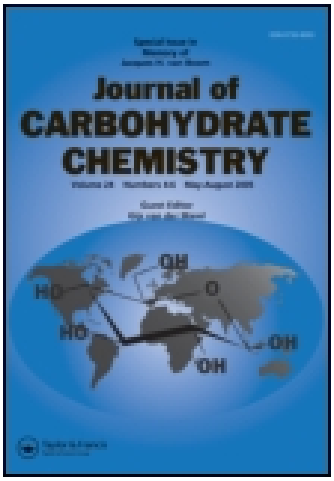


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

2,3,4,6-tetra-O-Acetyl-D-Gluconic Acid: Crystal Structure and Application in the Synthesis of N-(D-gluconyl) Derivatives of D-Glucosamine

Monika Norkowska [a], Henryk Myszka [a], Magdalena Cyman [a], Daria Grzywacz [a], Damian Trzybiński [a], Artur Sikorski [a] & Beata Liberek [a]
[a] Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, PL-80-308 Gdańsk, Poland
Published online: 24 Feb 2014.

To cite this article: Monika Norkowska, Henryk Myszka, Magdalena Cyman, Daria Grzywacz, Damian Trzybiński, Artur Sikorski & Beata Liberek (2014) 2,3,4,6-tetra-O-Acetyl-D-Gluconic Acid: Crystal Structure and Application in the Synthesis of N-(D-gluconyl) Derivatives of D-Glucosamine, Journal of Carbohydrate Chemistry, 33:1, 33-47, DOI: 10.1080/07328303.2013.872790

To link to this article: http://dx.doi.org/10.1080/07328303.2013.872790

Journal of Carbohydrate Chemistry, 33:33–47, 2014

ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328303.2013.872790

2,3,4,6-tetra-*O*-Acetyl-D-Gluconic Acid: Crystal Structure and Application in the Synthesis of *N*-(D-gluconyl) Derivatives of D-Glucosamine

Monika Norkowska, Henryk Myszka, Magdalena Cyman, Daria Grzywacz, Damian Trzybiński, Artur Sikorski, and Beata Liberek

Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, PL-80-308 Gdańsk, Poland

2,3,4,6-tetra-*O*-Acetyl-D-gluconic acid was synthesized and coupled with 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose and diosgenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside to afford *N*-gluconyl derivatives of diosgenyl 2-amino-2-deoxy-D-glucopyranoside using the methods of solution-phase peptide synthesis. Both coupling reactions suffered from acetyl $O \rightarrow N$ migration, which caused the *N*-acetyl derivatives to be formed together with the *N*-(D-gluconyl) derivatives of D-glucosamine. Additionally, single-crystal X-ray diffraction and high-resolution NMR spectral data for 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid were analyzed to reveal that this acyclic carbohydrate has adopted the ${}_2G^-$ conformation instead of a typical zigzag conformation. The planarity and *cis* geometry of the acetoxyl groups are demonstrated.

Keywords D-Glucosamine; D-Gluconic acid; X-ray critical structure; Conformation; Mesomeric effect

INTRODUCTION

Our research is aimed to synthesize and study *N*-substituted derivatives of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside **2**, a synthetic steroid saponin. Naturally occurring diosgenyl glycosides are widely distributed in plants and marine organisms.[1–3] The biological activities of these glycosides

Received September 24, 2013; accepted December 3, 2013.
Address correspondence to Beata Liberek, Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, PL-80-308 Gdańsk, Poland. E-mail: beata.liberek@ug.edu.pl

are manifold: anti-inflammatory, antibacterial, antiparasitic, antifungal, and antitumor,[4,5,6,7] making them attractive targets for synthetic and biological studies. For example, a number of synthetic analogs of diosgenyl glycosides have recently been synthesized and subjected to SAR studies.[8,9]

As we have demonstrated previously, diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside **2** showed promising antitumor and antimicrobial activities.[10,11] Unfortunately, its solubility in water is relatively poor. The presence of a free amino group in **2** provides the opportunity for further derivatization to discover new analogs with improved solubility and cytotoxic activity.[12] For this purpose, the *N*-gluconyl analog of **2** and related structures were designed and synthesized.

Here, the synthesis of *N*-gluconyl derivatives of 2-amino-2-deoxy-D-glucopyranose and its diosgenyl glycoside is presented. Coupling of 2-amino-2-deoxy-D-glucopyranose and its diosgenyl glycoside with D-gluconic acid required the acetylated forms of all the substrates. Therefore, 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid was synthesized. In the second part of this article, the crystal structure of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid is presented. Crystal data enabled us to disclose the conformation of the D-gluconic acid chain, orientation of the acetoxyl groups, and intra- and intermolecular interactions in the crystal lattice of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid monohydrate.

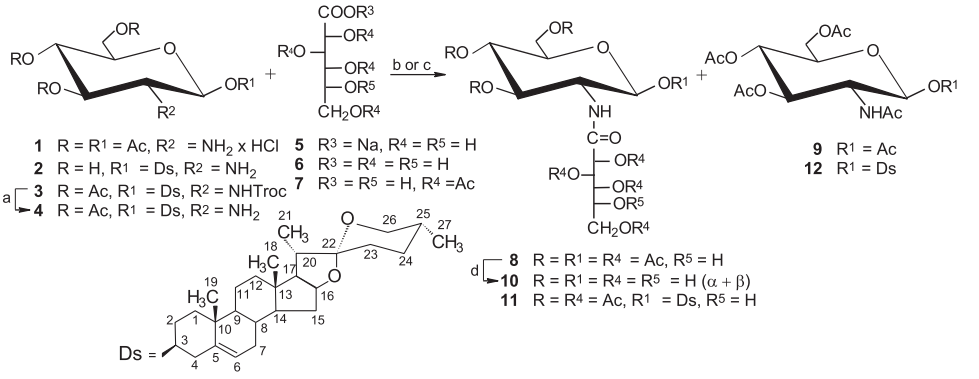
RESULTS AND DISCUSSION

Syntheses

1,3,4,6-tetra-*O*-Acetyl-D-glucosamine hydrochloride **1**,[10] diosgenyl 2-amino-2-deoxy-β-D-gluco-pyranoside **2,**[10] or its per-*O*-acetylated analog **4** was used to couple with sodium D-gluconate **5**, D-gluconic acid **6,** or its 2,3,4,6-tetra-*O*-acetyl derivative **7** by the method of solution-phase peptide synthesis (Sch. 1). Even though coupling of 1,3,4,6-tetra-*O*-acetyl-D-glucosamine hydrochloride **1** with sodium L-lactate in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) proceeded smoothly (unpublished data), the analogous reaction of **1** with commercially available sodium D-gluconate **5** failed to proceed efficiently. Similarly, the coupling of diosgenyl 2-amino-2-deoxy-D-gluco-pyranoside **2** with D-gluconic acid **6**, prepared from **5** upon treatment with Dowex H^+, in the presence of diisopropylcarbodiimide (DIC) and HOBt was unsuccessful. We therefore decided to use the acetylated form of D-gluconic acid **7**.

