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5,6-Epimino-D-glucofuranose and Synthesis of Nojirimycin (5-Amino-5-deoxyglucose)[1]

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Lithium aluminum hydride reduction of 6-azido-3-O-benzyl-6-deoxy-1,2-O-isopropylidene-β-L-idofuranose (**5b**) afforded 3-O-benzyl-5,6-dideoxy-5,6-epimino-1,2-O-isopropylidene-α-D-glucofuranose (**1a**). Its acetate (**1b**) was treated with acetic acid to give an 5-acetamido-6-O-acetyl-5-deoxy-derivative (**6**) which was transformed into a monosaccharide antibiotic, nojirimycin (**2**) which was produced by some strains of *Streptomyces* and showed activity against *Sarcina lutea* and *Xanthomonas oryzae*.

A previous paper[3] in this series presented syntheses of methyl 2,3-di-O-benzyl-5,6-dideoxy-5,6-epimino-α-L-altrofuranoside and 3-O-benzyl-5,6-dideoxy-5,6-epimino-1,2-O-isopropylidene-β-L-idofuranose, and their ring-opening reactions with nucleophiles. The present paper deals with not only the analogous synthesis and characterization of 3-O-benzyl-5,6-dideoxy-5,6-epimino-1,2-O-isopropylidene-α-D-glucofuranose (**1a**), but also with its conversion into a monosaccharide antibiotic, nojirimycin (5-amino-5-deoxy-D-glucose)[4] (**2**).

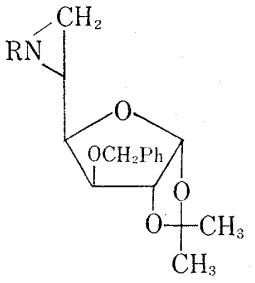
The starting material, 5,6-anhydro-3-O-benzyl-1,2-O-isopropylidene-β-L-idofuranose (**3**) was prepared by a modification of Whistler's procedure[5] as follows: Treatment of 3-O-benzyl-1,2-O-isopropylidene-5,6-di-O-tosyl-α-D-glucofuranose (**4a**)[3,6] with sodium acetate in dimethyl sulfoxide (DMSO) gave a 6-O-acetyl-5-O-tosylate (**4b**) of mp 131—132°, in good yield, which on treatment with sodium methoxide yielded **3**.

The epoxide (**3**) was treated with sodium azide in methylcellosolve to give a syrupy 6-azide (**5a**) which was converted into a 5-O-tosylate (**5b**). Following the previously described method,[3] lithium aluminum hydride reduction of **5b** in ether at a low temperature afforded the desired 5,6-dideoxy-5,6-epimine (**1a**) as a syrup. The infrared spectrum of **1a** did not indicate the presence of azide or tosyloxy group, but only that of an amine. The latter was so unstable that attempted purification by silica gel chromatography afforded only an unidentified gum. Therefore, **1a** was transformed into its acetate (**1b**) of mp 97—99°, whose infrared spectrum exhibited an absorption at 1700 cm^{-1} assigned to an amide I band, but no absorption corresponding to an amide II band. This suggested the presence of an N-acetylaziridine ring.[7] The acetate was also found to be unstable to bases. Thus treatment of **1b** with a catalytic amount of sodium methoxide gave the parent copmound **1a**.

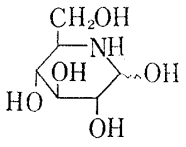
As in similar cases of other 5,6-dideoxy-5,6-epimines,[3] **1b** was treated with a warm acetic acid solution to yield 5-acetamido-6-O-acetyl-3-O-benzyl-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**6**) of mp 123.5—124.5° with epimine ring opening. The structure of **6** was

1) Partly presented as preliminary communications: H. Saeki, T. Iwashige, and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **16**, 188 (1968); H. Saeki and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **16**, 962 (1968).
2) Location: *Hiromachi, Shinagawa-ku, Tokyo.*
3) H. Saeki and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2471 (1968).
4) *a*) N. Ishida, K. Kumagai, T. Niida, T. Tsuruoka, and H. Yumoto, *J. Antibiotics.* (Tokyo), Ser A, **20**, 66 (1967); *b*) S. Inouye, T. Tsuruoka, and T. Niida, *J. Antibiotics* (Tokyo), Ser A, **19**, 288 (1966); *c*) S. Inouye, T. Tsuruoka, T. Ito, and T. Niida, *Tetrahedron*, **24**, 2125 (1968).
5) R.L. Whistler and R.E. Gramera, *J. Org. Chem.*, **29**, 2609 (1964).
6) A.S. Meyer and T. Reichstein, *Helv. Chim. Acta*, **29**, 152 (1946).
7) H.L. Spell, *Anal. Chem.*, **39**, 185 (1967).

