JOC*Article*

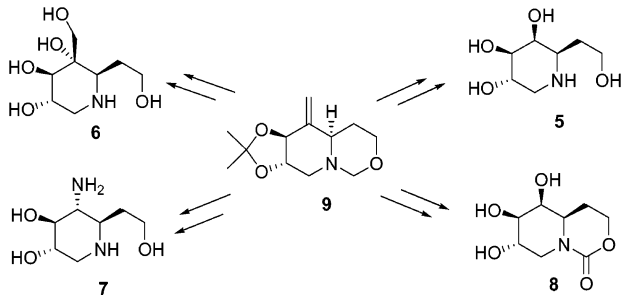
Convergent Approach toward the Synthesis of the Stereoisomers of C-6 Homologues of 1-Deoxynojirimycin and Their Analogues: Evaluation as Specific Glycosidase Inhibitors

Ganesh Pandey,*[,†] Shrinivas G. Dumbre,[†] M. Islam Khan,[‡] and M. Shabab[‡]

Division of Organic Chemistry (Synthesis) and Division of Biochemical Science, National Chemical Laboratory, Pune-411008, India

gp.pandey@ncl.res.in

Received July 13, 2006



A new and stereoselective strategy is developed to synthesize an appropriate template **9** to obtain C-6 homologues of 1-deoxyazasugars such as 1-deoxy-D-galactohomonojirimycin (**5**), 1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin (**6**), and their enantiomers. The template **9** is also used to obtain neutral nonbasic pseudo-glyconolactam (**8**), C-4 amino, and methyl analogues of 1-deoxy-homonojirimycin as new analogues of 1-deoxyhomoazasugars. Compound **5** is found to be a potent and specific inhibitor to α-galactosidase ($K_i = 1.7$ μM). Similarly compounds **6** ($K_i = 28$ μM), *ent*-**5** ($K_i = 129$ μM), and *ent*-**6** ($K_i = 12$ μM) exhibited specific inhibition of β-glucosidase.

Introduction

Recent years have witnessed an increasing interest in synthetic as well as naturally occurring azasugars (1-*N*-iminosugars or azahexoses[1]) due to their use as tools for studying the biological functions of oligosaccharides and emerging therapeutic potentials[2] for a variety of carbohydrate mediated diseases such as HIV,[3] diabetes,[4] hepatitis,[5] cancer,[4,6] Gaucher's disease,[7] and viral infections such as influenza.[8] Immediately after unraveling the potential of nojirimycin (NJ, **1**) as a strong α- and β-glucosidase inhibitor,[9] its 1-deoxy analogues such as 1-deoxynojirimycin (**2**, DNJ) and 1-deoxygalactonojirimycin (**3b**, DGJ), due to enhanced stability, attracted the attention of synthetic

† Division of Organic Chemistry (Synthesis).

‡ Division of Biochemical Science.

(1) McNaught, A. D. *Pure Appl. Chem.* **1996**, *68*, 1919.

(2) (a) Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265. (b) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Curr. Top. Med. Chem.* **2003**, *3*, 561. (c) Asano, N. *Glycobiology* **2003**, *13*. (d) Greimel, P.; Spreitz, J.; Stütz, A. E.; Wrodnigg, T. M. *Curr. Top. Med. Chem.* **2003**, *3*, 513.

(3) (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) Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; deGoede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedema, F.; Ploegh, H. L. *Nature* **1987**, *330*, 74. (c) Ratner, L.; Heyden, N. V.; Dedera, D. *Virology* **1991**, *181*, 180. (d) Bridges, G.; Ahmed, S. P.; Kang, M. S.; Nash, R. J.; Porter, E. A.; Tyms, A. S. *Glycobiology* **1995**, *5*, 243

(4) (a) Balfour, J. A.; McTavish, D. *Drugs* **1993**, *46*, 1025. (b) Bollen, M.; Stalmans, W. *Eur. J. Biochem.* **1989**, *181*, 775 (c) Taylor, R. H.; Barker, H. M.; Bowey, E. A.; Canfield, J. E. *Gut* **1986**, *27*, 1471. (d) Anzeveno, P. B.; Creemer, L. J.; Daniel, J. K.; King, C. H. R.; Liu, P. S. *J. Org. Chem.* **1989**, *54*, 2539.

(5) Zitzmann, N.; Mehta, A. S.; Carroue'e, S.; Butters, T. D.; Platt, F. M.; McCauley, J.; Blumberg, B. S.; Dwek, R. A.; Block, T. M. *PNAS* **1999**, *96*, 11878.

(6) (a) Ostrander, G. K.; Scibner, N. K.; Rohrschneider, L. R. *Cancer Res.* **1988**, *48*, 1091. (b) Gross, P. E.; Baptiste, J.; Fernandes, B.; Baker, M.; Dennis, J. W. *Cancer Res.* **1994**, *54*, 1450.

(7) (a) Kolter, T. *Angew. Chem., Int. Ed.* **1997**, *36*, 1955. (b) Asano, N.; Ishii, S.; Kizu, H.; Ikeda, K.; Yasuda, K.; Kato, A.; Martin, O. R.; Fan, J. Q. *Eur. J. Biochem.* **2000**, *267*, 4179 (c) Jeyakumar, M.; Butters, T. D.; Dwek, R. A.; Platt, F. M. *Neuropath. Appl. Neurobiol.* **2002**, *28*, 343. (d) Platt, F. M.; Neises, G. R.; Reinkensmeier, G.; Townsend, M. J.; Perry, V. H.; Proia, R. L.; Winchester, B.; Dwek, R. A.; Butters, T. D. *Science* **1997**, *276*, 428.

(8) Laver, W. G.; Bischofberger, N.; Webster, R. G. *Sci. Am.* **1999**, *Jan*, 78.

10.1021/jo061455v CCC: $33.50
Published on Web 09/26/2006

chemists.[10] Interestingly, intense research activities in this field have culminated in the launch of two new azasugar medicines such as Miglitol (*N*-hydroxethyl DNJ, Glyset or Diastabol)[11] for the treatment of type II diabetes and Miglustat (*N*-*n*Bu DNJ, Zavesca)[12] for the treatment of Gaucher's disease. In the search for the structure−activity relationship, strong synthetic efforts have also been directed toward the synthesis of homoazasugars with (CH_2)-homologation at C-6 of such 1-deoxyazasugars. The major challenge in designing a synthetic route for these types of azasugars has been the preparation of suitably polyhydroxylated piperidine moiety having a chiral alkyl group at C-5. In this context, the strategy involving intermolecular as well as intramolecular 1,4-conjugate addition of an amine to an α,β-unsaturated carbonyl group, derived from appropriate sugar substrates, has either resulted in poor yield[13] or poor diastereoselectivity.[14] Similarly, Szolcsányi's approach, which utilized Pd(II)-catalyzed aminocarbonylation of an amine tethered allylic alcohol, derived from D-glucose, for the synthesis of 1-deoxy-D-homonojirimycin and 1-deoxy-L-homoidonojirimycin, has resulted in poor diastereoselectivity.[15] Herdeis et al.[16] utilized crucial intramolecular tandem Wittig azide-olefin cycloaddition, though, in moderate yield as a key step for the synthesis of D-tallo and L-allo homo-1-deoxysugars. Another strategy utilizes optically active allenylstannane for the synthesis of 1-deoxy-D-galactohomonojirimycin (**5**); however, the use of toxic tin reagent in the key step limits its application.[17] Beyond the synthetic challenges, it was surprising to note that none of these studies have attempted the evaluation of the inhibitory potencies of synthesized C-6 homologues of 1-deoxyazasugars.Considering the unexplored potential of C-6 homologues of 1-deoxyazasugars as glycosidase inhibitors, we ventured into developing an entirely new and versatile strategy for the synthesis of C-6 homologues of 1-deoxyazasugars. We report herein the synthesis and glycosidase inhibition study of 1-deoxy-D-galactohomonojirimycin (**5**), 1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin (**6**), and related analogues **7** and **8** using template **9** through the retrosynthetic approach as depicted in Scheme 1. The key synthetic design for the template **9** involved cyclization of α-trimethylsilylmethylamine radical cation generated via photoinduced electron transfer (PET) reaction to the tethered π-functionality, a strategy developed earlier by our group.[18]

