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Bioorganic & Medicinal Chemistry 14 (2006) 5535–5539
Bioorganic & Medicinal Chemistry

Synthesis and evaluation of glycosidase inhibitory activity of *N*-butyl 1-deoxy-D-*gluco*-homonojirimycin and *N*-butyl 1-deoxy-L-*ido*-homonojirimycin

Shankar D. Markad,[a] Narayan S. Karanjule,[a] Tarun Sharma,[b] Sushma G. Sabharwal[b] and Dilip D. Dhavale[a,*]

[a]*Garware Research Centre, Department of Chemistry, University of Pune, Pune 411 007, India*
[b]*Division of Biochemistry, Department of Chemistry, University of Pune, Pune 411 007, India*

Received 17 March 2006; revised 19 April 2006; accepted 20 April 2006
Available online 8 May 2006

Abstract—Conjugate addition of *n*-butyl amine to D-glucose derived α,β-unsaturated ester **4** afforded β-amino esters **5a**,**b** that on reduction of ester group, 1,2-acetonide deprotection, and reductive amination led to the formation of corresponding *N*-butyl 1-deoxy-D-*gluco*-homonojirimycin **2c** and *N*-butyl 1-deoxy-L-*ido*-homonojirimycin **2d** which were found to be selective β-glucosidase inhibitors with an IC_{50} value in millimolar range.

1. Introduction

Amongst azasugars, nojirimycin **1a** and 1-deoxynojirimycin **1b** are known to be potent glycosidase inhibitors[1,2] having interesting antidiabetic,[3] anticancer,[4] and anti-HIV-I and HIV-II[5] properties. In general, the N-alkylated derivatives were found to be stronger glycosidase inhibitors than the corresponding non-alkylated derivatives.[6] For example, the *N*-butyl 1-deoxynojirimycin **1c** has been shown to possess potent inhibitory activity of glycosidase enzymes[7] and highest cytopathic effect (CPE), at a concentration which did not demonstrate cytotoxicity[5], thus making it under clinical evaluation as an agent for the chemotherapy of AIDS.[8] It is also useful for treatment of the Gaucher disease. Other N-alkylated derivatives, such as *N*-hydroxyethyl 1-deoxynojirimycin **1d** (miglitol) and emiglitate **3** (Fig. 1), have been reported to reduce postprandial elevations of blood glucose and plasma insulin in animals in loading tests with starch and sucrase.[9] Miglitol **1d** is a potent sucrase inhibitor[10] and antidiabetic drug in the market since 1996. The promising therapeutic potential of N-alkylated derivatives thus led to increased interest and development of new methodologies for their synthesis and biological evaluation. This led to a number of structural modifications in the basic skeleton of nojirimycin **1a** that mainly include (a) change of stereochemical orientation of substituents, (b) presence/absence of –OH, and (c) hydroxymethyl substituents at either end of the ring nitrogen atom.[11] In view of this and as a part of our continuing efforts in the area of azasugars[12] we are now reporting an efficient strategy for the synthesis of new azasugar analogues namely *N*-butyl 1-deoxy-D-*gluco*-homonojirimycin **2c**, and *N*-butyl 1-deoxy-L-*ido*-homonojirimycin **2d** and evaluation of their glycosidase inhibitory activity.

2. Results and discussion

D-Glucose was converted to α,β-unsaturated ester **4** in good yield as reported earlier by us.[12c,12d] Conjugate addition of *n*-butyl amine (1.2 equiv) to **4**, in the absence of solvent at room temperature for 24 h, furnished a diastereomeric mixture of D-*gluco*- and L-*ido*-configurated β-amino esters **5a** and **5b**, respectively, in the ratio of 1:9 (Scheme 1). The stereochemical assignment at the newly generated C-5 stereocenter was established by ^{1}H NMR spectral studies of **5a**,**b**. It is known that, for a given C5-epimeric pair derived from D-*gluco*-furanose, the $J_{4,5}$ in the L-*ido* isomer (*threo* relationship) is consistently larger than that of the corresponding D-*gluco*

Keywords: Conjugate addition; Glycosidase inhibitors; Iminosugars; Azasugars.
* Corresponding author. Tel.: +91 20 2560 1225x584; fax: +91 20 2569 1728; e-mail: ddd@chem.unipune.ernet.in

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doi:10.1016/j.bmc.2006.04.027

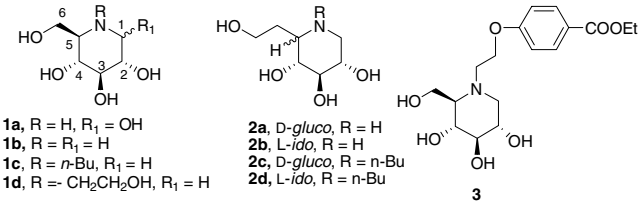
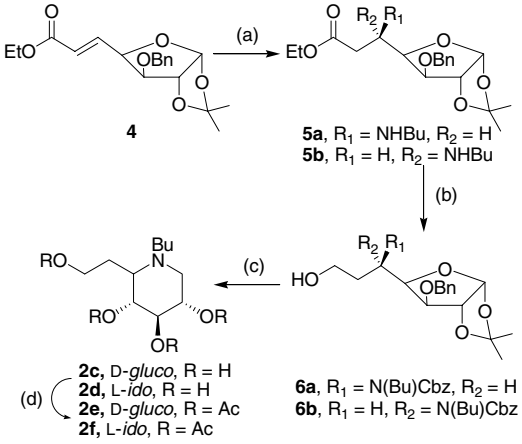


Figure 1. Nojirimycin and analogues.



