

Synthesis, Characterization, and Crystal Structure of α -Glucosimino-pyranose Anthranilic Acid

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Abstract The new secondary amine, build of glucose and anthranilic acid— α -glucosiminopyranose anthranilic acid (**1**)—has been synthesized and characterized by elemental analyses, FT-IR and ^1H NMR spectroscopy. Crystal and molecular structure of the hydrate of **1** has been determined by means of X-ray diffraction. $1\cdot\text{H}_2\text{O}$ crystallizes in monoclinic P2_1 space group, with $a = 15.584(3)$ Å, $b = 4.992(1)$ Å, $c = 18.928(4)$ and $\beta = 107.63(3)^\circ$. The studies prove that a cyclic secondary amine is formed instead of open chain Schiff base, and only one anomer, α is produced. There are two symmetry-independent molecules of $1\cdot\text{H}_2\text{O}$ in the asymmetric part of the unit cell, and these independent molecules create complicated hydrogen bonded structures—starting with the homomolecular chains which in turn are joined into three dimensional structure. The differences in the supramolecular structures are correlated with the differences in the bond angles patterns, which result from the different orientations of certain O–H bonds. The antibacterial activities of **1** were also tested but the results were negative.

Keywords Glucose · Anthranilic acid · Crystal structure · Secondary amine ligand · Hydrogen bonding

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Introduction

Schiff bases have versatile applications in biochemistry and medicine, for instance they show significant antifungal, antibacterial, and anticancer activities (e.g., [1, 2]). In recent years also the molecules designed on anthranilic acid have attracted great interest as they were shown to be able to act through different biological mechanisms that are involved in the development and maintenance of tumoral cells, and therefore possess significant preventive or inhibitory activity [3, 4] in such disorders. On the other hand, biological activity of sugars is well known since ancient times; for instance they were used in wound treatment—recently promising results have been reported regarding the treatment of infected wounds with granulated sugar, and also there are some reports about the efficiency of sugar in the treatment of surgical wounds [5–7]. Therefore one can expect that Schiff bases containing both sugar and anthranilic acid moieties would show enhanced biological activity. Here, we used D-(+)-glucose and anthranilic acid, and the resulting compound— α -glucosiminopyranose anthranilic acid (**1**, Scheme 1)—has been characterized with elemental analysis, FT-IR, ^1H NMR. X-ray single crystal diffraction. The results proved that a cyclic secondary amine is formed instead of an open chain Schiff base. Also the antibacterial activities of this compound against *Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative) and *Pseudomonas aeruginosa* (Gram-negative) were evaluated.

Results and Discussion

The amine (**1**) was readily synthesized in ethanol solution. This compound is stable in air at room temperature, is