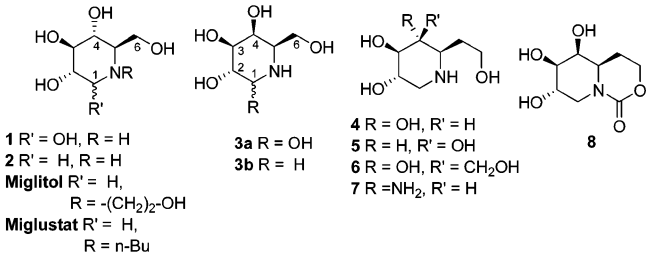
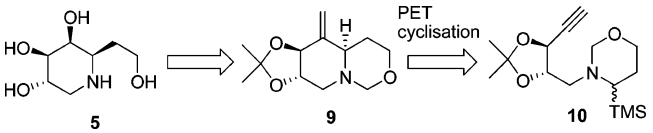
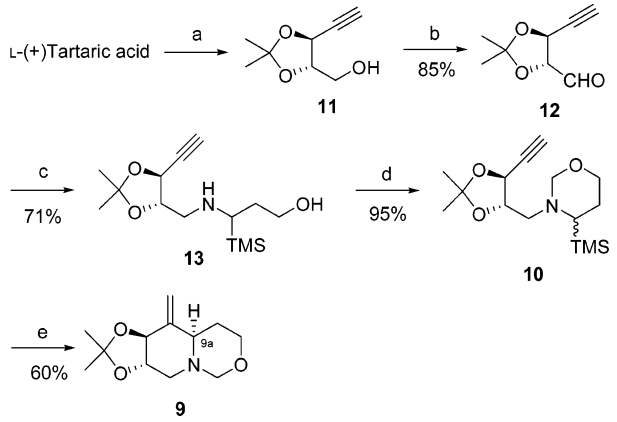


FIGURE 1. Nojirimycin and analogues.

SCHEME 1. Retroanalysis



SCHEME 2. Synthesis of Acetylene Tethered Cyclic Amine 10 and Its PET Cyclization[a]



[a] Reagents and conditions: (a) ref 21a; (b) IBX, EtOAc, reflux, 9 h; (c) **16**, $NaBH(OAc)_3$, DCE, rt, 12 h; (d) $(CH_2O)_n$, benzene, reflux, 4 h; (e) $h\nu$, DCN, CH_3CN:2-PrOH (3:1), 4 h.

Results and Discussion

Synthesis of Both Enantiomers of 1-Deoxygalactohomonojirimycin. We began our synthesis first by synthesizing the acetylene tethered amine **13** (71% yield) by coupling components **12** and **16** via reductive amination using sodium triacetoxyborohydride as a reducing agent[19] as shown in Scheme 2. The aldehyde **12** was obtained by the IBX oxidation[20] of alcohol **11**, prepared from the L-(+)-tartaric acid by following the reported procedure.[21a] The preparation of 3-amino-3-(trimethylsilyl)propan-1-ol (**16**) is depicted in Scheme 3.[22]

Since our PET cyclization strategy[18] required *N*-alkylated α-trimethylsilylmethylamine moiety, we transformed **13** into the

(9) (a) Niwa, T.; Inouye, S.; Tsuruoka, T.; Koaze, Y.; Niida, T. *Agric. Biol. Chem.* **1970**, *34*, 966. (b) Legler, G.; Julich, E. *Carbohydr. Res.* **1984**, *128*, 61. (c) Stütz, A. E. *Iminosugars as glycosidase inhibitors*: *nojirimycin and beyond*; Wiley-VCH: Weinheim, Germany, 1999.

(10) (a) Pearson, M. S. M.; Mathé-Allainmat, M.; Fargeas, V.; Lebreton, J. *Eur. J. Org. Chem.* **2005**, 2159. (b) Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036. (c) Afarinkia, K.; Bahar, A. *Tetrahedron*: *Asymmetry* **2005**, *16*, 1239.

(11) (a) Mitrakou, A.; Tountas, N.; Raptis, A. E.; Bauer, R. J.; Schulz, H.; Raptis, S. A. *Diabetic Med.* **1998**, *15*, 657. (b) Scott, L. J.; Spencer, C. M. *Drugs* **2000**, *59*, 521.

(12) (a) Sorbera, L. A.; Castañer, J.; Bayés, M. *Drugs Fut.* **2003**, *28*, 229. (b) McCormack, P. L.; Goa, K. L. *Drugs* **2003**, *63*, 2427. (c) Cox, T. M.; Aerts, J. M. F. G.; Andria, G.; Beck, M.; Belmatoug, N.; Bembi, B.; Chertkoff, R.; Vom Dahl, S.; Elstein, D.; Erikson, A.; Giralt, M.; Heitner, R.; Hollak, C.; Hrebicek, M.; Lewis, S.; Mehta, A.; Pastores, G. M.; Rolfs, A.; Sa Miranda, M. C.; Zimran, A. *J. Inherited Metab. Dis.* **2003**, *26*, 513.

(13) Kilonda, A.; Compernolle, F.; Toppet, S.; Hoornaert, G. J. *Tetrahedron Lett.* **1994**, *35*, 9047.

(14) (a) Saha, N. N.; Desai, V. N.; Dhavale, D. D. *Tetrahedron* **2001**, *57*, 39. (b) Patil, N. T.; Tilekar, J. N.; Dhavale, D. D. *J. Org. Chem.* **2001**, *66*, 1065. (c) Desai, V. N.; Saha, N. N.; Dhavale, D. D. *Chem. Commun.* **1999**, 1719−1720. (d) Dhavale, D. D.; Matin, M. M.; Sharma, T.; Sabharwal, S. G. *Bioorg. Med. Chem.* **2003**, *11*, 3295.

(15) (a) Szolcsányi, P.; Gracza, T.; Koman, M.; Prónayová, N.; Liptaj, T. *Tetrahedron*: *Asymmetry* **2000**, *11*, 2579. (b) Szolcsányi, P.; Gracza, T.; Koman, M.; Prónayová, N.; Liptaj, T. *Chem. Commun.* **2000**, 471.

(16) Herdeis, C.; Schiffer, T. *Tetrahedron* **1996**, *52*, 14745.

(17) Achmatowicz, M.; Hegedus, L. S. *J. Org. Chem.* **2004**, *69*, 2229.

(18) (a) Pandey, G.; Reddy, G. D.; Kumaraswamy, G. *Tetrahedron* **1994**, *50*, 8185. (b) Pandey, G.; Kumaraswamy, G.; Bhalerao, U. T. *Tetrahedron Lett.* **1989**, *30*, 6059.

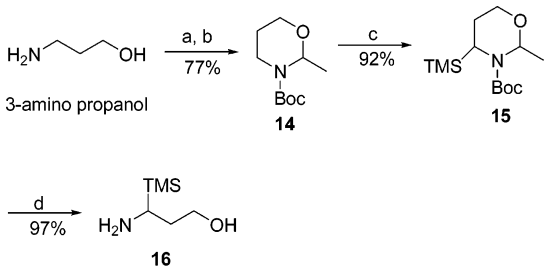
(19) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849

(20) More, J. D.; Finney, N. S. *Org. Lett.* **2002**, *4*, 3001.

(21) (a) Pandey, G.; Kapur, M. *Synthesis* **2001**, 1263. (b) Pandey, G.; Kapur, M.; Islam Khan, M.; Gaikwad, S. M. *Org. Biomol. Chem.* **2003**, *1*, 3321.

(22) Pandey, G.; Banerjee, P.; Kumar, R.; Puranik, V. G. *Org. Lett.* **2005**, *7*, 3713.

SCHEME 3. Synthesis of 3-Amino-3-(trimethylsilyl)-propan-1-ol (16)[a]



[a] Reagents and conditions: (a) $(Boc)_2O$, TEA, DCM, 0 °C to rt, 24 h; (b) $CH_3CH(OEt)_2$, PPTS, benzene, reflux, 24 h; (c) *s*BuLi, TMEDA, THF, 3 h, then TMSCl −78 °C to rt over 3 h; (d) 2 N HCl, dioxane, 80 °C, 45 min.

cyclic 1,3-oxazine derivative **10** by refluxing with paraformaldehyde. The thought of utilizing the open chain *N*-alkylated derivative of **13** for PET cyclization was abandoned considering our previous experience of poor diastereoselectivity.[18a] The cyclization of **10** was carried out by irradiating a dilute solution of **10** (3 mmol) and 1,4-dicyanonaphthalene (0.4 mmol) in a mixture of acetonitrile:2-propanol (3:1, 250 mL) in a Pyrex vessel, using 450-W Hanovia medium-pressure lamp. Usual workup and purification of the photolyzate gave **9** as a single diastereomer in 60% yield (Scheme 2). The cyclized product **9** was fully characterized by extensive ^{1}H NMR, ^{13}C NMR, and ^{1}H−^{1}H 2D spectral analyses.