Scheme 1. Reagents and conditions: (a) *n*-$BuNH_2$, neat, rt, 24 h, 90%; (b) (i) LAH, THF, 0 °C, 1 h; (ii) CbzCl, $NaHCO_3$, CH_3OH–H_2O, 0 °C, 4 h, 83%; (c) (i) TFA–H_2O (2:1), 0 °C to rt, 2.5 h, 97%; (ii) $HCOONH_4$, 10% Pd/C, MeOH, reflux, 45 min, 91%; (d) Ac_2O, pyridine, DMAP, 25 °C, 24 h, 98%.

isomer (*erythro* relationship). The higher value of $J_{4,5}$ (9.0 Hz) observed in the diastereomer **5b** as compared to **5a** (8.5 Hz) indicated L-*ido* configuration for **5b** and the D-*gluco* configuration for **5a**. This assignment was further supported by comparison of the chemical shifts of H3 in both the isomers. The chemical shift of H3 is reported to be diagnostic such that, in the L-*ido*-isomer, it is significantly upfield as compared to that in the D-*gluco*.[13] In **5b**, H3 appeared upfield at δ 3.86 as compared to **5a** at δ 3.98, further supporting the L-*ido* and D-*gluco* configuration at C5 in **5b** and **5a** with 5*S* and 5*R* absolute configuration, respectively.

In the next step, reduction of ester group in **5a** with LAH and selective amine protection afforded *N*-Cbz compound **6a**. Deprotection of 1,2-acetonide functionality in **6a** (TFA–water; 2:1) afforded anomeric mixture of hemiacetal, that on treatment with ammonium formate in the presence of 10% Pd/C in dry methanol gave *N*-butyl-1-deoxy-D-*gluco*-homonojirimycin **2c** (2.4% overall yield from D-glucose) as thick liquid. The same reaction sequence was performed with **5b** that afforded *N*-butyl-1-deoxy-L-*ido*-homonojirimycin **2d** in good yield (26.2% overall yield from D-glucose). Further characterization of **2c** and **2d** was made with corresponding acetyl derivatives. Thus, individual treatment of **2c** and **2d** with acetic anhydride and pyridine in the presence of DMAP gave peracetate derivatives **2e** and **2f**, respectively. Compounds **6a**, **6b** and **2c**–**f** were characterized by spectral

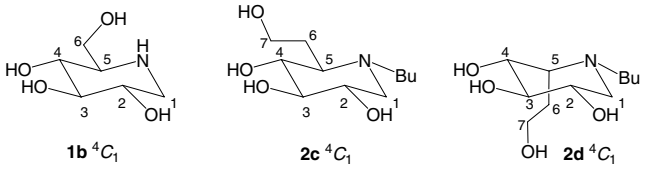


Figure 2. Conformations of azasugars.

and analytical techniques and data were found to be in agreement with their structures.

2.1. Conformational assignment

The conformations of azasugars play an important role in glycosidase inhibitory activity. The 1-deoxy-D-*gluco*-nojirimycin and 1-deoxy-L-*gluco*-nojirimycin are known to exist in $^4C_1$ and $^1C_4$ conformations, respectively.[12k] The presence of $-CH_2CH_2OH$ functionality at C5 in **2d** is expected to change the conformation. Therefore, we have studied the conformations of **2c** and **2d** by studying ^{1}H NMR wherein the coupling constant information was obtained by decoupling experiments. In the ^{1}H NMR spectrum of **2c**, the appearance of a triplet corresponding to H1a proton with $J_{1a,1e} = J_{1a,2a} =$ 11.7 Hz indicates geminal and axial–axial coupling due to the axial orientation of H2. The two triplets corresponding to H3 and H4 with $J_{2a,3a} = J_{3a,4a} = J_{4a,5a} = 9.3$ Hz indicated the *trans* di-axial disposition of H3–H4 and H4–H5 protons, and hence confirm the $^4C_1$ conformation of **2c** (Fig. 2). In the ^{1}H NMR spectrum of **2d**, the appearance of doublet of doublet corresponding to the H1a proton with $J_{1a,1e} = 12.3$ Hz and $J_{1a,2a} = 10.2$ Hz indicated the *trans* di-axial orientation of H1a and H2. In the intermediate **6b**, the H3 and H4 are *trans* and the same stereochemistry is retained in **2d** as evident from the appearance of triplet corresponding to H3 with large coupling constant ($J_{2a,3a} = 9.3$ Hz). The H4 appeared as a doublet of doublet with $J_{3a,4a} = 9.3$ Hz and $J_{5e,4a} = 5.2$ Hz wherein the small $J_{5,4}$ indicated the axial orientation of $-CH_2CH_2OH$ substituent with $^4C_1$ conformation of **2d**.

