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[c014]

Synthesis and antimicrobial activities of 3-*O*-Methyl-(*R*)-1,2-*O*-trichloroethylidene-*α*-D-*xylo*-furanuronic acid and 3-*O*-Methyl-(*S*)-1,2-*O*-trichloroethylidene-*α*-D-*xylo*-furanuronic acid

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Abstract: The preparation of 3-*O*-methyl-1,2-*O*-(*S*)-trichloroethylidene-α-**D**-*xylo*-furanuronic acid and 3-*O*-methyl-1,2-*O*-(*R*)-trichloroethylidene-α-**D**-*xylo*-furanuronic acid starting from β-chloralose and α-chloralose are described. All new products were characterized by ^{1}H NMR, ^{13}C NMR and FTIR. Antibacterial potency of the product (**6b**) was determined in term of inhibition zone diameter and results showed that this compound showed moderate activity against the tested microorganisms with inhibition zones ranging from 10 to 22 mm.

Keywords: Antimicrobial activity; uronic acid; trichloroethylidene acetals; β-chloralose; α-chloralose

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1. Introduction

It has been proved that carbohydrates and their derivatives are used in therapeutic strategies for the treatment of cancer, AIDS and diabetes[1]. It is also known that trichloroethylidene acetals of sugars, existed as protecting groups, are potentially active derivatives of carbohydrates such as α-chloralose, used as a hypnotic drug and anaesthetic surgeries in laboratory works[2]. But, β-chloralose (the monoacetal derivative which trichloromethyl group is in *exo*-position) is toxic compound[3].

In the last years, uronic acids, transformed their primary hydroxyl groups into a carboxyl group because of oxidation, are generally applied to monosaccaharides[4]. The structures of uronic acids have been both aldehyde and carboxyl groups. They have received much attention because of their biological activities. Uronic acids and their derivatives are of quite important in medicine, pharmacology and industrial area[5,6,7]. For example; glycosaminoglycans, carboxylic

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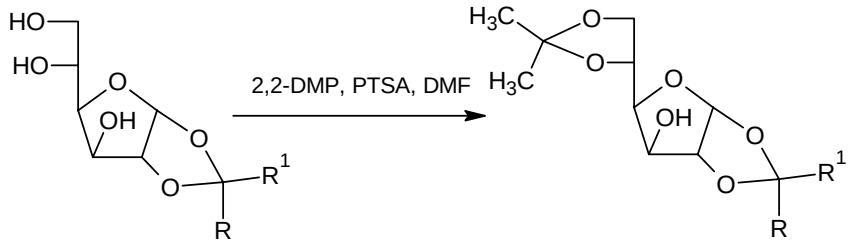
acid formations on the sugar skeletons, play a key role in biomedical processes including growth factor interactions, virus entry and angiogenesis[8,9].

Only a small proportion of pharmaceuticals derived from different sources have yet been examined for biologically active compounds, there is still enormous potential for the identification of many novel agents. In addition, almost all antibiotics used today are of microbial origin. In medicine, we experience an increasing problem with pathogenic microbes that becomes resistant to the most commonly used antibiotics. Therefore, the study reported herein was undertaken to determine the preparation and the synthesis of a new series of uronic acids derived from β-chloralose and α-chloralose as well as antimicrobial potency of them.

2. Results and discussion

2.1. Chemistry

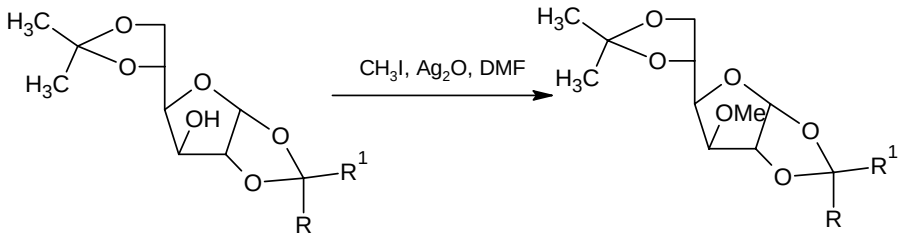
In the present study, glucuronic acid derivatives have been synthesized from α-chloralose (**1a**) and β-chloralose (**1b**) as starting sugars. The corresponding reactions can be represented according to the synthetic protocol outlined in **Scheme 1**.