verified by the following reactions. First, the thin-layer chromatogram of **6** was not identical with that of 6-acetamido-5-O-acetyl-3-O-benzyl-6-deoxy-1,2-O-isopropylidene-β-L-idofuranose (**7a**) which was supposed to be one of the possible products and prepared by lithium aluminum hydride reduction of the azide (**5a**), followed by acetylation of the resulting 6-amine (**7b**) of mp 123—125°. Therefore, **6** was thought to be a 5-acetamido-6-O-acetyl-5-deoxy-D-glucose derivative. Thus, hydrogenation of **6** over palladium charcoal, followed by saponification with a base, afforded a debenzylated amine, which was found to be identical with 5-amino-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**8**) prepared unequivocally by a modification of Whistler's procedure[5] as follows: Treatment of the epoxide (**3**) with sodium benzylate gave a 3,6-di-O-benzyl derivative (**9a**) of mp 74°, whose tosylation afforded 3,6-di-O-benzyl-5-O-tosylate (**9b**) of mp 89°. **9b** was treated with sodium azide to give 5-azido-3,6-di-O-benzyl-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**10**) of mp 71—72°, which on hydrogenation over palladium charcoal yielded **8** of mp 125—126°.[8]



1a : R=H
b : R=Ac



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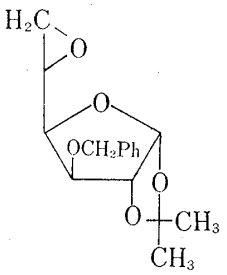

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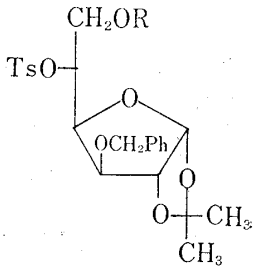
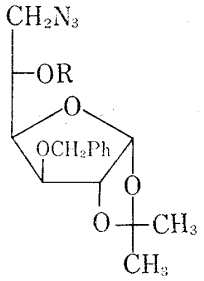
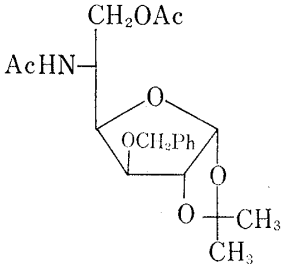


4a : R=Ts
b : R=Ac



5a : R=H
b : R=Ts



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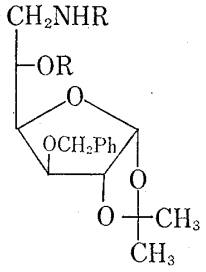
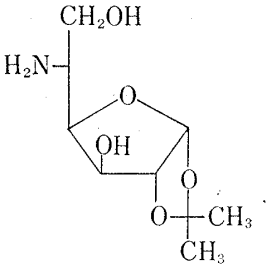
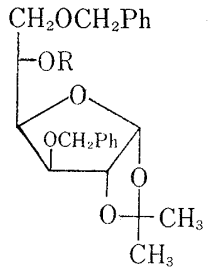


7a : R=Ac
b : R=H



8



9a : R=H
b : R=Ts

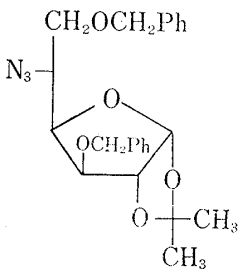


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Chart 1

8) Professor R.L. Whistler, Purdue University, Indiana, U.S.A., sent us a private communication that the melting point of **8** (mp 86°) recorded in the Journal (footnote 5) was listed incorrectly and it will be corrected in a forthcoming issue of the Journal. We greatly appreciate his kind help for sending the communication and a sample of **8** prepared by his group for identification.

Thereby, the nuleophilic attack of an acetate ion was shown to be exclusively effected at the terminal position of **1b** with epimine ring opening, as is consistent with other examples.[3)]

In 1966—1967, it was reported that several strains of *Streptomyces* such as *Str. roseochromogenes* R-468, *Str. lavendulae* SF-425 and *Str. nojiriensis n. sp.* SF-426 produced a new antibiotic, nojirimycin (**2**) which showed activity against *Sarcina lutea*, *Xanthomonas oryzae*, and a drug-resistant strain of *Shigella flexneri*.[4a)] The structure of **2** was designated as D-glucopiperidinose,[4b),4c)] which is not only of interest as the first member of "heterose" found in nature, but also as a structurally-simple hexose derivative active against bacteria. Therefore, we attempted the conversion of 5-amino-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**8**) thereby obtained into the antibiotic (**2**). Independently, Inouye, Tsuruoka, Ito and Niida[4c)] have recently accomplished the same conversion of **8** into **2** by de-O-acetonation with sulfurous acid and successive treatment with a base.

