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Note

Synthesis and antimicrobial activities of two novel amino sugars derived from chloraloses

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ABSTRACT

The synthesis of 5-amino-5-deoxy-1,2-*O*-(*S*)-trichloroethylidene-β-L-arabinofuranose and 6-amino-6-deoxy-1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose is described by a simple three- or four-step route. Antibacterial potency of the new compounds was determined using an inhibition zone diameter test. The results show that these compounds have a broad-spectrum activity against Gram-positive, Gram-negative bacteria and *Candida albicans*.

Carbohydrates and their derivatives are important synthetic targets because of their wide range of functions in living organisms.[1] These derivatives bearing an amino substituent in different ring positions on the sugar skeleton are called amino sugars[2] and are known constituents of several bio-active compounds such as antibiotics[3] and biopolymers.[4] On the cell surface, amino sugars play a key role as receptors for proteins and enzymes,[5] and they have been shown to interact with either RNA or the backbone phosphate of DNA.[6]

All this explains that certain series of amino sugars are regarded as major target molecules for many years, owing to their biological and medicinal importance.[7]

Acetals and ketals are common protecting groups for diols, and find numerous applications in sugar chemistry. Trichloroethylidene acetal rings, known as chloraloses, can be obtained via the reaction of sugars, preferably in their furanose form, with chloral. These acetals have not been widely used as protecting groups in carbohydrate chemistry,[8] notwithstanding they present some real advantages for further modifications of the sugar compounds. First, trichloroethylidene acetals are highly crystalline compounds and very stable under acidic and mildly basic conditions. Next, these acetals can be easily deprotected by hydrogenation on Raney nickel, followed by acidic hydrolysis.[9] It is also known that trichloroethylidene acetals have different activities in biological systems; for instance 1,2-*O*-(*R*)-trichloroethylidene-α-D-glucofuranose, namely α-chloralose, has found applications as a surgical anesthetic, sedative, and as hypnotic drug.[11] Also, 1,2-*O*-(*S*)-trichloroethylidene-α-D-arabinofuranose, namely arabinochloralose, has been used for the development of new antituberculosis drugs.[12] In this paper, we report the synthesis of novel amino sugar derivatives based on 1,2-*O*-(*S*)-trichloroethylidene-β-L-arabinofuranose and 1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose.

The synthesis of amino sugars and their derivatives is well documented in the literature[13] but there are no examples of the family of chloraloses and their amino derivatives. We first aimed at a simple synthesis of the 5- or 6-amino derivatives of 1,2-*O*-(*S*)-trichloroethylidene-β-L-arabinofuranose (β-L-arabinochloralose) and 1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose (β-chloralose) from their intermediate mono-azides, obtained in relatively good yields. These target molecules are available from a simple sequence involving protection of primary hydroxyl groups, nucleophilic substitution and reduction reactions. Under the action of catalytic concd H_2SO_4, D-arabinose reacts with chloral to yield the 1,2-*O*-trichloroethylidene acetal of D-arabinofuranose as described by Makinabakan and co-workers.[14] β-L-Arabinochloralose **1** was synthesized by using this method.[8a] 1,2-*O*-(*S*)-Trichloroethylidene-α-D-arabinofuranose (α-D-arabinochloralose), reported earlier in the literature[14] was also re-synthesized by us in order to make a comparison and determination of the acetal carbon configuration of compound **1**. The acetal carbon signals of β-L- and α-D-arabinochloraloses resonate at relatively lower fields such as 5.67 and 5.76 ppm in DMSO-d_6, respectively, when compared with the diastereoisomeric analogues of 1,2-*O*-trichloroethylidene derivatives

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R configuration of acetal carbons, which are in the range of δ 5.30–5.50. Based on this strong evidence, and the relatively low field of acetal carbon signal (δ 5.50–6.00), we can confidently assume that the acetal carbon configuration of **1** is (*S*)[8a,9,10] (Scheme 1). Initially, compound **1** was acetylated with acetic anhydride in pyridine to get its diacetylated form (**2**) that has chemical shift values at relatively low fields as expected from *O*-acetyl substitution.