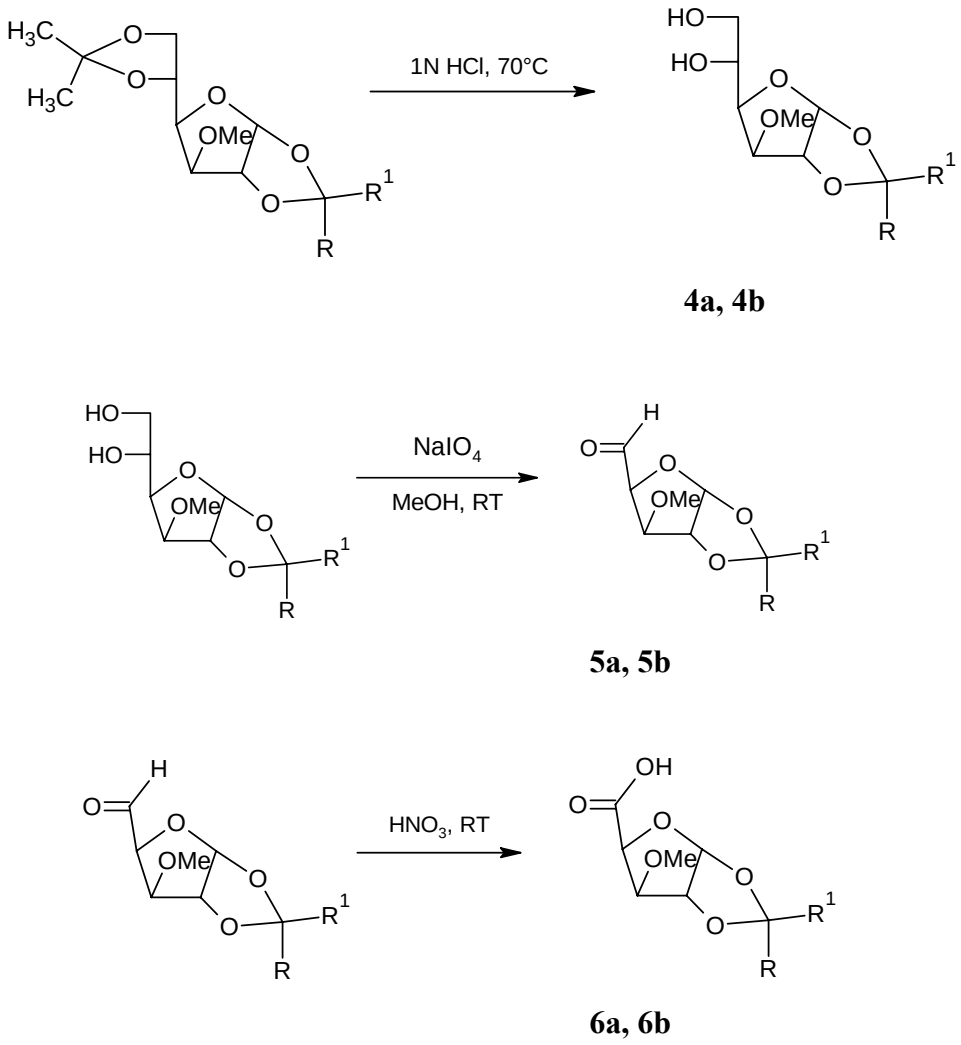
1a, 1b **2a, 2b**

α-chloralose: R=CCl_3, R^1=H **(1a)**

β-chloralose: R^1=CCl_3, R=H **(1b)**



3a, 3b

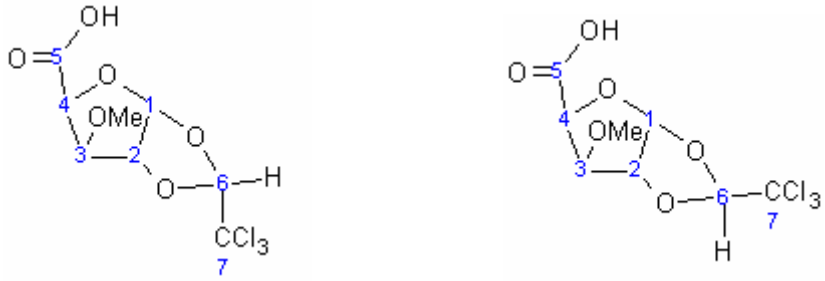


Scheme 1. Synthetic pathway for prepared compounds

The molecular structure of uronic acid derivatives (**6a**, **6b**) has been confirmed by using spectral methods as a FT-IR, ^{1}H-NMR, ^{13}C-NMR.

