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Synthesis, characterization, and antimicrobial activities of novel monosaccharide-containing Schiff base ligands

Fatma ÇETİN TELLİ, Stephen Thomas ASTLEY, Azize Yeşim SALMAN*
Department of Chemistry, Faculty of Science, Ege University, İzmir, Turkey

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Abstract: Four new chiral Schiff base ligands (**3a**–**4a**, **3b**–**4b**) have been prepared using aminoisopropylidene derivatives of glucose and aminochloralose derivatives of mannose. The synthesized compounds **1** and **2** were characterized by nuclear magnetic resonance (^{1}H and ^{13}C NMR), Fourier transform infrared spectroscopy (FTIR), UV/visible spectroscopy, and elemental analysis. A total of eight Schiff base compounds (**3a**–**6a**, **3b**–**6b**) were tested for antimicrobial activity against *Escherichia coli* ATCC 12228, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* CCM 2318, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, and *Candida albicans* ATCC 10239 and exhibited a range of activities.

Key words: Chloralose, ONO-tridentate chiral Schiff base, amino sugar, antimicrobial activity

1. Introduction

Schiff bases, named after Hugo Schiff,[1] are formed when a primary amine reacts with an aldehyde or a ketone. Structurally, a Schiff base is a nitrogen analogue of a carbonyl compound in which the carbonyl group has been replaced by an imine or azomethine group (Figure 1).

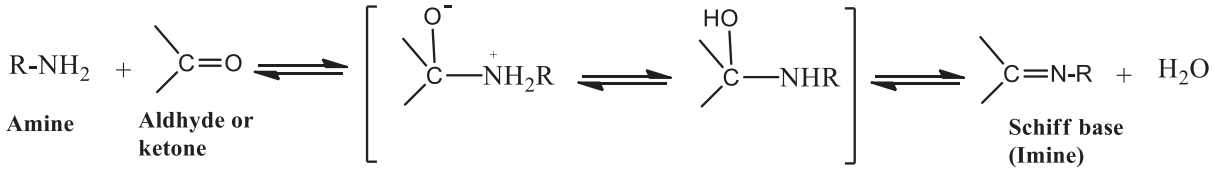


Figure 1. Mechanism of the synthesis of Schiff base (imine) from amine and aldehyde or ketone.

Schiff base ligands are easily synthesized and form complexes with almost all metal ions. Over the past few years, there have been many reports on their biological properties including antibacterial, antifungal, anticancer, antioxidant, antiinflammatory, antimalarial, antiviral, and antimicrobial activities.[2–7] In addition, they can act as catalysts in several reactions such as polymerization, reduction of thionyl chloride, oxidation of organic compounds, reduction reaction of ketones, aldol reaction, Henry reaction,[8,9] epoxidation of alkenes, hydrosilylation of ketones, and Diels–Alder reaction.

Monosaccharides mostly react in their furanose forms with chloral to give trichloroethylidene acetals. Chloraloses (β-chloralose or α-glucochloralose) have been prepared by the simple reaction of chloral and

*Correspondence: yesim.salman@ege.edu.tr

glucose.[10] Thus, 1,2-*O*-trichloroethylideneacetals of D-glucofuranose,[11] D-galactofuranose,[12] D-arabinofuranose,[13] and D-mannofuranose[14] are known. 1,2-O-(R)-trichloroethylidene-D-glucofuranose is a commercially available compound, also known as α-chloralose, which is used as an anesthetic for animals.[15] Unlike other acetals, 1,2-*O*-trichloroethylidene acetals are highly stable in the presence of acids. This is attributed to the electron-withdrawing ability of the trichloromethyl group. By contrast, they offer much less stability under even mildly basic conditions and they can be converted to synthetically useful ketene acetals in the presence of strong bases. Alternatively, the 1,2-*O*-trichloroethylidene acetal can be removed using a Raney nickel procedure.[11] They have proved to be suitable protecting groups for the synthesis of some biologically important compounds such as amines,[16] lactones,[17] orthoesters,[13,14] *spiro*endoperoxides,[18] *spiro*difuranose,[19] *O*-glycosides,[20] uranic acid,[21] oxime,[22] oxetanes,[23] Wittig products,[24] and Schiff base ligands.[25]

