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SYNTHESIS OF A NEW INHIBITOR OF α-FUCOSIDASE

Yasuhiro Igarashi, Mie Ichikawa, and Yoshitaka Ichikawa*

Department of Pharmacology and Molecular Sciences
The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Abstract: A new fucose-type iminosugar in which the nitrogen is placed in the anomeric position was synthesized from D-ribose and was shown to be a potent inhibitor of α-fucosidase ($Ki = 8.4 \ \mu M$).

Deoxyfuconojirimycin (**1**), one of the deoxynojirimycin analogues, is a potent inhibitor of α-L-fucosidases,[1] and its *N*-alkyl derivatives have been demonstrated to inhibit HIV cytopathicity.[2] These iminosugars have, therefore, been considered as a new class of drugs for use in a variety of infectious diseases and diabetes in which carbohydrates play a key role.[3-5] In the course of our study on developing more potent inhibitor of glycosidases, we proposed a new type of iminosugar in which a nitrogen atom is placed in the anomeric position. Several new iminosugars of the glucose- and galactose-type (**2**) have been synthesized by us[6-8] and others[9] and have proven to be more potent inhibitors of β-glycosidases than are the conventional deoxynojirimycin-type iminosugars. In an extension of this work, we herein report the stereoselective synthesis of a new fucose-type iminosugar (**3**) and its analogues (**4-8**) and an evaluation of biological activity as inhibitors of various glycosidases.
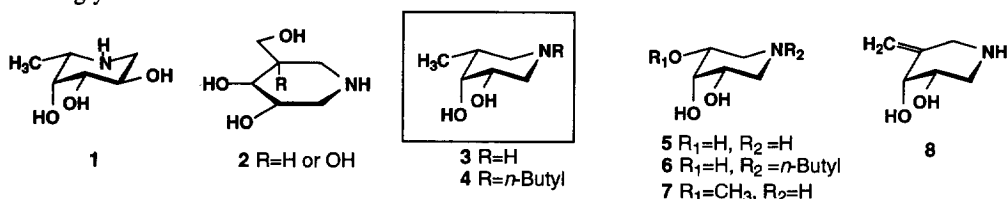