In FT-IR spectrums of 3-*O*-methyl-1,2-*O*-(*R*)-trichloroethylidene-α-**D**-*xylo*-furanuronic acid (**6a**) and 3-*O*-methyl-1,2-*O*-(*S*)-trichloroethylidene-α-**D**-xylo-furanuronic acid (**6b**), the hydroxyl absorption bands of carboxylic acid groups of them was observed at 3514 cm^{-1} and 3415 cm^{-1}, respectively. In regions 3015-2940 cm^{-1} and 3008-2834 cm^{-1}, the C-H bonds was appeared by some absorption bands. The absorption band of carbonyl bond (C=O) was also signed in region 1697 cm^{-1} and 1708 cm^{-1} (acid form), 1736 cm^{-1} (aldehyde form), respectively (**Table 1**). In the light of these IR data, it seems that the oxidation reaction of aldehyde groups of **5a** and **5b** can easily be done. However, the spectral results of NMR proved that the oxidation reaction of compound **5a** was not completed while comparing with the oxidation of compound **5b**. It is also to note that there is the aldehyde form, approximately 30% yield, in the mixture as remaining, after the oxidation of **5a**. ^{1}H NMR spectral data of compound **6a** and **6b** are given in

Table 2. The characteristic peak of these compounds is the acetal peak, H-6, shown in **Scheme 2**. This acetal peak of trichloroethylidene derivatives are observed at about 5.43-5.75 ppm. Contrary to expectation, compound **6a** have two singlets, which belong to H-6 peak, at about 5.42, and 5.43 ppm for two proton signals in its ^{1}H NMR spectra. In addition, ^{13}C NMR spectral data of compound **6a,** shown in **Table 3,** exhibits that there are two carbonyl peaks (aldehyde and carboxylic acid) at 169.3 and 169.4 ppm on its spectrum. For products, (**6a** and **6b**) hydrogen of carboxylic acid groups was not observed on their ^{1}H NMR spectrums. ^{1}H NMR and ^{13}C NMR spectral data of compound **6b** are consistent with the proposed structure.



6a **6b**

Scheme 2. The structural determination of compound **6a** and **6b** for NMR spectra

Table 1. IR spectral data of the compound **4a/4b**, **5a/5b** and **6a/6b** (KBr tablets)

	IR (cm^{-1}) of the compound		
	4a/4b	**5a/5b**	**6a/6b**
-OH	3425/3397	-/-	-/-
C-H	2940-2840/ 2992-2838	2834-2937/ 2959-2857	3008-2834
C=O (aldehyde)	-/-	1736/1727	-/-
C=O (acid)	-/-	-/-	1708/1697
C-O	1101/1102	1104/1101	1104/1101
C-Cl	806/809	806/814	809/840

Table 2. ^{1}H NMR spectral data of the compound **6a** and **6b** (400 MHz, in CD_3OD)

	Compound 6a		Compound 6b	
Number of atoms	**^{1}H NMR (δ)**	**H and J couplings (Hz)**	**^{1}H NMR (δ)**	**H and J couplings (Hz)**
H-1	6.14 d	1 H, $J_{1,2}$= 3.6	6.28 d	1 H, $J_{1,2}$= 4.0
H-6	5.42 s and 5.43 s	1 H	5.75 s	1 H
H-2	5.09 d	1 H	5.02 d	1 H
H-3	4.90 d	1 H, $J_{3,4}$= 4.0	4.85 d	1 H, $J_{3,4}$= 4.0
H-4	4.19 d	1 H	4.14 d	1 H
O$\underline{C}H_3$	3.43 s	3 H	3.48 s	3 H

Table 3. ^{13}C NMR spectral data of the compound **6a** and **6b** (400 MHz, in CD_3OD)

Number of atoms	**^{13}C NMR (δ) of Compound 6a**	**^{13}C NMR (δ) of Compound 6b**
C-1	107.0	109.3
C-2, C-3, C-4	84.6, 83.8, 80.8	83.8, 84.6, 79.9
C-5(carbonyl)	169.3, 169.4	169.3
C-6	106.6	107.0
C-7 ($\underline{C}Cl_3$)	97.2	99.6
O$\underline{C}H_3$	55.7	57.5