As part of ongoing studies into the chemistry of monosaccharide trichloroethylidene acetals, we recently synthesized some ONO-tridentate chiral Schiff bases. Based on previous observations,[9] it was expected that a bulky sugar moiety could be expected to have a significant effect on the catalytic activity of these ligands. Thus, when these ONO-tridentate Schiff base ligands were employed as chiral ligands for the Cu(II) catalyzed asymmetric Henry reaction, high yields and good enantioselectivities were obtained.[25]

It has recently been pointed out that a sugar moiety plays a major role in the biological activity of carbohydrate-based Schiff bases.[26] Considering that it is well known that tridentate Schiff base ligands themselves exhibit interesting biological activity[27–29] we decided that it could be beneficial to investigate the biological activity of a series of Schiff base ligands containing different sugar substituents. Herein we describe the preparation of aminoisopropylidene glucose Schiff bases (**3a**, **3b**) and aminochloralose Schiff base derivatives of mannose (**4a**, **4b**) (Figure 1). Their biological activities are compared with those of Schiff base ligands from aminochloralose-containing glucose (**5a**, **5b**) and galactose (**6a**, **6b**) derivatives (Figure 2).[25]

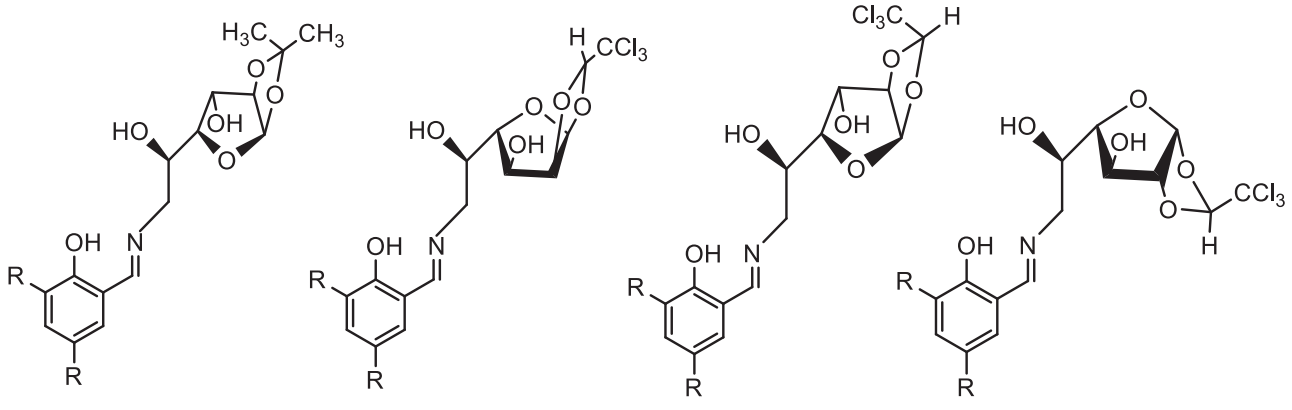
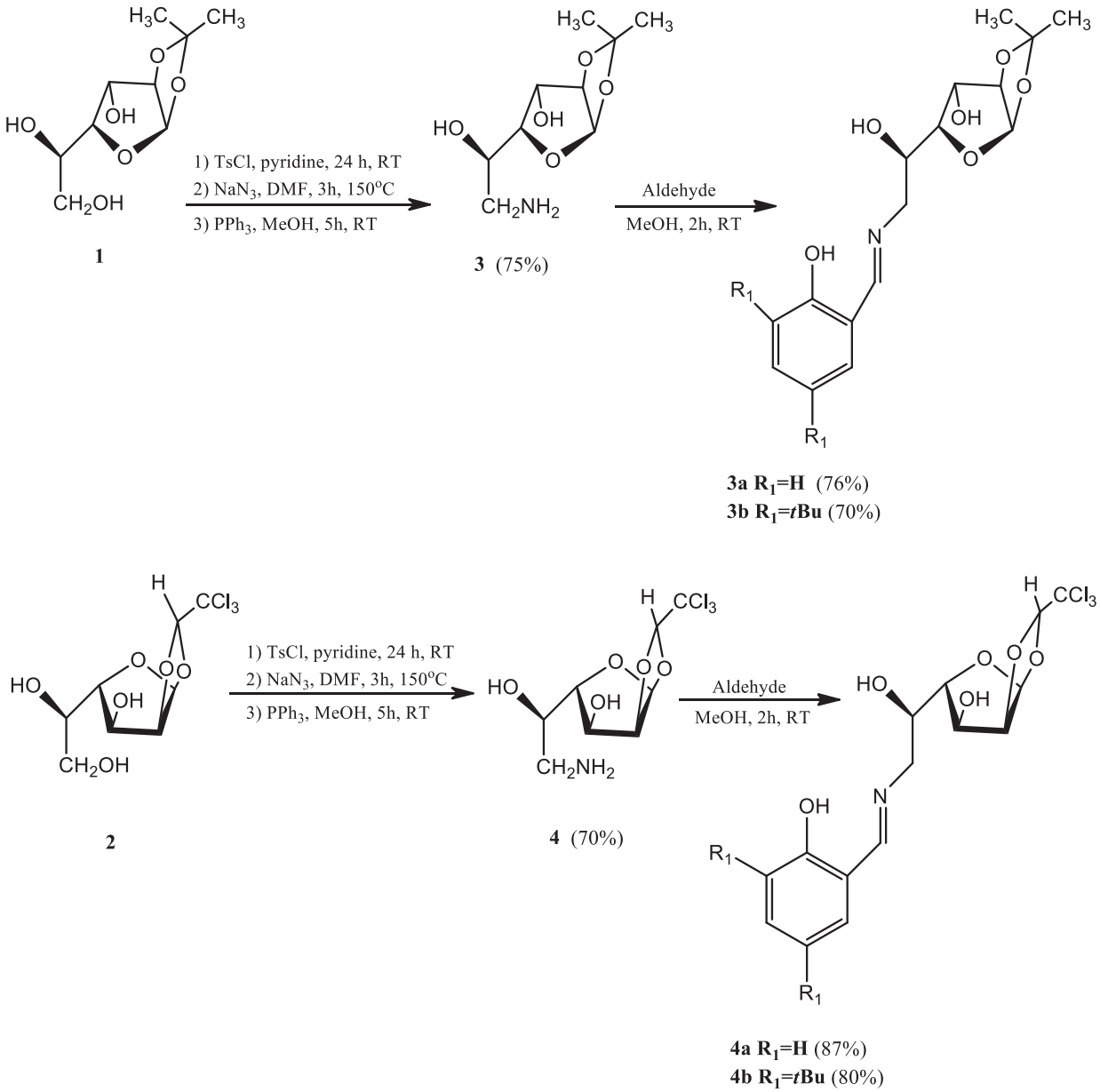