It is known that direct acetylation of D-gluconic acid **6** yields a complicated mixture of products. Thus, we employed the procedure where D-glucono-δ-lactone was acetylated to give 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid

7 monohydrate.[13] Coupling of 1,3,4,6-tetra-*O*-acetyl-D-glucosamine hydrochloride **1** with 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** in presence of DCC, HOBt, and Et_3N was successful, which led to two products: 1,3,4,6-tetra-*O*-acetyl-*N*-(2′,3′,4′,6′-tetra-*O*-acetyl-D-gluconyl)-2-amino-2-deoxy-β-D-glucopyranose **8** (28%) and 1,3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose **9** (42%). The latter was formed from acetyl $O \rightarrow N$ migration. Coupling of 1,3,4,6-tetra-*O*-acetyl-D-glucosamine hydrochloride **1** with 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** was also conducted in the presence of DIC and HOBt, giving the same products **8** and **9** with similar yields. Complete *O*-deacetylation of **8** with sodium methylate gave 2-amino-2-deoxy-*N*-(D-gluconyl)-D-glucopyranose **10** (85%) as a mixture of α and β anomers (α:β = 1.2:1.0), as identified by ^{1}H NMR.



Reaction condiitons: a: Zn-Cu / AcOH; b: DCC / HOBt / Et_3N; c: DIC / HOBt; d: MeONa / MeOH

Scheme 1: Coupling of 2-amino-2-deoxy-D-glucopyranose derivatives with 2,3,4,6-tetra-*O*-D-gluconic acid.

An attempt was also made to couple 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** with diosgenyl 2-amino-2-deoxy-D-gluco-pyranoside **2** in the presence of DIC and HOBt. Unfortunately, no desired reaction was observed. Therefore, we synthesized diosgenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-D-gluco-pyranoside **4**, which was less hydrophilic than **2**, from the previously reported diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloro-ethoxy-carbonyl-amino)–β-D-gluco-pyranoside (**3**).[14] Coupling of **4** with **7** in the presence of DIC and HOBt yielded diosgenyl 3,4,6-tri-*O*-acetyl-*N*-(2′,3′,4′,6′-tetra-*O*-acetyl-D-gluconyl)-2-amino-2-deoxy-β-D-glucopyranoside **11** (17%), as well as diosgenyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside **12** (34%). The result again demonstrated that the use of acetylated D-glucosamine and D-gluconic acid derivatives caused acetyl $O \rightarrow N$ migration to occur.

Table 1: Crystal data and structure refinement for **7** monohydrate

Empirical formula	$C_{14}H_{20}O_{11}\cdot H_2O$
Formula weight	382.32
Temperature (K)	295(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	
a (Å)	7.1732(4)
b (Å)	15.2991(8)
c (Å)	8.2095(4)
β (º)	94.976(5)
V (Å^3)	897.54(8)
Z	2
D_{calcd} (Mg m^{-3})	1.415
Absorption coefficient (mm^{-1})	0.126
Absorption correction type	"multiscan"
F(000)	404
Crystal size (mm)	0.40 × 0.15 × 0.05
Θ Range for data collection (å)	3.62–25.05
Limiting indices	$-5 \le h \le 8, -17 \le k \le 18, -9 \le l \le 9$
Reflections collected/unique	22208 / 3118 ($R_{int} = 0.0269$)
Completeness 2Θ = 25.05å (%)	99.7
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3118 / 4 / 247
Goodness-of-fit on F^2	1.040
Final R indices ($I>2\sigma(I)$)	$R_1 = 0.0450$ w$R_2 = 0.1078$
R indices (all data)	$R_1 = 0.0515$ w$R_2 = 0.1153$
Absolute structure parameter	−0.10(12)
Extinction coefficient	0.046(5)
Largest diff. peak and hole (a Å^{-3})	0.278 and −0.265

The Crystal Structure of 7 Monohydrate

A single crystal of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** was obtained and then subjected to X-ray analysis. The crystallographic data, data collection, and structure refinement are summarized in Table 1. The coordinates of atoms and their isotropic temperature factors are set out in Table 2, and selection of the crystal's important geometric parameters is given in Table 3. The hydrogen bonds and short contacts are summarized in Table 4.

The X-ray crystal structure of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** (Fig. 1) showed that it contained one molecule of water. The occluded water molecule created three hydrogen bonds (Fig. 2), which stabilized the crystal structure. These are H-bonds between the water hydrogen and the oxygen of the sugar 5-OH group, between the water hydrogen and the O7 carbonyl oxygen of the 2-OAc group, and between the water oxygen and the H11 hydrogen of

Table 2: Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters (Å^2 $\times$ 10^3) for **7** monohydrate

Atom	x	Y	z	U_{eq}
O-1W	593(4)	2502(2)	3440(3)	78(1)
C-1	9362(4)	1421(2)	9898(3)	39(1)
O-1	10757(3)	1766(2)	9479(2)	57(1)
O-2	8635(3)	891(1)	7183(2)	44(1)
C-2	7959(4)	926(2)	8761(3)	37(1)
O-3	5283(3)	1503(1)	10086(2)	37(1)
C-3	5991(4)	1331(2)	8526(3)	34(1)
O-4	7120(2)	2799(1)	8558(2)	37(1)
C-4	5927(4)	2192(2)	7611(3)	33(1)
O-5	2694(3)	1986(1)	6643(2)	47(1)
C-5	3975(4)	2602(2)	7411(3)	36(1)
O-6	4515(3)	3213(1)	4835(2)	45(1)
C-6	3934(4)	3426(2)	6416(3)	43(1)
C-7	9291(4)	124(2)	6658(3)	47(1)
O-7	9234(5)	−532(2)	7437(3)	88(1)
C-8	4815(5)	824(2)	10997(4)	48(1)
O-8	4919(5)	84(2)	10555(3)	87(1)
O-9	8662(3)	3167(2)	6393(3)	59(1)
C-9	8407(4)	3259(2)	7797(3)	41(1)
O-10	4960(4)	4622(2)	4333(3)	78(1)
C-10	5032(5)	3878(2)	3911(4)	50(1)
O-11	8920(3)	1408(2)	11418(2)	54(1)
C-11	9981(6)	217(2)	5031(4)	61(1)
C-12	4248(6)	1108(2)	12606(4)	63(1)
C-13	9433(5)	3866(2)	8953(4)	61(1)
C-14	5680(8)	3571(3)	2353(5)	81(1)