Compound **1** was next reacted with *p*-toluenesulfonyl chloride in pyridine and the resulting derivative was treated with sodium azide to afford the corresponding 5-azido-5-deoxy-1,2-(*S*)-*O*-trichloroethylidene-β-L-arabinofuranose **4**, in 65% yield. The reduction of **4** was more troublesome. Neither $LiAlH_4$ nor $NaBH_4$ can be used for the reduction of such sugars: the trichloroethylidene acetal ring turns into a ketene acetal due to the elimination of HCl.[14a] Milder reduction methods such as Staudinger[15] or catalytic Zn/NH_4Cl in acidic media[16] proved inefficient in the case of this derivative. Finally, using dry methanol instead of the tetrahydrofuran–water (4:1) generally employed in Staudinger's method, turned out to be the best route.[17] Thus, refluxing compound **4** with 1.5 equiv triphenylphosphine in methanol for 2 h afforded the expected amino derivative of β-L-arabinochloralose **5**.[18] The IR spectrum of **5** exhibits the characteristic amine peaks at 3366 and 3272 cm^{-1} as a double shoulder for both $-NH_2$ and –OH groups. Also, the N–H bending vibration of the primary amine is observed at 1590 cm^{-1}. Complementarily, a broad singlet at 2.37 ppm on the ^1H NMR spectrum is assigned to the $-NH_2$ and –OH groups. As expected, the acetylation of **5** led to the corresponding diacetylated compound **6**.

A comparable sequence was applied to the commercially available β-chloralose (**7**). Thus, the tosylation of the primary hydroxyl group, its transformation into the corresponding azide derivative **8** and reduction of the azido group led to 6-amino-6-deoxy-1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose (**10**). The IR spectrum of **10** exhibits the characteristic amine peaks at 3467 and 3437 cm^{-1} as a double shoulder while the –OH was observed as a broad band at 3200 cm^{-1}. Also, the N–H bending vibration of the primary amine group is observed at 1590 cm^{-1} similar to compound **5**. Complementarily, a broad singlet at 4.91 ppm on the ^1H NMR spectrum is assigned to the $-NH_2$ group plus the two –OH at positions 3 and 5. Finally, treating **10** in excess acetic anhydride led to the expected triacetylated derivative **11**.

Determination of the antimicrobial activities of the new compounds was carried out in vitro by the agar well diffusion method[19] against test microorganisms stated in Table 1. The antimicrobial activity was evaluated by measuring the inhibition zone diameter observed. In addition, commercial antibiotics such as nalidixic acid (30 μg), chloramphenicol (30 μg), and nystatin (10 μg) were used as positive controls to determine the sensitivity of the strains. It is obvious that our synthesized compounds showed significant activity against the tested microorganisms with inhibition zones ranging from 12 to 24 mm. However, the compounds differ significantly in their activity against test microorganisms. The most active compound was **5** that showed broad-spectrum antimicrobial activity against *Escherichia coli*, *Bacillus cereus*, *Enterococcus faecalis*, and *Candida albicans* 22, 22, 24, and 14 mm, respectively (Table 1).

