}(Å^2) [a]
O-3	0.4651(2)	0.5305(9)	0.2923(1)	0.0299(8)
O-4	0.2202(2)	0.3305(9)	0.2053(1)	0.0269(7)
O-5	0.4532(3)	0.02110(0)	0.1280(1)	0.0354(8)
O-6	0.5561(3)	0.3527(11)	0.0714(1)	0.0450(9)
O-11	0.7513(3)	0.2024(11)	0.3688(2)	0.0463(10)
O-22	−0.0855(4)	0.2188(10)	0.1365(2)	0.0589(14)
O-3′	−0.1427(3)	0.7865(10)	0.2923(2)	0.0422(10)
O-4′	0.0008(3)	0.8264(10)	0.4278(1)	0.0411(10)
O-5′	0.2046(2)	0.3883(9)	0.3246(1)	0.0279(7)
O-6′	0.1614(3)	0.2232(10)	0.4708(1)	0.0388(9)
N-11	0.6752(3)	0.2407(11)	0.2534(2)	0.0369(10)
N-22	−0.0121(3)	0.6325(11)	0.1735(2)	0.0291(10)
C-1	0.5685(4)	0.1755(11)	0.1312(2)	0.0329(14)
C-2	0.5803(3)	0.3436(11)	0.2002(2)	0.0291(11)
C-3	0.4528(3)	0.3395(12)	0.2343(2)	0.0276(10)
C-4	0.3422(3)	0.4064(11)	0.1807(2)	0.0245(10)
C-5	0.3594(4)	0.2369(12)	0.1126(2)	0.0294(11)
C-6	0.4212(4)	0.3903(14)	0.0540(2)	0.0390(14)
C-11	0.6676(4)	0.2805(11)	0.3236(2)	0.0344(13)
C-21	0.5464(3)	0.4107(11)	0.3487(2)	0.0309(11)
C-22	−0.0710(4)	0.4657(13)	0.1254(2)	0.0387(14)
C-23	−0.1206(6)	0.5901(15)	0.0564(3)	0.0511(18)
C-31	0.5769(4)	0.6248(13)	0.4053(2)	0.0421(14)
C-1′	0.1757(3)	0.5064(11)	0.2558(2)	0.0256(10)
C-2′	0.0301(3)	0.5424(11)	0.2448(2)	0.0243(10)
C-3′	−0.0076(3)	0.7453(12)	0.3011(2)	0.0293(11)
C-4′	0.0342(3)	0.6355(11)	0.3751(2)	0.0271(10)
C-5′	0.1778(4)	0.5780(11)	0.3804(2)	0.0283(13)
C-6′	0.2288(4)	0.4597(12)	0.4512(2)	0.0340(13)

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

the most frequent conformers led to the $^4C_1$ chair (Table 1). The δ-lactam ring changed from a half-chair to sofa. The energy of the conformer with the D-glucopyranose ring in a twisted ($^2S_3$) conformation is ca. 11 kcal/mol greater than that of the optimal energy conformer (Table 1).

The geometry optimisation of **3β** in vacuo did not reveal a change of the $B_{O,3}$ conformation of the β-D-glucopyranose ring. However, molecular dynamics simulations in vacuo at high temperature (1200 K) followed by the energy optimisation of the most frequent conformers showed that a conformer with the β-D-glucopyranose ring in the $^4C_1$ conformation is more stable than the distorted $^2S_3/B_{O,3}$ conformer (energy difference is ca. 10 kcal/mol). From these calculations, it also emerged that the conformational energy of the β anomer of the D-glucopyranose ring is ca. 10 kcal/mol lower than that of the α anomer, as discussed by Stoddart [18] for most of the D-aldohexopyranoses.