D-Ribose (**9**) was converted to a 2,3-*O*-isopropylidene derivative **10**,[10] which was subsequently treated with TsCl and BzCl to afford **11** as a single isomer in 48% overall yield. Treatment of **11** with NaN₃ gave the azido derivative (**12**) in 91% yield. The benzoyl group of **12** was removed with NaOMe and the subsequent intramolecular reductive amination with H₂-Pd(OH)₂ furnished a piperidine derivative **13** in 75% overall yield. To introduce a *C*-5 methyl group of L-fucose structure, we took advantage of the *cis*-fused bicyclic structure of **13**. The amino group of **13** was first protected with BocON[11] to give **14**. Swern oxidation[12] of **14** gave **15**, which was subjected to Wittig methylenation to give **16** in 63% overall yield. Catalytic hydrogenation of **16** preferentially occurred from the less hindered β-face to afford **17** exclusively in 82% yield.[13] Treatment of **17** with 1*N* HCl simultaneously removed both the 3,4-*O*-isopropylidene and the *t*-Boc group to furnish the desired fucose-type 1-*N*-iminosugar **3**. The overall yield was 12% from D-ribose in 11 steps.
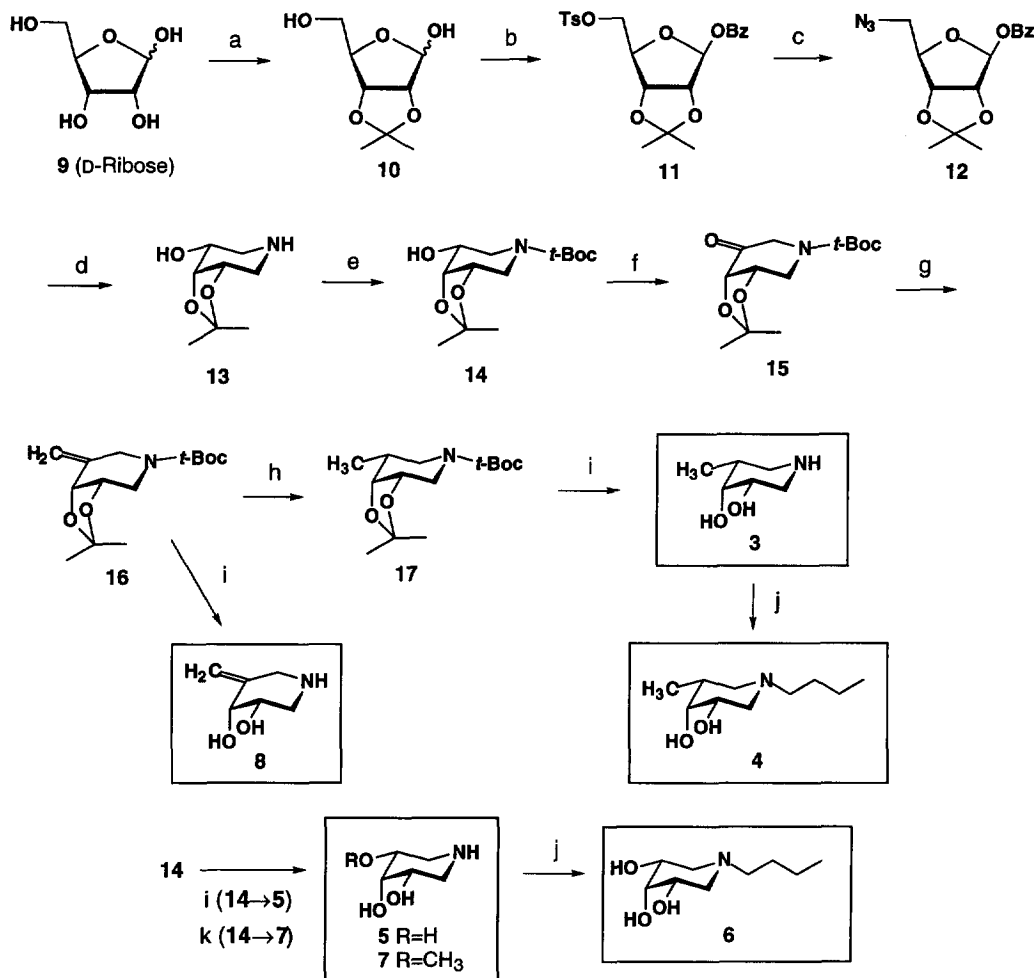
Scheme 1. Synthesis of fucose-type 1-*N*-iminosugar (3) and its analogues (4-8). Reagents and conditions: (a)2,2-Dimethoxypropane/TsOH/acetone/rt/12 h; (b)(i)TsCl/Pyr/0-5 °C/10 h, (ii)BzCl/Pyr/0-5 °C/1 h (48% in 3 steps); (c)NaN3/DMF/60-65 °C/4 h (91%); (d)(i)NaOMe/MeOH/rt/10 min; (ii)H2/Pd(OH)2/MeOH-H2O/rt/12 h (75% in 2 steps); (e)BocON/Et3N/H2O-dioxane/rt/8 h (73%); (f)(COCl)2/ DMSO/CH2Cl2/-70 °C/30 min then Et3N/-70 °C to 0 °C/30 min (80%); (g)CH3PPh3+Br−/*n*-BuLi/DME/0 °C to rt/8 h (77%); (h)H2/Pd-C/MeOH/rt/5 h (82%); (i)1*N* HCl/rt/12 h (90-95%); (j)*n*-Butylaldehyde/BH3·Pyr/MeOH-phosphate buffer (pH 7.2)/rt/12 h (80%); (k)MeI/NaH/DMF/0 °C to rt/12 h then 1*N* HCl/rt/12 h (90%).