Figure 2. Structure of Schiff base ligands (**3a**–**6a**, **3b**–**6b**) from aminoisopropylidene derivatives of glucose (**3**) and aminochloralose derivatives of mannose (**4**), glucose (**5**), and galactose (**6**).

2. Results and discussion

Our methods of formation of the ONO-tridentate chiral Schiff base ligands involved initial preparation of aminosugars via selective tosylation of the corresponding furanose. As shown in the Scheme, this was followed by azidation, reduction, and subsequent reaction with aldehyde (3,5-di-t-butylsalicylaldehyde or salicylaldehyde)

to give the targeted ONO-tridentate chiral Schiff bases. The yields of these reactions were very high (70% to 87%). All products were characterized by elemental analysis and by ^{1}H NMR, ^{13}C NMR, UV, and FTIR spectroscopic methods.



Scheme. Synthesis of aminosugar derivatives (**3**–**4**) and Schiff base derivatives (**3a**–**4a**, **3b**–**4b**).

In the UV/visible spectra, run in ethanol, three distinct bands were observed at 280 nm, 338 nm, and 375 nm. The absorption at 280 nm can be assigned to a $\pi \rightarrow \pi*$ transition involving the aromatic rings and the absorption at 338 nm can be assigned to the $\pi \rightarrow \pi*$ transition for the C = N chromophore.[30,31] The final band around 375 nm corresponds to the $n \rightarrow \pi$ * transitions of the nonbonding electrons present in the nitrogen of the azomethine group of the Schiff base.[32]

The infrared (IR) spectra of the ligands exhibit a broad absorption band at 3463 cm^{-1} that corresponds to the hydroxyl protons of the Schiff base ligands. The broadness and the low frequency values indicate the presence of significant intramolecular hydrogen bonding of the phenolic O − H group[33,34] and the hydroxyl groups in positions 6 and 3 of the ONO chiral Schiff base ligands. A strong absorption band dominates the spectrum between 1630 and 1640 cm^{-1}. This band is attributed to the C=N vibration characteristic of imines. As expected, there is no evidence of the characteristic band related to free aromatic aldehydes group near 1665 cm^{-1}.