Dihydroxylation of **9** with OsO_4 produced **17** in 90% yield. Single-crystal X-ray diffraction analysis unequivocally confirmed the stereochemistry of **17** at C_{10} and C_{9a}.[23] Sodium periodate oxidation of **17** afforded corresponding ketone which on sodium borohydride reduction provided **18** in 85% yield as an exclusive diastereomer (Scheme 4). The stereochemistry of **18** was also confirmed from ^{1}H and COSY NMR of the corresponding benzylated derivative **19**. Stereochemistry at C_{10} of **19** was ascertained by analyzing the coupling constants for H_{3a} (δ 4.24, dt, J = 4.4, 9.8 Hz), H_{10a} (δ 3.40, dd, J = 2.2, 9.3 Hz), H_{10} (δ 3.84, t, J = 2 Hz), and H_{9a} (δ 2.24, t, J = 3.3 Hz), which suggested the orientations for H_{3a}-axial, H_{10a}-axial, H_{10}-equatorial, and H_{9a}-axial, respectively. This stereochemical analysis was further confirmed by X-ray crystallography.[23] The acetonide and 1,3-oxazine ring moieties of **18** were removed by refluxing with 6 N HCl in dioxane−methanol for 12 h to obtain 1-deoxy-D-galactohomonojirimycin **5** in 95% yield.[24]

In a similar manner starting from D-(−)-tartaric acid, 1-deoxy-L-galactohomonojirimycin **21** (*ent*-**5**) was also synthesized.

Synthesis of Some Other Analogues of 1-Deoxyhomoazasugars. Having developed a novel strategy to obtain **17** in sufficiently good amount, it was visualized that removal of all the protecting groups from it would provide a new molecule that would have all the required structural features of an azasugar. Therefore, all the protecting groups from **17** (and *ent*-**17**) were removed by refluxing with 6 N HCl in dioxane−methanol (1:1) to obtain 1-deoxy-4-hydroxymethyl-D-glucohomonojirimycin (**6**) and 1-deoxy-4-hydroxymethyl-L-glucohomonojirimycin (*ent*-**6**, **20**), respectively, in 95% yield.

Generally NJ (**1**), DNJ (**2**), and their analogues are believed to exhibit their glycosidase inhibitory activities due to their binding with the glycosidases by mimicking the shape and charge of the postulated oxo-carbenium ion intermediate for the glycosidic bond cleavage reaction.[25] However, there has been intense interest, recently, in evaluating nonbasic neutral glyconolactams such as **22**,[26] **23**,[27] and **24**[28] as glycosidase inhibitors (Figure 2) and a considerable number of inhibitory activities have been recorded. These examples, where glycosidic oxygen is replaced by pseudo sp^2 ring nitrogen (e.g. **22**, K_i = 85 μM, β-glucosidase), mechanistically were originally believed to inhibit glycosidases by involving tautomeric iminol form. However, recent studies suggest that the glycosidase inhibition by these compounds may in fact be caused by the H-bonding of the lactam carbonyl moiety[29] with the enzyme as the tautomerization energy for the amide−iminol conversion[30] is of the order of 11 kcal mol^{-1} indicating the concentration of the corresponding iminol form in solution at a given time is very low.

In this context, we also envisioned that a hitherto unknown nonbasic neutral molecule **8** could easily be realized from **19** for evaluation as a new glycosidase inhibitor. Toward this end, the acetonide as well as 1,3-oxazine ring moiety of **19** were deprotected and protection of the resultant secondary amine as the *N*-Boc derivative gave **25** in 78% yield. Mesylation of the primary alcoholic moiety of **25** followed by reflux under basic conditions in acetonitrile[31] and debenzylation gave compound **8** in 85% yield (Scheme 5).

In continuation, another new analogue **7** having a basic amine moiety at C-4 was also visualized to be easily affordable from **18** for evaluation as glycosidase inhibitor**.** To this end alcohol **18** was first converted to corresponding mesylate derivative **27**, which on nucleophilic displacement with azide, followed by catalytic hydrogenation and all deprotection afforded **7** in 71% yield (Scheme 5).

Since we had earlier observed that 1-*N*-iminosugar **30** showed better inhibitory activity for β-glucosidase (K_i = 30 μM) than **29** (K_i = 90 μM),[21b] it also occurred to us that it would be pertinent to evaluate the enzyme inhibition activity of **31** too (Figure 3). In this context, we synthesized compound **31** by following the analogous route as described for **10** starting from alcohol **32**[21b] as shown in Scheme 6. The stereochemistry at C_{10} and C_{9a} of **31** was ascertained by analyzing the coupling constants for H_{3a} (δ 3.57, ddd, J = 4.1, 7.4, 9.5 Hz) and H_{10a} (δ 2.96, dd, J = 8.7, 10.5 Hz) and by ^{1}H−^{1}H NOESY spectrum.[32] The removal of the acetonide and 1,3-oxazine ring moiety from **34** gave **31** in 95% yield.

(23) CCDC-607587 and -607588 contains the supplementary crystallographic data for compounds **17** and **19**, respectively. These data can be obtained free of charge from the Cambridge Crystallographic data centre via http://www.ccdc.ac.uk/data_request/cif. ORTEP diagram of compounds **17** and **19** are given in the Supporting Information.

(24) The similar strategy as described for 1-deoxyhomonojirimycin in this paper is being carried out in our group toward the synthesis of 1-deoxynojirimycin starting from a PET precursor containing 4-(timethylsilyl)oxazolidine moiety.

(25) Sears, P.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2300.

(26) (a) Nishimura, Y.; Adachi, H.; Satosh, T.; Shitara, E.; Nakamura, H.; Kojima, F.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 4871. (b) Lillelund, V. H.; Liu, H.; Liang, X.; Søhoel, H.; Bols, M. *Org. Biomol. Chem.* **2003**, *1*, 282.

(27) García-Moreno, M. I.; Rodríguez-Lucena, D.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2004**, *69*, 3578.

(28) Díaz Pérez, P.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M. *Eur. J. Org. Chem.* **2005**, 2903.

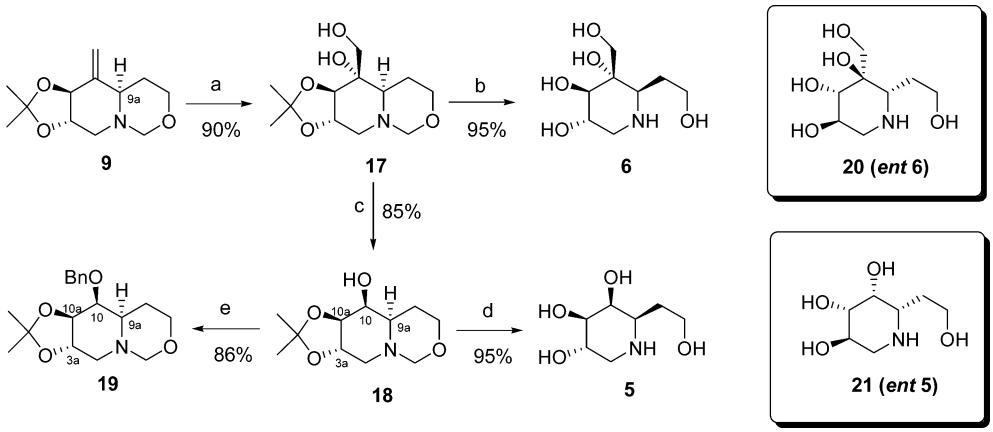
(29) Williams, S. J.; Notenboom, V.; Wicki, J.; Rose, D. R.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 4229.

(30) Sygula, A. *J. Chem. Res.* **1989**, 56.

(31) Campanini, L.; Dureault, A.; Depezay, J. C. *Tetrahedron Lett.* **1995**, *36*, 8015.

(32) Hydrogenation of **9** gave the diastereomeric mixture of **31**.

SCHEME 4. Synthesis of 1-Deoxy-D-galactohomonojirimycin (5) and 1-Deoxy-4-hydroxymethyl-D-glucohomonojirimycin (6)[a]



[a] Reagents and conditions: (a) OsO_4, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/water (1:1), rt, 16 h; (b) 6 N HCl, dioxane−MeOH, reflux, 12 h; (c) (i) $NaIO_4$, silica gel, DCM, 10 min, (ii) $NaBH_4$, MeOH, rt, 6 h; (d) 6 N HCl, dioxane−MeOH, reflux, 12 h; (e) BnBr, NaH, THF, reflux 10 h.