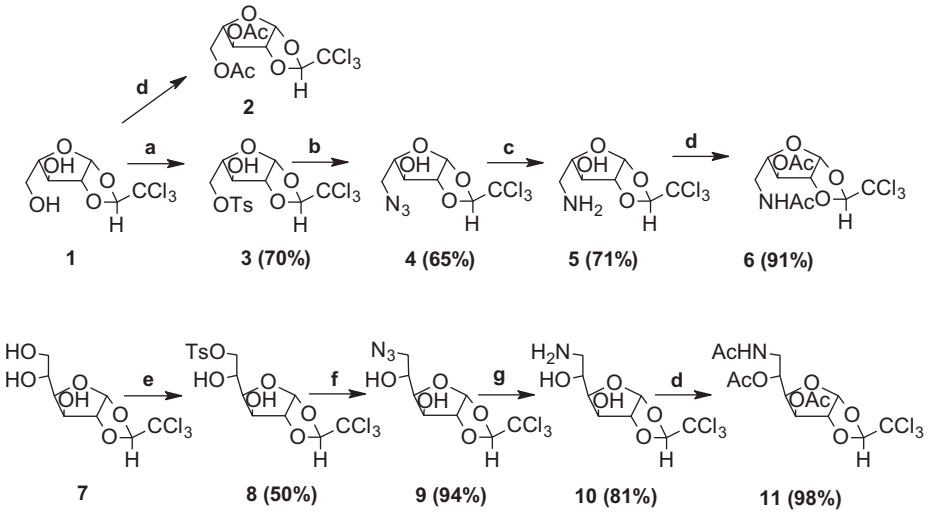
Significant antimicrobial effects, expressed as MIC of tested compounds against selected microorganisms are presented in Table 2. MIC values of the compounds ranging from 187 to 6670 μg/mL. Compound **5** showed very strong activity against *E. feacalis* with the best MIC (187 μg/mL). The lowest MIC for *Staphylococcus aureus* was obtained with **5** and was 1458 μg/mL, whereas the highest MIC was 6670 μg/mL for *C. albicans* by the compound **10**. MIC values for *E. coli* from **5** and **10** were 2083 and 4167 μg/mL, respectively. The results of the present investigation clearly indicate that the compounds of β-L-arabinochloralose and β-chloralose with amino groups are also important, since they are found in some antimicrobial active molecules.

In conclusion, we have achieved the synthesis of novel amino sugars (**5** and **10**) derived from 1,2-*O*-trichloroethylidene furanoses in good yields and purities. Their antimicrobial activities were studied against various microorganisms. Both amino sugars of β-L-arabinochloralose and β-chloralose exhibit moderate antimicrobial effects. The synthesis and antimicrobial activity of other stereoisomers of trichloroethylidene acetals of sugars are currently being investigated in our laboratories.

1. Experimental

1.1. General methods

Melting points were measured by using Electrothermal 9100 melting point apparatus in capillary and uncorrected. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian AS 400 NMR spectrometer. APCI positive polarity (70 eV) mass spectra were recorded on Agilent 1100 (LC–MSD) mass spectrometer. IR spectra were recorded on Perkin–Elmer Spectrum 100 FTIR



Scheme 1. Synthesis of aminosugar derivatives of β-L-arabinochloralose and β-chloralose, respectively (**5** and **10**). Reagents and conditions: (a) *p*-TsCl (1.5 equiv)/Py, in ice bath, 8 h; (b) NaN_3 (5.0 equiv)/DMF, 120 °C, 3 h; (c) PPh_3 (1.5 equiv)/MeOH, reflux, 2 h; (d) Ac_2O/Py, rt, overnight; (e) *p*-TsCl (1.2 equiv)/Py, in ice bath than at rt, 48 h; (f) NaN_3 (5.0 equiv)/DMF, 100 °C, 4.5 h; (g) PPh_3 (3 equiv)/MeOH, reflux, 1.5 h.

Table 1
Antimicrobial activity of synthesized compounds against test microorganisms

Compounds	Microorganisms[a]										
	SA[d]	EC	KR	BC	BS	STYP	PV	EF	EA	ECLO	CA
5	18	22	20	22	18	16	16	24	20	22	14
10	18[b]	12	22	16	13	10	16	20	22	14	12
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
NYS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22
NC (EtOH)	11	0	10	$8^{\pm}$	0	$10^{\pm}$	$12^{\pm}$	0	0	0	$13^{\pm}$

[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] Applied compounds dose, 1200 μg/well; standard antibiotics; NA—nalidixic acid (30 μg/disc); CHL—chloramphenicol (30 μg/disc); 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 (ethanol, 60 μL), ±, partially inhibition.