U_{eq} is defined as one-third of the trace of the orthogonalized U_{ij} tensor.

the carboxyl group (Table 4). The crystal structure of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid monohydrate was also stabilized by the intermolecular hydrogen bond between carbonyl O1 and the hydrogen of the 5-OH group. Additionally, several C—H···O contacts consolidated the crystal structure (Table 4).

Most acyclic carbohydrate derivatives in the solid state adopt a conformation having the carbon atoms in an extended, planar zigzag arrangement, unless there are parallel 1,3-steric interactions, written in abbreviated form as O//O interactions (Fig. 3).[15] The presence of O//O interactions may cause the carbon chain to twist in such a manner as to give a local *gauche* relationships between carbon atoms in their chains; these have been termed sickle conformers.[16] Nomenclature for *gauche* (sickle) conformations was developed by Horton and Wander.[17] According to their nomenclature of *gauche* conformations, a ${}_2G^-$ conformation is obtained from the planar zigzag conformation by a 120° clockwise rotation of the remote atom along the C2-C3 bond (Fig. 3). Analogously, a ${}_2G^+$ conformation is obtained from the planar zigzag conformation by a 120° counter-clockwise rotation of the remote atom along the C2-C3 bond.

Table 3: Selected bond lengths (Å), valence angles (°), and torsion angles (°) for **7** monohydrate

Bond lengths	(Å)
O-2—C-7	1.348(3)
O-2—C-2	1.423(3)
O-3—C-8	1.340(3)
O-3—C-3	1.442(3)
O-4—C-9	1.355(3)
O-4—C-4	1.444(3)
O-5—C-5	1.426(3)
O-6—C-10	1.340(4)
O-6—C-6	1.435(3)
C-7—O-7	1.193(4)
C-8—O-8	1.194(4)
C-9—O-9	1.191(3)
C-10—O-10	1.193(4)
Valence angles	(°)
C-7—O-2—C-2	118.7(2)
O-2—C-2—C-1	108.6(2)
C-8—O-3—C-3	118.6(2)
O-3—C-3—C-2	110.7(2)
C-9—O-4—C-4	118.76(19)
O-4—C-4—C-3	107.54(19)
C-10—O-6—C-6	117.1(2)
O-7—C-7—O-2	122.1(3)
O-8—C-8—O-3	122.8(3)
O-9—C-9—O-4	123.6(3)
O-10—C-10—O-6	122.6(3)
Torsion angles	(°)
C-1—C-2—C-3—C-4	67.9(3)
C-2—C-3—C-4—C-5	−178.8(2)
C-3—C-4—C-5—C-6	−176.6(2)
C-4—C-5—C-6—O-6	59.5(3)
C-2—O-2—C-7—O-7	3.9(5)
C-3—O-3—C-8—O-8	−1.8(5)
C-4—O-4—C-9—O-9	−3.0(4)
C-6—O-6—C-10—O-10	−2.7(5)

Chain twisting generally results in an oxygen atom extending the chain rather than a hydrogen atom. With reference to the terminal hydroxymethyl group, all rotamers are observed, but the preferred one has the oxygen atom extending the chain.[15]

The crystal data of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** monohydrate presented here corroborate the above-mentioned general rules concerning the conformation of the acyclic carbohydrate derivatives. Clearly, 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** adopts the ${}_2G^-$ conformation in order to avoid the 1,3-parallel interactions between the 2-OAc and 4-OAc groups (Figs. 1 and 3). The ${}_2G^-$ conformation is demonstrated by the C1-C2-C3-C4 torsion angle of 67.9° (Table 3). Interestingly, **7** does not twist to replace the O//O interactions by the O//H interactions (the ${}_2G^+$ conformation). Instead, it twists to replace the

Table 4: Hydrogen bonds and short contacts (*) for **7** monohydrate with distances (d): d(D···A) < R(D) + R(A) + 0.50Å; d(H···A) < R(H) + R(A) − 0.12 Å, and angle (<) <D-H···A > 100.0°

D—H	A	D(D-H)	d(H···A)	d(D···A	<D—H···A
O-1W—H-1W	O-7[a]	0.84(3)	2.32(3)	3.097(4)	153(5)
O-1W—H-2W	O-5	0.84(3)	2.20(3)	3.020(3)	165(5)
O-5—H-5	O-1[b]	0.82	2.06	2.833(3)	157
O-11—H-11	O-1W[c]	0.82	1.78	2.579(3)	166
*C-2—H-2	O-7	0.98	2.23	2.678(4)	106
*C-2—H-2	O-8	0.98	2.50	3.024(4)	113
*C-4—H-4	O-2	0.98	2.49	2.825(3)	100
*C-4—H-4	O-6	0.98	2.51	2.874(3)	102
*C-4—H-4	O-9	0.98	2.31	2.723(4)	105
C-11—H-11B	O-9[iv]	0.96	2.57	3.514(4)	167
C-14—H-14A	O-8[i]	0.96	2.47	3.326(5)	148

Symmetry codes: [a] –x + 1, y + 1/2, –z + 1; [b] x – 1, y, z; [c] x + 1, y, z + 1, z; (iv) –x + 2, y – 1/2, –z + 1.