2.2. Inhibition studies

Glycosidases namely β-glucosidase (E.C. 3.2.1.21), α-glucosidase (E.C. 3.2.1.20), and α-mannosidase (E.C. 3.2.1.24) were purchased from Sigma Chemicals Co. USA. β-Galactosidase (E.C. 3.2.1.23) was purified from sweet almonds. The glycosidase inhibitory activity was studied with different glycosidases and compared with *N*-butyl 1-deoxynojirimycin **1c**. As shown in Table 1, compounds **2c** and **2d** were found to be selective inhibitors against β-glucosidase. As compared to *N*-butyl 1-deoxynojirimycin **1c** (IC_{50} 0.57 μM against α-glucosidase),[14] it is observed that one carbon homologation at C6 resulted in the change in selectivity as **2c** and **2d** showed β-glucosidase inhibition in millimolar range.

In conclusion, we have demonstrated a short and an efficient methodology for the synthesis of **2c** and **2d**, which were found to be selective β-glucosidase inhibitors with IC_{50} values in millimolar range.

Table 1. IC_{50} values in mM

Compound	α-Glucosidase (yeast)	β-Glucosidase (sweet almonds)	β-Galactosidase (sweet almonds)	α-Mannosidase (jack bean)
2c	NI	24.22	NI	NI
2d	NI	0.24	NI	NI

NI, no Inhibition observed under assay conditions. The value is average of three sets of data.

3. Experimental

3.1. General methods

Melting points were recorded with Thomas Hoover melting point apparatus and are uncorrected. IR spectra were recorded with FTIR as a thin film or in nujol mull or using KBr pellets and are expressed in cm^{-1}. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded using $CDCl_3$ and/or D_2O as solvent(s). Chemical shifts were reported in δ unit (parts per million) with reference to TMS as an internal standard and J values are given in Hertz. Elemental analyses were carried out with C,H-analyzer. Optical rotations were measured using polarimeter at 25 °C. Thin-layer chromatography was performed on pre-coated plates (0.25 mm, silica gel 60 F_{254}). Column chromatography was carried out with silica gel (100–200 mesh). The reactions were carried out in an oven-dried glassware under dry N_2. Methanol, DMF, and THF were purified and dried before use. Petroleum ether (PE) that was used is a distillation fraction between 40 and 60 °C. LAH, CbzCl, and 10% Pd/C were purchased from Aldrich and/or Fluka. After decomposition of the reaction with water, work-up involves washing of combined organic layer with water, brine, drying over anhydrous sodium sulfate, and evaporation of solvent under reduced pressure. For enzyme inhibition studies substrates were purchased from Sigma Chemicals Co., USA. α-Glucosidase from yeast and α-mannosidase from jack bean were purchased from Sigma Chemicals Co., USA. β-Glucosidase, β-galactosidase were extracted and purified from sweet almonds and used.