Table 2
MIC values of the tested compounds and standard antibiotics against selected microorganisms

MO[a]	Tested compounds and standard antibiotics (μg/mL)				
	5	10	NEO[b]	GE	NYS
SA	1458 ± 510[c] (1250–2500)	2917 ± 1021 (2500–5000)	27.08 ± 9.88 (18.75–37.5)	3.907 ± 1.172 (2.344–4.688)	ND
BC	1125 ± 280 (625–1250)	2500 ± 0.00 (2500–2500)	7.292 ± 2471 (4.688–9.375)	5.730 ± 2.067 (4.688–9.375)	ND
KR	833 ± 323 (625–1250)	938 ± 342 (625–1250)	3.386 ± 1.235 (2.344–4.688)	2.865 ± 1.034 (2.344–4.688)	ND
EC	2083 ± 645 (1250–2500)	4167 ± 1291 (2500 –5000)	5.730 ± 2.067 (4.688–9.375)	6.250 ± 2.344 (4.688–9.375)	ND
EF	187 ± 70 (156–313)	438 ± 170 (313 –625)	6.250 ± 2.344 (4.688–9.375)	4.427 ± 0.781 (2.344–4.688)	ND
CA	5833 ± 2041 (5000–10,000)	6670 ± 2580 (5000–10,000)	ND	ND	0.488 ± 0.293 (0.293–1.172)

[a] MO: Microorganisms: SA—*Staphylococcus aureus*; BC—*Bacillus cereus*; EC—*Escherichia coli*; KR—*Kocuria rhizophila*; EF—*Enterobacter faecalis*; CA—*Candida albicans*.
[b] Standard antibiotics: NEO—neomycin; GE—gentamycin; NYS—nystatin. ND—not determined.
[c] Minimum and maximum values are shown in parentheses. Data presented as the mean value of six determinations ± standard deviation.

Spectrometer. Optical rotation measurements were carried out on an Autopol II polarimeter. Elemental analyses were carried out on LECO CHNS-932 elemental analyzer. TLC and column chromatography were performed on precoated aluminum plates (Merck 5554) and Silica Gel G-60 (Merck 7734), respectively. The chromatograms were detected with 5% aqueous sulfuric acid by heating the plates above 120 °C for about 3 min. All solvent removals were carried out under reduced pressure with rotary evaporator. L-Arabinose, β-chloralose, and chloral hydrate were purchased from Sigma–Aldrich Chem. Co. and β-chloralose was purified by crystallization from boiling MeOH. *p*-Toluenesulfonyl chloride, triphenylphosphine, and all solvents were purchased from Merck Co.

1.1.1. 1,2-*O*-(*S*)-Trichloroethylidene-β-L-arabinofuranose (1)

Compound **1** was prepared according to literature while using dried L-arabinose (50.0 g, 0.33 mol) as a starting material.[8a,14] The pure product was obtained as colorless crystals (39.6 g, 43%), mp 191–193 °C; $[\alpha]_D^{21}$ +22.55 (*c* 1.22, MeOH); IR cm^{-1} (KBr); 3417 (–OH), 814 745 (CCl_3). ^1H NMR δ (DMSO-d_6); 6.17 (d, 1H, $J_{1,2}$ 4.0, H-1), 5.67 (s, 1H, H-acetal), 4.84 (br s, 1H, H-3), 4.72 (d, 1H, H-2), 3.91 (t, 1H, $J_{4,5a}$ 6.8 Hz, $J_{5a,5b}$ 6.8 Hz, H-5a), 3.40 (m, 2H, H-4, H-5b); ^{13}C NMR; 108.8, 107.7 (HC–CCl_3 and C-1), 100.4 (HC–CCl_3), 89.9, 89.5, 75.0, 61.9 (C-2, C-3, C-4, C-5).

Anal. Calcd for $C_7H_9Cl_3O_5$: C, 30.08; H, 3.25. Found: C, 29.93; H, 3.17.

1.1.2. 3,5-Di-*O*-acetyl-(*S*)-trichloroethylidene-β-L-arabinofuranose (2)

Acetylation of compound **1** (1.04 g, 0.0038 mol) in pyridine (10 mL) with Ac_2O (3.0 mL) at room temperature afforded the syrupy diacetate **2** (1.30 g, 95%), ^1H NMR δ (DMSO-d_6): 6.30 (d, 1H, $J_{1,2}$ 4.0, H-1), 5.89 (s, 1H, H-acetal), 5.09 (br s, 1H, H-3), 5.03 (d, 1H, H-2), 4.32 (t, 1H, $J_{4,5a}$ 6.4 Hz, $J_{5a,5b}$ 6.8 Hz, H-5a), 4.16 (m, 2H, H-4, H-5b), 2.05 (s, 2H, 2 × CH_3); ^{13}C NMR: 170.6, 170.2 (2 × $OCOCH_3$) 109.1, 107.8 (HC–CCl_3 and C-1), 99.9 (HC–CCl_3), 86.7, 83.8, 77.2, 63.5 (C-2, C-3, C-4, C-5), 21.1, 21.0 (2 × CH_3).