^{1}H and ^{13}C NMR data of the Schiff base derivatives are given in Tables 1 and 2. The characteristic chemical shift of the azomethine (CH=N) protons is observed at 8.40 ppm as a singlet. In addition, the aromatic protons appear as multiplets of δ values in the range 6.84–7.90 ppm.[35,36] One sharp singlet appeared at δ 5.76 ppm in the spectrum assigned to the acetal proton of D-mannochloralose. The methyl protons of the isopropylidine group and tert-butyl groups were observed between δ 1.28 and 1.47 ppm as singlets.

Table 1. ^{1}H NMR (400 MHz) chemical shifts (δ ppm) in $CDCl_3$, for Schiff base derivatives (**3a**–**4a**, **3b**–**4b**).

Compd	CH=N	Aromatic protons	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H-CCl_3	CH_3
3a	8.37	7.33, 7.25, 6.93, 6.84	5.96	4.55	4.40	4.11	4.32	4.00	3.73	-	1.47, 1.30
3b	8.39	7.38, 7.21	6.06	4.60	4.32	4.06	4.21	3.99	3.75	-	1.45, 1.28
4a	8.40	7.64, 7.55, 6.95, 6.87	6.09	4.92	4.51	4.30	4.27	3.87	3.68	5.77	-
4b	8.42	7.90, 7.41	6.12	4.98	4.48	4.31	4.18	3.97	3.76	5.76	1.44, 1.32

As seen in Table 3, the Schiff base ligands showed antimicrobial activity against gram positive/negative bacteria and *C. albicans* at different concentrations. Schiff bases are known to have a variety of biological properties including antibacterial, antifungal, antimalarial, antiproliferative, and antiviral activities.[37] Surprisingly, however, none of the compounds exhibited activity against *S. aureus* ATCC 6538 except compound **4b**. In contrast, all of the compounds except **4b** and **6a** exhibited activity against *S. thyphimirium* CCM 5445. The Schiff base ligand from aminochloralose mannose (**4a**) exhibited activity against only *S. thyphimirium* CCM 5445 and *B. subtilis* ATCC 6633, whereas the other Schiff base ligand from aminochloralose mannose (**4b**) exhibited activity against only *E. coli* ATCC 12228 and *S. aureus* ATCC 6538. In addition, Schiff base ligands (**3b** and **6a**) in the concentration range 0.1–0.2 mg/mL proved to be effective against *E. faecalis* ATCC 29212. Having been a hospital pathogen and secondary infection agent, this antimicrobial activity against *P. aeruginosa* is a property of only Schiff base ligands from aminochloralose glucose (**5a** and **5b**). Thus, it may be concluded that Schiff base ligands from aminochloralose glucose (**5a** and **5b**) possess similar antimicrobial activity, which is uncommon among other Schiff base ligands. Comparing the overall activities of all eight Schiff bases, it appears that the glucose derivatives **3a**, **3b**, **5a**, and **5b** seem to have slightly greater activity than the mannose and galactose derivatives. This could suggest that the particular chiral configuration of glucose has an important role. To the best of our knowledge, this appears to be the first study of the antibacterial activities of Schiff base ligands from aminochloralose derivatives of glucose, mannose, and galactose and also aminoisopropylidene derivatives of glucose. The positive results reported here suggest that further work into the preparation and properties of this type of compound may prove beneficial.

Table 2. ^{13}C NMR chemical shifts (δ ppm) in $CDCl_3$, for Schiff base derivatives (**3a**–**4a**, **3b**–**4b**).

Compd	$C_{Azomethine}$	Aromatic carbones	C-1,C_{CHCl3}	C-2, C-3, C-4	C-5, C-6	Tertiary carbons C_{isop}, CCl_3, $C(CH_3)_3$	Me groups
3a	168.4	164.7, 135.4, 133.2, 119.9, 118.7, 116.9	105.3, -	85.9, 82.5, 74.1	68.7, 48.6	109.9, -, -, -	27.1,23.2
3b	167.3	159.1, 137.6, 132.9, 130.7, 118.3, 117.8	105.2, -	87.4, 82.4, 76.1	69.4, 62.5	107.5, -, 38.3, 36.7	35.1, 34.3, 31.7, 31.0, 29.4, 29.2, 23.1, 20.9
4a	167.5	161.6, 132.6, 132.1, 119.1, 118.6, 117.0	105.1, 109.4	83.2, 82.8, 69.6	67.7, 62.2.	-, 99.5, -, -	-
4b	167.3	159.1, 141.6, 137.6, 131.9, 130.0, 128.3	105.4, 107.3	87.4, 88.4, 76.7	69.3, 62.3	- , 97.6, 37.8, 36.9	35.0, 34.2, 31.6, 31.2, 29.4, 29.2.