The computer-designed compound **4** generated from **2** by opening of the δ-lactam ring was subjected to geometry optimisation by molecular mechanics in vacuo; in the conformer obtained, D-glucopyranose has adopted a distorted $B_{O,3}$ conformation. Molecular dynamics simulations (in vacuo, at 1200 K) followed by geometry optimisation revealed the distorted $^1C_4/E_O$ conformation of the D-glucopyranose ring. The energy of the conformer with distorted $B_{O,3}$ is ca. 6 kcal/mol higher than that for $^1C_4/E_O$. Table 1 lists the results of two such conformers obtained for **4**. From the Cambridge Structural Database [6] (version 5), eight crystal structures including the 1,6-anhydro-β-D-glucopyranose bicyclic skeleton with substituents of polarity and size close to those of the examined compounds were selected. Their asymmetry parameters were calculated and a conformation between distorted chair and sofa was determined. One example was selected and added for comparison to Table 1.

From the data available, the glucopyranose moiety having the [4.2.1.1] dodecane skeleton has a strict preference for the boat $B_{O,3}$ conformation; 1,6-anhydro and δ-lactam rings impose high rigidity [4] (**1** and **2**). However, in the structures involving only a 1,6-anhydro ring, a preference for a distorted chair/sofa conformation was found.

3. Experimental

X-ray structure determination of **1**.—Crystals suitable for X-ray analysis were grown from methanol–diisopropyl ether at room temperature during 3 to 6 days. The crystal data and a summary of the experimental details are listed in Table 6. The X-ray intensity data were collected with an Enraf–Nonius CAD4 diffractometer with graphite-monochromatised Cu$K\alpha$ 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 [19]. The structure was solved by SHELX86 [20]. Refinement was by full-matrix least-squares minimizing $\Sigma w(|F_o| - |F_c|)^2$ with the SHELX77 [21] system of programs using F values. 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. The N–H bond distance in the δ-lactam ring and the N–H and O–H bond distances of the *N*-acetylglucosamine moiety were normalised to the values obtained by neutron diffraction (N–H, 1.009 Å; O–H, 0.983 Å). Atomic scattering factors were those included in SHELX77 [21]. Details of the refinement procedures are given in Table 6. During the structure determinations, the D enantiomer was 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 the program package EUCLID [22]. Drawings were prepared by the program PLUTON incorporated in EUCLID [22] and ORTEP II [7]. The final atomic coordinates and equivalent isotropic thermal parameters are listed in Table 7 *.

Molecular mechanics and dynamics calculations.—All molecular dynamics calculations were carried out with the program DISCOVER [14], using the consistent valence force field based on Lifson and Warshel [23] and Hagler et al. [24,25], at a temperature of 1200 K in vacuo ($\varepsilon_r = 1.0$) in order to search the conformational space. Different conformations obtained in this way were energy-minimized by DISCOVER (molecular mechanics). For calculations performed on **1**, the initial molecular model was based on X-ray coordinates. Molecular mechanics geometry optimisation on **1** was also performed using the DISCOVER package. Since compound **1** has additional flexibility due to the glycosidic linkage, a complete (Φ, Ψ) molecular mechanics energy map, using the MM3(92) force field [16] and program [17] ($\varepsilon_r = 1.5$), has been calculated. To obtain the global minimum of all other degrees of freedom (e.g., hydroxyl group orientations) at each (Φ, Ψ), the method of "prudent ascent" has been used [15]. It involves the use of a completely automated procedure [26] as an interface to the MM3(92) driver option. This method leads to a smooth energy profile when driving two torsion angles.

Calculations were performed on Micro-VAX II and IRIS-4D25G computers of the X-ray Laboratory, Rudjer Bošković Institute (Zagreb, Croatia), and on the VAX-Cluster and Evans&Sutherland workstation of the Bijvoet Center for Biomolecular Research, Rijksuniversiteit Utrecht (The Netherlands).

Acknowledgments

This work was supported by Ministry of Science and Technology grants Nos. 1-07-179 and 1-07-192, and The Commission of the European Communities, Brussels, contract No. CI1*-CT91-0891.

* The observed and calculated structure factors, H-atom coordinates, and anisotropic thermal parameters have been deposited with the Cambridge Crystallographic Data Centre. The data may be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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