8 was quite unstable to acid; it was found that a reaction product obtained by a preliminary treatment of **8** with diluted hydrochloric acid exhibited absorption maxima at 225—226 mμ and 289—290 mμ which shifted to 245 mμ and 302—303 mμ in diluted sodium hydroxide solution. This suggested the presence of a pyridine derivative (**11**) or a similar substance which would be the same compound obtained by acid treatment of nojirimycin.[4b),4c)] However, the hydrolysis of **8** was successfully carried out by protecting O- and N-functions with the easily-removable trifluoroacetyl group before acid treatment. Thus treatment of **8** with trifluoroacetic anhydride in acetonitrile readily afforded a syrupy N- and O-trifluoroacetate (**12**). The infrared spectrum exhibited no amino or hydroxyl absorption, but absorptions of trifluoroacetoxy group at 1800 cm^{-1} and trifluoroacetamido group at 1740 and 1650 cm^{-1} were noted. **12** was also obtained by trifluoroacetylation of **8** in the presence of bases. Hydrolysis of **12** with 0.1 N hydrochloric acid at 70—80° for 1 hr, followed by removal of the protective group by adjusting to pH 7—8 with Dowex 1×4 (OH^-), afforded, in a good yield, an amorphous D-glucopiperidinose (**2**). The analytical sample, 95—115° (decomp.), was purified by passing through a column of Dowex 1×2 (OH^-) as described earlier.[4a)] **2** thus obtained was identified with the authentic sample of nojirimycin by infrared spectrometry and thin-layer chromatography. Moreover, hydrogenation of **2** over platinum gave a deoxy compound (**13**), mp 195°, which was also identical with the sample of deoxynojirimycin derived from the natural antibiotic (**2**) by comparison of infrared spectra and mixed melting point test. The synthesized nojirimycin (**2**) also showed the same activity against *Sarcina lutea* and *Xanthomonas oryzae* as natural nojirimycin.

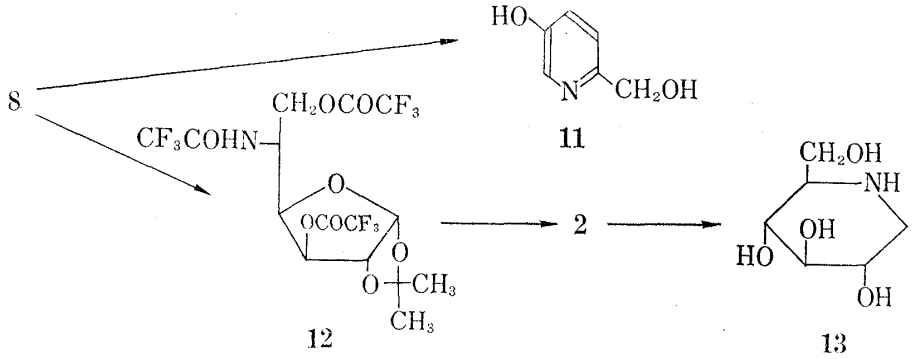

Chart 2

Experimental

Melting points are uncorrected. Infrared spectra were determined on Perkin-Elmer Model 21. Plates for thin-layer chromatography were prepared with Silica Gel G (E. Merck AG). Development of spots was effected by spraying a solution of NH_4VO_3 in 50% H_2SO_4, followed by heating. Column chromatography was carried out on a column packed with Silica Gel (Kanto Chemical Co., Tokyo). Evaporations done *in vacuo* were performed in a rotary evaporator.

5,6-Anhydro-3-O-benzyl-1,2-O-isopropylidene-β-L-idofuranose (3)——**3** was prepared by a modification of the Whistler's procedure[5] as follows; treatment of 3-O-benzyl-1,2-O-isopropylidene-5,6-di-O-tosyl-α-D-glucofuranose (**4a**)[3,6] with anhyd. NaOAc in DMSO at 110—120° for 2—3 hr afforded 6-O-acetyl-3-O-benzyl-1,2-O-isopropylidene-5-O-tosyl-α-D-glucofuranose (**4b**) as needles (from benzene–petr. ether) of mp 131—132°, $[\alpha]_D^{21}$ −7.1°(c=3.3, $CHCl_3$), in 74% yield. IR ν_{max}^{Nujol} cm^{-1}: 1750 (OAc). *Anal.* Calcd. for $C_{25}H_{30}O_9S$: C, 59.28; H, 5.97; S, 6.33. Found: C, 59.50; H, 5.93; S, 6.23.