Scheme 1 Reaction pathway to the compound **1**

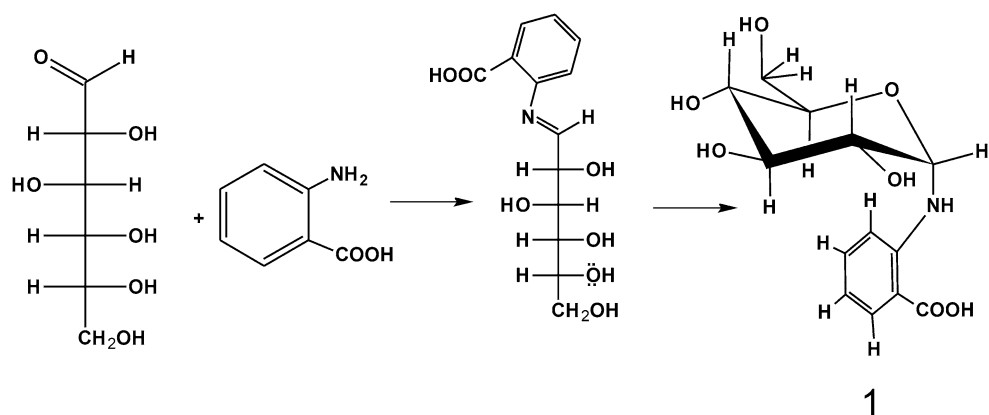
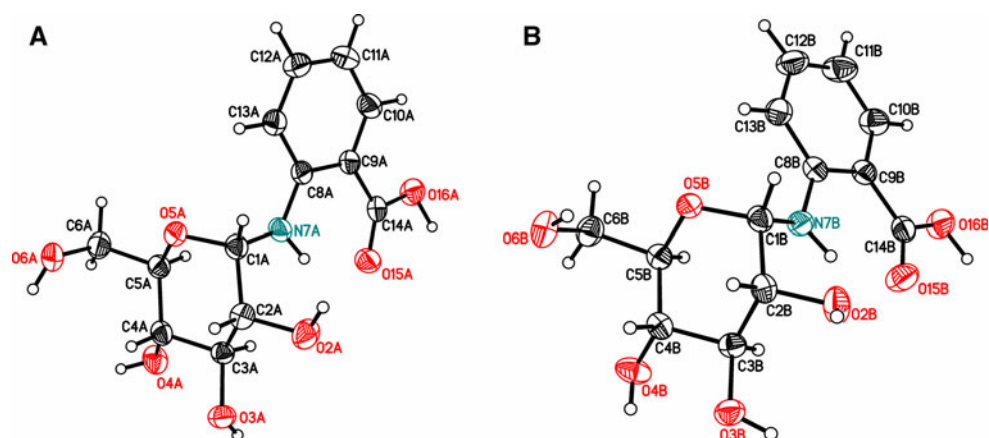


Fig. 1 Two symmetry-independent molecules of **1**, **A** (left) and **B** (right) [20]. Ellipsoids are drawn at 50 % probability level, hydrogen atoms are shown as spheres of arbitrary radii



soluble in methanol, ethanol, acetonitrile, DMF and DMSO. The FT-IR spectrum shows a broad absorption band of the COOH group in the 3,200–3,600 cm^{-1} region. The $\nu(\text{C}=\text{O})$ and $\nu(\text{C}-\text{N})$ bands are observed at 1,650 and 1,512 cm^{-1} , respectively. There are no stretching bands neither for C=O of D-(+)-glucose nor for C=N of corresponding open chain Schiff base. The ^1H NMR spectroscopic data are given in the experimental section. The appearance of a broad signal around 5.04 ppm (NH), multiplets around 6.67–8.40 ppm (H_{Ar}), multiplets around 4.33–5.36 ppm (OH), a broad signal around 12.07 ppm (COOH) and the signals due to the aliphatic C–H fragments strongly confirm the synthesis of (1). The biological test results indicate that this cyclic amine of D-(+)-glucose and anthranilic acid had no antibacterial activity against studied bacterial strains.

The structure of a hydrate of (1) was also confirmed by X-ray diffraction analysis. This compound crystallizes in the monoclinic chiral space group $P2_1$, with two symmetry-independent molecules of (1) and two water molecules in the asymmetric part of the unit cell. The general view of two symmetry-independent molecules of (1) is shown in Fig. 1. Selected geometrical parameters are given in Table 1.

This compound exists in its solid-state structure as the α -anomer (N7–C1–C2–C3 torsion angles are 51.7(2)° and 57.3(2)° in two independent molecules), and assumes the C-1 conformation. It might be noted that in the Cambridge Crystallographic Database [8; Version 5.32 of Nov. 2010, last update Aug. 2011] there are only two structures containing α -anomer of 1-*N*-substituted glycopyranose: (2,3,4,6-tetra-*o*-acetyl- α -D-glucopyranosyl)–(2,3,4,6-tetra-*o*-acetyl- β -D-glucopyranosyl) amine [9] and α -*N*-(4-methoxyphenyl)-2,3,4,6-tetra-*o*-acetyl-D-glucopyranosylamine [10], but as much as 48 of the β -anomer.