2.2. Antimicrobial activity

It is obvious that synthesized compound, **6b** showed moderate activity against the tested microorganisms with inhibition zones ranging from 12 to 22 mm. However, the compound differs significantly in its activity against test microorganisms. The most activity was showed against *E. aerogenes, S. aureus, B. cereus* and *P. vulgaris*, 22, 20, 18 and 14 mm, respectively (**Table 4**). In addition, this compound active against *C. albicans*, which are known to cause dermic and mucosal infections, beside other infections in humans with an inhibition zone of 10 mm. Furthermore, determination of antibacterial activity indicated that **6b** was less active against the Gram-negative bacteria. The results of the present investigation clearly indicate that uronic acids are also important, since they are found in some antimicrobial active molecules. Antimicrobial activity of standard antibiotics is summarized for comparison in **Table 4**.

Table 4. Antimicrobial activity of synthesized compound (6b) and some standard antibiotics against test microorganisms

Compound	*Microorganisms*										
	SA[a]	*EC*	*KR*	*BC*	*BS*	*STYP*	*PV*	*EF*	*EA*	*ECLO*	*CA*[d]
6b (ACT)	20[b]	0	10	18	0	0	14	12	22	0	10
SAN (NA)[c]	20	26	10^R	28	30	6^R	12^R	28	26	10^R	ND
CLH	20	26	30	26	28	40	16	28	12^R	28	ND
P	24	6^R	20^R	10^R	8^R	6^R	10^R	24	6^R	12^R	ND
NYS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22
NC (ACT)	0	0	8	0	0	$8^{\pm}$	0	8	8	0	8

[a]Test microorganisms: SA, *Staphylococcus aureus* ATCC 6538P; EC, *Escherichia coli* ATCC 39628; KR, *Kocuria rhizophila* ATCC 9341; BC, *Bacillus cereus* CM 99; BS, *Bacillus subtilis* ATCC 6633; STYP, *Salmonella typhimurium* CCM 5445; PV, *Proteus vulgaris* ATCC 8427; EF, *Enterococcus faecalis* ATCC 29212; EA, *Enterobacter aerogenes* ATCC 13048; ECLO, *Enterobacter cloacae* ATCC 13047D; CA, *Candida albicans* ATCC 10231. [b]Inhibition zone diameter in millimeters, including well and disc diameter (6 mm). [c]SAN - Standard antibiotics, NA - Nalidixic acid (30 µg/disc); CHL - Chloramphenicol (30 µg/disc); P – Penicilin (10 IU); NYS - Nystatin (10 µg/disc). [d]Bacteria tested in MHA medium, yeast in PDA; mean values, n=3; ND, not determined; [R], resistant, NC, negative control, ACT – Acetone (60 µL); [±] partially inhibition.

3. Experimental part

3.1. Chemistry

Melting points were measured by using Electrothermal 9100 melting point apparatus in capillary and uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian AS 400 NMR spectrometer. IR spectra were recorded on Perkin Elmer Spectrum 100 FTIR Spectrometer. Optical rotation measurements were carried out on a Schimidt-Haensch Polartronic E polarimeter. TLC was performed on precoated aluminum plates (Merck 5554). The chromatograms were detected with 5% aqueous sulphuric acid by heating the plates above 120 ^{o}C for about 3 minutes. All solvent removals were carried out under reduced pressure with rotary evaporator.

3.2. *5,6-O-Isopropylidene-1,2-O-(S)-trichloroethylidene-α-D-glucofuranose*[10] (2b)

The solution of 1,2-*O*-(*S*)-trichloroethylidene-α-**D**-glucofuranose, known as β-chloralose, (30 g, 0.097 mol) was dissolved in DMF (200 mL). Then, 2,2-dimethoxypropane (60 mL) and PTSA (30 mg) was added to this solution. This mixture was stirred at room temperature (RT) overnight. The reaction mixture was neutralized with saturated Na_2CO_3 solution after the reaction times.

Then solvent was evaporated under reduced pressure and the crude product was obtained as a solid. The crude product was crystallized from boiling methanol and the pure compound was obtained (30.27 g, 89%), mp 184-187°C.