Subsequent treatment of **4b** with NaOMe as described[5] earlier yielded **3** as a syrup, which was purified by column chromatography on silica gel (eluted with AcOEt–benzene (1:9, v/v)). **3** was also prepared from 6-O-benzoyl-5-O-tosyl derivative by the method of Meyer and Reichstein.[6]

6-Azido-3-O-benzyl-6-deoxy-1,2-O-isopropylidene-β-L-idofuranose (5a)——To a solution of 3.4 g of **3** in a mixture of 35 ml of methylcellosolve and 2 ml of H_2O, 1.5 g of NaN_3 and 0.85 g of NH_4Cl was added and the resulting mixture was refluxed for 1.5 hr. After filtration, the mixture was evaporated to dryness *in vacuo*. The residue was extracted with $CHCl_3$, and the extract was dried over anhyd. Na_2SO_4. Evaporation of the solvent from the extract *in vacuo* gave 4.3 g of a brown syrup which was chromatographed on 140 g of silica gel. Evaporation of solvent from fractions eluted with AcOEt–benzene (1:9, v/v) afforded 3.5 g of **5a** as a colorless syrup of $[\alpha]_D^{20}$ −68.8 (c=4.3, $CHCl_3$). IR $\nu_{max}^{liq.}$ cm^{-1}: 3500 (OH), 2100 (N_3). *Anal.* Calcd. for $C_{16}H_{21}O_5N_3$: C, 57.32; H, 6.31; N, 12.53. Found: C, 57.42; H, 6.44; N, 12.54.

6-Azido-3-O-benzyl-6-deoxy-1,2-O-isopropylidene-5-O-tosyl-β-L-idofuranose (5b)——A mixture of 7.01 g of **5a**, 4.42 g of TsCl, and 50 ml of pyridine was allowed to stand for 5 days at room temperature. Treatment in the usual manner gave 9.96 g of the crude **5b** which was chromatographed on a silica gel column (180 g). Evaporation of the solvent from fractions eluted with benzene gave 7.36 g (58%) of a colorless syrup of **5b**. *Anal.* Calcd. for $C_{23}H_{27}O_7N_3S$: C, 56.57; H, 5.56; N, 8.59; S, 6.55. Found: C, 56.58; H, 5.59; N, 8.45; S, 6.57.

3-O-Benzyl-5,6-dideoxy-5,6-epimino-1,2-O-isopropylidene-α-D-glucofuranose (1a) and Its Acetate (1b)——To an ice-cold solution of 7.08 g of **5b** in dry ether, 3 g of $LiAlH_4$ was added in small portions with stirring. The mixture was stirred at 0° for 1 hr and then at room temperature for 10 min. After the excess of the reagent was decomposed by dropwise addition of H_2O, the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*, and left 3.92 g of a crude **1a** which revealed one spot on thin-layer chromatogram. Further purification of **1a** was not successful.

To a solution of 3.92 g of **1a** in 38 ml of MeOH, 2.1 ml of Ac_2O was added with stirring and cooling, and the mixture was stirred at room temperature for 15 min. The mixture was diluted with H_2O to turbidity and then saturated with NaCl. The mixture was extracted with $CHCl_3$ and the extract was washed with 2N NaOH solution and H_2O, and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a syrup, which was dissolved in iso-PrOH and added dropwise hexane to turbidity. Recrystallization of crystals thereby obtained from iso-PrOH gave 3.49 g of **1b** as prisms of mp 97—99°, $[\alpha]_D^{22}$ −10.5° (c=3.9, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1700 (-NAc). *Anal.* Calcd. for $C_{18}H_{23}O_5N$: C, 64.85; H, 6.95; N, 4.20. Found: C, 64.68; H, 6.97; N, 4.27.

Treatment of **1b** with a catalytic amount of NaOMe in MeOH in a short time afforded **1a** in a good yield.