3.1.1. Ethyl 1,2-*O*-isopropylidene-3-*O*-benzyl-5-(*N*-butylamino)-5,6-dideoxy-α-D-*gluco*-heptofuranuronate (5a) and ethyl 1,2-*O*-isopropylidene-3-*O*-benzyl-5-(*N*-butylamino)-5,6-dideoxy-β-L-*ido*-heptofuranuronate (5b). Compound **4** (3 g, 8.62 mmol) and 0.76 g of *n*-butyl amine (10.34 mmol) were mixed and stirred at room temperature under N_2 for 24 h. The reaction was directly loaded on column. First elution with *n*-hexane–ethyl acetate 98:2 afforded β-amino ester **5a** (0.33 g, 09%) as a thick liquid; R_f 0.58 (20% ethyl acetate–*n*-hexane); $[\alpha]_D$ −25.37 (*c* 1.025, $CHCl_3$); IR (Nujol) 3600–3220 (broad band), 1729, 1379 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + D_2O$) δ: 0.79 (3H, t, J = 7.2 Hz, $-CH_2CH_3$), 1.18 (3H, t, J = 7.2 Hz, $-OCH_2CH_3$), 1.24 (7H, br s, $-CH_2CH_2-$ and CH_3), 1.41 (3H, s, CH_3), 2.38–2.62 (3H, m, $-NCH_2$ and *H*6a), 2.70 (1H, dd, J = 15.6, 4.2 Hz, *H*6b), 3.30–3.37 (1H, m, *H*5), 3.98 (1H, d, J = 3.3 Hz, *H*3), 4.04 (1H, dd, J = 8.5, 3.3 Hz, *H*4), 4.06 (2H, q, J = 7.2 Hz, $-OCH_2CH_3$), 4.48 (1H, d, J = 11.4 Hz, OCH_2Ph), 4.53 (1H, d, J = 3.9 Hz, *H*2), 4.63 (1H, d, J = 11.4 Hz, $-OCH_2Ph$), 5.82 (1H, d, J = 3.9 Hz, *H*1), 7.27 (5H, br s, Ar–H's); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 14.1 ($-CH_2CH_3$), 20.3 ($-OCH_2CH_3$), 26.2, 26.6 ($2\times CH_3$), 32.5 ($-NCH_2CH_2CH_2-$), 35.5 (C6), 46.4 (NCH_2), 52.4 (C5), 60.1 ($-OCH_2Ph$), 71.8 ($-OCH_2Ph$), 81.4, 81.6, 82.0 (*C*2/*C*3/*C*4), 104.5 (C1), 111.4 (OCO), 127.6 (s), 127.6, 127.7, 128.3, 137.4 (Ar–*C*'s), 172.4 (*C*O); Anal. $C_{23}H_{35}NO_6$ requires C, 65.53; H, 8.37. Found: C, 65.58; H, 8.40. Further elution with *n*-hexane–ethyl acetate 97:3 gave β-amino ester **5b** (2.94 g, 89%) as a white solid; mp 50–52 °C (from *n*-hexane); R_f 0.48 (20% ethyl acetate–*n*-hexane); $[\alpha]_D$ −26.37 (*c* 1.365, $CHCl_3$); IR (Nujol) 3600–3220 (broad band), 1730, 1375 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + D_2O$) δ: 0.81 (3H, t, J = 7.2 Hz, $-CH_2CH_3$), 1.16 (3H, t, J = 7.2 Hz, $-OCH_2CH_3$), 1.21–1.31 (2H, m, $-CH_2CH_2CH_3$), 1.25 (3H, s, CH_3), 1.31–1.39 (2H, m, $-CH_2CH_2CH_3$), 1.41 (3H, s, CH_3), 2.20 (1H, dd, J = 15.0, 6.6 Hz, *H*6a), 2.30 (1H, dd, J = 15.0, 4.5 Hz, *H*6b), 2.45–2.63 (2H, m, $-NCH_2$), 3.34 (1H, ddd, J = 9.0, 6.6, 4.5 Hz, *H*5), 3.86 (1H, d, J = 3.3 Hz, *H*3), 4.05 (2H, q, J = 7.2 Hz, $-OCH_2CH_3$), 4.11 (1H, dd, J = 9.0, 3.3 Hz, *H*4), 4.37 (1H, d, J = 11.7 Hz, OCH_2Ph), 4.57 (1H, d, J = 3.6 Hz, *H*2), 4.63 (1H, d, J = 11.7 Hz, $-OCH_2Ph$), 5.87 (1H, d, J = 3.6 Hz, *H*1), 7.26 (5H, br s, Ar–H's); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 14.0 ($-CH_2CH_3$), 20.3 ($-OCH_2CH_3$), 26.2, 26.6 ($2\times CH_3$), 32.2 ($-NCH_2CH_2CH_2-$), 36.0 (C6), 46.7 (NCH_2), 53.8 (*C*5), 60.2 ($-OCH_2CH_3$), 71.3 ($-OCH_2Ph$), 81.4, 81.6, 81.9 (*C*2/*C*3/*C*4), 104.6 (OCO), 114.4 (*C*1), 127.8 (s), 127.9, 128.3 (s), 136.9 (Ar–*C*'s), 171.7 (*C*O); Anal. $C_{23}H_{35}NO_6$ requires C, 65.53; H, 8.37. Found: C, 65.54; H, 8.38.

3.1.2. 3-*O*-Benzyl-5,6-dideoxy-5-(*N*-butyl-*N*-benzyloxycarbonylamino)-1,2-*O*-isopropylidene-α-D-*gluco*-hepto-1,4-furanose (6a). To an ice-cooled solution of 0.16 g LAH (4.28 mmol) in dry THF (4 mL) was added 0.3 g of β-amino ester **5a** (0.71 mmol) in dry THF (10 mL) at 0 °C and stirred for 1 h. Reaction was quenched by adding ethyl acetate (20 mL), followed by aqueous solution of ammonium chloride (3 mL). The reaction mixture was filtered through Celite and the filtrate was concentrated under vacuum. To an ice-cooled solution of 0.27 g of amino alcohol (0.71 mmol) in methanol–water (10 mL, 9:1) were added 0.15 g of benzyl chloroformate (0.85 mmol) and 0.18 g of sodium bicarbonate (2.13 mmol) at 0 °C and stirred for 12 h. Methanol was evaporated under reduced pressure and the residue was extracted with chloroform (3×15 mL). Usual work-up and purification by column chromatography (*n*-hexane–ethyl acetate 9:1) gave **6a** (0.3 g, 82% overall) as a thick liquid; R_f 0.51 (40% ethyl acetate–*n*-hexane); $[\alpha]_D$ −40.0 (*c* 1.45, $CHCl_3$); IR (Nujol) 3200–3600 (broad band), 1688, 1560, 1456, 1375 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, t, J = 7.5 Hz, $-CH_2CH_3$),

