Synthesis of some nucleosides derivatives from L-rhamnose with expected biological activity

Amira Atef Ghoneim

Abstract
Practical procedures for production of variously blocked compounds from L-rhamnose have been developed. These compounds are highly useful as indirect β-L-rhamnosyl donors. This approach represents a new method for the synthesis of aromatic nucleoside analogues and the synthesis of (3S, 4S, 5S, 6R)-3, 4, 5-triacyetoxy-2-methyl-7,9-diaza-1-oxa-spiro[4,5]decane-10-one-8-thione (7).

Introduction
Rhamnose is a component of the outer cell membrane of acid-fast bacteria in the Mycobacterium genus, which includes the organism that causes tuberculosis [1-3]. Rhamnose has no role in mammalian metabolism so that compounds which interfere specifically with rhamnose metabolism should not have any deleterious effect on humans. It is possible that a chemotherapeutic approach to the treatment of diseases induced by mycobacteria, such as tuberculosis and leprosy, would be to find compounds which inhibit either the biosynthesis of Tdp-rhamnose or its subsequent incorporation into the cell wall. The term spironucleoside was introduced in 1990 to designate a class of spiranic sugar derivatives in which the anomeric carbon belongs to both the sugar ring and to a heterocyclic base [4-6].

Data on this type of compound were reported before 1990 but, as far as we are aware, without using the term spironucleoside. Of the different classes of nucleosides, the spironucleosides are probably the least well known. In the last eight years, other syntheses of hydantocidin [5], spirofuranoid derivatives of different heterocycles [7], pyranoid analogues of hydantocidin [8].

Results and Discussion
2, 3, 4-Tri-O-acetyl-β-L-rhamnopyranose bromide 3 (Scheme 1) obtained by a known procedure from tetra-O-acetyl-L-rhamnopyranose 2 with HBr in acetic acid [9] was treated with mercury(II) cyanide in dry nitromethane at room temperature to give 2,3,4-tri-O-acetyl-β-L-rhamnopyranose cyanide 4.

For the partial hydrolysis of the nitrile moiety in 4 HBr in acetic acid [10] afforded the corresponding (2, 3, 4-tri-O-acetyl-β-L-rhamnopyranose) formamide 5 after 3 h reaction time in 94% yield as an almost analytically pure crystalline product. The anomeric configuration of compounds 4 and 5 has been found to be β according to the measured coupling constants between H-1 and H-2 (J₁,₂ ~ 6.3 Hz).

Photobromination of 5 with bromine in refluxing chloroform [11] gave 6 as an essentially pure product in an almost quantitative yield. Reaction of 6 with ammonium thiocyanate in nitromethane in the presence of elemental sulfur to suppress radical-mediated pathways [12] under nitrogen atmosphere gave spiro-thiohydantoin 7. The structures of the new heterocyclic derivatives have been unequivocally established by ¹H NMR spectroscopy.

2, 3-O-Isopropylidene-L-rhamnopyranose 8 was synthesized according to the reported procedure [13]. L-rhamnose 1 was reaction with dry acetone in the presence of p-toluene sulfonic acid to give 8. The 2, 3-O-isopropylidene group is used extensively as a blocking group in nucleosides synthesis as a means of enhancing the volatility of polar nucleosides. The reaction of 2, 3-O-isopropylidene-L-rhamnopyranose 8 with malononitrile in the presence of ammonium hydroxide in methanol afforded the corresponding 3-(2, 3-O-isopropylidene-β-L-rhamnopyranose) iminopropanenitrile 9 (Scheme 2).

The structure of compound 9 was confirmed by their elemental analyses and spectral (IR, 1H NMR and MS) data (see Experimental Section). For example, their 1H
NMR spectra in DMSO-d6 revealed in each case, a characteristic signal in the region $\delta = 7.28-7.34$ assignable to the azamethine N=CH proton. This was reacted with thioglycolic acid in the presence of absolute ethanol yielded the corresponding 2-(6-(2,3-O-isopropylidene-$\beta$-L-rhamnopyranose)-2-acetonitrile-3H-1,3-thiazol-4-one 10 (Scheme 3).