O//O interactions by the C//O interactions (the ${}_2G^-$ conformation). It may be due to the fact that 1,3-diaxial interactions in the case of the planar carboxyl group are not as strong as in the case of the other groups (e.g., hydroxymethyl group).[18]

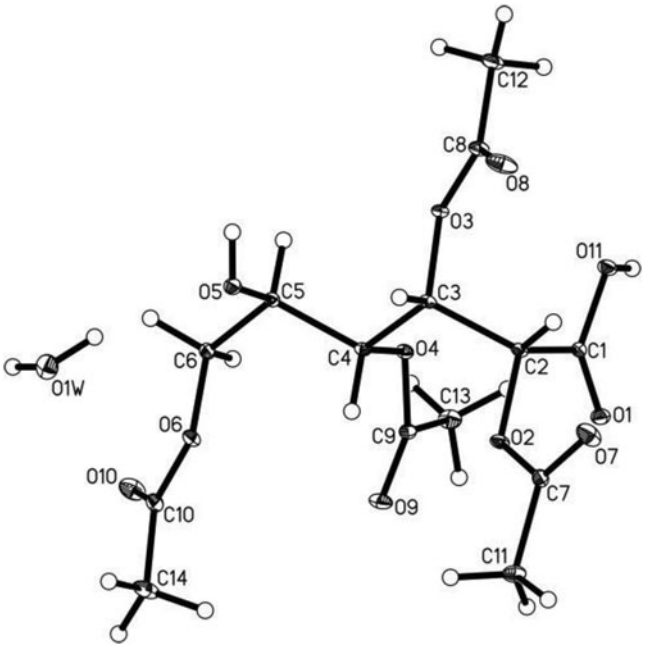


Figure 1: Structure of **7** monohydrate showing 25% probability displacements for ellipsoids.

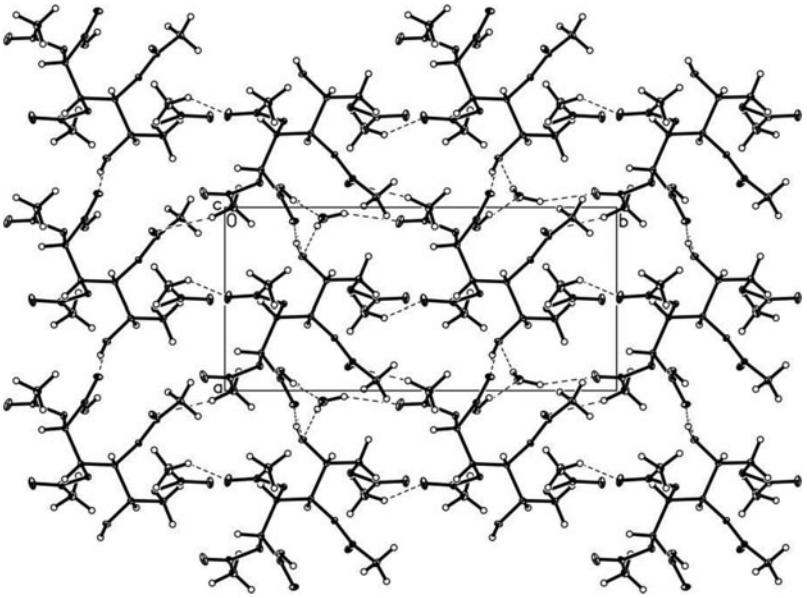


Figure 2: Molecular packing of **7** monohydrate (view along *c*-axis).

Contrary to the presented results, Köll et al. demonstrated that 1,3-parallel O//O arrangements in planar zigzag chains of 2,3,4,5,6-penta-*O*-acetyl-D-gluconic acid and its ester derivatives are tolerated.[19] This means that steric 1,3-parallel interactions between 2-OAc and 4-OAc are not very strong and other factors (e.g., crystal packing or intermolecular hydrogen bonds) can easily change the conformational preferences of aldonic acids. Importantly, our

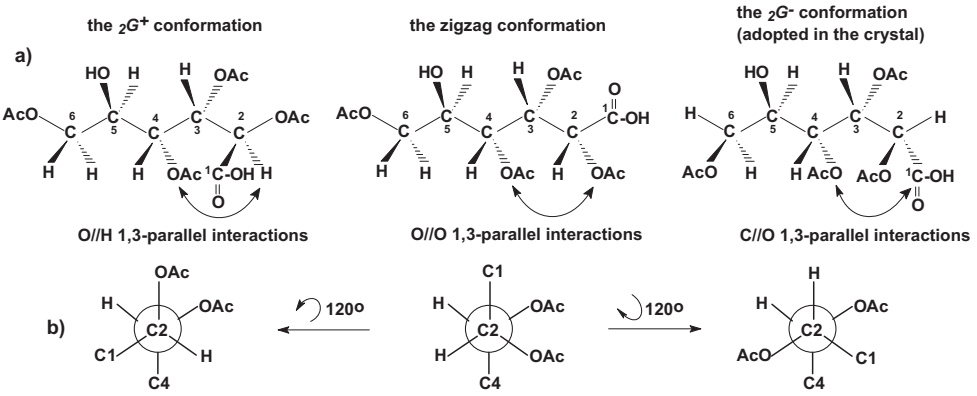


Figure 3: (a) Considered conformations of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7.** (b) Newman projections along the C2-C3 bond showing the interconversion of the zigzag conformation into the $_2G^-$ and $_2G^+$ conformations.

data are in accordance with Köll's statement that gluconic acid derivatives are prone to adopt the zigzag conformation in the solid state unless these are monohydrates. It seems that to be in the form of monohydrate, D-gluconic acid derivative needs at least one unprotected hydroxyl group. Such kind of group is present in 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** (5-OH). Typically, D-gluconic acid derivatives, found in the crystals in a sickle conformation, in almost all cases have unprotected hydroxy groups.[19]

With reference to the C5-C6 bond rotations, 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** adopts the conformation with the hydrogen atom instead of the O6 oxygen atom extending the chain. This is demonstrated by the C4-C5-C6-O6 torsion angle of 59.5° (Table 3). Thus, the carbon zigzag chain of the presented gluconic acid from both sides is extended by the hydrogen atoms, not by the respective oxygen atoms. In this way, the molecule is probably better packed in the crystal.

In our previous crystallographic[20] and theoretical studies,[18,21] we have consistently demonstrated that the acetoxyl groups were planar starting from the respective pyranose carbon atom. Here, we have been able to demonstrate that the acetoxyl groups are also planar in acyclic compounds. The planarity of the acetoxyl group is required due to the mesomeric effect, which causes the delocalization of the noncarbonyl oxygen lone pair of electrons onto the carbonyl oxygen atom. Such delocalization stabilizes the compound and increases the CO-O rotational barrier. On the other hand, the mesomeric effect requires the noncarbonyl oxygen atom to be sp^2 hybridized. Indeed, in 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** monohydrate, the respective C7-O2-C2, C8-O3-C3, C9-O4-C4, and C10-O6-C6 valence angles of 118.7°, 118.6°, 118.76°, and 117.1° (Table 3) are better suited to sp^2 than to sp^3 hybridization of the noncarbonyl oxygen atoms. Comparison of the O-C (carbonyl) and O-C (noncarbonyl) bond lengths additionally points to the action of the mesomeric effect. The former bonds (O2-C7, O3-C8, O4-C9, and O6-C10, Table 3) are shorter than the latter bonds (O2-C2, O3-C3, O4-C4, and O6-C6, Table 3) by 0.075–0.102 Å.