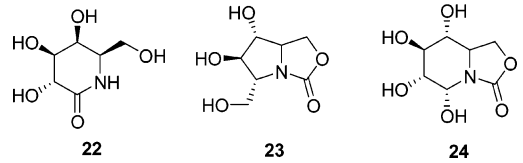
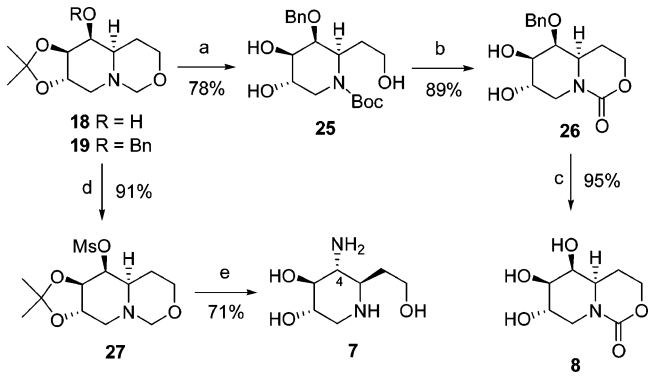


FIGURE 2. Nonbasic azasugars.

SCHEME 5. Synthesis of 7 and 8[a]



[a] Reagents and conditions: (a) (i) 6 N HCl, dioxane−MeOH, reflux, 16 h; (ii) $(Boc)_2O$, TEA, DCM, 8 h; (b) (i) MsCl, TEA, DCM, 0 °C, 10 min, (ii) K_2CO_3, CH_3CN, reflux, 6 h; (c) H_2, Pd/C, EtOH, 9 h; (d) MsCl, py, rt, 4 h; (e) (i) LiN_3, DMF, 110 °C, 16 h, (ii) H_2, Pd/C, MeOH, 7 h, (iii) 6 N HCl, MeOH, reflux, 18 h.

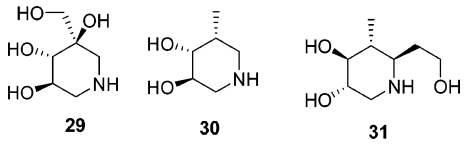
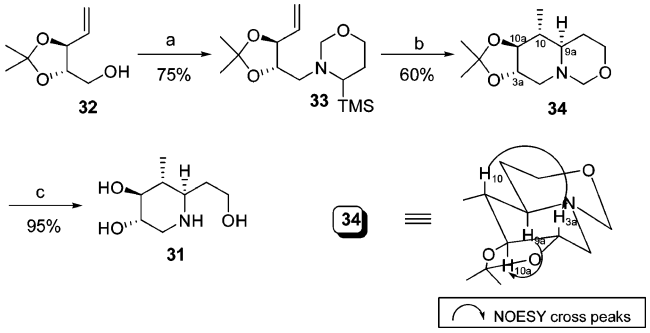


FIGURE 3. Structural comparison between DNJ-type iminosugar and 1-*N*-iminosugar.

Enzyme Inhibition Study. The inhibitory activities of **5**, **6**, **7**, **8**, **20**, **21**, and **31** were screened against *β*-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), *β*-glucosidase/*β*-mannosidase (almonds), α-glucosidase (yeast), and α-mannosidase (jack beans). The results are summarized in the Table 1.

None of the prepared compounds inhibited α- and *β*-mannosidases. The compounds **7** and **31** in which C-4 hydroxy functionality of 1-deoxy-D-glucohomonojirimycin is replaced by an amino group and methyl, respectively, showed no inhibition against any of the enzymes under study. In the case of **7**, the noninhibitory activity could be attributed to the more basic amino group at the C-4 position than the ring nitrogen thereby forbidding it from binding to the active site of α-/*β*-glucosidase in correct orientation. Similarly, inactivity of **31** in comparison to **30** may be correlated to their differences in structural class. Compound **31** is a structural analogue of DNJ-

SCHEME 6. Synthesis of (3*S*,4*S*,5*R*,6*R*)-6-(2-Hydroxyethyl)-5-methylpiperidine-3,4-diol (31)[a]



[a] Reagents and conditions: (a) (i) IBX, EtOAc, reflux, 9 h, (ii) **16**, $NaBH(OAc)_3$, DCE, (iii) $(CH_2O)_n$, benzene, reflux; (b) *hν*, DCN, CH_3CN: 2-PrOH (3:1), 4 h; (c) 6 N HCl, dioxane−MeOH, reflux, 16 h.

TABLE 1. Inhibition (K_i in μM) of Various Glycosidases by 5, 6, 7, 8, 20, 21, and 31[a]

	glycosidase					
inhibitor	*β*-Gal	α-Gal	*β*-Glc	α-Glc	*β*-Man	α-Man
1-DNJ (**2**)[b]	−	−	47	25	−	270
1-DGJ (**3b**)[c]	0.16	0.0016	540	1000	−	−
5	1100	1.7	n.i.	n.i.	n.i.	n.i.
6	1000	8%[d]	28	n.i.	n.i.	n.i.
7	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
8	1900	800	n.i.	n.i.	n.i.	n.i.
20	n.i.	10%[e]	12	n.i.	n.i.	n.i.
21	n.i.	n.i.	129	n.i.	n.i.	n.i.
31	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

[a] The enzyme inhibition study was carried out under similar conditions as reported in ref 34 and the same is detailed in the Supporting Information; n.i., no inhibition at 1 mM; −, not measured; [b] The data for 1-DNJ are from ref 33. [c] The data for 1-DGJ are from ref 34. [d] Percent inhibition at 0.6 mM; [e] Percent inhibition at 1 mM.

type azasugar whereas **30** represents the 1-*N*-iminosugar class.[35] Therefore, on the basis of our observations it suggests the necessity of a C-4 hydroxy functionality for DNJ-type azasugars for the substrate−enzyme interactions. Compound **6 (**D-gluco configured) exhibited anomer specificity for β-glucosidase (K_i = 28 μM) as it showed no inhibition against α-glucosidase and weak inhibition for β-galactosidase (K_i = 1000 μM). The corresponding enantiomer **20** exhibited strong inhibition against β-glucosidase (K_i = 12 μM) but no inhibition against any other enzyme studied. 1-Deoxy-D-galactohomonojirimycin (**5)** exhibited potent inhibition (K_i = 1.7 μM) against α-galactosidase and 650 times weak inhibition (K_i = 1100 μM) against β-galactosidase, whereas its corresponding enantiomer **21** (L-galacto/L-fuco-configured) showed moderate (K_i = 129 μM) inhibition against β-glucosidase and no inhibition against any of the enzyme under study. Compound **8** with pseudoamide type nitrogen exhibited weaker inhibition against both α- and β-galactosidases.

In conclusion, we have developed a general synthetic strategy to access stereoisomers of the C-6 homologue of 1-deoxynojirimycin. Some of the synthesized molecules such as **5**, **6**, **20**, and **21** exhibited enzyme specific inhibition against glycosidases.

Experimental Section

3-{[(4*S*,5*S*)-5-Ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl]methylamino}-3-(trimethylsilyl)propan-1-ol (13). To a solution of alcohol **11** (6.23 g, 39.93 mmol) in ethyl acetate (250 mL) was added IBX (19 g, 67.89 mmol). The resulting suspension was immersed in an oil bath set at 80 °C and stirred vigorously open to the atmosphere. After 9 h (GC monitoring), the reaction was cooled to room temperature and filtered through a filter paper. The filter cake was washed with 3 × 100 mL of ethyl acetate and combined filtrates were concentrated to yield 5.8 g of **12** (85% yield, >85% pure by GC). The aldehyde **12** was found to be unstable and used immediately for the next step. To the solution of this aldehyde in dry 1,2-dichloroethane (180 mL) was added amine **16** (6.45 g, 43.92 mmol) followed by sodium triacetoxyborohydride (16.62 g, 78.78 mmol). The mixture was stirred at room temperature under argon atmosphere for 12 h. The reaction mixture was ice cooled and quenched by adding 1 N NaOH until the aqueous layer was basic and stirred for additional 3 h. The reaction mixture was extracted with ethyl acetate (2 × 100 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary evaporation and the residue was purified by column chromatography (silica, petroleum ether−ethyl acetate, 7:3) to afford a 1:1 diastereomeric mixture of **13** (8.2 g, 71%) as a colorless oil. IR (neat) υ 3404, 3309, 2987, 2950, 2900, 2119, 1662, 1456, 1380, 1373, 1249 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.98 (dd, 1H J = 6.2, 13.2 Hz), 1.32 (s, 3H), 1.36 (s, 3H), 1.45−1.85 (m, 2H), 2.19 (dt, 1H, J = 3.8, 8.6 Hz), 2.47 (d, 1H, J = 1.6 Hz), 2.60−3.00 (m, 2H), 3.68−3.82 (m, 3H), 4.00−4.13 (m, 1H), 4.27 + 4.35 (dd, 1H, J = 2.0, 7.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ −2.8 (CH_3), 25.9 + 26.0 (C-*Me*$_2$), 26.7 + 26.9 (C-*Me*$_2$), 31.1 + 31.3 (CH_2), 48.8 + 49.8 (CH), 49.6 + 51.5 (CH_2), 64.0 + 64.4 (CH_2), 67.6 + 68.2 (CH), 74.8 (CH), 80.5 + 81.1 (CH), 80.5 (C), 110.4 + 110.6 (C); MS 286 (MH^+). Anal. Calcd for $C_{14}H_{27}NO_3Si$: C, 58.91; H, 9.53; N, 4.91. Found: C, 58.65; H, 9.62; N, 4.81.

