A convenient new synthesis, characterization and antibacterial activity of double headed acyclo-C-nucleosides from unprotected D-glucose

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Abstract Double headed acyclo-C-nucleosides, 1,4-bis(3-mercapto-1H-1,2,4-triazol-5-yl)butane-1,2,3,4-tetrol (6), 5,5’-(1,2,3,4-tetrahydroxybutane-1,4-diyl)bis(1,3,4-oxadiazole-2(3H)-thione) (7), and 1,4-bis(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)butane-1,2,3,4-tetrol (8) have been synthesized from D-glucose (1) without protecting hydroxyl groups. Two steps synthesis leading to formation of 2,3,4,5-tetrahydroxyhexanedihydrazide (4) which is regarded as starting intermediates for the synthesis of 6,7 and 8. Synthetic intermediates and final products were appropriately characterized by IR, 1H NMR and 13C NMR. The products were tested in vitro against gram positive bacteria Staphylococcus aureus, Listeria innocua and gram negative bacteria Klebsiella pneumoniae, Salmonella sp., Escherichia coli and compared with the known antibiotic: amoxicillin + clavulanic acid (AMC) and showed variable effects.

1. Introduction

The modern structural features of nucleosides are somewhat different from those present in poly-ribonucleotides RNA and DNA (see Fig. 1), by means of variations in sugar, heterocyclic moieties and the various modes of attachments between the two major components (sugar and heterocycle) (Watson and Crick, 1954).

The cyclic sugar residues have shown a lot of variations such as the ring size (Nakamura et al., 1986; Furuta et al., 1979; Singha et al., 2003) and oxygen’s ring has been replaced by N, Se, CH2 and other elements (Evans et al., 2003; Prichard et al., 2009; Brown et al., 2001). Moreover the open chain sugars are now appearing quite often in the literature (Poonian and Nowoswiat, 1977; Yosuzawa et al., 1987; Tsuchiya et al., 1995) since the discovery of acyclovir (Schaeffer et al., 1978) as acyclic (Rashad et al., 2005; Rybak et al., 2000) and seco-acyclo-nucleosides (Ashry and Kilany, 1998) to expand the scope of the terms: nucleosides and nucleosides analogues may be schematically presented in Fig. 2.
In this communication we would like to present a convenient method for the synthesis of double headed nucleoside using the spontaneous lactonization reaction as a step protecting method within the synthetic pathway.

2. Experimental

2.1. General

All reactions were monitored by TLC analysis (silica gel for TLC supplied by MERIC), iodine was used for visualization. The melting points were measured with a BUCHI 540 melting point apparatus and are uncorrected. The IR spectra exhibited as wave number (ν cm⁻¹) were recorded using KBr discs in a JASCO V-530 spectrophotometer at the University of Oran, Es-Senia, Algeria. The ¹H NMR and ¹³C NMR (250 MHz) spectra in DMSO-d₆ exhibited as ppm, were recorded at the University of Oran, Es-Senia (Algeria). Microorganisms in this study were supplied by the university hospital of Oran and identified by the laboratory of applied microbiology, University of Oran Es-Senia (Algeria). The Mueller Hinton medium was supplied by Difco.

2.2. Chemical synthesis

2.2.1. D-Gluaro-1,5:6,3-dilactone (3)

Fuming nitric acid (31.0 mL) was heated to 55–60 °C and D-Glucose (I) (10.0 g, 55.5 mmol) was added in small batches over a period of 10 min with an aid of magnetic stirring. A vigorous exothermic reaction started taking place. The reaction vessel was immerged into an ice-bath until vigorous reaction seized. The reaction mixture then heated to 55–60 °C for 90 min. After cooling, the excess of nitric acid was removed under vacuum and ether was added to the remainder of the solution. A crystalline material was formed, filtered and washed with ether/dichloromethane 3:1 (v:v). The solid was recrystallized from methanol to give D-glucaro-1,5:6,3-dilactone (3). Colourless crystals, yield 45%, mp 80 °C, IR (νmax, cm⁻¹): 3490 (OH), 1783 (C=O, γ-lactone), 1747 (C=O, δ-lactone). ¹H NMR (250 MHz, DMSO-d₆), δH (d, 1H, C-5-H), 4.47 (dd, 1H, C-4–H), 4.42 (dd, 1H, C-3–H), 4.32 (d, 1H, C-2–H), 4.25 (dd, 1H, C-4–H), 4.21 (s, 1H, OH), 4.18 (s, 1H, OH). ¹³C NMR (250 MHz, DMSO-d₆), δC 176.42 (C-6), 172.18 (C-1), 80.51 (C-5), 72.49 (C-3), 70.74 (C-2), 69.86 (C-4).