3.3. ***5,6-O-Isopropylidene-3-O-methyl-1,2-O-(S)-trichloroethylidene-α-D-glucofuranose***[10] **(3b)**

5,6-*O*-Isopropylidene-1,2-*O*-(*S*)-trichloroethylidene-α-**D**-glucofuranose (10 g, 0.028 mol) was dissolved in DMF (50 mL). Then, Ag_2O (20 g) and CH_3I (15 mL) was added to this solution. This reaction mixture was stirred at RT overnight. The solution filtered off and the solvent was washed with saturated $Na_2S_2O_3$. The residue was crystallized from boiling methanol (9.47 g, 91%), mp 116-120°C.

3.4. ***3-O-Methyl-1,2-O-(S)-trichloroethylidene-α-D-glucofuranose***[10] **(4b)**

5,6-*O*-Isopropylidene-3-*O*-methyl-1,2-*O*-(*S*)-trichloroethylidene-α-**D**-glucofuranose (10 g, 0.027 mol) was dissolved in ethanol (38 mL) and heated at 70°C with 1N HCl (25 mL) for 1h. At the end of this time, water (200 mL) was added to the reaction medium and the organic compound was extracted with chloroform (5x25 mL). The organic layer was washed with saturated Na_2CO_3 solution, dried with Na_2SO4, and filtered off. Solvent was evaporated under reduced pressure and the pure product (8.77 g, 98%) was obtained as a white solid, mp 116-117°C.

3.5. ***3-O-Methyl-1,2-O-(S)-trichloroethylidene-α-D-xylo-1,4-furanodialdose*** [10] **(5b)**

3-*O*-methyl-1,2-*O*-(*S*)-trichloroethylidene-α-**D**-glucofuranose (4 g, 0.012 mol) was dissolved in methanol (120 mL) and $NaIO_4$ (2.65 g, 0.012 mol), in water (75 mL), was added to this solution. The reaction mixture was stirred at RT overnight. The solution was dried and filtered off. Crude product was obtained as a syrupy (2.85 g, 79%).

3.6. ***3-O-Methyl-1,2-O-(S)-trichloroethylidene-α-D-xylo-furanuronic acid*** **(6b)**

3-*O*-methyl-1,2-*O*-(*S*)-trichloroethylidene-α-**D**-*xylo*-1,4-furanodialdose (1.5 g, 0.051 mol) and concentrated HNO_3 (20 mL, 65%) were mixed at RT for 36 hours. The residue was filtered off and washed with water. Crude product was obtained in quite yields (1.28 g, 81%), mp 144-150°C, $[\alpha]_D^{27}$ -5.41 (*c* 0.02 in MeOH).

3.7. *5,6-O-Isopropylidene-1,2-O-(R)-trichloroethylidene-α-D-glucofuranose* (2a)

Commercially available 1,2-*O*-(*R*)-trichloroethylidene-*α*-D-glucofuranose (*α*-chloralose; **1a**) which is containing *β*-chloralose was dissolved in cold methanol in order to separate β-chloralose. Pure *α*-chloralose was obtained after removing the solvent under reduced pressure. Periodate oxidation of *α*-chloralose was carried out according to literature[8] as following procedures.

The solution of 1,2-*O*-(*R*)-trichloroethylidene-α-**D**-glucofuranose (20 g, 0.065 mol) was dissolved in DMF (50 mL). This solution was stirred with 2,2-dimethoxypropane (25 mL) and PTSA (20 mg) at RT overnight. Reaction mixture was neutralized with saturated Na_2CO_3 solution. Then the solvent was evaporated under reduced pressure and the crude product was crystallized from boiling methanol to get the white solid product (20.30 g, 90%), mp 102-104°C.

3.8. *5,6-O-Isopropylidene-3-O-methyl-1,2-O-(R)-trichloroethylidene-α-D-glucofuranose* (3a)

5,6-*O*-Isopropylidene-1,2-*O*-(*R*)-trichloroethylidene-α-**D**-glucofuranose (20 g, 0.057 mol) was dissolved in DMF (100 mL). Then this solution was stirred with Ag_2O (20 g) and CH_3I (30 mL) at RT overnight. The solution filtered off and the filtrate was washed with saturated $Na_2S_2O_3$ solution and dried. The crude product was obtained as syrup (20.1 g, 96%).