1.16–1.42 (2H, m), 1.34 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.72–1.91 (1H, m, D_2O exchangeable, –O*H*), 1.92–2.14 (2H, m), 2.18–2.34 (2H, m), 3.00–3.30 (2H, m), 3.45–3.58 (1H, m), 3.60–3.75 (1H, m), 3.91 (1H, d, J = 3.3 Hz, *H*3), 4.30–4.52 (1H, m), 4.55–4.70 (1H, m), 4.47 (1H, d, J = 12.3 Hz, –OCH_2Ph), 4.58 (1H, d, J = 3.9 Hz, *H*2), 4.60 (1H, d, J = 12.3 Hz, –OCH_2Ph), 5.18 (2H, Abq, J = 12.6 Hz, –OCH_2Ph), 5.95 (1H, d, J = 3.9 Hz, *H*1), 7.25–7.34 (10H, m, Ar–*H*'s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.7, 20.3, 26.1, 26.7 (*C*6/–$CH_2CH_2CH_2$–), 32.1, 58.8, 67.3 (*C*5/*C*7/N–*C*H_2), 77.2 (2× –O*C*H_2Ph), 80.2, 81.6, 82.1 (*C*2/*C*3/*C*4), 104.8 (*C*1), 111.5 (O*C*O), 127.6, 127.7, 127.8, 128.0 (s), 128.1, 128.2, 128.4 (s), 136.1, 136.5, 137.0 (Ar–*C*'s), 156.0 (CO); Anal. $C_{29}H_{39}NO_7$ requires C, 67.81; H, 7.65. Found: C, 67.82; H, 7.68.

3.1.3. 3-*O*-Benzyl-5,6-dideoxy-5-(*N*-butyl-*N*-benzyloxycarbonylamino)-1,2-*O*-isopropylidene-β-L-*ido*-hepto-1,4-furanose (6b). Compound **5b** (0.3 g, 0.71 mmol) was reacted with 0.16 g LAH (4.28 mmol) followed by 0.15 g of benzyl chloroformate (0.85 mmol) and 0.18 g of sodium bicarbonate (2.13 mmol) as described for the preparation of **6a** afforded **6b** (0.32 g, 88% overall) as a white solid; mp 109–111 °C (from 40% ethyl acetate–*n*-hexane); R_f 0.48 (40% ethyl acetate–*n*-hexane); $[\alpha]_D$ −22.86 (*c* 0.7, $CHCl_3$); IR (Nujol) 3200–3600 (broad band), 1686, 1560, 1456, 1375 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (3H, t, J = 7.5 Hz, –CH_2CH_3), 1.20–1.32 (4H, m), 1.36 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.58–1.71 (2H, m), 1.72–1.80 (1H, br s, D_2O exchangeable, O*H*), 3.00–3.35 (2H, m), 3.40–3.68 (2H, m), 3.89 (1H, d, J = 3.3 Hz, *H*3), 4.02–4.40 (2H, m), 4.54 (1H, d, J = 11.7 Hz, OCH_2Ph), 4.69 (1H, d, J = 3.9 Hz, H2), 4.75 (1H, d, J = 11.7 Hz, OCH_2Ph), 5.21 (2H, Abq, J = 12.3 Hz, –OCH_2Ph), 5.97 (1H, d, J = 3.9 Hz, *H*1), 7.25–7.52 (10H, m, Ar–*H*'s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.6, 20.8, 26.1, 26.6 (*C*6/–$CH_2CH_2CH_2$–), 31.2, 58.1, 66.9 (*C*5/*C*7/N–*C*H_2), 69.5, 71.6 (2× –O*C*H_2Ph), 79.0, 80.9, 81.2, 81.4 (*C*2/*C*3/*C*4), 104.6 (*C*1), 111.3 (O*C*O), 127.8, 127.4, 127.7, 128.0, 128.29 (s), 128.3, 128.4 (s), 136.6, 136.7, 136.9 (Ar–*C*'s), 156.2 (CO); Anal. $C_{29}H_{39}NO_7$ requires C, 67.81; H, 7.65. Found: C, 67.78; H, 7.64.

3.1.4. *N*-Butyl-1-deoxy-D-*gluco*-homonojirimycin (2c). Compound **6a** (0.1 g, 0.194 mmol) in TFA–H_2O (3 mL, 2:1) was stirred at 25 °C for 2.5 h. Trifluoroacetic acid was co-evaporated with benzene to furnish a thick liquid. To a solution of the above product in methanol (10 mL) were added 0.073 g of ammonium formate (1.16 mmol) and 0.05 g of 10% Pd/C, and the reaction mixture was refluxed for 1 h. The catalyst was filtered through Celite and washed with methanol. The filtrate was concentrated to get a syrup, which on purification by silica gel column chromatography (chloroform–methanol–25% aq NH_3 9:1:0.01) gave **1d** (0.04 g, 89%) as a thick liquid; R_f 0.58 (50% chloroform–methanol); $[\alpha]_D$ −16.0 (*c* 0.75, CH_3OH); IR (neat) 3200–3600 (broad band) cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ: 0.77 (3H, t, J = 7.2 Hz, –CH_2CH_3), 1.19 (2H, sextet, J = 7.2 Hz, –CH_2C$H_2$$CH_3$), 1.35–1.59 (2H, m, –C$H_2$$CH_2$$CH_3$), 1.80–2.05 (2H, m, *H*6a,b), 2.47 (1H, t, J = 11.7 Hz, *H*1a), 2.60–2.80 (2H, m, –NC$H_2$$CH_2$–), 2.85–3.15 (1H, m, *H*5), 3.19 (1H, dd, J = 11.7, 4.8 Hz, *H*1e), 3.20 (1H, t, J = 9.3 Hz, *H*3), 3.30 (1H, t,J = 9.3 Hz, *H*4), 3.53 (1H, ddd, J = 11.1, 9.3, 4.8 Hz, *H*2), 3.58 (2H, t, J = 6.9 Hz, –CH_2OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 9.7 (–CH_2CH_2*C*H_3), 16.3 (–CH_2*C*H_2CH_3), 22.3 (–*C*$H_2CH_2CH_3$), 26.1 (*C*6), 48.8, 50.9 (N–*C*H_2/*C*5), 55.1, 59.5 (*C*1/*C*2), 63.8, 68.1 (*C*3/*C*4), 73.7 (*C*H_2OH); Anal. $C_{11}H_{23}NO_4$ requires C, 56.63; H, 9.94. Found: C, 56.61; H, 9.95.