In this communication we would like to present a convenient method for the synthesis of double headed nucleoside using the spontaneous lactonization reaction as a step protecting method within the synthetic pathway.
2.2.2. 2,3,4,5-Tetrahydroxyhexanedihydrazide (4)
The dilactone (3) (1.0 g, 5.74 mmol) was dissolved in ethanol (40.0 mL). Hydrazine hydrate 64\% (5.0 mL) was added dropwise, a precipitate was formed immediately. The colloidal mixture was agitated for further 30 min. The precipitate was filtered, washed with an iced cooled ethanol and recrystallized from water. Yellow crystals, yield 86\%, mp 72.80°C.

2.2.3. 2,2′-((2,3,4,5-Tetrahydroxy-1,6-dioxohexane-1,6-diyl)dihydrazinecarbothioamide (5)
The dihydrazide (4) (1.0 g, 4.2 mmol) was dissolved in H2O with stirring. Ammonium thiocyanate (1.40 g, 18.6 mmol) and HCl (30 mL, 37\%) were added to the mixture. The solution was refluxed on a water bath for 8 h at 80°C. Excess solvent was evaporated to almost dryness and the crystalline solid was filtered off and recrystallized from methanol to afford compound 5. Brown crystals, yield 88.6\%, mp 100°C.

2.2.4. 1,4-Bis(3-mercapto-1H-1,2,4-triazol-5-yl)butane-1,2,3,4-tetrol (6)
2.2.4.1. Method A: Synthesis in ethanol. Dihydrazinecarbothioamide (5) (1.0 g, 2.8 mmol) was dissolved in ethanol (40.0 mL) and added to solution of NaOH (0.33 g, 8.25 mmol) in H2O (40.0 mL). A precipitate was formed immediately. The colloidal mixture was agitated for further 30 min. The precipitate was filtered, and recrystallized from methanol to afford compound 6. Yellow crystals, yield 84.4\%, mp 72°C.

2.2.4.2. Method B: synthesis in water. The compound 7 (1.0 g, 3.1 mmol) dissolved in EtOH (80.0 mL) and hydrazine hydrate 64\% (5.0 mL) were heated under reflux on a water bath for 8 h at 90°C. Excess solvent was evaporated and the remaining solid/liquid mixture was filtered off and washed with ethyl acetate to afford compound 7. The product was collected and crystallized from ethyl acetate and recrystallized from methanol to afford compound 6. Yellow crystals, yield 88.0\%, mp 72°C.

Figure 3  Nucleosides with multiple fragments.
A convenient new synthesis, characterization and antibacterial activity

4.031 (d, 1H, C-1–H or C-4–H); 3.870 (t, 1H, C-2-H or C-3-H); 3.462 (d, 1H, C-1–H or C-4–H); 3.403 (dd, 1H, C-2–H or C-3-H). $^{13}$C NMR (250 MHz, DMSO-$d_6$), $\delta$ 181.598 (C-SH); 165.942 (C=–N); 142.457 (C-2 and C-3); 140.917 (C-1 and C-4).

2.3. Antibacterial susceptibility testing

A disc diffusion assay according to the standard protocols (CLSI, 2006; NCCLS, 2003, 2005) was used to determine the susceptibility of two gram positive bacteria Staphylococcus aureus ATCC 25923, Listeria innocuit ATCC 19119 and gram negative bacteria Klebsiella pneumoniae ATCC 700603, Salmonella sp., Escherichia coli ATCC 25922 and using the known antibiotic amoxicillin + clavulanic acid combination (AMC 30/10 mg) as the reference. The bacterial suspension turbidity was adjusted to 0.5 McFarland, and then the suspensions were five times in test tubes to 15, 7.50, 3.75, 1.875, and 0.94 mg mL$^{-1}$. Filter paper discs (5 mm diameter) saturated with solution of each compound (concentration 30 µg/mL) were placed on the indicated agar media. The incubation time was 24 h at 37°C. The blank test disc with DMSO was used. Inhibitory activity was evaluated by measuring the diameter of clear zone observed around the disc (in mm).