The compound 9 underwent nucleophilic addition with benzylidene malononitrile in refluxing ethanol in the presence of piperidine afforded the corresponding substituted pyridine derivative 11 in good yield. The structure of 11 was confirmed on the basis of elemental analysis and spectral data. The formation of 11 is assumed to occur via initial formation of the Michael addition of the amino group in 9 to activate the double bond in benzylidene malononitrile followed by intramolecular cyclization, and then it loses hydrogen cyanide to afford the Pyridine derivative 11 [14-16].

The formation of compound 11 may be proceeding through the following mechanism (Scheme 4). The reaction of compound 9 with carbon disulfide in the presence of pyridine afforded the corresponding 4-(2,3-O-isopropylidene-$\beta$-L-rhamnopyranose)-6-amino-2H-1,3-thiazine-2-thione 12 (Scheme 3). The structure of compound 12 was established by IR, $^1$H NMR and mass spectrometry. IR spectra showed a peak at 1224 cm$^{-1}$ indicative of C=S.

**Antibacterial activity**

The antibacterial activities of the synthesized compounds were determined by the well diffusion method [17]. In this work, Escherichia coli (ATCC-25922), Klebsiella pneumoniae (ATCC 10031), Bacillus cereus (ATTC-10702), Salmonella typhimurium (ATTC-23564) were used to investigate the antibacterial activities. The prepared compounds were tested against the Gram positive bacteria (B. cereus) and Gram negative bacteria (E. coli). The bacterial liquid cultures were prepared in infusion broth for their activity tests. The compounds were dissolved in DMSO at concentration of 1 mg ml$^{-1}$. Antibacterial activity of DMSO against the test organisms was investigated, and was found to be nil. Approximately 1 cm$^3$ of a 24 h broth culture containing $10^6$ cfu cm$^{-3}$ was placed in sterile Petri dishes. Molten nutrient agar (15 cm$^3$), kept at 45°C, was then poured into the Petri dishes and allowed to solidify. Six millimeter diameter
holes were then punched carefully using a sterile cork borer and completely filled with the test solutions. The plates were incubated for 24 h at 37°C. After 24 h, the inhibition zone that appeared around the holes in each plate was measured. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin and sulphamethoxazole as standards [18,19].

These results are summarized in Table 1. The examination data (Table 1) reveals that most of compounds showed excellent antibacterial activity when compared with ciprofloxacin and sulphamethoxazole. From the results, it is obvious that compound 7 showed the highest degree of inhibition against Salmonella typhi, Klebsiella Pneumoniae Escherichia coli EC and Bacillus subtilis BS. Moreover, compounds 9 and 11 have weak inhibition against Escherichia coli and Bacillus Cereus. While
had a considerable degree of inhibition against *Bacillus Cereus* and *Klebsiella Pneumoniae*, compounds 9 and 10 had only weak inhibition against *Escherichia coil* EC.

**Experimental Section**
Melting points were determined with an Electro Thermal Mel-Temp II apparatus and are all uncorrected.

IR spectra were obtained in the solid state as potassium disc using a Perkin-Elmer model 1430 Spectrometer. $^1$H NMR were recorded on a Varian/Gemini 200/MHZ spectrometer in DMSO-$d_6$ as a solvent and TMS as an internal standard (chemical shift in $\delta$, ppm). Mass spectra were measured on an instrument “VG-7035” spectra were recorded at 70 or 15 electron volt. Elemental

### Table 1 Results of antibacterial activity of the tested compounds

| Compound | *Salmonella typhi* | *Klebsiella Pneumoniae* | *Bacillus subtilis* | *Escherichia coil* | *Bacillus Cereus* |
|----------|-------------------|-------------------------|-------------------|-------------------|-----------------|
| 7        | 9                 | 8                       | 7                 | 8                 | 8               |
| 9        | 6                 | 7                       | 6                 | 2                 | 5               |
| 10       | 4                 | 7                       | 4                 | 4                 | 6               |
| 11       | 8                 | 3                       | 8                 | 3                 | 3               |
| 12       | 7                 | 5                       | 6                 | 8                 | 7               |
| Sulphamethoxazol | 23         | 23                      | 21                | 19                | 18              |
| Ciprofloxacin | 8             | 10                      | 10                | 9                 | 15              |
analysis was performed at the Micro analytical centre, Cairo University, Giza, Egypt.