Six-membered pyranose rings are close to ideal chair conformation—they adopt $^4\text{C}_1$ conformations, as in some similar structures (e.g. [11]); substituents at the positions 2, 3, 4, and 5 are all in equatorial orientations, while the *N*-substituent at position 1 is in the axial orientation (cf. torsion angles in Table 1). In both independent molecules, the intramolecular N–H \cdots O(carboxyl) hydrogen bonds close six-membered rings (hydrogen bond data are given in Table 2). These intramolecular bonds influence almost coplanar orientation of the carboxylic group and the phenyl ring, the dihedral angles between these two planes are 8.6(3)° in molecule A and 4.5(3)° in B.

Table 1 Selected geometrical parameters for I (Å, °) with esd's in parentheses

	Molecule A	Molecule B
C1–C2–O2	112.08(15)	110.54(15)
C3–C2–O2	108.08(15)	110.28(15)
C2–C3–O3	108.95(15)	110.96(15)
C4–C3–O3	110.5(14)	107.7(14)
C3–C4–O4	106.56(15)	112.65(15)
C5–C4–O4	109.94(16)	107.27(14)
C1–N7–C8	123.46(15)	122.41(16)
C1–C2–C3–C4	–51.6(2)	–56.61(18)
C2–C3–C4–C5	50.0(2)	53.82(19)
C3–C4–C5–O5	–52.98(19)	–53.90(19)
C4–C5–O5–C1	60.04(18)	58.96(17)
C5–O5–C1–C2	–60.87(19)	–60.72(18)
O5–C1–C2–C3	56.00(19)	58.43(18)
O5–C1–C2–O2	177.33(15)	–179.22(15)
C1–C2–C3–O3	–177.33(14)	–175.29(13)
C2–C3–C4–O4	169.83(14)	174.00(14)
C3–C4–C5–C6	–173.04(17)	–170.83(15)
C3–C2–C1–N7	–69.64(19)	–65.07(18)
C5–O5–C1–N7	63.25(19)	61.57(18)
C8–C9–C14–O15	–8.3(3)	–4.8(3)
C8–C9–C14–O16	172.27(16)	176.24(16)
C1–C2–O2–H2	42(2)	129(1)
C2–C3–O3–H3	–139(1)	–85(2)
C3–C4–O4–H4	146(1)	63(2)
C5–C6–O6–H6	–95(2)	37(2)

The two symmetry independent molecules are similar at the level of bond lengths, which are comparable to typical values [8]. The normal probability plot, which shows the deviations from the statistical nature of the differences [12, 13], is almost linear, with the correlation factor R^2 between the theoretical (calculated for the normal distribution) and experimentally determined functions of the differences between bond lengths as high as 0.97. On the other hand, the significant differences begin at the level of bond angles ($R^2 = 0.82$), and are especially large within the pyranose ring. These differences can be attributed to the different orientation of certain O–H bonds (Fig. 2), and further to the different pattern of the hydrogen bonds in which the two molecules take part (cf. Tables 1 and 2).

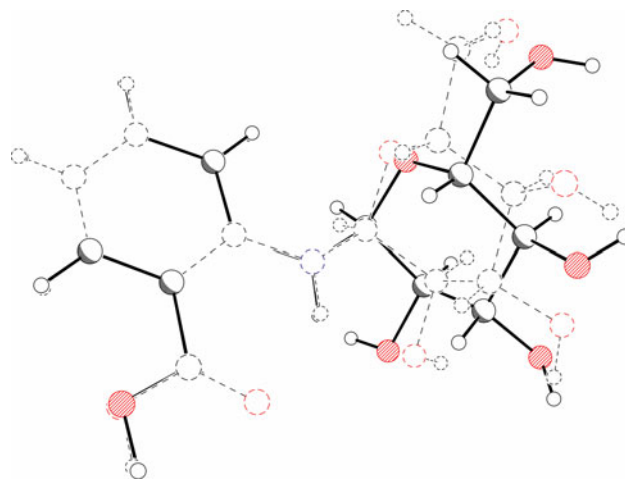
In the crystal structure the complicated network of relatively strong O–H···O hydrogen bonds connect molecules into a three-dimensional network. All hydrogen bond donors and acceptors, including the water molecules are involved in this network.