Being planar, the acetoxyl group may adopt two orientations with regard to the Ac-O bond rotation, in which the carbonyl oxygen is oriented *trans* or *cis* to the respective sugar carbon atom (Fig. 4). Our studies of peracetylated glycals using DFT calculations have indicated that the *trans* orientation of the acetoxyl group is unfavorable, due to both electronic and steric interactions.[18] This work has demonstrated that in acyclic compounds, too, the acetoxyl group prefers the *cis* over the *trans* orientation. Thus, the C2-O2-C7-O7 (3.9°), C3-O3-C8-O8 (−1.8°), C4-O4-C9-O9 (−3.0°), and C6-O6-C10-O10 (−2.7°) torsion angles (Table 3) confirm that the acetoxy groups are almost planar and *cis* oriented in the crystal lattice of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** monohydrate. Besides the previously discussed steric and electronic factors, X-ray analysis presented here also reveals that the *cis* orientation of

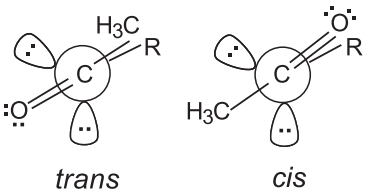


Figure 4: Newman projections along the Ac-O bond showing planarity and two possible conformations of the acetoxy group (R = sugar residue).

the acetoxyl groups enable the carbonyl oxygen atoms to interact preferentially with the chain hydrogen atoms. These intramolecular C-H···O interactions, namely, C2-H2···O7, C2-H2···O8, and C4-H4···O9, are illustrated in Table 4.

To conclude, coupling 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** with 1,3,4, 6-tetra-*O*-acetyl-D-glucosamine **1** or diosgenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-gluco-pyranoside **4** by the methods of solution-phase peptide synthesis was proved to be not very efficient. Not only the desired *N*-(D-gluconyl) derivatives but also the *N*-acetyl derivatives of D-glucosamine as side products were formed, demonstrating that acetyl $O \rightarrow N$ migration occurred readily under the condition. The crystal structure of 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** mono-hydra-te gave some very interesting results. It was shown that this acyclic carbohydrate adopted the ${}_2G^-$ conformation instead of the extended zigzag conformation. In this way, **7** could avoid the 1,3-parallel interactions between 2-OAc and 4-OAc groups. Based on the crystal data, we also found that the acetoxyl groups in this linear compound are planar starting from the respective chain carbon. Being planar, the acetoxyl groups adopt the *cis* orientation.

EXPERIMENTAL

General Methods

Melting points were uncorrected. IR spectra were recorded as a Nujol mull with a Bruker IFS 66 spectro-pho-to-meter, and the ^{1}H and ^{13}C NMR spectra ($CDCl_3$, DMSO or D_2O, internal Me_4Si) were recorded on a Varian Mercury 400 (400/100 MHz) instrument; positive-ion mode MALDI-TOF mass spectra were obtained on a Bruker Biflex III spectrometer. Thin-layer chromatography (TLC) was performed on E. Merck Kieselgel 60 F-254 plates using the following eluent systems (v/v): A, 1:3 $CHCl_3$-MeOH, or B, 1:6 toluene-AcOEt; and column chro-ma–tography was performed on MN Kieselgel 60 (<0.08 mm) with the eluent system B.

Diosgenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-D-gluco-pyranoside 4

Previously obtained diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloro-ethoxy-carbonyl-amino)–β-D-gluco-pyranoside **9**[14] (373.4 mg, 0.427 mmol) was dissolved in glacial acetic acid (10 mL), followed by addition of Zn-Cu dust (3 g). The mixture was stirred at rt and detected by TLC (solvent B). After 24 h, the Zn-Cu dust was filtered off and the filtrate was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over $MgSO_4$. Concentration under reduced pressure gave **11** (210.3 mg, 70%, white powder): R_f 0.47 (solvent B); IR: ν 3392 (N—H), 2950, 2905, 2870 (C—H), 1749 (ester C=O), 1243 cm^{-1} (ester C—O); MALDI TOF-MS: m/z 702.9 $(M)^+$, 724.7 $(M+Na)^+$, 740.7 $(M+K)^+$.

2,3,4,6-tetra-O-Acetyl-D-gluconic acid 7

Compound **7** (71%) was synthesized according to the literature procedure.[13] Literature mp, 110–113°C,[13] observed, 113–117°C; R_f 0.87 (solvent A); IR: ν 3445 (O—H,), 2964 (C—H), 1746 (ester C=O), 1715 (acid C=O), 1234 cm^{-1} (ester C—O); ^{1}H NMR (400 MHz, DMSO): δ 5.59 (dd, $J_{3,4}$=5.2 Hz, H3), 5.55 (bd, 1 H, $J_{5,OH}$ = 5.2 Hz, OH), 5.09 (d, 1 H, $J_{2,3}$ = 3.6 Hz, H2), 5.08 (dd, 1H, $J_{4,5}$ = 7.2 Hz, H4), 3.93 (d, 2 H, $J_{5,6}$ = 5.2 Hz, 2 × H6), 3.75 (m, 1 H, H5), 2.07 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 1.98 (s, 6 H, 2 × OAc); ^{13}C NMR (100 MHz, DMSO): δ 170.88 (acid C=O), 170.20, 170.14, 169.78, 168.74 (4 × ester C=O), 71.86, 71.75 (C2, C4), 69.68 (C3), 67.49 (C5), 65.36 (C6), 21.27, 21.21, 21.00 (4 × ester CH_3); MALDI TOF-MS: m/z 365.0 $(M+H)^+$, 387.1 $(M+Na)^+$, 403.1 $(M+K)^+$.