1.1.3. 5-*O*-Tosyl-1,2-*O*-(*S*)-trichloroethylidene-β-L-arabinofuranose (3)

A solution of **1** (22.52 g, 0.0806 mol) in pyridine (100 mL) was cooled with ice bath and *p*-toluenesulfonyl chloride (18.43 g, 0.0967 mol) in pyridine (40 mL) was added dropwise and stirred for 8 h. TLC (toluene–MeOH, 9:1) showed that a very small amount of starting sugar remained. The reaction mixture was concentrated to half volume and poured into ice-water (600 mL). Then, it was extracted with CH_2Cl_2 (3 × 300 mL). Organic phase was washed with water and dried over anhydrous Na_2SO_4, filtered off, evaporated under reducing pressure. The crude syrupy product was purified by column chromatography (CH_2Cl_2–MeOH, 100:1) giving **3** (24.47 g, 70%), as a white solid, mp 182–184 °C, $[\alpha]_D^{24}$ −30.0 (*c* 0.4, MeOH); IR cm^{-1} (KBr); 3450 (–OH), 2854–3092 (aliphatic and aromatic C–H). ^1H NMR δ (DMSO-d_6): 7.83 (d, 2H, benzene, *J* 8.4 Hz), 7.50 (d, 2H, benzene), 6.24 (d, 1H, $J_{1,2}$ 4.0, H-1), 5.42 (s, 1H, H-acetal), 4.73 (d, 1H, H-2), 5.75 (d, 1H, $J_{3,4}$ 4.4 Hz, H-3), 4.15 (m, 1H, H-4), 4.15 (m, 1H, H-5a), 3.94 (dd, 1H, $J_{5a,5b}$ 10.2, $J_{4,5b}$ 7.2 Hz, H-5b), 2.42 (s, 3H, CH_3); ^{13}C NMR: 145.9, 132.5, 130.9, 128.5 (6 × C, benzene), 108.7, 107.9 (HC–CCl_3 and C-1), 100.1 (HC–CCl_3), 88.9, 85.9, 74.9, 70.1 (C-2, C-3, C-4, C-5), 21.9 (CH_3).

Anal. Calcd for $C_{14}H_{15}Cl_3O_7S$: C, 38.77; H, 3.49; S, 7.39. Found: C, 39.04; H, 3.45; S, 7.28.

1.1.4. 5-Azido-5-deoxy-1,2-*O*-(*S*)-trichloroethylidene-β-L-arabinofuranose (4)

To a solution of **3** (20.53 g, 0.0473 mol) in DMF (100 mL) was added NaN_3 (15.37 g, 0.2365 mol). The mixture was heated with stirring in an oil bath at 120 °C for 3 h. TLC (toluene–MeOH, 9:1) showed completion of the reaction. The reaction mixture was poured into ice-water (500 mL). The solid product was filtered off and dried at room temperature. The product was crystallized from MeOH–H_2O to get the pure compound **4** (9.36 g, 65%), mp 124–126 °C; $[\alpha]_D^{21}$ +40.0 (*c* 0.4, MeOH); IR cm^{-1} (KBr); 3474 (–OH), 2852–3008 (aliphatic and aromatic C–H), 2114 (–N_3). ^1H NMR δ (DMSO-d_6): 6.27 (d, 1H, $J_{1,2}$ 3.6, H-1), 5.97 (d, 1H, H-2), 5.68 (s, 1H, H-acetal), 4.77 (d, 1H, $J_{3,4}$ 4.4 Hz, H-3), 4.06 (m, 1H, H-4), 3.92 (dd, 1H, $J_{4,5a}$ 9.2 Hz, H-5a), 3.48 (dd, 1H, $J_{5a,5b}$ 10.2 Hz, $J_{4,5b}$ 5.2 Hz, H-5b). ^{13}C NMR: 108.8, 107.8 (H*C*–CCl_3, C-1), 100.4 (HC–*C*Cl_3), 52.1, 75.7, 87.8, 89.1 (C-2, C-3, C-4, C-5).