Table 3. MIC value (mg/mL) of compounds **3a**–**6a** and **3b**–**6b** against test microorganisms.

Compounds	*E.coli* ATCC 12228	*S. aureus* ATCC 6538	*K. pneumoniae* CCM 2318	*P. aeruginosa* ATCC 27853	*S. thyphimirium* CCM 5445	*E. faecalis* ATCC 29212	B. *subtilis* ATCC 6633	*C. albicans* ATCC 10239
3a	-	-	0.2	-	0.2	-	0.1	-
3b	0.2	-	0.1	-	0.2	0.1	-	-
4a	-	-	-	-	0.2	-	0.4	-
4b	0.4	0.4	-	-	-	-	-	-
5a	-	-	-	0.1	0.1	-	-	0.2
5b	-	-	-	0.2	0.1	-	-	0.2
6a	0.4	-	0.2	-	-	0.2	-	-
6b	0.8	-	-	-	0.4	-	0.2	0.4

3. Experimental

3.1. Materials

All ^{1}H NMR and ^{13}C NMR spectra were recorded using a Varian AS 400+ Mercury FT NMR spectrometer at ambient temperature. IR spectra were recorded on a PerkinElmer 100 FTIR spectrometer. UV/vis spectra were obtained using a Shimadzu UV-1601 spectrophotometer. Optical rotations were determined using a Rudolph Research Analytical Autopol I automatic polarimeter with a wavelength of 589 nm. The concentration 'c' has units of g/100 mL. Elemental analyses were performed on a PerkinElmer PE 2400 elemental analyzer. TLC and column chromatography were performed on precoated aluminum plates (Merck 5554) and silica gel G-60 (Merck 7734), respectively. All solvent removals were carried out under reduced pressure.

3.2. General procedure for the preparation of aminosugar derivatives (3 and 4)

The preparation of amino monosaccharides has been well reported in the recent literature.[25] Recent studies by Yenil and Astley have described the preparation of a series of aminosugar chloralose derivatives.[16,25] In the present study the main objective was to develop a simple method for the preparation of the 6-amino derivatives of 1,2,5,6-*O*-diisopropylidene-α-D-glucofuranose (diacetone-D-glucose) (**1**) and 1,2-*O*-(*R*)-trichloroethylidene-β-D-mannofuranose (**2**). Initial work included the synthesis of the starting compound, specifically the trichloroethylidene acetal of D-mannose, according to literature procedures.[13] Then the target molecules (**3** and **4**) were synthesized in a three-step sequence. First of all, the hydroxyl group in position 6 of the monosaccharide derivatives (0.10 mol) (**1** or **2**) was tosylated using p-toluenesulfonyl chloride (0.11 mol) in pyridine at –5 °C for 12 h. The reaction mixture was concentrated to half volume and then poured into ice-water (250 mL) to remove the pyridine. Then it was extracted with 150 mL of dichloromethane three times and then the organic phase was treated with 100 mL of water (three times). Subsequently, the dichloromethane phase was dried by adding anhydrous sodium sulfate (Na_2SO_4) and then filtered. The dichloromethane was evaporated and then the residue, containing the monotosyl furanose derivative, was obtained in the pure state using column chromatography. The eluting solvents were a dichloromethane and methanol mixture (CH_2Cl_2:MeOH, 50:2). In the second reaction, we performed nucleophilic substitution of the tosyl group with an azide group. To a solution of the 6-*O*-tosyl derivative of the monosaccharide (0.10 mol) in dimethylforamide (DMF) (50 mL) was added sodium azide (0.125 mol). The reaction mixture was then stirred in an oil bath at 150 °C for 3 h. At this point, TLC (CH_2Cl_2:MeOH, 8:2) indicated that the reaction was complete and so the reaction mixture was decanted into ice-water (250 mL). Afterwards, the water phase was extracted with 100 mL of dichloromethane (three times) and the dichloromethane phase was collected and washed with 100 mL of water three times. Drying of the dichloromethane phase was carried out by adding anhydrous sodium sulfate (Na_2SO_4) and then filtering. The dichloromethane was evaporated under reduced pressure and the 6-azide derivative of monosaccharide was obtained. The final step was to reduce the azide group to an amine functionality using triphenyl phosphine (PPh_3).[25] The aminoisopropylidene derivatives of glucose (**3**) and aminochloralose derivatives of mannose (**4**) were prepared in high yields of 70% and 75%.