3-{[(4*S*,5*S*)-5-Ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}-4-(trimethylsilyl)-1,3-oxazinane (10). Paraformaldehyde (1.16 g, 38.94 mmol) and **13** (9.2 g, 32.45 mmol) in benzene (70 mL) were refluxed for 4 h under Dean−Stark condition. The reaction mixture was cooled and benzene was removed under reduced pressure. The crude mixture upon column chromatography (silica, petroleum ether−ethyl acetate, 9:1) afforded pure **10** (9.17 g, 95%) as a colorless liquid. IR (neat) υ 3309, 3265, 2985, 2952, 2858, 2360, 1739, 1458, 1380, 1375, 1249, 1076 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.10 (two s, 9H), 1.35−1.50 (three peaks 3:1.5:1.5, 6H), 1.55−1.65 (br s, 1H), 1.75−1.85 (m, 1H), 2.53 (two d, 1H, J = 2 Hz), 2.60 (ddd, 1H, J = 3.9, 9.3, 13.2 Hz), 2.71−2.81 (two dd, 1H, J = 7.6, 13.7 Hz and J = 3.8, 13.7 Hz), 3.23−3.39 (two dd, 1H, J = 7.0, 13.7 Hz and J = 3.0, 13.7 Hz), 3.73−3.82 (m, 1H), 3.95−4.05 (m, 1H), 4.12−4.22 (m, 1H), 4.28−4.36 (m, 2H), 4.62 (app t, 1H, J = 9.9, 10.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ −1.9 (CH_3), 22.2 (CH_2), 25.7 + 26.2 (CH_3), 26.9 + 27.2 (CH_3), 51.6 + 52.1 (CH_2), 52.7 + 53.0 (CH), 68.2 + 68.3 (CH_2), 68.4 + 68.5 (CH), 74.5 + 74.7 (CH), 80.7 + 82.5 (CH), 81.0 (C), 84.3 + 85.2 (CH_2), 110.5 + 110.7 (C);. GC-MS (*m*/*z* %) 297 (M^+, 0.5%), 224 (80%), 166 (20%), 100 (65%), 73 (100%). Anal. Calcd for $C_{15}H_{27}NO_3Si$: C, 60.57; H, 9.15; N, 4.71. Found: C, 60.62; H, 9.25; N, 4.55.

(3a*S*,9a*R*,10a*S*)-2,2-Dimethyl-10-methylenehexahydro-4*H*-[1,3]dioxolo[4,5]pyrido[1,2-*c*][1,3]oxazine (9). A solution containing **10** (1.0 g, 3.3 mmol) and 1,4-dicyanonaphthalene (0.12 g, 0.67 mmol) in acetonitrile:2-propanol (3:1, 250 mL) mixture was irradiated in an open vessel with use of a 450-W Hanovia medium pressure mercury vapor lamp. The lamp was immersed in a Pyrex water-jacketed immersion well to allow only wavelengths greater than 280 nm to pass through. After about 4 h of irradiation, the consumption of the starting material was found to be almost complete (monitored by GC) and at this stage the irradiation was discontinued. The solvent was removed under reduced pressure and the residue was column chromatographed (silica, petroleum ether−acetone, 9:1) to afford cyclized product **9** (0.450 g, 60%) as a yellow liquid. $[\alpha]^{27}_D$ +52.4 (*c* 0.44, $CHCl_3$), *ent*-**9** $[\alpha]^{27}_D$ −44.3 (*c* 1.08, CH_2Cl_2); IR (neat) υ 3097, 3053, 2985, 2931, 2846, 2732, 1627, 1456, 1371, 1226 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48 (s, 3H), 1.50 (s, 3H), 1.74 (dd, 1H, J = 1.6, 13.2 Hz), 1.90−2.00 (m, 1H), 2.33 (t, 1H, J = 9.9 Hz), 2.66 (br d, 1H, J = 10.7 Hz), 3.19 (dd, 1H, J = 4.0, 9.4 Hz), 3.52−3.60 (m, 2H), 3.80 (td, 1H, J = 1.9, 9.1 Hz), 3.86 (d, 1H, J = 8.0 Hz), 4.17 (dd, 1H, J = 4.8, 11.4 Hz), 4.48 (d, 1H, J = 8.0 Hz), 4.88 (t, 1H, J = 1.7 Hz), 5.14 (t, 1H, J = 1.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.6 (CH_3), 26.8 (CH_3), 27.7 (CH_2), 51.2 (CH_2), 60.4 (CH), 67.1 (CH_2), 76.5 (CH), 81.5 (CH), 86.5 (CH_2), 103.6 (CH_2), 111.0 (C), 142.7 (C); GC-MS (*m*/*z* %) 225 (M^+, 5%), 196 (35%), 167 (100%), 149 (35%), 81 (90%). Anal. Calcd for $C_{12}H_{19}NO_3$: C, 63.98; H, 8.50; N, 6.22. Found: C, 63.86; H, 8.63; N, 6.39.

(3a*S*,9a*R*,10*S*,10a*R*)-10-(Hydroxymethyl)-2,2-dimethylhydro-4*H*-[1,3]dioxolo[4,5]pyrido[1,2-*c*][1,3]oxazine-10-ol (17). To a mixture of potassium ferricynide (2.61 g, 7.94 mmol) and potassium carbonate (1.09 g, 7.94 mmol) in water (28 mL) at 5 °C was added **9** (0.59 g, 2.64 mmol) dissolved in *t*-BuOH (28 mL) followed by osmium tetroxide (2 mL of a 1% solution of OsO_4 in *t*-BuOH). The reaction mixture was allowed to warm to room temperature and stirred for 24 h. Solid Na_2SO_3 (0.4 g) was added to the stirring solution and a clear separation of two layers was noticed. The aqueous layer was extracted with ethyl acetate (5 × 20 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, petroleum ether−ethyl acetate, 3:7) to afford **17** (0.59 g, 90%) as a colorless crystalline solid. $[\alpha]^{27}_D$ +36.2 (*c* 0.95, CH_2Cl_2), *ent*-**17** $[\alpha]^{27}_D$ −34.4 (*c* 1.0, CH_2Cl_2); mp 165−168 °C; IR (in $CHCl_3$) υ 3490, 2964, 2860, 1382, 1373, 1226, 1107 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 3H), 1.43 (s, 3H), 1.64 (m, 1H), 1.82 (dd, 1H, J = 1.6, 13.1 Hz), 2.07 (t, 1H, J = 9.9 Hz), 2.15 (dd, 1H, J = 2.7, 11.3 Hz), 3.03 (dd, 1H, J = 4.3, 9.3 Hz), 3.38 (dt, 1H, J = 2.1, 12.0 Hz), 3.44 (d, 1H, J = 9.5 Hz), 3.64 (d, 1H, J = 8.0 Hz), 3.68−

(33) (a) Dong, W.; Jespersen, T.; Bols, M.; Skrydstrup, T.; Sierks, M. R. *Biochemistry* **1996**, *35*, 2788. (b) Andersen, S. M.; Ebner, M.; Ekhart, C. W.; Gradnig, G.; Legler, G.; Lundt, I.; Stütz, A. E.; Withers, S. G.; Wrodnigg, T. *Carbohydr. Res.* **1997**, *301*, 155.

(34) Legler, G.; Pohl, S. *Carbohydr. Res.* **1986**, *155*, 119.

(35) Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998**, *120*, 3007.

