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Note

X-ray crystallographic and high-resolution NMR spectroscopy characterization of 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl sulfamide

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ABSTRACT

Single crystal X-ray diffraction and high-resolution ^{1}H and ^{13}C NMR spectral data for 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl sulfamide, a selective inhibitor of carbonic anhydrase isozyme IX, are reported. The $^{0}H_{5}$ was found to be the preferred form for this glycosyl sulfamide, both in the crystal lattice and in solution.

The carbonic anhydrases (CAs) are widespread metalloenzymes, present in mammals in a multitude of isoforms, and catalyze the interconversion between carbon dioxide and bicarbonate at the physiological pH. It was found that isozyme CA IX is overexpressed in a variety of tumor types and plays an important role in the growth and survival of tumor cells.[1] Thus their inhibition is the target for the development of novel antitumor therapies. Very recently, we have described the interaction of novel *N*-glycosyl sulfamides with five CA isoforms.[2] One of them, 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl sulfamide (**1**), showed selectivity for inhibiting the tumor-associated isoforms hCA IX and XII over the ubiquitous isozyme hCA II. This isoform is an abundant housekeeping enzyme in most human cells and thus the selective inhibition of other isoforms than CA II may lead to drugs with less severe side effects. Compound **1** was ten times less effective hCA II inhibitor than its *threo* epimer, 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosyl sulfamide (**2**). A clash between 4-*O*-acetyl moiety of **1** and an amino acid residue, within the hCA II active site, was proposed as an explanation of its decreased affinity. This interaction is not present in its *threo* epimer **2**, which shows a good binding to the enzyme. Thus, resolving the X-ray crystal structure and studying the conformational behavior in solution of the glycosyl sulfamide **1** are highly relevant for understanding in detail the drug design of such enzyme inhibitors. This paper reports on single crystal X-ray diffraction and high-resolution NMR spectral data for **1** and discusses the conformation of the *N*-glucosyl sulfamide in the crystal lattice and in solution.

Figure 1 shows an ORTEP[3] drawing of the molecule. Selected intramolecular bond lengths, angles, and torsional angles are given

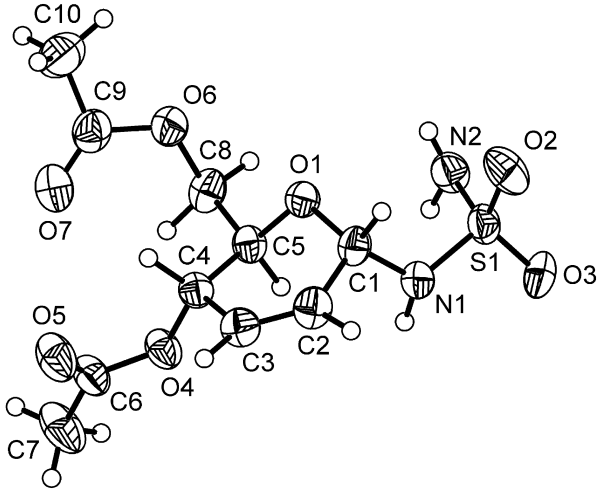

Figure 1. Ortep diagram of 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl sulfamide including the labeling scheme. Displacement ellipsoids are drawn at 50% probability.

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Table 1
Selected intramolecular bond distances (Å), bond angles (°), and torsional angles (°)[a]