1,3,4,6-tetra-O-Acetyl-N-(2′,3′,4′,6′-tetra-O-acetyl-D-gluconyl)-2-amino-2-deoxy-β-D-glucopyranose 8 and 1,3,4,6-tetra-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose 9

1,3,4,6-tetra-*O*-Acetyl-D-glucosamine hydrochloride **1**[10] (301.6 mg, 0.785 mmol) and Et_3N (0.109 mL, 0.785 mmol) were dissolved in dry CH_2Cl_2 (7 mL). Next, 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** (341.9 mg, 0.942 mmol), HOBt (106 mg, 0.785 mmol), DCC (161.7 mg, 0.785 mmol), and anhydrous DMF (3 mL) were added. The mixture was stirred at rt and detected by TLC (solvent A). After 120 h, the reaction mixture was diluted with CH_2Cl_2; washed with water, 1 M aq. HCl, brine, and saturated $NaHCO_3$; and then dried over $MgSO_4$. Concentration under reduced pressure led to the crude product, which was chromatographed (solvent B) to yield first **8** (155 mg, 28%, syrup) and then **9** (120.3 mg, 42%, syrup). **8**: R_f 0.41 (solvent B); IR: ν 3356, 3469 (O—H, N—H), 2959, 3023 (C—H), 1753 (ester C=O), 1538 (amide II), 1225 cm^{-1} (ester C—O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.56 (d, 1 H, NH), 5.74 (d, 1 H, $J_{1,2}$ = 8.8 Hz, H1), 5.61 (dd, 1 H, $J_{3',4'}$ = 2.4 Hz, H3′), 5.20 (t, 1 H, $J_{3,4}$ = 10.0 Hz, H3), 5.08 (t, 1 H,

$J_{4,5}$ = 9.6 Hz, H4), 5.01 (dd, 1 H, $J_{4',5'}$ = 8.4 Hz, H4′), 5.00 (d, 1H, $J_{2',3'}$ = 6.8 Hz, H2′), 4.27 (q, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{2,NH}$ = 9.2 Hz, H2), 4.25 (dd, 1 H, $J_{6A,6B}$ = 12.4 Hz, $H6_A$), 4.11 (dd, 1 H, $H6_B$), 4.09 (d, 2 H, 2 × H6′), 3.83 (ddd, 1 H, $J_{5,6A}$ = 2.4, $J_{5,6B}$ = 4.8 Hz, H5), 3.79 (m, 1H, $J_{5',OH}$ = 6.0 Hz, H5′), 3.29 (d, 1H, OH), 2.12–2.01 (8 s, 8 H, 8 × OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171,67–169.53 (8 × ester C=O), 166.92 (amide C=O), 92.26 (C1), 73.01 (C5), 72.62 (C3), 72.58 (C2′), 71.02 (C4′), 69.57 (C3′), 68.41 (C5′), 68.34 (C4), 65.10 (C6′), 61.96 (C6), 53.06 (C2), 21.07–20.64 (8 × ester CH_3); MALDI TOF-MS: m/z 716.2 $(M+Na)^+$, 732.2 $(M+K)^+$. **9**: R_f 0.25 (solvent B); IR: ν 3330 (N−H), 2850, 2928 (C−H), 1748 (ester C=O), 1666 (amide I), 1536 (amide II), 1224 cm^{-1} (ester C−O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.69 (d, 1 H, $J_{1,2}$ = 8.8 Hz, H1), 5.61 (d, 1 H, $J_{2,NH}$ = 9.2 Hz, NH), 5.13 (m, 2 H, $J_{4,5}$ = 9.6 Hz, H3, H4), 4.30 (m, 1 H, H2), 4.26 (dd, 1 H, $J_{6A,6B}$ = 12.4, $J_{5,6A}$ = 4.8 Hz, $H6_A$), 4.12 (dd, 1H, $J_{5,6B}$ = 2.4 Hz, $H6_B$), 3.79 (m, 1 H, H5), 2.11 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 1.93 (s, 3 H, NAc); MALDI TOF-MS: m/z 412.1 $(M+Na)^+$, 428.1 $(M+K)^+$.

2-Amino-2-deoxy-*N*-(D-gluconyl)-D-glucopyranose 10

O-Deacetylation of **8** (30.6 mg, 0.044 mmol) was achieved with 0.1 N MeONa in MeOH (0.442 mL) to give **10** (α:β ∼ 1:1) (13.3 mg, 85%, white powder). IR: ν 3389, 3307 (O−H, N−H), 2929 (C−H), 1647 (amide I), 1553 cm^{-1} (amide II); ^{1}H NMR (400 MHz, D_2O): δ 5.17 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H1α), 4.73 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H1β), 4.30 (d, 1 H, $J_{3',4'}$ = 4.4 Hz, 2 × H-3′), 4.05 (bs, 2 H, $J_{2',3'}$ = 6.0 Hz, 2 × H2′), 3.88 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, H2α), 3.70 (m, 1 H, H2β); MALDI TOF-MS: m/z 357.2 $(M)^+$, 379.2 $[(M-1)+Na)]^+$.

Diosgenyl 3,4,6-tri-O-acetyl-N-(2′,3′,4′,6′-tetra-O-acetyl-D-gluconyl)-2-amino-2-deoxy-β-D-glucopyranoside 11 and diosgenyl 3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside 12

Diosgenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-D-gluco-pyranoside **4** (205.3 mg, 0.293 mmol) was dissolved in dry CH_2Cl_2 (10 mL). Next, 2,3,4,6-tetra-*O*-acetyl-D-gluconic acid **7** (213.3 mg, 0.586 mmol), HOBt (39.6 mg, 0.293 mmol), DIC (0.0456 mL, 0.293 mmol), and anhydrous DMF (1 mL) were added. The mixture was stirred at rt and detected by TLC (solvent A and B). After 24 h, the reaction mixture was concentrated and chromatographed (solvent B) to yield first **11** (44.6 mg, 27%, syrup) and then **12** (39.2 mg, 34%). **11**: R_f 0.67 (solvent B); IR: ν 3477, 3351 (O−H, N−H), 2950, 2872 (C−H), 1749 (ester C=O), 1687 (amide I), 1538 (amide II), 1227 cm^{-1} (ester C−O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25 (d, 1 H, $J_{2,NH}$ = 8.4 Hz, NH), 5.69 (dd, 1 H, $J_{3',4'}$ = 2.4 Hz, H3′), 5.38 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ = 9.2, 10.4 Hz, H4), 5.28 (d, 1 H, $J_{5',OH}$ =