This hydrogen bonding pattern deserves closer inspection. The process of the crystal structure formation can be rationalized as a stepwise procedure. At the first level the

Table 2 Hydrogen bond data for compound (I)

D	H	A	D–H	H···A	D···A	D–H···A
C2A	H2A	O4A ^a	1.00(2)	2.51(2)	3.454(2)	157.9(16)
O2A	H2A1	O15A ^a	0.83(3)	1.97(3)	2.795(2)	175(3)
O3A	H3A1	O1 W ^b	0.89(3)	1.94(3)	2.829(2)	171(2)
O4A	H4A1	O3A ^b	0.85(3)	2.09(3)	2.910(2)	162(3)
O6A	H6A1	O1 W	0.85(3)	1.87(3)	2.721(2)	179(4)
N7A	H7A	O15A	0.89(2)	1.99(2)	2.692(2)	134(2)
O16A	H16A	O6B ^c	1.05(3)	1.60(3)	2.622(2)	163(3)
O2B	H2B1	O2 W ^d	0.90(3)	1.89(3)	2.787(2)	173(3)
O3B	H3B1	O2 W	0.88(3)	1.94(3)	2.808(2)	167(3)
O4B	H4B1	O3B	0.84(3)	2.09(3)	2.807(2)	144(3)
C4B	H4B	O3B ^d	1.01(2)	2.48(2)	3.465(2)	165.4(16)
O6B	H6B1	O4B ^a	0.90(3)	1.93(3)	2.787(2)	157(3)
N7B	H7B	O15B	0.89(2)	1.98(2)	2.684(2)	135.0(19)
O16B	H16B	O6A ^c	0.96(3)	1.67(3)	2.629(2)	173(3)
O1 W	H1W1	O3A ^b	0.88(4)	2.00(4)	2.855(2)	163(3)
O1 W	H1W2	O2B ^f	0.93(3)	1.95(3)	2.8085(19)	154(2)
O2 W	H2W1	O15A	0.81(4)	2.44(4)	3.080(2)	136(3)
O2 W	H2W2	O2A	0.93(4)	1.85(4)	2.773(2)	171(3)

Symmetry codes: ^a $x, -1+y, z$; ^b $-x, -y, 1-z$; ^c $x, 1+y, z$; ^d $-x, -1-y, -z$; ^e $x, 1+y, -1+z$; ^f $x, y, 1+z$

**Fig. 2** A comparison of two symmetry independent molecules 1 fitted onto their phenyl rings [20]

symmetry-independent molecules form chains of molecules related by a twofold screw axis along the b axis (Fig. 3). Using the graph set notation [14], these chains can be described as C(5). Within the chains the pairs separated by one molecule (i.e. 1st with 3rd, 2nd with 4th, 3rd with 5th etc.) are connected by another hydrogen bonds but these bonds are different for each symmetry-independent molecule. For molecules A these are O2···O15($x, -1+y, z$) bonds, while for molecules B O6···O4($x, -1+y, z$) ones. The

Fig. 3 The hydrogen-bonded chains of molecules A (*left*) and B (*right*), related by twofold screw axis [20]. Dashed lines depict intermolecular hydrogen bonds