Pyranose ring							
C1–C2	1.477(6)	[1.496(2)]	O1–C1–C2	112.0(4)	[112.0(3)]	C1–C2–C3–C4	3.0(7)
C2–C3	1.314(6)	[1.315(6)]	C1–C2–C3	122.8(4)	[122.8(4)]	C2–C3–C4–C5	12.3(6)
C3–C4	1.499(6)	[1.499(6)]	C2–C3–C4	121.6(4)	[121.5(4)]	C3–C4–C5–O1	−44.1(4)
C4–C5	1.511(5)	[1.512(5)]	C3–C4–C5	111.2(3)	[111.2(3)]	C4–C5–O1–C1	63.8(4)
C5–O1	1.419(5)	[1.419(4)]	C4–C5–O1	110.5(3)	[110.5(3)]	C5–O1–C1–C2	−47.2(5)
O1–C1	1.435(5)	[1.435(5)]	C5–O1–C1	113.1(3)	[113.1(3)]	O1–C1–C2–C3	13.4(6)
4-O-Acetyl							
			C5–C4–O4	104.0(3)			
C4–O4	1.443(4)		O4–C6–C7	111.3(4)		C3–C4–O4–C6	90.5(4)
C6–O5	1.189(5)		C4–O4–C6	118.1(3)		C5–C4–O4–C6	−150.1(4)
O4–C6	1.342(5)		O5–C6–C7	125.7(5)		C4–O4–C6–O5	−6.3(7)
C6–C7	1.489(6)		C3–C4–O4	110.6(3)		C4–O4–C6–C7	172.8(4)
			O4–C6–O5	122.9(4)			
Acetoxymethyl							
			O1–C5–C8	108.3(3)			
C5–C8	1.506(6)		C4–C5–C8	113.9(3)		O1–C5–C8–O6	−55.3(4)
C8–O6	1.457(5)		C5–C8–O6	112.3(3)		C4–C5–C8–O6	68.1(5)
O6–C9	1.347(5)		C8–O6–C9	118.0(4)		C5–C8–O6–C9	−101.4(4)
C9–O7	1.189(6)		O6–C9–C10	110.3(5)		C8–O6–C9–O7	10.2(6)
C9–C10	1.484(8)		O6–C9–O7	124.2(5)		C8–O6–C9–C10	−169.5(4)
			O7–C9–C10	125.5(5)			
Sulfamide							
			O1–C1–N1	112.7(3)			
			C2–C1–N1	109.8(4)			
C1–N1	1.463(5)		C1–N1–S1	119.5(3)		O1–C1–N1–S1	67.7(4)
N1–S1	1.622(3)		N1–S1–N2	112.0(2)		C2–C1–N1–S1	−166.7(3)
S1–N2	1.616(4)		N1–S1–O2	106.9(2)		C1–N1–S1–N2	−71.3(3)
S1–O2	1.420(3)		N1–S1–O3	105.6(2)		C1–N1–S1–O2	45.1(3)
S1–O3	1.422(3)		N2–S1–O2	106.6(2)		C1–N1–S1–O3	174.7(3)
			N2–S1–O3	105.3(2)			
			O2–S1–O3	120.7(2)			

[a] Between brackets are listed average endocyclic bond lengths and angle values obtained from the CSD search.

in Table 1. All endocyclic bond distances and angles of the pyranosyl ring are close to the mean values (Table 1) of 19 compounds, which contain the 6-*O*-acetyl-2,3-dideoxy-2-enopyranosyl fragment, retrieved from a search in the Cambridge Structural Database (CSD).[4]

According to Cremer and Pople,[5] the ring puckering values calculated from the X-ray atomic coordinates of the unsaturated pyranosyl ring, QT = 0.469(4)°, θ = 52.1(5)°, and ϕ = 330.1(7)°, show that its conformation can be described mainly as a distorted $^{0}H_{5}$ half-chair.[6] The $^{0}H_{5}$ ring conformation of **1**, in the crystal state, can be additionally confirmed by the inspection of the torsion angles values listed in Table 1. A small value of the C1–C2–C3–C4 torsion angle, 3.0(7)°, which indicates an almost planar orientation of the respective atoms, together with a C4–C5–O1–C1 torsion angle of 63.8(4)°, which indicates that the C5 and O1 atoms do not lay in the plane of those atoms can be obtained. The calculated distance of the C5 and O1 atoms from the mean plane defined by the C1, C2, C3, and C4 atoms (C1–C2–C3–C4 plane) are −0.351(4) Å and 0.360(3) Å, respectively. These values are similar and rather small but with opposite sign, showing that C5 and O1 atoms are located, at similar distances, on each side of the mean plane. Furthermore, the O1–C1–C2–C3, 13.4(6)°, and C2–C3–C4–C5, 12.3(6)°, torsion angles also show that the deflection of C5 and O1 atoms from the C1–C2–C3–C4 plane is similar and rather small.

The acetoxymethyl group equatorially bonded to the C5 ring atom, is arranged with the *O*-acetyl group approximately perpendicular to the C1–C2–C3–C4 plane (the C9–C10 bond line deviates from the normal to the plane at an angle of 21.0(4)°), resulting in a gauche conformation with respect to the pyranosyl ring (the O1–C5–C8–O6 and C4–C5–C8–O6 torsion angles are −55.3(4)° and 68.1(5)°, respectively) and a value of the C5–C8–O6–C9 torsion angle equal to −101.4(4)°.