We also synthesized the structurally related fucose-type iminosugar analogues **4, 5, 6, 7,** and **8**. For *N*-alkylation, **3** was treated with *n*-butylaldehyde and BH3·Pyr in MeOH-phosphate buffer (pH 7.2)[14] to give *N*-butyl iminosugar **4** in 80% yield. For preparation of a triol iminosugar, **14** was deprotected with 1*N* HCl to afford **5** and *N*-butylation of **5** was performed in the same manner described above to give the *N*-butyl derivative (**6**). An iminosugar (**7**) with a *C*-5 O-CH3 derivative instead of a methyl group was synthesized by methylation

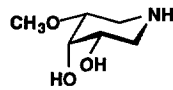## Table 1. Inhibition of glycosidases by iminosugars (3-8).

| Glycosidase	IC$_{50}$ (µM) for Inhibition by Compound					
	3	4	5	6	7	8
α-Fucosidase from bovine kidney (Sigma F 5884)	26	270	>1000	>1000	>1000	>1000
α-Glucosidase from yeast (Sigma G 7256)	>1000	>1000	>1000	>1000	>1000	>1000
β-Glucosidase from almonds (Sigma G 4511)	500	80	8.8	530	>1000	>1000
α-Galactosidase from green coffee beans (Sigma G 8507)	>1000	>1000	40	725	>1000	>1000
β-Galactosidase from Aspergillus oryzae (Sigma G 7256)	>1000	>1000	40	43	>1000	>1000
α-Mannosidase from jack beans (Sigma M 7257)	>1000	>1000	360	>1000	>1000	>1000
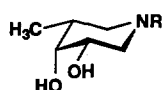
3 R=H
4 R=n-Butyl
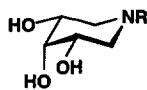
5 R=H
6 R=n-Butyl

7
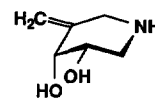
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of **14** with MeI and NaH. Another iminosugar derivative (**8**) with a *C-5 exo*-methylene group was prepared by deprotection of **16**.

The inhibitory potency of these iminosugars (**3-8**) was examined[15] against several glycosidases (Table 1). As we anticipated, among the iminosugars tested, **3** was the most potent inhibitor of α-fucosidase from bovine kidney, showing a Ki of 8.4 µM (IC$_{50}$ of 26 µM) at pH 6.8; its *N*-butyl analogue **4** also inhibited the enzyme with an IC$_{50}$ of 260 µM. All other analogues (**5-8**) showed no significant inhibition at 1 mM concentration. Contrary to our expectations, **8** did not inhibit the α-fucosidase although its half-chair conformation has been thought to mimic the transition state of the glycosidic bond-cleaving reaction. This lack of inhibitory activity suggests that the enzyme strictly recognizes the substituent at *C-5*.

It was also surprising that the trihydroxypiperidine **5**, in which the methyl group at *C-5* was replaced with an OH group potently inhibited β-glucosidase with an IC$_{50}$ value of 8.8 µM. Moreover, **5** and its *N*-butyl derivative **6** inhibited both α- and β-galactosidase with an IC$_{50}$ of 40 µM, suggesting that the galactosidases recognize the 3- and 4-OH groups of **5** and **6** as those of a galactoside. To our knowledge, this is the first demonstration of the inhibitory potency of **5** (the *meso*-type 3,4,5-trihydroxypiperidine) against glycosidases; the synthesis and biological activities of other stereoisomers of 3,4,5-trihydroxypiperidine have been reported by others.[16]

In summary, we have synthesized a new fucose-type 1-*N*-iminosugar (**3**) from D-ribose with (i) Wittig methylenation and (ii) stereoselective hydrogenation as the key steps and have shown this iminosugar to be a potent inhibitor of the α-fucosidase.