2, 3, 4-Tri-O-acetyl-β-L-rhamnopyranosyl cyanide (4)
Acetobromo-rhamnose 3 (1.89 g, 1.5 mmol) was dissolved in dry nitromethane (40 ml) and mercury (II) cyanide (0.79 g, 1.5 mmol) was added. The mixture was stirred at r.t for 2 days. The solids were then filtered off, washed with nitromethane, and the solvent was removed from the combined filtrate and washings. The residue was dissolved in chloroform, the solution filtered if necessary and washed with aqueous potassium bromide solution (2 × 50 ml). The unified CHCl₃ phases were washed with saturated aqueous sodium bicarbonate (2 × 50 ml), then extracted with chloroform (2 × 50 ml); 1H NMR (500 MHz, CDCl₃) δ (ppm) 6.63 (br s, 1H, CONH₂), 6.14 (d, J1,2 = 6.3 Hz, 1H, H-1), 4.31 (dd, 1H, H-4), 4.36 (dd, 1H, H-2), 4.72 (ddd, 1H, J = 6.1, 6.2, 10.1 Hz, H-5), 1.73 (s, 9H; CH₃COO), 1.24 (d, J = 5.9 Hz, 3H, CH₃). Anal. Calcd. for C₁₃H₁₇NO₇ (299.28): C, 52.17; H, 5.67; N, 4.54. Found: C 52.05; H 5.73; N, 4.68.

(2, 3, 4-Tri-O-acetyl-β-L-rhamnopyranosyl) formamide (5)
Rhamnosyl cyanide 4 (1.1 g, 0.20 mmol) was suspended in a solution of hydrogen bromide in acetic acid (5 ml, 20% m/m) and the mixture was stirred at r.t for 3 h. The resulting solution was poured into ice-water (50 ml), which was then extracted with chloroform (2 × 50 ml). The unified CHCl₃ phases were washed with saturated aqueous sodium bicarbonate (2 × 50 ml), then with water (20 ml), dried, and the solvent removed and give the crystalline residue 5 (94%, yield). M.p. 226-228°C. IR (KBr) νmax: 2926 (aliphatic CH), 1702 (C = O), 1078 (C-O) cm-1; 1H NMR (500 MHz, CDCl₃) δ (ppm) 6.52 (br s, 2H, CONH₂), 4.26 (d, J1,2 = 6.3 Hz, 1H, H-1), 3.91 (d, 1H, H-3), 1.38 (d, J = 6.2 Hz, 3H, CH₃). Anal. Calcd. for C₁₄H₁₉NO₈ (317.29): C, 49.21; H, 6.04; N, 4.41. Found: C 49.39; H 5.59; N 4.37.

(2, 3, 4-Tri-O-acetyl-1-bromo-1-deoxy-β-L-rhamnopyranosyl) formamide (6)
Rhamnosyl formamide 5 (200 mg, 0.32 mmol) was dissolved in chloroform (6 ml), bromine (0.07 ml, 1.28 mmol) and some barium carbonate were added, and the mixture was irradiated and refluxed by a heat lamp. After 1 h the mixture decolorized and 0.1 ml Br₂ was added again. This was repeated after another 0.5 h. After TLC had shown complete transformation (~2 h from the start) the mixture was filtered, washed with 5% aqueous sodium bisulphate and saturated aqueous sodium bicarbonate solutions, dried, and the solvent removed. The residual syrup (264 mg) crystallized on addition of diethyl ether to give 6. M.p. 181-183°C. IR (KBr) νmax: 3343 (NH), 1735 (C = O ester), 1672 (C = O amide), 1088 (C-O) cm⁻¹; 1H NMR (500 MHz, CDCl₃) δ (ppm) 6.63 (br s, 1H, CONH₂), 6.14 (d, J1,2 = 6.3 Hz, 1H, H-1), 4.31 (dd, 1H, H-4), 4.36 (dd, 1H, H-2), 4.72 (ddd, 1H, J = 6.1, 6.2, 10.1 Hz, H-5), 1.73 (s, 9H; CH₃COO), 1.24 (d, J = 5.9 Hz, 3H, CH₃). Anal. Calcd. for C₁₃H₁₉NO₈Br (396.19): C, 39.58; H, 4.46; N, 3.43. Found: C 39.58; H 4.46; N 3.43.