3.2.1. Synthesis of 6-amino-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (3)

The aminoisopropylidene derivative of D-glucose (**3**) was prepared in 70% yield. $[\alpha]_D^{23} = -6.2$, (c 0.65, EtOH); IR cm^{-1} (KBr); 3356 (–NH_2 and –OH), 1578 (N–H). ^{1}H NMR (δ ppm, DMSO-d_6): 5.75 (d, 1H, J = 4.0 Hz, H-1), 4.35 (d, 1H, J = 4.0 Hz, H-2), 4.10 (br s, 4 H, –NH_2, –OH), 4.01 (s, 1H, H-3), 3.99 (d, 1H, J = 8.0 Hz ,

H-4), 3.74 (dd, 1H, J = 12.6, 8.0 Hz, H-6a), 3.65 (m, 1H, H-5), 2.74 (dd, 1H, J = 12.6, 8.0 Hz, H-6b), 1.34 and 1.19 (2s, CH_3-isopropylidene); ^{13}C NMR: 110.8, 104.8 ($C_{isopropylidene}$, C-1), 85.1, 82.3, 73.5 (C-2, C-3, C-4), 68.2 (C-5), 46.0 (C-6), 27.1, 26.5 (Me group).

Anal. Calc. for $C_9H_{17}NO_5$: C, 49.31; H, 7.82; N, 6.39. Found: C, 49.25; H, 7.65; N, 6.49.

3.2.2. Synthesis of 6-amino-6-deoxy-1,2-*O*-(*R*)-trichloroethylidene- β-D-mannofuranose (4)

The aminochloralose derivative of D-mannose (**4**) was prepared in 75% yield. $[\alpha]_D^{23}$ = + 11.7, (c 0.60, EtOH). IR cm^{-1} (KBr); 3366 (–NH_2 and –OH), 1594 (N–H). ^{1}H NMR (δ ppm, DMSO-d_6,): 6.17 (d, J = 3.6 Hz, 1 H, H-1), 5.72 (s, 1 H, HC-CCl_3), 5.00 (d, J = 3.6 Hz, 1 H, H-2), 4.66 (s, 1 H, H-3), 4.28 (m, 1 H, H-4), 3.62 (m, 1 H, H-5), 3.15, 2.78 (dd, J = 16.0, 4.0 Hz, 2 H, H-6), 2.30 (br s, 4 H, –NH_2, –OH); ^{13}C NMR: 109.3, 108.5 (HC-CCl_3, C-1), 99.6 (HC-$C$$Cl_3$), 90.8, 90.3, 83.2 ($C$-2, C-3, C-4), 71.7 (C-5), 49.0 (C-6).

Anal. Calc. for $C_8H_{12}Cl_3NO_5$: C, 31.14; H, 3.92; N, 4.54. Found: C, 31.45; H, 3.95; N, 4.49.

3.3. General procedure for the synthesis of the ONO-tridentate chiral Schiff bases (3a–4a, 3b–4b)

Ethanol (5 mL) solution of aldehyde (salicylaldehyde or 3,5-ditbutylsalicylaldehyde) (10 mmol) was added dropwise into an ethanol (5 mL) solution of the appropriate amino sugar derivative (10 mmol). After stirring for 3 h at room temperature, the solvent was evaporated and the remaining residue was crystallized from diethylether and light petroleum ether to afford yellow crystals in yields of 70%–87%.