3.9. *3-O-Methyl-1,2-O-(R)-trichloroethylidene-α-D-glucofuranose* (4a)

5,6-*O*-Isopropylidene-3-*O*-methyl-1,2-*O*-(*R*)-trichloroethylidene-α-**D**-glucofuranose (24.5 g, 0.067 mol) was dissolved in ethanol (92 mL). This solution was stirred and heated at 70°C with 1N HCl (61 mL) for 1h. Water (200 mL) was added to the reaction medium and the product extracted with chloroform (5 X 25 mL). The organic layer was washed with saturated Na_2CO_3 solution and dried. Then the solvent was evaporated under reduced pressure and the crude product was obtained as syrup (8.8 g, 91%).

3.10. *3-O-Methyl-1,2-O-(R)-trichloroethylidene-α-D-xylo-1,4-furanodialdose* [8] (5a)

3-*O*-methyl-1,2-*O*-(*R*)-trichloroethylidene-α-**D**-glucofuranose (4.3 g, 0.013 mol) was dissolved in methanol (100 mL) and $NaIO_4$ (5 g, 0.023 mol), in water (100 mL), was added. The reaction mixture was stirred at RT overnight. The solution was filtered off and dried. The crude product was obtained as syrup (2.99 g, 78%).

3.11. *3-O-Methyl-1,2-O-(R)-trichloroethylidene-α-D-xylo-furanuronic acid* (6a)

3-*O*-methyl-1,2-*O*-(*R*)-trichloroethylidene-α-**D**-*xylo*-1,4-furanodialdose (4 g, 0.014 mol) and concentrated HNO_3 (50 mL, 65%) were mixed at RT for 36 hours. After that time, the residue filtered off and washed with water. The crude product was obtained as a solid (2.89 g, 69%) (This product contains aldehyde form and uronic acid form of related sugar compounds).

3.12. Determination of antimicrobial activity of synthesized compound (6b)

In vitro antimicrobial activity of compound was evaluated by the agar diffusion technique[11] against test microorganisms. Sample solutions were prepared by dissolving the **6b** (20 mg) in acetone (1 mL). Bacterial strains grown on nutrient agar at 37 ^oC for 24 h (yeast grown on PDA at 28 ^oC for 48 h) were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards [10^6 Colony Forming Units (CFU)/mL]. Briefly, 50-µL inoculum (containing approximately 10^5 bacteria per milliliter and 10^4 yeast per milliliter) was added to 25 mL melted Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium cooled at 45 ^oC. This was then poured into 90 mm diameter Petri dishes and maintained for 1 h at room temperature. Small wells (6 mm diameter) were cut in the agar plate using a cork borer; 60 µL of compound concentration (1.2 mg) with a negative control (Acetone, 60 µL) was loaded in the wells. The dishes were preincubated at 4 ^oC for 2 h to allow uniform diffusion into the agar. After preincubation, for bacteria the plates were incubated at 37 ^oC for 24 h and 30 ^oC for 48 h for yeast. The antimicrobial activity was evaluated by measuring the inhibition zone diameter observed. In addition, commercial antibiotics [Nalidixic acid (30 µg), Penicillin G (10 IU), Chloramphenicol (30 µg) and Nystatin (10 µg)] were used as positive control to determine the sensitivity of the strains[12]. These studies were performed in triplicate.

4. Conclusion

Unlike other acetals and ketals, trichloroethylidene acetal group of *α*- and β- chloraloses are not hydrolysed with concentrated HNO_3 in oxidation reaction. 3-*O*-Methyl-1,2-*O-(S)*-trichloroethylidene-α-**D**-*xylo*-furanuronic acid **(6b)** has been synthesized from *β*-chloralose with excellent yields. Its molecular structure has been confirmed using modern spectroscopic methods. In addition, antimicrobial activity test was applied to this compound. To the synthesis of 3-*O*-Methyl-1,2-*O-(S)*-trichloroethylidene-α-D-*xylo*-furanuronic acid **(6a)**, same synthetic routes was applied but the spectral analysis show that last step (oxidation of aldehyde group to the carboxylic acid form) was not succeed completely. Based on the present study, we have

found new uronic acid derivatives with inhibitory activity comparable to standard antibiotics, which are used as reference inhibitors.

5. Acknowledgements

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Sample Availability: Samples of the compounds are available from the authors.

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