A comparison of the acetoxymethyl geometry observed in compound **1** with those obtained from the CSD search reveals that while all distances and angles in **1** are, within experimental error, close to the mean values, the C5–C8–O6 angle, equal to 112.3(3)°, deviates toward a higher value. From a structural point of view, it might be rationalized by the fact that those acetoxymethyl groups that are equatorially bonded with the *O*-acetyl fragment approximately perpendicular to the C1–C2–C3–C4 plane would exert a bigger steric effect upon the pyranosyl ring when the C5–C8–O6–C9 torsion angle decreases, causing an increment of the C5–C8–O6 angle value.

To investigate this hypothesis, five compounds were selected from the previous CSD search, which contain an acetoxymethyl group in an arrangement similar to **1**, but with different values of C5–C8–O6–C9 torsion angles. The result is shown in Figure 2. From the curve it can be seen that as the C5–C8–O6–C9 torsion angle increases, the C8–O6–C9 angle decreases. Both the 4-*O*-acetyl and sulfamide substituents are located (opposite to the acetoxymethyl group) on the same side of the C1–C2–C3–C4 plane. The angles formed by the C4–O4 and C1–N1 bonds with the normal to the plane are 42.9(3)° and 29.1(3)°, respectively.

An inspection of the interactions exhibited by molecules of **1** in the crystalline phase, based on somewhat flexible geometrical criterion, reveals that these substituent arrangements promote various types of weak non-bonded intra- and intermolecular contacts that might help to stabilize the molecular conformation and assembly. In Table 2 some selected non-bonded distances are listed. The geometry analysis of the molecule shows that a three-center intramolecular short contact interaction is established

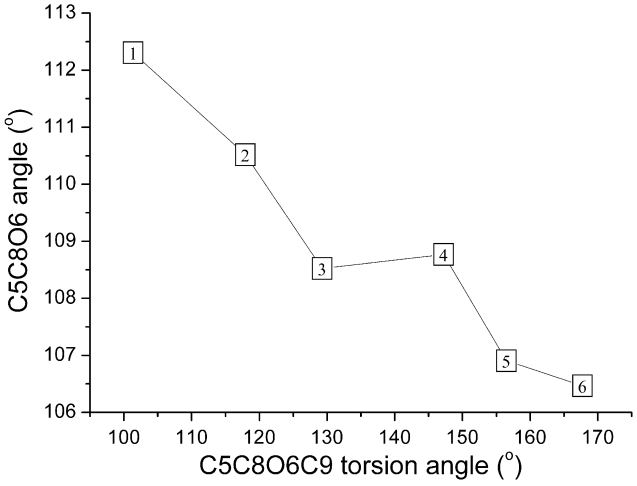


Figure 2. The curve shows the correlation between the C5–C8–O6 angle and the modulus of the C5–C8–O6–C9 torsion angle for the five structures obtained from the CSD search, with the acetoxymethyl group equatorially bonded to the pyranose ring and the *O*-acetyl fragment approximately perpendicular to the C1–C2–C3–C4 plane. Each point of the curve characterizes one system: (1) this work, (2) TAYCIF, (3) NUBLOL, (4) GAHTEO, (5) NUBLUR, and (6) CIZBAO, where the CSD codes are used.