3.3.1. Synthesis of 6-deoxy-1,2-*O*-(*S*)-isopropylidene-6-[(2'-ylimino)methyl] phenol-α-D-glucofuranose (3a)

The title product was prepared in 76% yield. mp = 79–81 °C, $[\alpha]_D^{22}$ = –12.7 (c 0.5, CH_2Cl_2); IR (KBr), 3390, 2936, 1634, 1582, 1497, 1462, 1280, 1160, 1060, 828, 805, 757 cm^{-1}. UV/vis (EtOH) λmax (nm): 280, 338, 375. ^{1}H NMR ($CDCl_3$, δ ppm) 8.37 (s, 1H, –CH=N–), 7.33 (m, 1H, Ar-H), 7.25 (dd, J = 12.0 Hz, 4.0 Hz, 1H, Ar-H), 6.93 (d, J = 8 Hz, 1H, Ar-H), 6.84 (m, 1H, Ar-H), 5.96 (d, 1H, J = 4.0 Hz, H-1), 4.55 (d, 1H, J = 4.0 Hz, H-2), 4.40 (d, J = 3.0 Hz, 1H, H-3), 4.32 (m, 1H, H-5), 4.11 (dd, J = 9.0 Hz, 3.0 Hz, H-4), 4.00 (dd, 1H, H-6a), 3.73 (dd, 1H, J = 12.0 Hz, 8.0 Hz, H-6b), 1.47 and 1.30 (2s, CH_3– isopropylidene); ^{13}C NMR: 168.4, 164.7, 135.4, 133.2, 119.9, 118.7, 116.9, 109.9, 105.3, 85.9, 82.5, 74.1, 68.7, 48.6, 27.1, 23.2.

Anal. Calcd for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.64; H, 6.70; N, 4.21.

3.3.2. Synthesis of 6-deoxy-1,2-*O*-(*S*)-isopropylidene-6-[2',4'-ter-butyl-(6'-ylimino) methyl]phenol-α-D-glucofuranose (3b)

The title product was prepared in 70% yield. mp = 59–61 °C, $[\alpha]_D^{22}$ = +10.8 (c 0.50, CH_2Cl_2); IR (KBr), 3412, 2958, 1634, 1470, 1442, 1273, 1162, 1011, 828, 855, 749 cm^{-1}. UV/vis (EtOH) λmax (nm): 282, 335, 376. ^{1}H NMR ($CDCl_3$, δ ppm) 8.39 (s, 1H, –CH=N–), 7.38 (d, J = 4 Hz, 1H, Ar-H), 7.21 (m, 1H, Ar-H), 6.06 (d, 1H, J = 3.6 Hz, H-1), 4.60 (d, 1H, J = 3.6 Hz, H-2), 4.32 (d, 1H, J = 4.0, H-3), 4.21 (m, 1H, H-5), 4.06 (d, 1H, H-3), 3.99 (dd, 1H, J = 12.0 Hz, 8.0 Hz, H-6a), 3.75 (d, 1H, H-6b), 1.45 and 1.28 (s, 24H, CH_3); ^{13}C NMR: 167.3, 159.1, 137.6, 132.9, 130.7, 118.3, 117.8, 107.5, 105.2, 87.4, 82.4, 76.1, 69.4, 62.5, 38.3, 36.7, 35.1, 34.3, 31.7, 31.0, 29.4, 29.2, 23.1, 20.9.

Anal. Calcd for $C_{24}H_{37}NO_6$: C, 66.18; H, 8.56; N, 3.22. Found: C, 66.27; H, 8.69; N, 3.28.

3.3.3. 6-Deoxy-1,2-*O*-(*S*)-trichloroethylidene-6-[(2'-ylimino)methyl]phenol-β-D-mannofuranose (4a)

The title product was prepared in 87% yield. mp = 97–99 °C, $[\alpha]_D^{22}$ = +17.4 (c 0.40, CH_2Cl_2); IR (KBr), 3410, 2955, 1631, 1470, 1438, 1368, 1276, 1156, 1032, 829, 811, 751 cm^{-1}. UV/vis (EtOH) λmax (nm): 288, 340, 370. ^{1}H NMR ($CDCl_3$, δ ppm) 8.40 (s, 1H, –CH=N–), 7.64 (m, 1H, Ar-H), 7.55 (dd, J = 8.0 Hz, 6.2 Hz, 1H, Ar-H), 6.95 (d, J = 8.0 Hz, 1H, Ar-H), 6.87 (m, 1H, Ar-H), 6.09 (d, 1H, J = 3.6 Hz, H-1), 5.77 (s, 1H, $HCCl_3$), 4.92 (d, J = 3.6 Hz, 1H, H-2), 4.51 (d, 1H, J = 4.0 Hz , H-3), 4.30 (d, J = 2.8 Hz, H-4), 4.27 (m, 1H, H-5), 3.87 (dd, , J = 12.6, 8.0 Hz, 1H, H-6b), 3.68 (d, 1H, J = 12.6, 8.0 Hz, H-6a); ^{13}C NMR: 167.5, 161.6, 132.6, 132.1, 119.1, 118.6, 117.0, 109.4, 105.1, 99.5, 83.2, 82.8, 69.6, 67.7, 62.2.