The minimum inhibition concentrations (MIC) test. Each 1 mL of the original concentration (30 µg mL$^{-1}$) in DMSO of the compounds 4, 6, 7 and 8 was diluted with DMSO for five times in test tubes to 15, 7.50, 3.75, 1.875, and 0.94 µg mL$^{-1}$. The optical density at 600 nm was measured at 0 h, 18 h, 24 h and 48 h.

3. Results and discussion

3.1. Synthesis

The double headed acyclo-C-nucleosides 6, 7 and 8 have been synthesized by a common pathway as described in scheme 1. Oxidation of d-glucose (1) by fuming nitric acid revealed the formation of d-glucaro-1,5,6,3-dilactone (3) as shown by IR spectrum. It exhibited two carbonyl absorption bands at 1783 cm$^{-1}$ attributed to five member ring lactone and another one at 1747 cm$^{-1}$ assigned to six member ring lactone (Pretch et al., 2000a,b,c, p. 265). Further support for the thione form came from $^{1}H$ NMR spectrum. For the preparation of the bis-oxadiazole (7), the dihydrazide (4) was heated with an aqueous solution of NaOH and CS$_2$ under reflux conditions followed by acidification with HCl to give a yellowish crystalline product 7 in a moderate yield 76.3%. The IR spectrum exhibited the absorptions at 1618 cm$^{-1}$ for (C=–N), 1381 cm$^{-1}$ characteristic of (C=S), and 1042 cm$^{-1}$ for (C–O–C) (Pretch et al., 2000a,b,c, p. 265). The position of the (C=–N) band suggested that the oxadiazole existed as the thione tautomer rather than the ene-thiol form. Further support for the thione form came from $^{1}H$ NMR spec-

<table>
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<tr>
<th>Compounds$^*$</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
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<tr>
<td></td>
<td>S. aureus</td>
<td>L. inovanii</td>
</tr>
<tr>
<td>AMC</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
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For: AMC (amoxicillin + clavulanic acid combination) $S \geq 18$ mm; $I = 14–17$ mm; $R \leq 13$ mm.

$^*$ Concentration 30 µg mL$^{-1}$. Abbreviations: S, susceptible; I, intermediate; R, resistant.
trum which showed a singlet at 8.91 ppm assigned to N–H proton (Pretch et al., 2000a,b,c, p. 186).

The bis-4-amino-triazole (8) was prepared in moderate yield 72.2% by heating the compound 7 with hydrazine hydrate under reflux conditions for 8 h. The IR spectrum showed the characteristic band at 3431 cm\(^{-1}\), 3315 cm\(^{-1}\), 2621 cm\(^{-1}\) and 1622 cm\(^{-1}\) for (OH), (NH), (SH) and (C\(\equiv\)N) respectively (Pretch et al., 2000a,b,c, p. 265). The 1H NMR supported the structure 8 by exhibiting four singlets between 8.87 \(-\) 8.22 ppm attributed to two NH\(_2\) groups. (OH) groups appeared as singlet at 5.64 ppm. In addition, SH signal at 4.19 ppm was also present as singlet (Pretch et al., 2000a,b,c, p. 186). 13C NMR confirmed the structure of 8 by revealing signals at 181.59 ppm attributed to (C–SH) and 165.94 ppm for (C\(\equiv\)N) (Pretch et al., 2000a,b,c, p. 104).