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17. Selected ^{1}H and ^{13}C NMR data for the new compounds.

1-*O*-Benzoyl-2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribose 11: ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.35 and 1.57 (s, 3H each, CH$_3$ of isopropylidene group), 2.39 (s, 3H, ArCH$_3$), 4.05 (dd, 1H, *J* 6.9, 10.3 Hz, H-5a), 4.10 (dd, 1H, *J* 5.1, 10.3 Hz, H-5b), 4.48 (dd, 1H, *J* 5.1, 6.9 Hz, H-4), 4.87 (s, 2H, H-2 and -3), 6.43 (s, 1H, H-1), 7.22 (d, 2H, *J* 8.2 Hz, aromatic H of tosyl group), 7.48 (dd, 2H, *J* 7.7 Hz, aromatic H of benzoyl group), 7.62 (dd, 1H, *J* 7.3 Hz, aromatic H of benzoyl group), 7.66 (d, 2H, *J* 8.2 Hz, aromatic H of tosyl group), 8.00 (d, 2H, *J* 7.3 Hz, aromatic H of benzoyl group); ^{13}C NMR (75 MHz, CDCl$_3$): δ 21.47, 24.87, 26.33, 68.84, 81.09, 84.92, 85.09, 102.73, 113.32, 127.78, 128.45, 129.73, 129.80, 133.48, 145.04, 164.61.

5-Azido-1-O-benzoyl-5-deoxy-2,3-O-isopropylidene-β-D-ribose 12: ^{1}H NMR (300 MHz, CDCl₃) 1.37 and 1.59 (s, 3H each, CH₃ of isopropylidene group), 3.31 (dd, 1H, J 6.8, 12.7 Hz, H-5a), 3.55 (dd, 1H, J 7.0, 12.7 Hz, H-5b), 4.48 (dt, 1H, J 0.93, 6.9 Hz, H-4), 4.76 (dd, 1H, J 0.93, 6.0 Hz, H-3), 4.90 (d, 1H, J 6.0 Hz, H-2), 7.46 (dd, 2H, J 7.4, 7.9 Hz, m-aromatic H), 7.60 (tt, 1H, J 1.3, 7.4 Hz, p-aromatic H), 8.01 (dd, 2H, J 1.3, 7.9 Hz, o-aromatic H); ^{13}C NMR (75 MHz, CDCl₃) 24.9, 26.4, 53.0, 81.7, 85.2, 86.6, 103.1, 113.4, 128.5, 129.6, 133.5, 164.7.

(3S, 4R, 5R)-3,4-O-Isopropylidene-3,4,5-trihydroxypiperidine 13: ^{1}H NMR (300 MHz, CDCl₃) 1.38, 1.55 (s, 3H each, CH₃ of isopropylidene group), 2.74-3.15 (m, 6H, H-2, H-6, O$\underline{H}$ and N$\underline{H}$), 3.79 (m, 1H), 4.15 (m, 1H), 4.27 (m, 1H); ^{13}C NMR (75 MHz, CDCl₃) 25.5, 26.9, 46.6, 47.2, 64.9, 71.8, 74.2, 108.7.

(3S, 4R, 5R)-1-N-t-Butoxycarbonyl-3,4-O-isopropylidene-3,4,5-trihydroxypiperidine 14: ^{1}H NMR (300 MHz, CDCl₃) 1.38 and 1.51 (s, 3H each, CH₃ of isopropylidene group), 1.46 (s, 9H, t-Bu of t-Boc group), 2.79 (m, 1H), 3.18 (dd, 1H, J 9.9, 12.2 Hz), 3.45 (dd, 1H, J 4.0, 14.0 Hz), 3.56 (m, 2H), 3.90 (m, 1H), 4.38 (m, 2H); ^{13}C NMR (75 MHz, CDCl₃) 24.7, 26.6, 28.3, 65.6, 72.2, 79.8, 109.3.

