Carbohydrate Research 419 (2016) 29–32

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

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Alternative synthesis and antibacterial evaluation of 1,5-dideoxy-1,5-imino-L-rhamnitol

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ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 27 October 2015
Accepted 28 October 2015
Available online 4 November 2015

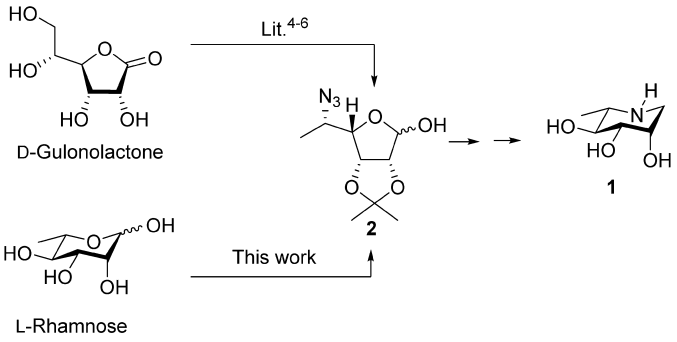
Keywords:
L-1-deoxyrhamnojirimycin
Pseudomonas aeruginosa
L-rhamnofuranose
Glycosyl transferase inhibitors
Antibacterial

ABSTRACT

A convenient synthesis is described of 5-azido-5-deoxy-2,3-O-isopropylidene-L-rhamnofuranose from L-rhamnose in seven steps and 17% overall yield. A key feature of the synthesis is the selective oxidation of the secondary alcohol in 2,3-O-isopropylidene-L-rhamnofuranose in the presence of the hemiacetal to give the corresponding ketone in good yield using the Parikh–Doering reagent. 5-Azido-5-deoxy-2,3-O-isopropylidene-L-rhamnofuranose is then converted by a literature protocol to 1,5-dideoxy-1,5-imino-L-rhamnitol, which was found to have no significant antimicrobial activity against *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and *Escherichia coli*.

1. Introduction

The recent discovery that in *Pseudomonas aeruginosa* rhamnosylation of a conserved arginine residue in Elongation Factor-P (EF-P) circumvents ribosome stalling at X-PP-X sequences and is essential for bacterial fitness and pathogenicity of this nosocomial pathogen suggests the responsible glycosyl transferase (EarP) and dTDP-L-rhamnose synthesizing enzymes as possible new targets for antibiotic development.[1] Iminosugars have a long and fruitful history as glycosidase and glycosyl transferase inhibitors.[2,3] Pertinently, 1,5-dideoxy-1,5-imino-L-rhamnitol **1** and its N-alkyl and aryl derivatives were prepared by Davis and coworkers and studied as inhibitors of RhamT, a rhamnosyl transferase involved in the assembly of the bridging region linking arabinogalactan units to the peptidoglycan of *Mycobacterium tuberculosis* cell wall.[4] As EarP and RhamT employ the same glycosyl donor dTDP-L-Rha, **1** is a possible inhibitor of EarP and therefore of *P. aeruginosa*. Following earlier work by Fleet and coworkers,[5] Davis and coworkers prepared **1** and its N-substituted analogs by two routes,[4] both employing 5-azido-5-deoxy-2,3-O-isopropylidene-L-rhamnofuranose **2** as a key intermediate assembled in seven steps by a literature protocol[5,6] from D-gulonolactone via its 2,3-acetonide[7] (Scheme 1). 1,5-Dideoxy-1,5-imino-L-rhamnitol **1**, alternatively called L-deoxyrhamnonojirimycin, also has been prepared by chemoenzymatic synthesis,[8] and has been shown to have only weak inhibitory properties for various glycosidase enzymes.[5,8]



Scheme 1. Existing and new routes to 1,5-dideoxy-1,5-imino-L-rhamnitol.

We describe an alternative route in 17% overall yield and seven steps to the key intermediate **2** from commercial L-rhamnose (Scheme 1), its conversion to **1** by the Davis protocol, and the finding that **1** displays no significant activity against clinical isolates of *P. aeruginosa*.