3.76 (m, 2H), 3.98 (d, 1H, J = 11.5 Hz), 4.08 (dd, 1H, J = 4.3, 11.3 Hz), 4.43 (d, 1H, J = 7.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7 (CH_2), 26.6 (CH_3), 51.5 (CH_2), 62.3 (CH_2), 66.5 (CH), 67.9 (CH_2), 72.0 (CH), 72.1 (C), 86.49 (CH), 86.50 (CH_2), 110.1 (C); MS 260 (MH^+). Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.71; H, 8.36; N, 5.45.

1-Deoxy-4-hydroxymethyl-D-glucohomonojirimycin or (2*R*,3*S*,4*R*,5*S*)-2-(2-Hydroxyethyl)-3-(hydroxymethyl)piperidine-3,4,5-triol (6). To a solution of **17** (25 mg, 0.097 mmol) in distilled dioxane−methanol (1:1, 3 mL) was added 2 mL of 6 N HCl and the reaction mixture was refluxed for 12 h. The solvent was evaporated to dryness to afford **7** as its HCl salt as a white foam, which was further purified by column chromatography as a free base [silica, chloroform−methanol−aq NH_3 (8.0:2:0.5) and finally eluted with MeOH−chloroform (4:6)] to afford **6** (19 mg, 95%) as a colorless gummy liquid. $[\alpha]^{27}_D$ +17.4 (*c* 1, MeOH), *ent*-**6** $[\alpha]^{27}_D$ −18.1 (*c* 0.8, MeOH); ^{1}H NMR (200 MHz, D_2O) δ 1.40−1.61 (m, 1H), 2.03 (dq, 1H, J = 2.5, 7.2 Hz), 2.41 (dd, 1H, J = 10.6, 12.5 Hz), 2.60 (dd, 1H, J = 2.4, 10.1 Hz), 3.10 (dd, 1H, J = 5.4, 12.5 Hz), 3.39 (d, 1H, J = 9.8 Hz), 3.96−3.86 (m, 5H); ^{13}C NMR (50 MHz, D_2O) δ 30.6 (CH_2), 49.2 (CH_2), 60.0 (for two CH_2), 60.7 (CH), 68.9 (CH), 73.6 (C), 80.8 (CH); MS (*m*/*z* %) 230 (M + Na^+, 32%), 208 (MH^+, 100%), 174 (20%). Anal. Calcd for $C_8H_{17}NO_5$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.19; H, 8.46; N, 6.39.

(3a*S*,9a*R*,10*S*,10a*S*)-2,2-Dimethylhexahydro-4*H*-[1,3]dioxolo-[4,5]pyrido[1,2-*c*][1,3]oxazin-10-ol (18). A solution of **17** (0.33 g, 1.28 mmol) in DCM (10 mL) was added to a suspension of silica gel supported sodium periodate [prepared by dissolving $NaIO_4$ (0.55 g, 2.56 mmol) in 1.3 mL of water and 2.52 g of flash silica gel] in DCM (5 mL). The suspension was stirred for 15 min and filtered. The solvent was evaporated off and the brownish pasty mass was extracted with ethyl acetate (3 × 3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The crude mixture, keto **17(i)** (crude weight 0.28 g, 95%), was pure enough and was used as such for the next step. $[\alpha]^{27}_D$ +68.6 (*c* 0.42, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 3H), 1.41 (s, 3H), 1.67−1.87 (m, 2H), 3.45 (app t, 1H, J = 9.7, 9.9 Hz), 3.35 (dd, 1H, J = 4.4, 9.8 Hz), 3.45 (dt, 1H, J = 3.5, 11.6 Hz), 3.82 (dt, 1H, J = 4.4, 10.0 Hz), 3.93 (d, 1H, J = 8.3 Hz), 4.75 [two sets of dd, 1H, J = 1.52, 4.8 Hz and J = 2.20, 4.3 Hz], 4.19 (dd, 1H, J = 1.5, 10.3 Hz), 4.42 (d, 1H J = 8.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.2 (CH_2), 26.2 (CH_3), 26.7 (CH_3), 50.5 (CH_2), 64.9 (CH), 66.8 (CH_2), 75.1 (CH), 83.1 (CH), 85.5 (CH_2), 112.9 (C), 197.6 (C). This ketone is unstable and should be used immediately for the next step.

Sodium borohydride (0.09 g, 2.26 mmol) was added to a solution of ketone **17(i)** (0.257 g, 1.13 mmol) in methanol (4 mL). The resulting mixture was stirred for 6 h and then quenched by adding an excess of the saturated solution of NaCl. This brownish suspension was stirred overnight and extracted with ethyl acetate (4 × 3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica, petroleum ether−ethyl acetate, 1:4) to afford **18** (0.22 g, 85%) as a colorless oil. $[\alpha]^{27}_D$ +28.4 (*c* 0.45, $CHCl_3$), *ent*-**18** $[\alpha]^{27}_D$ −24.4 (*c* 1.75, CH_2Cl_2); IR (in $CHCl_3$) υ 3438, 2987, 2929, 2856, 1674, 1456, 1382, 1215 cm^{-1}; ^{1}H NMR (500 MHz, $CHCl_3$) δ 1.42 (s, 4H), 1.43 (s, 3H), 2.12 (app t, 1H, J = 9.5, 9.9 Hz), 2.19−2.27 (m, 2H), 3.09 (dd, 1H, J = 4.3, 9.4 Hz), 3.29 (dd, 1H, J = 2.5, 9.2 Hz), 3.45 (dt, 1H, J = 2.8, 11.0 Hz), 3.72 (d, 1H, J = 7.9 Hz), 4.01 (dd, 1H, J = 4.0, 9.9 Hz), 4.02(s, 1H), 4.08 (dd, 1H, J = 4.4, 10.7 Hz), 4.44 (d, 1H, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.2 (CH_3), 26.5 (CH_3), 27.1 (CH_2), 51.2 (CH_2), 61.7 (CH), 66.8 (CH_2), 68.1 (CH), 69.6 (CH), 81.4 (CH), 86.1 (CH_2), 112.8 (C); MS (*m*/*z* %) 228 (MH^+, 100%). Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.39; H, 8.34; N, 6.30.

1-Deoxy-D-galactohomonojirimycin or (2*R*,3*S*,4*R*,5*S*)-2-(2-Hydroxyethyl)piperidine-3,4,5-triol (5). To a solution of **18** (25 mg, 0.109 mmol) in distilled dioxane−methanol (1:1, 3 mL) was added 2 mL of 6 N HCl and the reaction mixture was refluxed for 12 h. The solvent was evaporated to dryness to afford **6**·HCl as a white foam that was further purified by column chromatography as a free base [silica, chloroform−methanol−aq NH_3 (8.0:2:0.5) and finally eluted with MeOH−chloroform (4:6)] to afford **5** (17 mg, 94%) as a white solid. Mp 166−168 °C (lit.[17] mp 167.5−168.5 °C); $[\alpha]^{27}_D$ +25.2 (*c* 1, MeOH) (lit.[17] +24.8 (*c* 1, MeOH)), *ent*-**5** $[\alpha]^{27}_D$ −24.6 (*c* 1.20, MeOH); IR (KBr) υ 3360, 2958, 2825, 1077, 1024 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.94−2.02 (m, 2H), 2.83 (dd, 1H, J = 11.6, 12.4 Hz), 3.36−3.49 (m, 2H), 3.61 (dd, 1H, J = 3.0, 9.7 Hz), 3.66−3.78 (m, 2H), 3.91−4.06 (m,1H), 4.08 (dd, 1H, J = 1.2, 2.8 Hz); ^{13}C NMR (100 MHz, D_2O) δ 30.4 (CH_2), 46.2 (CH_2), 56.9 (CH), 57.5 (CH_2), 64.2 (CH), 68.1 (CH), 72.8 (CH); MS (*m*/*z* %) 178 (MH^+, 100%), 160 (10%), 142 (8%), 132.15 (12%).