Anal. Calcd for $C_7H_8Cl_3N_3O_4$: C, 27.61; H, 2.65; N, 13.80. Found: C, 27.76; H, 2.62; N, 12.78.

1.1.5. 5-Amino-5-deoxy-1,2-*O*-(*S*)-trichloroethylidene-β-L-arabinofuranose (5)

To a solution of **4** (5.30 g, 0.0174 mol) in MeOH (100 mL) was added triphenylphosphine (6.85 g, 0.0261 mol). The reaction mixture was refluxed for 1.5 h. TLC (CH_2Cl_2–MeOH, 9:1) showed the completed reaction. The solvent was evaporated under reduced pressure. The syrupy product was purified by column chromatography with eluting solvent CH_2Cl_2–MeOH (100:1) to give **5** (3.44 g, 71%) as a white solid, mp 172 °C (decomp.); $[\alpha]_D^{21}$ −40.51 (*c* 1.233, CH_3OH); IR cm^{-1} (KBr); 3366–3272 (–NH_2 and –OH), 1590 (N–H); ^1H NMR δ ($CDCl_3$): 6.27 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.58 (s, 1H, H-acetal), 4.92 (d, 1H, H-2), 4.69 (t, 1H, $J_{4,5a}$ 5.2, $J_{4,5b}$ 3.2 Hz, H-4), 4.23 (s, 1H, H-3), 2.87 (dd, 1H, $J_{5a,5b}$ 7.2 Hz), H-5a), 2.85 (dd, 1H, H-5b), 2.37 (br s, 3H, –NH_2 and –OH); ^{13}C NMR: 108.7, 107.6 (H*C*–CCl_3 and C-1), 100.6 (HC–*C*Cl_3), 43.9, 75.4, 89.5, 90.5 (C-2, C-3, C-4, C-5); LC/MS-APCI: *m/z* 278 (100%, M^+), 279 (8%, M^++1), 280 (82%, M^++2).

Anal. Calcd for $C_7H_{10}Cl_3NO_4$: C, 30.18; H, 3.62; N, 5.03. Found: C, 30.39; H, 3.74; N, 4.68.

1.1.6. 5-Acetamido-5-deoxy-3-*O*-acetyl-1,2-*O*-(*S*)-trichloroethylidene-β-L-arabinofuranose (6)

A solution of **5** (1.01 g, 0.0036 mol) in pyridine (20 mL) was acetylated with Ac_2O (1.1 mL, 0.0108 mol) at room temperature for 24 h. The usual work-up procedure and purification on a silica gel column eluting with CH_2Cl_2–MeOH (100:1) gave the white solid **6** (1.31 g, 91%), mp 128–130 °C; ^1H NMR δ (DMSO-d_6): 8.06 (t, 1H, –N*H*C(O)CH_3), 6.22 (d, 1H, H-1), 5.82 (s, 1H, H-acetal), 4.71 (d, 1H, $J_{1,2}$ 3.6 Hz, H-2), 4.12 (s, 1H, H-3), 3.94 (t, 1H, $J_{4,5a}$ 6.8, $J_{4,5b}$ 5.6 Hz, H-4), 3.19 (dd, 1H, $J_{5a,5b}$ 13.0 Hz, H-5a), 3.14 (dd, 1H, H-5b), 3.16 (s, 6H, –OC(O)*CH*$_3$, –NHC(O)*CH*$_3$).