3.6 Hz, OH), 5.15 (d, 1 H, $J_{2',3'} = 7.6$ Hz, H2′), 5.01 (m, 2 H, H3, H4′), 4.86 (d, 1 H, $J_{1,2} = 8.4$ Hz, H1), 4.25 (dd, 1 H, $J_{5,6B} = 4.8$ Hz, $H6_B$), 4,12 (m, 2 H, 2 × H6′), 4.09 (dd, 1 H, $J_{5,6A} = 2.4$, $J_{6A,6B} = 12.4$ Hz, $H6_A$), 3.83 (m, 1 H, H5′), 3.70 (m, 2 H, H2, H5), 3.14, 2.11–2.00 (7s, 21 H, 7 × OAc); diosgenyl protons: 5.28 (d, $J_{6,7} = 6.0$ Hz, H6), 4.40 (q, $J = 7.6$ Hz, H16), 3.49 (m, H3), 3.46 (dd, $J_{25,26'} = 4.0$, $J_{26,26'} = 10.8$ Hz, H26′), 3.36 (t, $J_{25,26} = 10.8$ Hz, H26), 1.00 (s, H19), 0.96 (d, $J_{20,21} = 6.8$ Hz, H21), 0.78 (d, $J_{25,27} = 6.4$ Hz, H27), 0.77 (s, H18); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.36–169.66 (7 × ester C=O), 166.70 (amide C=O), 98.66 (C1), 72.22 (C2′), 72.09 (C5), 71.82 (C4), 71.05 (C3), 69.65 (C3′), 69.24 (C4′), 68.78 (C5′), 65.08 (C6′), 62.51 (C6), 55.78 (C2), 21.05–20.76 (7 × acetyl CH_3); diosgenyl carbons: 140.64 (C5), 122.03 (C6), 109.49 (C22), 81.02 (C16), 79.48 (C3), 67.07 (C26), 62.35 (C17), 56.72 (C14), 50.28 (C9), 41.83–38.72 (C4, C12, C13, C20), 37.35–37.06 (C1, C10), 32.28–31.61 (C7, C8, C15, C23), 30.51–29.03 (C2, C24, C25), 21.83 (C11), 19.53 (C19), 17.33 (C27), 16.46 (C18), 14.70 (C21); MALDI TOF-MS: m/z 1048.4 $(M+H)^+$, 1070.3 $(M+Na)^+$, 1086.4 $(M+K)^+$. **12**: R_f 0.59 (solvent B); IR: ν 3415, 3272 (N−H), 2939, 2906, 2877 (C−H), 1747 (ester C=O), 1652 (amide I), 1577 (amide II), 1245 cm^{-1} (C−O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.46 (d, 1 H, $J_{2,NH} = 8.4$ Hz, NH), 5.37 (dd, 1 H, $J_{4,5} = 10.4$ Hz, H4), 5.03 (t, 1 H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H3), 4.84 (d, 1 H, $J_{1,2} = 8.4$ Hz, H1), 4.25 (dd, 1 H, $J_{5,6B} = 5.2$ Hz, $H6_B$), 4.10 (dd, 1H, $J_{5,6A} = 2.8$, $J_{6A,6B} = 12.0$ Hz, $H6_A$), 3.68 (m, 2 H, H-2, H-5), 2.06, 2.02, 2.01 (3 s, 9 H, 3 × OAc), 1.95 (s, 3 H, NAc); diosgenyl protons: 5.34 (d, $J_{6,7} = 6.4$ Hz, H6), 4.40 (q, $J = 7.6$ Hz, H16), 3.50 (m, H3), 3.47 (dd, $J_{25,26'} = 4.0$, $J_{26,26'} = 10.8$ Hz, H26′), 3.37 (t, $J_{25,26} = 10.8$ Hz, H26), 1.00 (s, H19), 0.96 (d, $J_{20,21} = 7.2$ Hz, H21), 0.79 (d, $J_{25,27} = 5.2$ Hz, H27), 0.78 (s, H18); MALDI TOF-MS: m/z 744.2 $(M+H)^+$, 766.3 $(M+Na)^+$, 782.3 $(M+K)^+$.

Description of the Crystal Structure of 2,3,4,6-tetra-O-Acetyl-D-gluconic Acid 7 Monohydrate

Diffraction data were collected at rt (295 K) on a Gemini R Ultra four circle diffractometer[22] with Mo$K\alpha$ radiation ($\lambda = 0.71073$ Å). The initial phase angle determination was performed by the *SHELXS-97*.[23] The H atoms of the water molecule were located on a Fourier difference map, restrained by DFIX command 0.85 for O−H distances and by DFIX 1.39 for H···H distance, and refined as riding with $U_{iso}(H) = 1.5U_{eq}(O)$. All other H atoms were placed geometrically and refined using a riding model with C−H = 0.96-0.98 Å, and $U_{iso}(H) = 1.2U_{eq}(C)$ (C−H = 0.96 Å and $U_{iso}(H) = 1.5U_{eq}(C)$ in the case of the methyl H atoms) and O−H = 0.82 Å and $U_{iso}(H) = 1.5U_{eq}(O)$.

The crystal structure was refined to $R_1 = 0.0515$ (5721 reflections) and $R_1 = 0.0450$ (3118 reflections with $F > 2\sigma(F_0)$) by full-matrix least-squares method using the *SHELXL*-97 program[24] based on 247 parameters. The compound structure showing the conformations and atom numbering system is

illustrated in Figure 1.[25] Molecular packing in the crystal, illustrated in Figure 2, was prepared by *ORTEP-3*[25] The computational material for publication was prepared using the *PLATON* program.[26]

FUNDING

This research was financed by the European Union within the European Regional Development Fund (grant UDA-POIG.01.01.02-14-102/09-04) and by Ministry of Science and Higher Education (grant DS/530-8451-D-382-13).

SUPPLEMENTAL MATERIALS

Full crystallographic details, excluding structural features, have been deposited (deposition No. CCDC 935806) with the Cambridge Crystallographic Data Center. These data may be obtained, on request, from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (tel.: +44-1223-336408; fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk).

REFERENCES

1. Sparg, S.G.; Light, M.E.; van Staden, J. Biological activities and distribution of plant saponins. *J. Ethnopharmacol.* **2004**, *94*, 219–243.