Anal. Calcd for $C_{15}H_{16}Cl_3NO_6$: C, 43.66; H, 3.91; N, 3.39. Found: C, 43.87; H, 3.83; N, 3.45.

3.3.4. 6-Deoxy-1,2-*O*-(*S*)-trichloroethylidene-6-[2',4'-ter-butyl-(6'-ylimino)methyl] phenol-β-D-mannofuranose (4b)

The title product was prepared in 80% yield. mp = 65–67 °C, $[\alpha]_D^{22}$ = +10.8 (c 0.50, CH_2Cl_2); IR (KBr), 3377, 2961, 1640, 1467, 1439, 1273, 1169, 1026, 828, 801, 750 cm^{-1}. UV/vis (EtOH) λmax (nm): 284, 335, 380. ^{1}H NMR ($CDCl_3$, δ ppm) 8.44 (s, 1H, –CH=N–), 7.90 (d, J = 2.0 Hz, 1H, Ar-H), 7.41 (m, 1H, Ar-H) 6.12 (d, 1H, J = 3.6 Hz, H-1), 5.76 (s, 1H, $HCCl_3$), 4.98 (d, 1H,J = 3.6 Hz, H-2), 4.48 (d,1H, J = 4.0, H-3), 4.31 (m, 1H, H-4), 4.18 (dd, 1H, J = 12.0, 8.0 Hz, H-5), 3.97 (dd, 1H, J = 12.0, 8.0 Hz, H-6a), 3.76 (d, J = 12.0 Hz, 1H, H-6b) 1.44 and 1.32 (s, 18H, CH_3); ^{13}C NMR: 167.3, 159.1, 141.6, 137.6, 131.9, 130.0, 128.3, 107.3, 105.4, 97.6, 87.4, 88.4, 76.7, 69.3, 62.3, 37.8, 36.9, 35.0, 34.2, 31.6, 31.2, 29.4, 29.2.

Anal. Calcd for $C_{23}H_{32}Cl_3NO_6$: C, 52.63; H, 6.15; N, 2.67. Found: C, 52.78; H, 6.31; N, 2.75.

3.4. Antimicrobial activities of the compounds (3a–6a and 3b–6b)

The in vitro antimicrobial activity of the synthesized compounds (**3a**–**6a** and **3b**–**6b**) was studied against the following bacterial strains of gram-positive organisms: *Staphylococcus aureus* ATCC6538-P, *Bacillus subtilis* ATCC 6633, and *Enterococcus faecalis* ATCC 29212, and gram-negative organisms: *Escherichia coli* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, and *Klebsiella pneumoniae* CCM 2318 and unicellular yeast *Candida albicans* ATCC 10239, by microdilution method. Briefly, all compounds were dissolved in 10% dimethylsulfoxide (DMSO). Then serial dilutions of each sample were performed in 10% DMSO. Inocula for assays were prepared from activated cultures in Mueller-Hinton broth (MHB) by dilution to give a final viable cell count of 4.0–5.5 $\times$ 10^5 CFU/mL. Each sample solution (25 μL) and inoculum of test microorganism (25 μL) were added into each well of a flat-bottom, 96-well microtiter plate prefilled with 200 μL of MHB to give a total volume of 250 μL. Microtiter plates were incubated at 37 °C for 24 h for bacteria and 48–72 h for *C. albicans*. The solvent, 10% DMSO, was used as the negative control for all experiments. After incubation, the MIC value was detected by adding 50 μL of 0.5% triphenyltetrazolium chloride (TTC) aqueous solution.[38,39] MIC was defined as the lowest concentration of extract that inhibited visible growth as indicated by the TTC reduction. In the presence of bacterial growth by reduction reactions, TTC changes the color of microbial cells from colorless to red. This provides clearly defined and easily readable endpoints. All tests were repeated three times to confirm the results.

4. Conclusions

Our studies results are in line with the literature although there are very limited studies on antimicrobial activities of similar compounds. For example, it was reported that the Schiff base derivatives of D-glucose amine showed biological activity when evaluated for antimicrobial activity against gram-positive and gram-negative bacterial and fungi strains.[36] In our studies, four new chiral Schiff base ligands (**3a**–**4a**, **3b**–**4b**) were prepared from aminoisopropylidene derivatives of glucose (**3**) and aminochloralose derivatives of mannose (**4**). The chiral Schiff base ligands containing the aminofuranose moiety from glucose, mannose, and galactose showed a range of antimicrobial activities.

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