2. Results and discussion

Following Khan et al.,[9] L-rhamnose was treated with tetrabutylammonium tribromide in acetone at room temperature to give acetonide **3**, in 89% yield after work up. Parikh–Doering oxidation[10] of **3** gave the ketone **4a** in 58% yield together with 3% of the ketolactone **4b** and 16% of starting material **3**. This selective oxidation of the secondary alcohol in the presence of a hemiacetal is noteworthy and contributes significantly to the success of this

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http://dx.doi.org/10.1016/j.carres.2015.10.015
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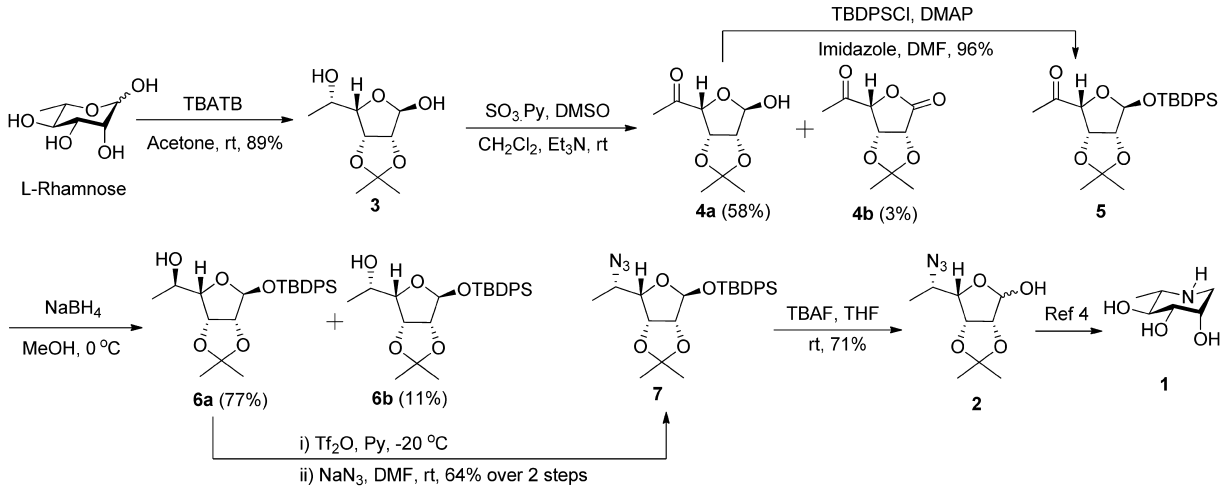

Scheme 2. Synthesis of **2** and **1** from L-rhamnose.

synthesis. Subsequent reaction with *tert*-butyldiphenylsilyl chloride, DMAP and imidazole then afforded the silyl glycoside **5** in 96% yield with excellent selectivity in favor of the 1,2-*trans*-configuration. The anomeric configuration of compounds **3**–**7**, all of which are essentially single isomers, is assigned on the basis of the minimal value of $^{3}J_{1,2}$ in the ^{1}H NMR spectra, which is indicative of the 1,2-*trans*-configuration.[11] Reduction of **5** with sodium borohydride in methanol at 0 °C gave 77% of the desired D-gulofuranoside **6a** and 11% of the L-rhamno-isomer **6b**. The selectivity of this reduction is consistent with that reported previously in the reaction of sodium borohydride with the corresponding methyl glycoside.[12] Reaction of **6a** with triflic anhydride and pyridine in dichloromethane at –20 °C afforded an intermediate triflate that on treatment with sodium azide in DMF gave the 5-deoxy-5-azido-L-rhamnose derivative **7** in 64% yield for the two steps. Finally, cleavage of the silyl glycosides with tetrabutylammonium fluoride gave **2** in 71% yield (Scheme 2), whose spectral data were in full agreement with the literature.[4] Overall, the synthesis of key intermediate **2** is achieved in six steps and 17% overall yield from the readily available L-rhamnose, thereby providing a useful alternative to the existing synthesis that proceeds in a total of seven steps and 37% yield from the more costly and less available D-gulonolactone. Finally, following the method of Davis and coworkers **2** was then converted to the target **1** in 82% yield (over 2 steps) by reductive cyclization with H_2 over Pd/C and deprotection of acetonide group with aqueous trifluoroacetic acid.

Iminoglycoside **1** was screened for activity against several strains of *P. aeruginosa*. No activity was observed at concentrations of 128 μg/mL or less. Similarly, no activity was observed against the Gram-positive bacterium methicillin resistant *Staphylococcus aureus*, and the Gram-negative bacterium, *Escherichia coli*.