3.1.5. *N*-Butyl-1-deoxy-L-*ido*-homonojirimycin (2d). Compound **6b** (0.1 g, 0.194 mmol) was reacted with trifluoroacetic acid–water (3 mL, 2:1), followed by 0.073 g of ammonium formate (1.16 mmol) and 0.05 g of 10% Pd/C as described for the preparation of **2c** affording **2d** (0.042 g, 93%) as a thick liquid; R_f 0.51 (50% chloroform–methanol); $[\alpha]_D$ −16.5 (*c* 1.7, CH_3OH); IR (neat) 3200–3600 (broad band) cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ: 0.72 (3H, t, J = 7.2 Hz, –CH_2CH_3), 1.13 (2H, sextet, J = 7.2 Hz, –CH_2C$H_2$$CH_3$), 1.28 (2H, quintet, J = 7.2 Hz, –CH_2C$H_2$$CH_2$–), 1.58 (2H, dt, J = 10.2, 7.2 Hz, *H*6a,b), 2.27 (1H, dd, J = 12.3, 10.2 Hz, *H*1a), 2.39 (2H, t, J = 7.2 Hz, –NC$H_2$$CH_2$–), 2.63 (1H, dd, J = 12.3, 5.1 Hz, *H*1e), 2.90 (1H, q, J = 10.2, 5.1 Hz, *H*5), 3.28 (1H, t, J = 9.3 Hz, *H*3), 3.43 (1H, ddd, J = 10.2, 9.3, 5.1 Hz, *H*2), 3.50 (2H, t, J = 7.2 Hz, –CH_2OH), 3.53 (1H, dd, J = 9.3, 5.1 Hz, *H*4); ^{13}C NMR(75 MHz, D_2O) δ: 13.2 (–CH_2CH_2*C*H_3), 20.0 (–CH_2*C*H_2CH_3), 24.1 (–*C*$H_2CH_2CH_3$), 28.7 (*C*6), 50.3 (*C*1), 53.0 (N–*C*H_2), 58.4 (*C*5), 61.5 (*C*3), 69.3 (*C*2), 70.7 (*C*7), 74.2 (*C*4); Anal. $C_{11}H_{23}NO_4$ requires C, 56.63; H, 9.94. Found: C, 56.65; H, 9.97.

3.1.6. Tetraacetyl-*N*-butyl-1-deoxy-D-*gluco*-homonojirimycin (2e). To an ice-cooled solution of 0.03 g of **2c** (0.129 mmol) in 0.5 mL of dry pyridine were added 2.65 g of acetic anhydride (26.11 mmol) and 5 mg DMAP. After stirring for 12 h at room temperature, ice water was added and extracted with chloroform (3 × 10 mL). Usual workup and chromatographic purification (*n*-hexane–ethyl acetate 9:1) afforded tetraacetyl derivative **2e** (0.05 g, 96%) as a thick liquid; R_f 0.56 (40% ethyl acetate–*n*-hexane); $[\alpha]_D$ +33.3 (*c* 0.3, $CHCl_3$); IR (Nujol) 1740 (broad) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (3H, t, J = 7.2 Hz, –CH_2CH_3), 1.24–1.36 (2H, m, –CH_2C$H_2$$CH_3$), 1.37–1.54 (2H, m, –C$H_2$$CH_2$$CH_2$–), 1.74–1.94 (2H, m, *H*6a,b), 2.02 (3H, s, –COCH_3), 2.04 (3H, s, –COCH_3), 2.05 (3H, s, –COCH_3), 2.08 (3H, s, –COCH_3), 2.24–2.36 (1H, m, NC*H*1a), 2.42–2.52 (1H, m, H5), 2.60–2.78 (2H, m, NCHb and H1b), 3.25 (1H, dd, J = 11.7, 4.2 Hz, 1H_e), 4.06–4.20 (2H, m, CH_2OAc), 4.94–5.02 (3H, br s, *H*2/*H*3/*H*4); ^{13}C NMR(75 MHz, $CDCl_3$) δ: 14.0 (–CH_2CH_2*C*H_3), 20.4 (–CH_2*C*H_2CH_3), 20.8 (s), 20.9, 21.0 (4 × CO*C*H_3), 26.6 (–*C*$H_2CH_2CH_3$), 28.4 (*C*6), 49.4, 52.1 (*C*1/*C*5), 59.9, 60.8 (*C*H_2OAc and N–*C*H_2), 68.7, 71.2, 75.0 (*C*2/*C*3/*C*4), 170.0, 170.1, 170.4, 171.1 (4 × *C*O); Anal. $C_{19}H_{31}O_8$ requires C, 56.84; H, 7.78. Found: C, 56.86; H, 7.81.