### 3.2. Antibacterial tests

The inhibitory effect of the compounds 3, 4, 5, 6, 7 and 8 in DMSO (10% v:v) was tested upon \textit{in vitro} against gram positive bacteria \textit{S. aureus} ATCC 25923, \textit{L. inovanii} ATCC 19119 and gram negative bacteria \textit{K. pneumoniae} ATCC 700603, \textit{Salmonella} sp., \textit{E. coli} ATCC 25922. DMSO which is known as bacterial static in the above mentioned concentration was used as negative control and standard discs (Mast Diagnostics, UK) saturated with known antibiotic: amoxicillin + clavulanic acid (AMC) as positive control were applied. After incubation at 37 °C for 24 h, the zone of inhibition of growth around each disc was measured in millimetres and zone diameters were interpreted in accordance with CLSI and NCCLS guidelines (National Commit-
The compound E. coli against the tested bacteria. The averages are shown in Table 2.

The minimum inhibitory concentrations (MIC) were determined for the active compounds 4, 6, 7, and 8 in triplicates against the tested bacteria. The averages are shown in Table 2.

The bis-triazole (6) showed an intermediate effect on K. pneumoniae.

The bis-oxadiazole (7) exhibited an intermediate activity against gram positive bacteria S. aureus and L. inovanii.

The bis-4-amino-triazol (8) has a better inhibition effect than AMC against S. aureus, a similar effect than AMC against L. inovanii, K. pneumoniae and an intermediate activity against the other bacteria tested.

The bis-triazole (6) showed an intermediate activity against K. pneumoniae.

The compound 4 has a better inhibition effect than AMC against S. aureus, a similar effect than AMC against L. inovanii, and an intermediate activity against E. coli.

The minimum inhibitory concentrations (MIC) were determined for the active compounds 4, 6, 7, and 8 in triplicates against the tested bacteria. The averages are shown in Table 2.

The bis-triazole (6) showed an intermediate activity on S. aureus and at 1.875 μg mL⁻¹ on other bacteria under test.

The bis-triazole (6) has exhibited its MIC at 1.875 μg mL⁻¹ on K. pneumoniae.

The bis-oxadiazole (7) has shown its (MIC) at 1.875 μg mL⁻¹ on the two gram positive bacteria S. aureus and L. inovanii.

The bis-4-amino-triazol (8) has exhibited its MIC at 0.94 μg mL⁻¹ on S. aureus, and at 1.875 μg mL⁻¹ on E. coli.

All the newly synthesized compounds showed moderate antibacterial activity, except dihydrazide (4) and bis-4-amino-triazol (8) which showed the highest effect at lowest concentration upon S. aureus. These findings concluded that the titled compounds have the property to kill the microbes in some extent when compared with standard drug; it gives a future scope to study the mechanism of action and would be worthy of further investigation.

4. Conclusion

In conclusion, double headed C-nucleosides from unprotected D-glucose were synthesized and their structures were determined and also assayed for their in vitro antibacterial activity. This study should extend on anti-viral, antifungal and anticancer tests because the literature gives enormously interesting results on these subjects. Also tests on other bacteria should also be included to widen the investigation.

References


Belkadi, M., Othman, A.A., 2006. A common route to the synthesis of 1,3,4-oxadiazole-2-thione and 1,2,4-triazole -3-thiols derivatives of trioses and pentoses as models for acyclic C-nucleosides. Arkivoc 183 (xi).


Demirbas, N., 2005. Synthesis and characterization of new triheterocyclic compounds consisting of 1,2,4-triazol-3-one, 1,3,4-thiadiazole and 1,3,4-oxadiazole rings. Turk. J. Chem. 29, 125.


Holla, B.S., Shivanada, M.K., Akberalli, P.M., Baliga, S.S., 1996. Studies on arylluran derivatives-Part VI. Synthesis, characterization and antibacterial activities of some 6-(5-aryl-2-furyl)-1,2,4-triazolo-[3,4-bj,1,3,4-thiadiazoles and 6-(5-nitro-2-furyl)-1,2,4-triazolo-[3,4-bj,1,3,4-thiadiazoles. Farmaco 51 (12), 785.


Kalluraya, B., Chimbalkar, R., Gunaga, P., 1996. Synthesis and biological activities of some 1,2,4-triazoles and 1,3,4-oxadiazoles. Ind. J. Heterocycl. Chem. 6, 103.


Paolo, F., Daniele, S., Franca, S., Noemi, P., 1996. 3,6-Disubstituted 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles: synthesis, antimicrobial and antiviral activity. Farmaco 51 (10), 659.