1.1.7. 6-*O*-Tosyl-1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose (8)

β-Chloralose (**7**), commercially available, (17.62 g, 0.0569 mol) was reacted with *p*-toluenesulfonyl chloride for 48 h as described in Section 1.1.3. Compound **8** (13.19 g, 50%) was obtained as a white solid, mp 145–147 °C, $[\alpha]_D^{21}$ −20.0 (*c* 0.4, MeOH); IR cm^{-1} (KBr); 3507 (–OH), 1598 (–SO_2–); ^1H NMR δ (DMSO-d_6): 7.81 (d, 2H, benzene, *J* 8.4 Hz), 7.48 (d, 2H, benzene), 6.16 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.81 (s, 1H, H-acetal), 4.70 (d, 1H, H-2), 4.15 (m, 1H, H-3), 4.15 (m, 1H, H-4), 3.90 (m, 1H, H-5), 3.94 (d, 1H, $J_{6a,6b}$ 7.2 Hz, H-6a), 3.87 (d, 1H, H-6b), 2.36 (s,3H, C*H*$_3$); ^{13}C NMR: 145.5, 132.9, 130.8, 128.3 (6 × C, benzene), 108.7, 106.7 (HC–CCl_3 and C-1), 100.5 (HC–*C*Cl_3), 87.6, 81.3, 73.7, 73.3, 65.7 (C-2, C-3, C-4, C-5, C-6), 21.8 (CH_3).

Anal. Calcd for $C_{15}H_{17}Cl_3O_8S$: C, 38.85; H, 3.70; S, 6.92. Found: C, 38.75; H, 3.63; S, 6.75.

1.1.8. 6-Azido-6-deoxy-1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose (9)

A solution of compound **8** (10.76 g, 0.0232 mol) in DMF was reacted with NaN_3 for 4.5 h at 100 °C as described in Section 1.1.4. Compound **9** (7.30 g, 94%) was obtained as a white solid, mp 165–168 °C, $[\alpha]_D^{21}$ −5.0 (*c* 0.4, MeOH); IR cm^{-1} (KBr); 3478–3399 (–OH), 2124 (–N_3); ^1H NMR δ (DMSO-d_6): 6.18 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.84 (s, 1H, H-acetal), 3.90 (br s, 2H, 2 × OH), 4.70 (d, 1H, H-2), 5.43 (d, 1H, H-3), 5.34 (m, 1H, H-5), 4.14 (dd, 1H, H-4), 3.38 (dd, 1H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.4 Hz H-6a), 3.23 (m, 1H, H-6b); ^{13}C NMR: 108.7, 106.8 (H*C*–CCl_3, C-1), 100.6 (HC–*C*Cl_3), 87.8, 82.2, 73.4, 67.2 (C-2, C-3, C-4, C-5), 54.9 (–*C*H_2N_3).

Anal. Calcd for $C_8H_{10}Cl_3N_3O_5$: C, 28.72; H, 3.01; N, 12.56. Found: C, 28.82; H, 2.94; N, 11.87.

1.1.9. 6-Amino-6-deoxy-1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose (10)

A solution of compound **9** (5.65 g, 0.0169 mol) in MeOH was reacted with triphenylphosphine for 2 h as described in Section 1.1.5. The title product was obtained as a white solid (4.22 g, 81%) after re-crystallization in boiling water, mp 163–166 °C; $[\alpha]_D^{27}$ −5.14 (*c* 2.432, DMF); IR cm^{-1} (KBr); 3467–3437 (–NH_2), 3200 (–OH), 1590 (N–H); ^1H NMR δ (DMSO-d_6): 6.19 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.80 (s, 1H, H-acetal), 4.91 (br s, 4H, –NH_2, 2 × OH), 4.70 (d, 1H, H-2), 4.17 (br s, 1H, H-3), 3.83 (dd, 1H, H-6a), 3.72 (br s, 1H, H-4), 2.88 (dd, 1H, $J_{6a,6b}$ 11.2 Hz, H-6b); ^{13}C NMR: 108.7, 106.8 (H*C*–CCl_3, C-1), 100.5 (HC–*C*Cl_3), 87.8, 83.2, 83.0 (C-2, C-3, C-4), 68.6 (C-5), 44.5 (C-6); LC/MS-APCI: *m/z* 308 (100%, M^+), 309 (9%, M^++1), 310 (87%, M^++2).