3.1.7. Tetraacetyl-*N*-butyl-1-deoxy-L-*ido*-homonojirimycin (2f). Compound **2d** (0.028 g, 0.12 mmol) was reacted

with 0.5 mL of dry pyridine, 2.56 g of acetic anhydride (26.11 mmol) and 5 mg DMAP as described in the synthesis of **2e** affording **2f** (0.045 g, 94%) as a thick liquid; R_f 0.48 (ethyl acetate–*n*-hexane); $[\alpha]_D$ −33.3 (*c* 0.37, $CHCl_3$); IR (Nujol) 1738 (broad) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 0.94 (3H, t, $J = 7.2$ Hz, $-CH_2CH_3$), 1.52 (2H, sextet, $J = 7.2$ Hz, $-CH_2CH_2CH_3$), 1.43 (2H, quintet, $J = 7.2$ Hz, $-CH_2CH_2CH_2-$), 1.92 (2H, apparent q, $J = 12.9$, 5.4 Hz, *H*6a), 2.06 (3H, s, $-COCH_3$), 2.07 (3H, s, $-COCH_3$), 2.08 (3H, s, $-COCH_3$), 2.09 (3H, s, $-COCH_3$), 2.60–2.78 (3H, m, NCH_2 and H1a), 2.99 (1H, dd, $J = 12.3$, 5.4 Hz, H1e), 3.28 (1H, dd, $J = 12.9$, 5.4 Hz, H5), 4.06–4.22 (2H, m, CH_2OAc), 5.04 (1H, ddd, $J = 10.2$, 9.3, 5.4 Hz, H2), 5.15 (1H, dd, $J = 10.2$, 5.4 Hz, H4), 5.30 (1H, dd, $J = 10.2$, 9.3 Hz, H3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8 ($-CH_2CH_2CH_3$), 19.9 ($-CH_2CH_2CH_3$), 20.7, 20.79 (s), 20.8 ($4 \times COCH_3$), 23.2 ($-CH_2CH_2CH_3$), 30.8 (*C*6), 46.7 ($N-CH_2$), 53.8, 55.5 (*C*1/*C*5), 62.2 (C7), 69.3, 70.2, 71.3 (*C*2/*C*3/*C*4), 169.9, 170.1, 170.2, 170.9 ($4 \times CO$); Anal. $C_{19}H_{31}O_8$ requires C, 56.84; H, 7.78. Found: C, 56.83; H, 7.80.

3.1.8. General procedure for inhibition assay. Inhibition potencies of the *N*-butyl 1-deoxynojirimycin analogues **2c**,**d** were determined by measuring the residual hydrolytic activities of the glycosidases. The substrates (Purchased from Sigma Chemicals Co., USA.) namely *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, and *p*-nitrophenyl-β-D-galactopyranoside, of 2 mM concentration, were prepared in 0.025 M citrate buffer with pH 6.0, *p*-nitrophenyl-α-D-mannopyranoside of 2 mM was prepared in 0.025 M citrate buffer with pH 4.0. The test compound was preincubated with the respective enzyme for 1 h at 37 °C. The enzyme reaction was initiated by the addition of 100 μL substrate. Controls were run simultaneously in absence of test compound. The reaction was terminated at the end of 10 min by the addition of 0.05 M borate buffer (pH 9.8) and absorbance of the liberated *p*-nitrophenol was measured at 405 nm using Shimadzu Spectrophotometer UV-1601. One unit of glycosidase activity is defined as the amount of enzyme that hydrolyzed 1 μmol of *p*-nitrophenyl pyranoside per minute at 25 °C.[15]

Acknowledgments

We are grateful to Prof. M. S. Wadia for helpful discussion. We are thankful to CSIR, New Delhi, for the financial support. S.D.M. and N.S.K. are thankful to CSIR, New Delhi, for the Senior Research Fellowships. We gratefully acknowledge UGC, New Delhi, for the grant to purchase high field (300 MHz) NMR facility.