(2*R*,3*S*,4*R*,5*S*)-*tert*-Butyl-3-(benzyloxy)-4,5-dihydroxy-2-(2-hydroxyethyl)piperidine-1-carboxylate (25). To a solution of the substrate **19** (0.15 g, 0.469 mmol) in dioxane:methanol (3:1, 8 mL) was added 3 mL of 6 N HCl and the reaction mixture was refluxed for 16 h. The solvent was evaporated to dryness to afford the hydrochloride salt of (3*S*,4*R*,5*S*,6*R*)-5-(benzyloxy)-6-(2-hydroxyethyl)piperidine-3,4-diol as a white foam that was further purified by column chromatography as a free base (silica, chloroform-methanol-aq.NH_3, 8.0:2:0.5) and finally eluted with MeOH-chloroform (4:6) to afford (3*S*,4*R*,5*S*,6*R*)-5-(benzyloxy)-6-(2-hydroxyethyl)piperidine-3,4-diol **19(i)** (0.11 g, 88%) as a semisolid. $[\alpha]^{27}_D$ +17.35 (*c* 0.65, MeOH); ^{1}H NMR (500 MHz, D_2O) δ 1.70−1.81 (m, 2H), 2.54 (t, 1H, J = 11.9 Hz), 3.01 (t, 1H, J = 6.8 Hz), 3.22 (dd, 1H, J = 5.4,12.9 Hz), 3.58 (t, 2H, J = 6.4 Hz), 3.66 (dd, 1H, J = 2.6, 9.7 Hz), 3.89−3.96 (m, 2H), 4.70 (d, 1H, J = 11.1 Hz), 4.96 (d, 1H, J = 11.1 Hz), 7.40−7.55 (m, 5H); ^{13}C NMR (125 MHz, D_2O) δ 32.5 (CH_2), 48.2 (CH_2), 55.6 (CH), 58.3 (CH_2), 67.2 (CH), 75.3 (CH), 75.4 (CH_2), 78.4 (CH), 128.2 (CH), 128.5 (CH), 128.6 (CH), 137.8 (C).

A solution of $(Boc)_2O$ (0.11 mL, 0.48 mmol, in 1 mL of DCM) was slowly added to a stirring solution of hydrochloride salt of **19(i)** (0.12 g, 0.40 mmol) and Et_3N (0.13 mL, 1.00 mmol) in DCM (4 mL) at 0 °C. The reaction mixture was stirred for 8 h at room temperature. The reaction mixture was diluted with DCM (5 mL) and washed with water (3 × 100 mL) and brine (1 × 100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resultant colorless residue was purified by column chromatography (silica, petroleum ether−ethyl acetate 2:8) to afford **25** (0.134 g, 92%) as a white solid. Mp 128−130 °C; $[\alpha]^{27}_D$ −8.5 (*c* 2.25, $CHCl_3$); IR ($CHCl_3$) υ 3421, 3008, 2979, 2931, 2902, 1666, 1658, 1423, 1367, 1220, 1163, 1072 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.02 (br s, 1H), 2.67 (br s, 1H), 3.21 (d, 1H, J = 13.9 Hz), 3.42 (br s, 1H), 3.57−3.64 (m, 1H), 3.74−4.04 (m, 4H), 4.47−4.75 (m, 3H), 7.22−7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.3 (CH_3), 29.6 (CH_2), 40.4 (CH_2), 48.2 (CH), 58.5 (CH_2), 68.7 (CH), 70.2 (CH), 71.3 (CH_2), 73.2 (CH), 80.9 (C), 127.8 (CH), 128.0 (CH), 128.5 (CH), 137.6 (C), 157.0 (C); MS (*m*/*z* %) 390 (M + Na^+, 100%), 368 (MH^+, 46%), 312 (20%), 268 (12%). Anal. Calcd for $C_{19}H_{29}NO_6$: C, 62.11; H, 7.96; N, 3.81. Found: C, 62.28; H, 8.05; N, 3.79.

(4a*R*,5*S*,6*R*,7*S*)-5-(Benzyloxy)-6,7-dihydroxyhexahydropyrido-[1,2-*c*][1,3]oxazin-1-one (26). To a solution of **25** (0.12 g, 0.33 mmol) in DCM (5 mL) at 0 °C was added triethylamine (0.0314 g, 0.31 mmol, in 0.5 mL of DCM) followed by dropwise addition of mesyl chloride (0.035 g, 0.31 mmol, in 0.5 mL of DCM). The reaction was found to be complete in 10 min, monitored by TLC. The reaction mixture was diluted with dichloromethane (5 mL), washed with water (3 × 5 mL) and brine solution (5 mL), and then dried over Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was dissolved in dry acetonitrile. Solid K_2CO_3 (0.227 g, 1.65 mmol) was added to the solution. The reaction

mixture was refluxed for 6 h and cooled to room temperature. Filtration of the reaction mixture was done to separate solid K_2CO_3. Removal of solvent gave **26** (0.085 g, 89%) as a white cryastalline solid. Mp 80−83 °C; $[\alpha]^{27}_D$ +36.5 (*c* 1.65, MeOH); IR ($CHCl_3$) υ 3385, 2927, 1682, 1481, 1454, 1229 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.95−2.10 (m, 2H), 2.71 (dd, 1H, J = 11.0, 13.2 Hz), 3.61 (app t, 1H, J = 6.4, 8.2 Hz), 3.69 (dd, 1H, J = 3.3, 9.9 Hz), 3.88 (dt, 1H, J = 5.5, 10.3 Hz), 3.96 (s, 1H), 4.15 (ddd, 1H, J = 3.3, 9.6, 11.3 Hz), 4.23−4.32 (m, 2H), 4.73 (d, 1H, J = 11.7 Hz), 4.95 (d, 1H, J = 11.7 Hz), 7.20−7.31 (m, 5H); ^{13}C NMR (125 MHz, D_2O) δ 23.4 (CH_2), 40.0 (CH_2), 54.9 (CH), 65.4 (CH_2), 65.9 (CH), 75.3 (CH), 75.7 (CH_2), 78.8 (CH), 128.2 (CH), 128.5 (CH), 128.5 (CH), 137.2 (C), 156.7 (C); MS (*m*/*z* %) 316 (M + Na^+, 100%), 294 (MH^+, 34%), 268 (27%), 242 (20%). Anal. Calcd for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.69; H, 6.81; N, 4.42.

(4a*R*,5*S*,6R,7*S*)-5,6,7-Trihydroxyhexahydropyrido[1,2-*c*][1,3]-oxazin-1-one (8). A solution of **26** (85 mg, 0.29 mmol) in ethanol (5 mL) was hydrogenated at atmospheric pressure in the presence of Pd on charcoal (10%, 3 mg) for 9 h. The reaction mixture was passed through a short pad of Celite and the solvent was removed under reduced pressure to afford **8** (56 mg, 95%) as a white solid. Mp 210−212 °C dec; $[\alpha]^{27}_D$ +29.5 (*c* 0.125, MeOH); ^1H NMR (500 MHz, D_2O) δ 2.11−2.27 (m, 2H), 2.72 (dd, 1H, J = 11.2, 13.0 Hz), 3.56 (dd, 1H, J = 3.2, 9.6 Hz), 3.66 (app t, 1H, J = 7.3, 7.8 Hz), 3.79 (dt, 1H, J = 5.5, 10.5 Hz), 3.94 (d, 1H, J = 1.8 Hz), 4.22−4.34 (m, 2H), 4.36−4.42 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 23.3 (CH_2), 47.8 (CH_2), 55.0 (CH), 65.4 (CH_2), 65.5 (CH), 70.5 (CH), 74.2 (CH), 156.8 (C); MS (*m*/*z* %) 609 (M + M + M^+, 15%), 204 (MH^+, 5%), 150 (100%). Anal. Calcd for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89. Found: C, 46.99; H, 6.63; N, 6.79.

(3*S*,4*S*,5*R*,6*R*)-5-Amino-6-(2-hydroxyethyl)piperidine-3,4-diol (7). To a solution of **27** (0.11 g, 0.358 mmol) in DMF (3 mL) was added LiN_3 (0.175 g, 3.58 mmol) and the mixture was heated to 110 °C for 16 h. When TLC revealed the absence of starting material, and the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The ethyl acetate layer was washed with water, dried over Na_2SO_4, and concentrated to give the azide derivative of **27** [0.08 g, 88% after chromatographic purification (ethyl acetate−petroleum ether 5:15)]. $[\alpha]^{27}_D$ +41.25 (*c* 1.8, $CHCl_3$); IR ($CHCl_3$) υ 2987, 2928, 2856, 2252, 2108, 1662, 1373, 1269, 1230, 1147 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 6H), 1.58−1.76 (m, 1H), 1.86−2.07 (m, 2H), 2.17 (dd, 1H, J = 9.8, 10.0 Hz), 3.03 (dd, 1H, J = 4.0, 9.5 Hz), 3.24−3.34 (m, 2H), 3.41 (dt, 1H, J = 2.4, 11.9 Hz), 3.52−3.66 (m, 1H), 3.74 (d, 1H, J = 7.8 Hz), 4.09 (dd, 1H, J = 4.8, 11.6 Hz), 4.41 (d, 1H, J = 7.9 Hz); MS (*m*/*z* %) 255 (MH^+, 100%), 212 (25%), 200 (50%), 180 (80%), 158 (25%).