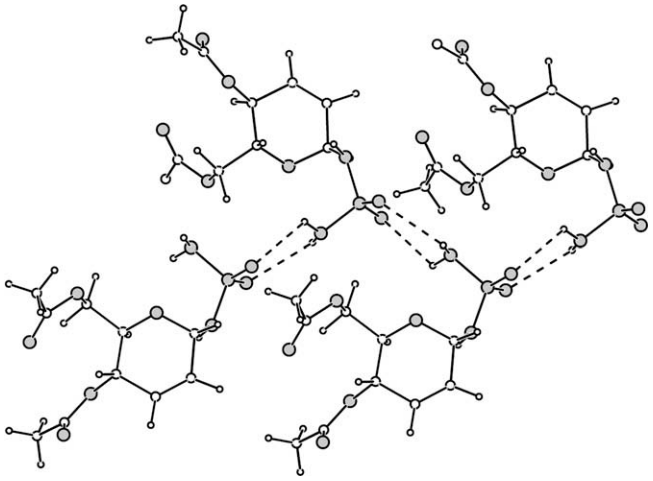


Figure 3. Packing diagram of 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-erythro-hex-2-eno-pyranosyl, viewed down along the *b* axis, showing the extended N–H···O sulfamide chains (dashed lines) along the *a* axis. For clarity, carbon atoms are indicated by small empty circles while oxygen, nitrogen, and sulfur atoms are indicated by bigger gray circles.

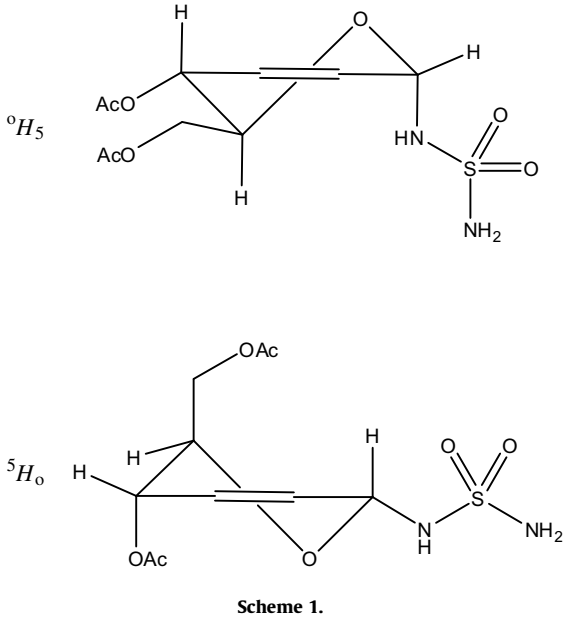
Table 2
Selected non-bonded contacts (Å) and angles (°)

	D–H	H···A	D···A	D–H···A
C1–H1···O7^{ii}	1.10	2.50	3.330(6)	131
C4–H4···O7	1.05	2.51	3.208(5)	123
C4–H4···O5	1.05	2.32	2.686(5)	98
C5–H5···O5^{i}	1.15	2.26	3.308(5)	151
C8–H82···O4	1.07	2.48	2.990(6)	108
N1–H11···O5^{i}	0.96	2.45	3.126(5)	128
N2–H22···O5^{i}	0.98	2.07	2.963(5)	150
N2–H21···O3^{iii}	0.85	2.62	2.934(5)	103
N2–H22···O2^{iii}	0.98	2.77	3.421(5)	125

Symmetry codes: (i) $1-x, -1/2+y, 1/2-z$; (ii) $1+x, y, z$; (iii) $-1/2+x, 3/2-y, -z$.

between the pyranosyl C4 carbon, and the 4-*O*-acetyl and the acetoxymethyl carbonyl oxygen atoms. Although some intermolecular distances are larger than the sum of van der Waals atomic radii, molecular assembly analysis suggests that various types of weak contacts might be recognized between neighboring molecules. One of the most notable intermolecular contacts involves the two hydrogen atoms from the amino group and the two oxygen atoms of another symmetry-related sulfamide group, see Table 2, which form the pattern represented in Figure 3. Then sulfamide groups link each other by building extended chains along the *a* axis. The contact planes are arranged approximately in a zigzag manner forming an angle between contiguous planes of 73.4(5)°.

Although compound **1** was previously characterized by ^{1}H and ^{13}C NMR spectroscopy,[8] high-resolution NMR spectroscopy (COSY, HSQC, and HMBC) was used in this report to study the conformation of **1** in solution. The α anomers of the 2,3-enopyranosyl systems could be present in two equilibrium conformations ($^{0}H_5$ and $^{5}H_0$) in solution, (Scheme 1). The most diagnostic coupling constants, $J_{3,4}$ = 1.6 Hz and $J_{4,5}$ = 9.0 Hz indicate that in acetonitrile the equilibrium between the two half-chair forms lies significantly toward the $^{0}H_5$ conformation of **1**, with pseudoaxial position for H4; a $^{1}J_{C1,H1}$ = 169 Hz confirms the pseudoaxial position of the sulfamide group.[7] Also, a long range coupling ($^{4}J_{2,4}$ = 1.6 Hz) with the pseudoaxial H4 was observed. In the related α-D-*threo* isomer **2**,