3. Experimental section

3.1. General experiment

All experiments were performed in oven dried glassware under an argon atmosphere. The visualization of spots on TLC plates was effected by exposure to iodine or spraying with 10% H_2SO_4 and charring. ^{1}H NMR spectra were recorded in $CDCl_3$ solution unless otherwise stated at 400 MHz. ^{13}C NMR spectra were recorded in $CDCl_3$ solution unless otherwise stated at 100 MHz. Mass spectra were recorded in the +ve ion mode using electrospray ionization (ESI-TOF). Specific rotations were recorded in dichloromethane solution at room temperature.

3.2. 6-Deoxy-2,3-O-isopropylidene-α-L-lyxo-5-hexulofuranose (*4a*) and L-lyxo-6-deoxy-5-hexulosono-1,4-lactone (*4b*)

To a stirred solution of **3**[9,13] (5.0 g, 24.4 mmol) in dimethyl sulfoxide (55.0 mL) and dichloromethane (82.5 mL) were added triethylamine (8.5 mL, 61.2 mmol) and sulfur trioxide pyridine complex (7.8 g, 48.9 mmol) at room temperature. After stirring for 1.5 h the reaction mixture was washed with water and extracted with ethyl acetate. The ethyl acetate layer was dried over Na_2SO_4 and concentrated under high vacuum. Column chromatography over silica gel (eluent: 30% ethyl acetate in hexane) afforded **4a** (2.4 g, 58%), **4b** (0.12 g, 3%), and recovered substrate (0.8 g, 16%).

4a: Mp = 147–148 °C; $[\alpha]_D^{22}$ = +45.5 (*c* 0.55, CH_2Cl_2); IR (neat) ν_{max} 3346 (—O—H), 1696 (—C═O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (d, *J* = 2.1 Hz, 1H, H-1), 5.06 (dd, *J* = 5.7, 4.2 Hz, 1H, H-3), 4.66–4.62 (m, 2H, H-2, H-4), 2.75 (s, 1H, O—*H*), 2.23 (s, 3H, H-6), 1.42 (s, 3H, —C(C*H*$_3$)$_2$), 1.28 (s, 3H, —C(C*H*$_3$)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.4, 113.1, 101.4, 85.2, 84.7, 80.8, 27.8, 25.8, 24.5; HRMS (ESI) m/z calcd for $C_9H_{14}O_5Na$ [M + Na]$^+$, 225.0739; found, 225.0732.

4b: Mp = 124–125 °C; $[\alpha]_D^{22}$ = –5.7 (*c* 2.60, CH_2Cl_2); IR (neat) ν_{max} 1792 (O—C═O), 1736 (—C═O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 (t, *J* = 4.6 Hz, 1H, H-3), 4.88–4.81 (m, 2H, H-2, H-4), 2.28 (s, 3H, H-6), 1.44 (s, 3H, —C(C*H*$_3$)$_2$), 1.36 (s, 3H, —C(C*H*$_3$)$_2$; ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3, 172.9, 114.7, 81.4, 76.5, 75.5, 27.6, 26.6, 25.6; HRMS (ESI) m/z calcd for $C_9H_{12}O_5Na$ [M + Na]$^+$, 223.0582; found, 223.0575.

3.3. tert-Butyldiphenylsilyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-5-hexulofuranoside (*5*)

To a stirred solution of **4a** (0.230 g, 1.14 mmol) in DMF (3.0 mL) were added *tert*-butylchlorodiphenylsilane (0.44 mL, 1.71 mmol), imidazole (0.155 g, 2.3 mmol), and dimethylaminopyridine (0.014 g, 0.11 mmol). The reaction mixture was stirred for 5 h at room temperature before TLC (40% ethyl acetate in hexane) showed reaction completion. The reaction mixture was washed with water and extracted with ethyl acetate. The ethyl acetate layer was dried over Na_2SO_4 and concentrated. Column chromatography over silica gel (eluent: 10% ethyl acetate in hexane) afforded **5** (0.480 g, 96%) as a colorless oil. $[\alpha]_D^{22}$ = –2.5 (*c* 1.00, CH_2Cl_2); IR (neat) ν_{max} 1722 (—C═O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67–7.61 (m, 4H, Ar*H*), 7.50–7.32 (m, 6H, Ar*H*), 5.51 (s, 1H, H-1), 5.13–5.05 (m, 1H, H-3), 4.67 (d,