(4S, 5S)-1-N-t-Butoxycarbonyl-4,5-O-isopropylidene-4,5-dihydroxy-3-piperidone 15: ^{1}H NMR (300 MHz, CDCl₃) δ 1.40 and 1.47 (s, 3H each, CH₃ of isopropylidene group), 1.45 (s, 9H, t-Bu of t-Boc group), 3.51 (m, 1H, H-6), 3.60 (m, 1H, H-6), 4.65 (m, 1H, H-2), 4.83 (m, 2H, H-4 and H-5), 4.95 (m, 1H, H-2); ^{13}C NMR (75 MHz, CDCl₃) δ 24.9, 26.3, 80.5, 111.2, 154.2, 202.8.

(3S, 4R)-1-N-t-Butoxycarbonyl-3,4-O-isopropylidene-5-methylene-3,4-dihydroxypiperidine 16: ^{1}H NMR (300 MHz, CD₃OD) δ 1.34 and 1.40 (s, 3H each, CH₃ of isopropylidene group), 1.46 (s, 9H, t-Bu of t-Boc group), 2.94 (m, 1H, H-2), 3.74 (m, 1H, H-6), 3.89 (dd, 1H, J 2.4, 14.4 Hz, H-2), 4.24-4.36 (m, 2H, H-3 and -6), 4.71 (d, 1H, J 7.5 Hz, H-4), 5.20 (s, 1H, C=CH₂), 5.27 (s, 1H, C=CH₂); ^{13}C NMR (75 MHz, CD₃OD) δ 24.94, 27.05, 28.76, 76.05, 77.33, 80.87, 110.25, 141.24, 156.91.

(3S, 4R, 5R)-1-N-t-Butoxycarbonyl-3,4-O-isopropylidene-5-methyl-3,4-dihydroxypiperidine 17: ^{1}H NMR (300 MHz, CDCl₃) δ 1.05 (d, 3H, J 6.9 Hz, C-5 C$\underline{H}$₃), 1.34 and 1.44 (s, 3H each, CH₃ of isopropylidene group), 1.46 (s, 9H, t-Bu of t-Boc group), 1.90 (m, 1H, H-5), 2.99 (t, 1H, J 12.3 Hz), 3.34 (m, 2H), 3.68 (m, 1H), 4.20 (dd, 1H, J 2.5, 6.9 Hz), 4.27 (br.s, 1H); ^{13}C NMR (75 MHz, CDCl₃) δ 24.7, 26.5, 28.4, 30.7, 72.7, 74.9, 108.2, 164.1.

(3S, 4R, 5R)-3,4-Dihydroxy-5-methylpiperidine 3 (HCl salt): ^{1}H NMR (300 MHz, D₂O) δ 0.93 (d, 3H, J 6.75 Hz, C-5 C$\underline{H}$₃), 1.96 (m, 1H, H-5), 2.73 (dd, 1H, J 12.6 Hz, H-2ax or 6ax), 2.91 (dd, 1H, J 11.7 Hz, H-6ax or 2ax), 3.01 (dd, 1H, J 3.65, 12.6 Hz, H-2eq or 6eq), 3.17 (dd, 1H, J 4.22, 11.3 Hz,

H-6eq or 2eq), 3.85 (s, 1H, H-4), 3.87 (dm, 1H, J 12 Hz, H-3); ^{13}C NMR (75 MHz, D$_2$O) δ 13.89, 31.88, 41.85, 43.22, 66.25, 69.70.