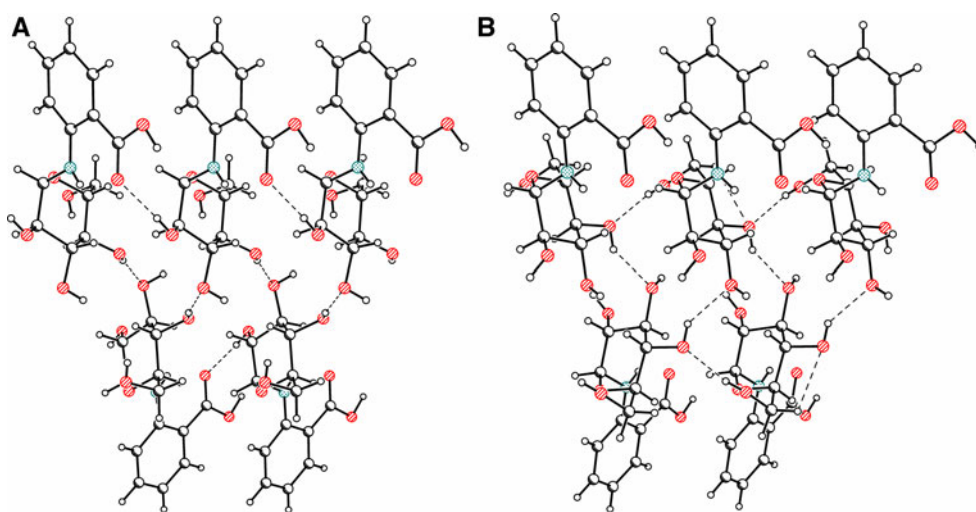
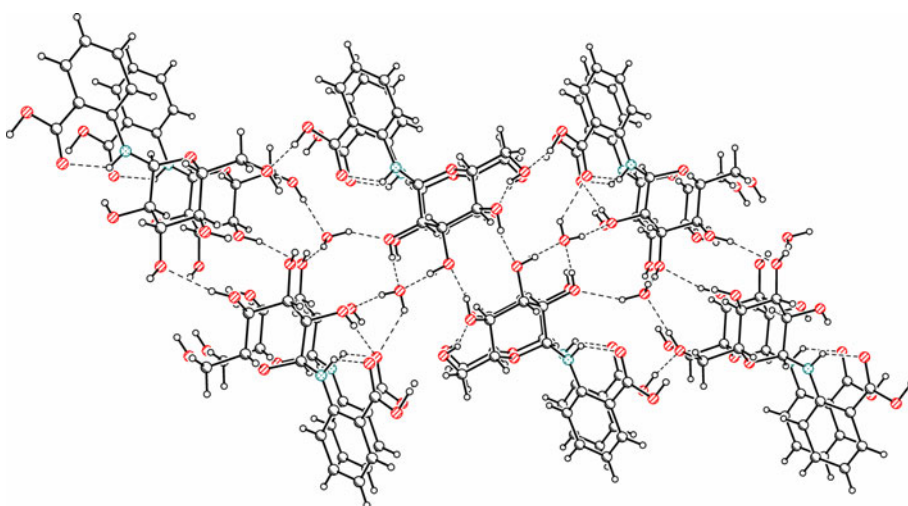


Fig. 4 The crystal packing of (1) as seen along [010] direction [20]. Dashed lines denote hydrogen bonds



graph set descriptors connected with these bonds are C(9) and C(6) for molecules A and B, respectively.

At the next level of organization the individual chains are connected into three dimensional structure by means of “direct” \sim ABABAB \sim O16 \cdots O6 hydrogen bonds (C₂²(22) chains) and “indirect”, involving water molecules (cf. Table 2). All these bonds create complicated three dimensional structure (Fig. 4) with a number of ring structures (for instance R₂³(10) and R₃³(14), cf. Fig. 4, Table 3).

Experimental

All solvents and chemicals were used as received, except for the amines which were distilled under reduced pressure prior to use. Elemental analyses were performed by using a Perkin-Elmer 2400II CHNS-O elemental analyzer. IR spectra were obtained as KBr plates using a Bruker FT-IR instrument. ¹H NMR spectra were recorded on a 500 MHz

Bruker FT-NMR spectrometer using CDCl₃ as solvent; chemical shifts (δ) are given in ppm.

Preparation of the Compound 1

To a stirring solution of anthranilic acid (0.68 g, 5 mmol) in ethanol was added an equimolar amount of *D*-(+)-glucose (0.63 g, 3.5 mmol). The reaction mixture was refluxed for longer than 6 h. The resulting solution was left in open air, and slow evaporation of the solvent at room temperature provided colorless crystals. The product was washed several times with acetonitrile. The yield of reaction was 92 %.

For C₁₃H₁₇NO₇ anal. calcd., C, 52.17; H, 5.73; N, 4.68; found, %: C, 53.95; H, 5.50; N, 7.80.