2. Hostettman, K.; Maraton, A. *Saponins*. Cambridge University Press: New York, **1995**; pp. 287–299.

3. Yu, B.; Zhang, Y.; Tang, P. Carbohydrate chemistry in the total synthesis of saponins. *Eur. J. Org. Chem.* **2007**, 5145–5161.

4. Tran, Q.L.; Tezuka, Y.; Banskota, A.H.; Tran, Q.K.; Saiki, I.; Kadota, S. New spirostanol steroids and steroidal saponins from roots and rhizomes of *Dracaena angustifolia* and their antiproliferative activity. *J. Nat. Prod.* **2001**, *64*, 1127–1132.

5. Iorizzi, M.; Lanzotti, V.; Ranalli, G.; De Marino, S.; Zollo, F. Antimicrobial furostanol saponins from the seeds of *Capsicum annuum* L. Var. *acuminatum*. *J. Agric. Food Chem.* **2002**, *50*, 4310–4316.

6. Raju, J.; Bird, R.P. Diosgenin, a naturally occurring furostanol saponin suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression and induces apoptosis in HCT-116 human colon carcinoma cells. *Cancer Lett.* **2007**, *255*, 194–204.

7. Dave, S.; Tarafdar, J.Ch. Stimulatory synthesis of saponin by mycorrhizal fungi in safed musli (*Chlorophytum borivilianum*) tubers. *Int. Res. J. Agric. Sci. Soil Sci.* **2011**, *1*, 137–141.

8. Pérez-Labrada, K.; Brouard, I.; Estévez, S.; Marrero, M.T.; Estévez, F.; Bermejo, J.; Rivera, D.G. New insights into the structure-cytotoxicity relationship of spirostan saponins and related glycosides. *Bioorg. Med. Chem.* **2012**, *20*, 2690–2700.

9. Wang, B.; Chun, J.; Liu, Y.; Han, L.; Wang, Y.; Joo, E.-J.; Kim, Y.-S.; Cheng, M. Synthesis of novel diosgenyl saponin analogues and apoptosis-inducing activity on A549 human lung adenocarcinoma. *Org. Biomol. Chem.* **2012**, *10*, 8822–8834.

10. Myszka, H.; Bednarczyk, D.; Najder, M.; Kaca, W. Synthesis and induction of apoptosis in B cell chronic leukemia by diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and its derivatives. *Carbohydr. Res.* **2003**, *338*, 133–141.

11. Cirioni, O.; Myszka, H.; Dawgul, M.; Ghiselli, R.; Orlando, F.; Silvestri, C.; Brescini, L.; Kamysz, W.; Guerrieri, M.; Giacometti, A. In vitro activity and in vivo efficacy of the saponin diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (HSM1) alone and in combination with daptomycin and vancomycin against Gram-positive cocci. *J. Med. Microbiol.* **2011**, *60*, 1337–1343.

12. Kaskiw, M.J.; Tassotto, M.L.; Mok, M.; Tokar, S.L.; Pycko, R.; Th'ng, J.; Jiang, Z.-H. Structural analogues of diosgenyl saponins: synthesis and anticancer activity. *Bioorg. Med. Chem.* **2009**, *17*, 7670–7679.

13. Braun, C.E.; Cook, C.D. 2,3,4,5,6-Penta-*O*-acetyl-D-gluconic acid and 2,3,4,5,6-penta-*O*-acetyl-D-gluconyl chloride. *Org. Synth. Coll.* **1973**, *5*, 887.

14. Bednarczyk, D.; Walczewska, A.; Grzywacz, D.; Sikorski, A.; Liberek, B.; Myszka, H. Differently *N*-protected 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-D-glucopyranosyl chlorides and their application in the synthesis of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside. *Carbohydr. Res.* **2013**, *367*, 10–17.

15. Grindley, T.B. Structure and conformation of carbohydrates. In: *Glycoscience: Chemistry and Chemical Biology*, B. Fraser-Reid, K. Tatsuta, J. Thiem, Eds. Springer: Heidelberg, **2001**, pp. 13–14.

16. Stoddart, J.F. *Stereochemistry of Carbohydrates.* J. Wiley & Sons: Toronto, **1971**, p. 94.

17. Horton, D.; Wander, J.D. Conformation of acyclic derivatives of sugars. XI. Conformations of the D-aldopentose diethyl and diphenyl dithioacetals in solution. *J. Org. Chem.* **1974**, *39*, 1859–1863.

18. Nowacki, A.; Walczak, D.; Liberek, B. Fully acetylated 1,5-anhydro-2-deoxypent-1-enitols and 1,5-anhydro-2,6-dideoxyhex-1-enitols in DFT level theory conformational studies. *Carbohydr. Res.* **2012**, *352*, 177–185.

19. Köll, P.; Bruns, R.; Kopf, J. Crystal and molecular structures of the pentaacetates of D-gluconic acid and derived *tert*-butyl- and *tert*-butylperoxyesters and the tetra-*O*-acetyl-1,2-*O*-*tert*-butyl-peroxyorthoacetyl derivative. *Carbohydr. Res.* **1998**, *305*, 147–154.

20. Tuwalska, D.; Sikorski, A.; Liberek, B. Synthesis and geometry of methyl (methyl 4-*O*-acetyl-3-azido-2,3-dideoxy-α/β-D-*arabino*- and α/β-D-*ribo*-hexopyranosid)uronates. *Carbohydr. Res.* **2008**, *343*, 404–411.

21. Nowacki, A.; Liberek, B. Methyl 4-*O*-Acetyl-3-azido- and 3-azido-4-*O*-methylsulfonyl-2,3,6,-trideoxy-hex-5-enopyranosides in DFT Level Theory Conformational Studies. *J. Phys. Chem. A.* **2007**, *111*, 4397–4403.

22. CrysAlis CCD, *Version 1.171.32.15.* Oxford Diffraction Ltd., 2008.

23. Sheldrick, G.M. A short history of SHELX. *Acta Cryst.* **2008**, *A64*, 112–122.

24. Sheldrick, G.M. *SHELXL-97. Program for Crystal Structure Refinement*. University of Göttingen: Göttingen, Germany, **1997**.

25. Farrugia, L.J. *ORTEP*-3 for Windows - a version of *ORTEP*-III with a Graphical User Interface (GUI). *J. Appl. Cryst.* **1997**, *30*, 565.

26. Spek, A.L. Structure validation in chemical crystallography. *Acta Cryst.* **2009**, *D65*, 148.