References and notes

1. (a) Ishida, N.; Kumagai, K.; Niida, T.; Hamamoto, K.; Shomura, T. *J. Antibiot.* **1967**, *20*, 62; (b) Niwa, T.; Inouye, S.; Tsuruoka, T.; Koaze, Y.; Niida, T. *Agric. Biol. Chem.* **1970**, *34*, 966; (c) Koyama, M.; Sakamura, S. *Agric. Biol. Chem.* **1974**, *38*, 1111.
2. Evans, S. V.; Hayman, A. R.; Fellows, L. E.; Shing, T. K. M.; Derome, A. E.; Fleet, G. W. J. *Tetrahedron Lett.* **1985**, *26*, 1465.
3. (a) Anzeveno, P. B.; Creemer, L. J.; Daniel, J. K.; King, C. H. R.; Liu, P. S. *J. Org. Chem.* **1989**, *54*, 2539; (b) Robinson, K. M.; Begovic, M. E.; Rhinehart, B. L.; Heineke, E. W.; Ducep, J. B.; Kastner, P. R.; Marshall, F. N.; Danzin, C. *Diabetes* **1991**, *40*, 825.
4. (a) Spearman, M. A.; Jameson, J. C.; Wright, J. A. *Exp. Cell Res.* **1987**, *168*, 116; (b) Tsukamoto, K. T.; Uno, A.; Shrimada, S.; Imokaw, G. *Clin. Res.* **1989**, *37A*, 722.
5. (a) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. *FEBS Lett.* **1988**, *237*, 128; (b) Khanna, I. K.; Mueller, R. A.; Weier, R. M.; Stealey, M. A. U.S. Patent 5216168, 1993; (c) Karpas, A.; Fleet, G. W.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9229; (d) Montefiori, D. C.; Robinson, W. E.; Mitchel, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9248.
6. (a) Stutz, A. E., Ed.; *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Wiley-VCH: Weinheim, Germany, **1999**; (b) Elbein, A. D. *FASEB J.* **1991**, *5*, 3055–3063; (c) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645.
7. (a) Platt, F. M.; Neises, G. R.; Dwek, R. A.; Butters, T. D. *J. Biol. Chem.* **1994**, *269*, 8362; (b) Block, X.; Lu, X.; Platt, F. M.; Foster, G. R.; Gerlich, W. H.; Blumberg, B. S.; Dwek, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2235.
8. Mann, J.; Davidson, R. S.; Hobbs, J. B.; Banthorpe, D. V.; Harborne, J. B. *Natural Products*; Longman Scientific and Technical: England, 1994, p 442.
9. Rhinehart, B. L.; Robinson, K. M.; Liu, P.; Payne, A. J.; Wheatley, M. E.; Wagner, S. R. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 915.
10. Bayer, A. G. *Drugs Future* **1986**, *11*, 1039.
11. Asano, N.; Nishida, M.; Kato, A.; Kizu, H.; Matsui, K.; Shimada, Y.; Itoh, T.; Baba, M.; Watson, A. A.; Nash, R. J.; Lilley, P. M. Q.; Watkin, D. J.; Fleet, G. W. J. *J. Med. Chem.* **1998**, *41*, 2565.
12. (a) Dhavale, D. D.; Desai, V. N.; Sindkhedkar, M. D.; Mali, R. S.; Castellari, C.; Trombini, C. *Tetrahedron: Asymmetry* **1997**, *9*, 1475; (b) Dhavale, D. D.; Saha, N. N.; Desai, V. N. . *J. Org. Chem.* **1997**, *62*, 7482; (c) Dhavale, D. D.; Desai, V. N.; Saha, N. N. *J. Chem. Soc., Chem. Commun.* **1999**, 1719; (d) Patil, N. T.; Tilekar, J. N.; Dhavale, D. D. *J. Org. Chem.* **2001**, *66*, 1065; (e) Saha, N. N.; Desai, V. N.; Dhavale, D. D. *Tetrahedron* **2001**, *57*, 39; (f) Patil, N. T.; Tilekar, J. N.; Dhavale, D. D. *Tetrahedron Lett.* **2001**, *42*, 747; (g) Patil, N. T.; John, S.; Sabharwal, S. G.; Dhavale, D. D. *Bioorg. Med. Chem.* **2002**, *10*, 2155; (h) Dhavale, D. D.; Desai, V. N.; Saha, N. N.; Tilekar, J. N. *Arkivoc* **2002**, 91; (i) Dhavale, D. D.; Matin, M. M.; Sharma, T.; Sabharwal, S. G. *Bioorg. Med. Chem.* **2003**, *11*, 3295; (j) Dhavale, D. D.; Matin, M. M. *Tetrahedron* **2004**, *60*, 4275; (k) Dhavale, D. D.; Markad, S. D.; Karanjule, N. S.; Prakashareddi, J. *J. Org. Chem.* **2004**, *69*, 4760; (l) Dhavale, D. D.; Matin, M. M.; Sharma, T.; Sabharwal, S. G. *Bioorg. Med. Chem.* **2004**, *12*, 4039.
13. Cornia, M.; Casiraghi, G. *Tetrahedron* **1989**, *45*, 2869.
14. Durantel, D.; Branza-Nichita, N.; Carrouee-Durantel, S.; Butters, T. D.; Dwek, R. A.; Zitzman, N. *J. Virol.* **2001**, *75*, 8987.
15. Li, Y.-T.; Li, S.-C. In *Methods in Enzymology*; Ginsberg, Victor, Ed.; Academic, 1972; p 702.