The solution of azide derivative of **27** (0.08 g, 0.34 mmol) in methanol (3 mL) was hydrogenated for 7 h at atmospheric pressure in the presence of Pd on charcoal (10%) (0.003 g). The reaction mixture was passed through a short pad of Celite and the solvent was removed under reduced pressure to afford the amine derivative of **27** (68 mg, 95%) as a syrup. $[\alpha]^{27}_D$ +16 (*c* 1.75, MeOH); IR ($CHCl_3$) υ 3285, 2987, 2928, 1665, 1373, 1270, 1234, 1024 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 6H), 1.50−1.71 (m, 1H), 1.75−2.03 (m, 2H), 2.17 (app t, 1H, J = 9.6, 10.0 Hz), 2.76 (dd, 1H, J = 8.3, 10.0 Hz), 3.02−3.17 (m, 2H), 3.44 (dt, 1H, J = 2.5, 12.0 Hz), 3.55 (ddd, 1H, J = 4.0, 7.5, 10.2 Hz), 3.75 (d, 1H, J = 7.8 Hz), 4.06−4.17 (dd, 1H, J = 4.8, 11.4 Hz), 4.42 (d, 1H, J = 7.8 Hz); MS (*m*/*z* %) 228 (M^+, 100%).

To a solution of the amine derivative of **27** (68 mg, 0.097 mmol) in distilled methanol (3 mL) was added 2 mL of 6 N HCl and the reaction mixture was refluxed for 18 h. The solvent was evaporated to dryness to afford **7**·HCl as a white foam that was further purified by column chromatography as a free base [silica, chloroform−methanol−aq NH_3 (8.0:2:0.5) and finally eluted with MeOH−chloroform (4:6)] to afford **7** (45 mg, 86%) a light yellow gummy liquid. $[\alpha]^{27}_D$ +4.8 (*c* 1.33, MeOH); ^1H NMR (200 MHz, D_2O) δ 1.96−2.06 (m, 1H), 2.13−2.22 (m, 1H), 3.00 (app t, 1H, J = 11.9, 12.4 Hz), 3.37 (app t, 1H, J = 10.4, 11.3 Hz), 3.56 (dd, 1H, J = 5.0, 12.8 Hz), 3.63 (ddd, 1H, J = 3.7, 9.27, 11.1 Hz), 3.71 (t, 1H, J = 9.6 Hz), 3.82−3.93 (m, 3H); ^{13}C NMR (50 MHz, D_2O) δ 30.9 (CH_2), 46.2 (CH_2), 53.3 (CH), 55.4 (CH), 57.4 (CH_2), 67.1 (CH), 72.2 (CH); MS (*m*/*z* %):176 (MH^+, 100%), 161 (22%), 127 (7%). Anal. Calcd for $C_7H_{16}N_2O_3$: C, 47.71; H, 9.15; N, 15.90. Found: C, 47.57: H, 9.43: N, 16.23.

3-{[(4*S*,5*S*)-2,2-Dimethyl-5-vinyl-1,3-dioxolan-4-yl]methyl}-4-(trimethylsilyl)-1,3-oxazinane (33). The alcohol **32** was transformed into **33** (1:1, diastereomer) by using a procedure similar to the one used for conversion of alcohol **11** to **10**. IR (neat) υ 3018, 2989, 2956, 2399, 1425, 1380, 1251, 1215, 1068 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.03 + 0.09 (singlet s, 9H), 1.35−1.55 (m, 7H), 1.70−1.95 (m, 1H), 2.55−2.70 (m, 2H), 3.17−3.37 (m, 1H), 3.65−3.90 (m, 2H), 3.97−4.07 (m, 2H), 4.27 (d, 1H, J = 10.5 Hz), 4.62 (two sets of d, 1H, J = 10.6 and 10.4 Hz), 5.20−5.40 (m, 2H), 5.71−5.91 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ −2.31 and −2.28 (Si−CH_3), 21.89 + 21.94 (CH_2), 26.4 + 26.6 (CH_3), 26.92 + 26.99 (CH_3), 51.0 + 51.2 (CH_2), 52.4 (CH), 68.1 + 68.2 (CH_2), 78.7 + 80.64 (CH), 80.80 + 81.82 (CH), 83.7 + 85.0 (CH_2), 108.9 (C), 118.4 (CH_2), 135.0 + 135.3 (CH); MS (*m*/*z* %) 322 (M + Na^+, 45%), 300 (MH^+, 100%), 288 (63%). Anal. Calcd for $C_{15}H_{29}NO_3Si$: C, 60.16; H, 9.76; N, 4.68. Found: C, 60.28; H, 9.52; N, 4.60.

(3a*S*,9a*R*,10*S*,10a*S*)-2,2,10-Trimethylhexahydro-4*H*-[1,3]dioxlo-[4,5]pyrido[1,2-*c*][1,3]oxazine (34). Compound **33** was transformed to **34** by using a similar protocol as used for the conversion of **10** to **9**. $[\alpha]^{27}_D$ +11.8 (*c* 3.15, $CHCl_3$); IR (neat) υ 3018, 2987, 2933, 2856, 2399, 2360, 1382, 1373, 1215, 1097 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.93 (d, 3H, J = 6.42 Hz), 1.41(s, 3H), 1.42 (s, 3H), 1.50−1.70 (m, 2H), 1.75 (br d, 1H J = 13.3 Hz), 1.80 (dt, 1H, J = 2.7, 10.1 Hz), 2.15 (app t, 1H, J = 9.6, 10.1 Hz), 2.96 (dd, 1H, J = 8.7, 10.5 Hz), 3.08 (dd, 1H, J = 4.1, 9.2 Hz), 3.42 (dt, 1H, J = 2.7, 11.9 Hz), 3.57 (ddd, 1H, J = 4.1, 7.3, 10.4 Hz), 3.74 (d, 1H, J = 8.2 Hz), 4.08 (dd, 1H, J = 4.6, 11.7 Hz), 4.40 (d, 1H, J = 7.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.3 (CH_3), 26.7 (CH_3), 26.9 (CH_3), 29.4 (CH_2), 39.4 (CH), 51.5 (CH_2), 65.0 (CH), 67.6 (CH_2), 75.4 (CH), 84.3 (CH), 86.4 (CH_2), 109.9 (C); MS (*m*/*z* %) 228 (MH^+, 100%). Anal. Calcd for $C_{12}H_{21}NO_3$: C, 63.41; H, 9.31; N, 6.16. Found: C, 63.66; H, 9.51; N, 5.98.

(3*S*,4*S*,5*S*,6*R*)-6-(2-Hydroxyethyl)-5-methylpiperidine-3,4-diol (31). Compound **34** was transformed to **31** by using a similar protocol as used for the conversion of **18** to **5**. Mp 180−183 °C; $[\alpha]^{27}_D$ +4.0 (*c* 1.15, MeOH); ^1H NMR (500 MHz, D_2O) δ 1.13 (d, 3H, J = 6.4 Hz), 1.71−1.80 (m, 1H), 1.81−1.89 (m, 1H), 2.12−2.20 (m, 1H), 2.93 (t, 1H, J = 11.9 Hz), 3.21 (ddd, 1H, J = 2.3, 9.7, 11.1 Hz), 3.31 (t, 1H, J = 9.6 Hz), 3.51 (dd, 1H, J = 5.0, 12.4 Hz), 3.72−3.88 (m, 3H); ^{13}C NMR (125 MHz, D_2O) δ 12.4 (CH_3), 31.0 (CH_2), 38.3 (CH), 46.1 (CH_2), 57.9 (CH_2), 59.2 (CH), 67.8 (CH), 75.4 (CH); MS (*m*/*z* %) 176 (MH^+, 100%), 149 (25%). Anal. Calcd for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99. Found: C, 54.86; H, 9.58; N, 8.06.

Acknowledgment. We thank Dr. P. R. Rajmohan and Mrs. U. D. Phalgune for special NMR experiments. S.G.D. and M.S thank UGC and CSIR, respectively, New Delhi for the award of Research Fellowships. Financial support by the DBT, New Delhi is gratefully acknowledged.

Supporting Information Available: Experimental procedures for compounds **19** and **27**, copies of ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of all new compounds, enzyme assay procedures, Lineweaver−Burk plots, and X-ray crystallographic data for compounds **17** and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO061455V