Anal. Calcd for $C_8H_{12}Cl_3NO_5$: C, 31.15; H, 3.89; N, 4.54. Found: C, 30.90; H, 3.87; N, 3.79.

1.1.10. 6-Acetamido-6-deoxy-3,5-di-*O*-acetyl-1,2-*O*-(*S*)-trichloroethylidene-α-D-glucofuranose (11)

Compound **10** (1.04 g, 0.0034 mol) was acetylated with Ac_2O as described in Section 1.1.6. Compound **11** was obtained (1.45 g, 98%) as syrup. It was solidified at room temperature, mp 92–95 °C; ^1H NMR δ (DMSO-d_6): 7.96 (t, 1H, –N*H*C(O)CH_3), 6.33 (d, 1H, $J_{1,2}$ 3.6, H-1), 5.94 (s, 1H, H-acetal), 5.17 (d, 1H, $J_{3,4}$ 2.8 Hz, H-3), 4.95 (d, 1H, H-2), 4.93 (br s, 1H, H-4), 3.01 (m, 1H, H-5), 4.37 (dd, 1H, $J_{6a,6b}$ 8.8, $J_{5,6a}$ 2.8 Hz, H-6a), 3.65 (dd, 1H, $J_{5,6b}$ 5.6 Hz, H-6b), 1.96, 1.92, 1.76 (s, 9H, 3 × –OC(O)*CH*$_3$).

2. Microbiological procedures

2.1. Agar well diffusion assay

In vitro antimicrobial studies were carried out by the agar well diffusion method[19] against test microorganisms. Bacterial strains grown on nutrient agar (37 °C for 24 h) and *C. albicans* grown on Potato Dextrose Agar (30 °C for 48 h) were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 McFarland standards (10^6 Colony Forming units/mL). Then, 50 μL (microliters) inoculum was added to 25 mL melted Mueller Hinton Agar (MHA) for bacteria and Potato Dextrose Agar (PDA) (Oxoid, Basingstoke, UK) for *C. albicans* medium cooled at 45 °C. These were then poured into 90 mm diameter Petri dishes and maintained for 1 h at room temperature. 6 mm diameter wells were cut in the agar plate and 60 μL of compound (1200 μg/well) and pure extraction solvent as a negative control (ethanol, 60 μL) were loaded individually in the wells. The dishes were preincubated at 4 °C for 2 h to uniform diffusion into the agar. After preincubation, the plates were

incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for yeast. The antimicrobial activity was determined by measuring the inhibition zone diameter around the wells. In addition, commercial antibiotics such as nalidixic acid (30 μg), chloramphenicol (30 μg), and nystatin (10 μg) were used as positive control to determine the sensitivity of the strains. Experiments were repeated three times, and the results were expressed as average values.

2.2. Determination of minimum inhibition concentration (MIC)

The microtiter broth dilution technique was performed by using the CLSI standards.[20,21] A sterile 96 round-bottomed well plate was labeled. A volume of 100 μL of antimicrobial compound solution was pipetted into the first row of the plate. To all other wells 50 μL of double strength Mueller Hinton broth or Potatoes Dextrose broth was added. Serial dilutions were performed using a micropipette (A1–A10). Tips were discarded after use such that each well had 50 μL of the test material in serially descending concentrations. Then, 50 μL of broth containing bacterial suspension (5×10^6 cfu/mL) or yeast (5×10^5 cfu/mL) was added to each well. Each column of wells contained a single antimicrobial agent in progressive dilutions and was inoculated with a single microorganism. Plates were sealed with clean film to ensure that microorganisms did not become dehydrated. Positive and negative controls were present on each microtiter plate. The plates were prepared in triplicate, and placed in an incubator set at 37 °C for 18–24 h for bacteria and 30 °C for 24–36 h for *C. albicans*, respectively. After incubation, was added 12 μL of 0.18% TTC (2,3-5-triphenyl tetrazolium chloride) solution to each well of microtitre plate and incubated at 37 °C for 1 h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. The average of six values was calculated and that was the MIC for the test material and microorganisms.

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