(2S, 4S, 6R) 3, 4, 5-triacectoxy-2-methyl-7, 9-diazia-1-oxa-spiro [4,5] decane-10-one-8-thione (7)
1-Bromorhamnosyl formamide 6 (2 g, 3.69 mmol) was dissolved in dry nitromethane (23 ml). Molecular sieves (3 Å), ammonium thiocyanate (0.732 g, 1.79 mmol) and elemental sulfur (4 mg, 0.26 mmol) were added, and the mixture was stirred in an 80°C bath under nitrogen atmosphere for 7 h. The syrup residue obtained after filtration and solvent removal was dissolved in dichloromethane, the solution filtered, washed with saturated aqueous NH₄Cl solution, dried, and concentrated. The remaining syrup was separated by silica gel column chromatography with ethyl acetate: hexane 2:5 eluent, the first fraction which crystallized from methanol to give 0.99 g of thiohydantoin 7. M.p. 199-202°C. IR (KBr) νmax: 3342 (NH), 1694 (C = O amide), 1078 (C-O) cm⁻¹; 1H NMR (500 MHz, CDCl₃) δ (ppm) 9.08 (s, 1H, NH), 3.67 (d, 1H, H-4), 5.95 (ddd, 1H, J = 6.0, 9.8, 10.1 Hz, 1H, H-5), 3.91 (d, 1H, H-3), 1.38 (d, J = 6.2 Hz, 3H, CH₃). Anal. Calcd. for C₁₄H₁₈N₂O₈S (374.37): C, 44.92; H, 4.85; N, 7.48. Found: C, 44.78; H, 4.76; N, 7.34.

2, 3-O-isopropylidene-L-rhamnopyranosyl (8)
The reaction mixture of L-rhamnose monohydrate (1 g; 5.5 mmol), dry acetone (50 ml), toluene-4-sulphonic acid monohydrate (100 mg) and 2, 2-dimethoxypropane (6.8 ml; 55 mmol) was stirred for 6 h. The reaction mixture was neutralized by the addition of sodium carbonate. The neutral mixture was filtered, washed with methanol and evaporated. A syrupy isopropylidene derivative was purified by column chromatography on silica gel. TLC indicated one major product 8 isolated as syrup. Yield 0.73 g (65%); RF = 0.55. IR (KBr) νmax: 3432-3530 (OH), 1078 (C-O) cm⁻¹; 1H-NMR (500 MHz, CDCl₃) δ (ppm) 5.65 (d, 1H, H1), 4.56 (s, 1H, OH), 4.16-3.79 (dd, 4H, H-2, 3, 4, 5), 1.53 (s, 6H, 2CH₃O), 1.36 (3H, J = 6.2 Hz, CH₃). Anal. Calcd. for C₁₉H₂₆O₅S (204.22): C, 52.91; H, 7.90. Found: C, 52.89; H, 7.86.