FT-IR (KBr, cm⁻¹): ν_{\max} 3,200–3,600 (s, COOH), 1,650 (s, C=O), 1,512 (s, C–N). ¹H NMR. (500 MHz, CDCl₃, δ , ppm): 3.28–3.53 (6H), 4.33–5.36 (5H), 6.67–8.40 (5H), 12.70 (1H).

Table 3 Crystal and experimental data

Formula (monomer)	C ₁₃ H ₁₉ NO ₈ 370.86
Formula weight	317.29
Crystal system	Monoclinic
Space group	P2 ₁
a(Å)	15.584(3)
b(Å)	4.992(1)
c(Å)	18.928(4)
β(°)	107.63(3)
V(Å ³)	1,403.4(5)
Z	4
d _x (g cm ⁻³)	1.50
F(000)	672
μ(mm ⁻¹)	0.13
Crystal size (mm)	0.3 × 0.15 × 0.1
Θ range (°)	1.13–26.26
hkl range	-18 ≤ h ≤ 13 -6 ≤ k ≤ 6 -23 ≤ l ≤ 23
Reflections:	
Collected	7,931
Unique (R _{int})	4,441 (0.021)
with I > 2σ(I)	4,286
Number of parameters	549
R(F) [I > 2σ(I)]	0.029
wR(F ²) [I > 2σ(I)]	0.073
R(F) [all data]	0.030
wR(F ²) [all data]	0.075
Goodness of fit	1.06
Max/min Δρ (e Å ⁻³)	0.17/−0.20

Antibacterial Activity

Evaluation of the antimicrobial activity of synthesized compound **1** was carried out by disk diffusion assay [15] and a twofold dilution method [16]. The Gram negative and Gram positive standard strains namely *Bacillus subtilis* (*B. subtilis*; PTCC no: 1023; ATCC 6633); *Staphylococcus aureus* (*S. aureus*; PTCC no: 1431; ATCC 25923), *Escherichia coli* (*E. coli*; PTCC no:1399; ATCC 25922), and *Pseudomonas aeruginosa* (*P. aeruginosa*; PTCC no: 1430; ATCC 27853) were purchased from Iranian Research Organization for Science and Technology (IROST). All bacteria were grown on Muller–Hinton Agar plates (37 °C, 24 h) and the zones of inhibition were measured after 24 h. Each organism was tested in duplicate on different days to measure the reproducibility of the test. Ampicillin, Chloramphenicol, Kanamycin, and Penicilin were purchased from PadtanTeb Company (Iran) and used as reference antibacterial agents.

Crystal Structure Determination

Diffraction data for (**1**) were collected at room temperature by the ω-scan technique on an Agilent Technologies Xcalibur four-circle diffractometer with Eos CCD-detector and graphite-monochromatized MoK_α radiation source (λ = 0.71073 Å). The data were corrected for Lorentz-polarization as well as for absorption effects [17]. Precise unit-cell parameters were determined by a least-squares fit of 4,366 reflections of the highest intensity, chosen from the whole experiment. The calculations were mainly performed within the WinGX program system [18]. The structure was solved with SIR92 [19] and refined with the full-matrix least-squares procedure on F² by SHELXL97 [20]. The scattering factors incorporated in SHELXL97 were used. The function $\sum w(|F_o|^2 - |F_c|^2)^2$ was minimized, with $w^{-1} = [\sigma^2(F_o)^2 + (0.0413 \cdot P)^2 + 0.1771 \cdot P]$ ($P = [\text{Max}(F_o^2, 0) + 2F_c^2]/3$). All non-hydrogen atoms were refined anisotropically, the hydrogen atoms were placed geometrically in idealized positions and refined as rigid groups with their Uiso's as 1.2 times Ueq of the appropriate carrier atom. Relevant crystal data are listed in Table 1, together with refinement details.

Crystallographic data (excluding structure factors) for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, no. CCDC 852983. Copies of this information may be obtained free of charge from: The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK. Fax:+44(1223)336-033, e-mail:deposit@ccdc.cam.ac.uk, or www: www.ccdc.cam.ac.uk.

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