(3S, 4R, 5R)-1-N-n-Butyl-3,4-dihydroxy-5-methylpiperidine   4 (HCl salt): ^{1}H NMR (300 MHz, D$_2$O) δ 0.83 (t, 3H, J 7.4 Hz, -CH$_2$CH$_3$ of N-Bu), 0.93 (d, 3H, J 6.9 Hz, C-5 CH$_3$), 1.29 (tq, 2H, J 7.4 Hz, -CH$_2$CH$_3$ of N-Bu), 1.63 (tt, 2H, J 7.4, 8.0 Hz, -CH$_2$CH$_2$CH$_3$ of N-Bu), 1.97 (m, 1H, H-5), 2.70 (dd, 1H, J 12.4 Hz, H-6ax), 2.87 (dd, 1H, J 11.6 Hz, H-2ax), 3.08 (dd, 1H, J 8.1 Hz, N-CH$_2$-), 3.14 (dd, 1H, J 3.5, 12.4 Hz, H-6eq), 3.27 (dd, 1H, J 4.2, 11.7 Hz, H-2eq), 3.82 (s, 1H, H-4), 3.87 (dd, 1H, J 4.1, 11.3 Hz, H-3); ^{13}C NMR (75 MHz, D$_2$O) δ 15.40, 15.95, 21.82, 28.03, 34.26, 52.17, 53.93, 59.77, 68.70, 71.36.

1,5-Dideoxy-1,5-iminoribitol 5 (HCl salt): ^{1}H NMR (300 MHz, D$_2$O) δ 3.23 (m, 4H, H-2 and -6), 4.03 (dd, 1H, J 2.7 Hz, H-4), 4.08 (m, 2H, H-3 and -5); ^{13}C NMR (75 MHz, D$_2$O) δ 46.71, 67.93, 70.73.

N-n-Butyl-1,5-dideoxy-1,5-iminoribitol 6 (free amine): ^{1}H NMR (300 MHz, D$_2$O) δ 0.86 (t, 3H, J 7.3 Hz, -CH$_3$ of N-Bu), 1.25 (tq, 2H, J 7.3 Hz, -CH$_2$CH$_3$ of N-Bu), 1.44 (tt, 2H, J 7.3, 8.0 Hz, -CH$_2$CH$_2$CH$_3$ of N-Bu), 2.20 (dd, 2H, J 10.6, 10.7 Hz, H-2ax and -6ax), 2.41 (t, 2H, J 8.0 Hz, N-CH$_2$), 2.67 (dd, 2H, J 3.8, 10.7 Hz, H-2eq and -6eq), 3.72 (dd, 2H, J 3.0, 4.0, 10.6 Hz, H-3 and -5), 3.96 (s, 1H, H-4).

(3S, 4S, 5R)-3,4-Dihydroxy-5-methoxypiperidine 7 (HCl salt): ^{1}H NMR (300 MHz, D$_2$O) δ 3.20 (m, 3H, H-2ax or -6ax, H-2eq and -6eq), 3.29 (dd, 1H, J 7.4, 12.9 Hz, H-6ax or -2ax), 3.38 (s, 3H, -OCH$_3$), 3.70 (m, 1H, H-5), 4.01 (m, 1H, H-3), 4.10 (dd, 1H, J 2.9 Hz, H-4); ^{13}C NMR (75 MHz, D$_2$O) δ 44.00, 47.30, 59.57, 67.81, 68.94, 77.16.

(3S, 4R)-3,4-Dihydroxy-5-methylenepiperidine 8 (HCl salt): ^{1}H NMR (300 MHz, D$_2$O) δ 3.28 (dd, 1H, J 2.1, 13.2 Hz, H-2), 3.35 (ddd, 1H, J 1.2, 4.8, 13.3 Hz, H-2), 3.64 (d, 1H, J 13.5 Hz, H-6a), 3.83 (d, 1H, J 13.5 Hz, H-6b), 4.12 (m, 1H, H-3), 4.37 (s, 1H, H-4), 5.31 (s, 1H, C-5 C=CH$_2$), 5.34 (s, 1H, C=CH$_2$); ^{13}C NMR (75 MHz, D$_2$O) δ 47.36, 47.84, 66.73, 70.45, 116.89, 136.94.