3-(2, 3-O-isopropylidene-β-L-rhamnopyranosyl) iminopropanenitrile (9)
A mixture of 2, 3-O-isopropylidene-β-L-rhamnopyranosyl 8 (0.25 g, 1.65 mmole), malononitrile (0.48 g, 0.73 mmole), methanol (10 ml) and 6M NH₄OH (2 ml) was heated at 50°C for 3 days. The reaction was cooled to
10°C and kept at this temperature overnight. The solvent was evaporated under reduced pressure and the residue recrystallized by methanol/diethyl ether to afford a brown crystals (67% yield). M. p. 220-224°C. IR (KBr) νmax: 3412 (OH), 3150 (NH), 2021 (CN), 1634 (C = N), 1078 (C-O) cm⁻¹; ¹H-NMR (500 MHz, CDCl₃) δ (ppm) 7.28 (s, 1H, NH), 5.65 (d, 1H, H1), 4.56 (s, 1H, OH), 4.16-3.79 (dd, 4H, H-2, 3, 4, 5), 2.53 (s, 2H, CH₂-CN), 1.27 (d, J = 5.9 Hz, 3H, CH₃), 1.53 (s, 6H, 2CH₃). Anal. Calcd. for C₁₂H₁₈N₂O₅S (270.28): C, 53.33; H, 6.71; N, 10.36. Found: C, 53.42; H, 6.52; N, 10.17.

2-(6-(2, 3-O-isopropylidene-β-L-rhamnopyranos)-2-acetonitrile)-3H-1, 3-thiazol-4-one (10)

Compound 9 (0.3 g, 1.1 mmole), absolute ethanol (50 ml) and thioglycolic acid (2 ml) were refluxed 6 hrs. The solvent was evaporated and the residue dissolved in chloroform the solution washed with sodium bicarbonate solution. The organic layer was evaporated to give compound 10 (57% yield). M. p. 205-208°C. IR (KBr) νmax: 3386 (OH), 3250 (NH), 2205 (CN), 1645 (C = O), 1078 (C-O) cm⁻¹; ¹H-NMR 500 MHz δ 7.25 (s, 1H, NH), 6.13 (s, 1H, H1), 5.98 (s, 1H, OH), 4.21 (s, 2H, CH₂), 3.91-4.23 (m, 3H, H-2, 3, 4), 2.6 (s, 2H, CH₂ -S). Anal. Calcd. for C₁₂H₁₈N₂O₅S (270.28): C, 53.33; H, 6.71; N, 10.36. Found: C, 53.12; H, 6.62; N, 10.12.

6-(2’, 3’-O-isopropylidene-β-L-rhamnopyranos)-4-amino-2-phenylpyridine-3-carbonitrile (11)

A mixture of 9 (1.48 g, 5.48 mol) and benzylidemalononitrile (0.54 g, 6 mol) in piperidine (10 ml) was refluxed for 3 h. The reaction mixture was then allowed to cool at room temperature. The solid product so formed was collected by filtration and recrystallized from methanol/diethyl ether to afford a white precipitate, filtered and washed with ethanol. The precipitate recrystallized from chloroform/ethanol to give white needles (84% yields). M.p. 236-238°C. IR (KBr) νmax: 3347-3258 (NH₂, OH), 1580, 1238, 1224 (C = S) cm⁻¹; ¹HNMR (500 MHz, DMSO) δ 5.65 (s, 1H, H-1), 4.29-3.64 (d, 4H, H-2, 3, 4, 5), 4.16 (s, 2H, NH₂), 2.54 (s, 1H, OH), 1.23 (d, J = 6.1 Hz, 3H, CH₃). Anal. Calcd. for C₂₀H₂₇N₂O₅S₂ (439.57): C, 54.65; H, 6.19; N, 6.37; S, 14.59. Found: C, 54.62; H, 6.32; N, 6.24; S, 14.53.

Conclusion

In conclusion, the preparation procedure follow in this work for synthesis of some nucleosides derivatives from L- rhamnose with expected biological activity show operation simplicity, cleaner reaction, easy work-up and improved yields. Spectroscopic and elemental analysis confirms the proposed structures of these compounds. The prepared compounds showed promising antibacterial activity against Gram-positive bacteria Bacillus cereus.

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Competing interests

The author declares that they have no competing interests.

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