Scheme 1.

long range coupling with the pseudoequatorial proton was not observed. According to the Karplus curve, the measured coupling constants $J_{3,4}$ = 1.6 Hz and $J_{4,5}$ = 9.0 Hz are in good agreement with the H3–C3–C4–H4 and H4–C4–C5–H5 torsion angle values, 52.3° and −154.7°, respectively, gleaned from the crystallographic data. Thus, the glucosyl sulfamide **1** adopts essentially the same conformation in the crystal lattice and in solution.

1. Experimental

1.1. General

NMR spectra were recorded at room temperature on a Varian Mercury 200 spectrometer. ^{1}H NMR spectra were measured at 200 MHz and ^{13}C spectra at 50 MHz in CD_3CN solution, using the standard pulse sequence and procedures.

Table 3
Crystal data, structure determination, and refinement summary

Empirical formula	$C_{10}H_{16}N_2O_7S$
Formula weight	308.31
Temperature (K)	293(2)
λ [Cu(Kα)] (Å)	1.54184
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	8.4924(8)
b (Å)	9.470(3)
c (Å)	17.003(3)
V (Å^3)	1367.5(4)
ρ_{calc} (g/cm^3)	1.498
Z	4
μ[Cu(Kα)] (mm^{-1})	2.443
F(000)	648
Crystal size (mm)	0.12 × 0.24 × 0.24
θ Range for data collection (°)	5.20–69.90
Limiting indices	$0 \leqslant h \leqslant 10$ $0 \leqslant k \leqslant 11$ $-20 \leqslant l \leqslant 1$
Reflections collected/unique with $I > 2\sigma(I)$	1614/1594
Completeness to $\theta = 69.90°$ (%)	99.9
Data/restraints/parameters	1594/0/182
Goodness-of-fit on F^2	1.049
R	0.0520
R_w	0.1405
Absolute structure parameter	−0.03(4)
Extinction coefficient	0.006(1)
$(\Delta/\rho)_{max}/(\Delta/\rho)_{min}$ (e/Å^{-3})	0.32/−0.31

1.2. Preparation of 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl sulfamide

4,6-Di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl sulfamide (**1**) was prepared from 3,4,6-tri-*O*-acetyl-D-glucal in the presence of boron trifluoride etherate, according to our recently reported procedure.[8]

1.3. X-ray data

Good-quality single crystals, suitable for X-ray analysis, were obtained upon crystallization from a solution of **1** in EtOAc–hexane.

Single-crystal X-ray diffraction data, at room temperature (293(2) K), were taken on an automatic four-circle Enraf-Nonius CAD-4 diffractometer equipped with a rotating anode generator using a graphite-monochromated Cu Kα (λ = 1.54184 Å) radiation. Crystals belong to the acentric orthorhombic $P2_12_12_1$ space group with four molecules per unit cell. Unit cell parameters and the orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 25 reflections in the θ range 12–45°. The data collection and reduction were performed with the CAD-4[9] and XCAD4[10] software, respectively. Crystal data, additional details of data collection, and structure refinement are given in Table 3.

The structure was solved by direct methods with SHELXS-97.[11] The model was refined by full-matrix least squares on F^2 with SHELXL-97.[11] All the hydrogen atoms were stereochemically positioned and refinements riding on bound atoms. Hydrogen atoms were set isotropic with a thermal parameter 20% greater than the equivalent isotropic displacement parameter of the atom to which each one was bonded.

The programs SHELXL97,[11] PLATON,[12] PARST,[13] and ORTEP-3[14] were used within WINGX[14] for structure analysis and to prepare materials for publication. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited by the Cambridge Crystallographic Data Centre (see below).

1.4. Crystallographic data search

Organic crystal structure data of good quality, R-factor less than 0.10, were obtained from the 2008 release edition of the Cambridge Structural Database 3D Graphics Search System (CSD),[15] using a fragment-based method which included the 6-*O*-acetyl-2,3-dideoxy-2-enopyranosyl residue.

Acknowledgments

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Supplementary data

Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 695102. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via: www.ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.08.035.

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