5-Acetamido-6-O-acetyl-3-O-benzyl-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (6)——A solution of 2.28 g of **1b** in 30 ml of AcOH was warmed at 60—70° for 1.5 hr. The mixture was diluted with H_2O and halfly neutralized with solid Na_2CO_3. The mixture was extracted twice with $CHCl_3$ in 100 ml portions and the extract was washed with dil. Na_2CO_3 solution and H_2O, then dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline mass which was recrystallized from iso-PrOH–hexane to give 2.19 g (81.4%) of **6** as prisms of mp 123.5—124.5°, $[\alpha]_D^{22}$ −25.9° (c=6.6, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3350 (NH), 1740 (-OAc), 1650, 1540 (-NHAc). *Anal.* Calcd. for $C_{20}H_{27}O_7N$: C, 61.05; H, 6.92; N, 3.56. Found: C, 61.01; H, 6.98; N, 3.75.

6-Amino-3-O-benzyl-6-deoxy-1,2-O-isopropylidene-β-L-idofuranose (7b)——To a solution of 2.7 g of **5a** in 30 ml of dry ether, 0.75 g of $LiAlH_4$ was added with cooling, and the mixture was stirred for 1 hr at room temperature. After decomposition of the excess of the reagent by dropwise addition of H_2O, the solid was filtered and washed with AcOEt. The combined washings and filtrate were evaporated to dryness *in vacuo* and afforded 1.93 g of crystals which were recrystallized from AcOEt–hexane to give **7b** as needles of mp 123—125°, $[\alpha]_D^{22}$ −43.2° (c=5.0, $CHCl_3$). *Anal.* Calcd. for $C_{16}H_{23}O_5N$: C, 62.12; H, 7.49; N, 4.53. Found: C, 61.92; H, 7.39; N, 4.64.

5-Amino-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (8)——i) Hydrogenation of **6** (500 mg) was carried out over 0.2 g of 10% Pd-C in 25 ml of EtOH at 70—80 kg/cm^2 at 60—70° for 5 hr. After the catalyst was filtered off, the mixture was evaporated to dryness *in vacuo* and left 406 mg of a crude debenzylated acetate. The infrared spectrum showed no absorption at 1500 and 690—730 cm^{-1} corresponding to a benzyl group.

A mixture of the debenzylated product thereby obtained and 10 ml of a saturated $Ba(OH)_2$ solution was heated on a steam bath for 4 hr and then, saturated with CO_2 and centrifuged. The supernatant was evaporated to dryness *in vacuo* to give a syrup, which was dissolved in 1 ml of conc. NH_4OH and extracted with $CHCl_3$. Evaporation of the solvent gave 158 mg of a crystalline mass which was recrystallized from

EtOH–ether to give **8** as needles of mp 125—126°, $[\alpha]_D^{20}$ −17.0° (reported mp 86°, $[\alpha]_D$ −12.2°).[5,8] *Anal.* Calcd. for $C_9H_{17}O_5N$: C, 49.30; H, 7.81; N, 6.39. Found: C, 49.64; H, 7.79; N, 6.39.

ii) One gram of **10** was hydrogenated over 0.7 g of 10% Pd-C at 80 kg/cm² at 70—80° for 6 hr. Treatment in the usual manner gave 313 mg of **8**, which was recrystallized from EtOH–ether and also identical with the sample obtained above by mixed mp and infrared spectrometry.

3,6-Di-O-benzyl-1,2-O-isopropylidene-5-O-tosyl-β-L-idofuranose (9b)——Working up as described previously,[5] **3** was treated with sodium benzylate to give 3,6-di-O-benzyl-1,2-O-isopropylidene-β-L-idofuranose (**9a**) as needles (from MeOH–H_2O) of mp 74°, $[\alpha]_D^{21}$ −48.9° (c=4.4, $CHCl_3$) (reported[5] mp 89—90°, $[\alpha]_D^{25}$ −44.0°). *Anal.* Calcd. for $C_{23}H_{28}O \cdot \frac{1}{2}H_2O$: C, 67.46; H, 7.15. Found: C, 67.57; H, 6.99.

9a thereby obtained was tosylated as reported earlier[5] furnishing **9b** in 92% yield as prisms of mp 89°, $[\alpha]_D^{20}$ −13.7° (c=2.6, $CHCl_3$) (reported[5] mp 75—76°, $[\alpha]_D^{25}$ −15.3°) (from MeOH). *Anal.* Calcd. for $C_{30}H_{34}O_8S$: C, 64.97; H, 6.18; S, 5.78. Found: C, 65.02; H, 6.21; S, 5.78.

5-Azido-3,6-di-O-benzyl-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (10)——A mixture of 7.91 g of **9b**, 6.44 g of NaN_3, and 80 ml of DMSO was heated at 120—140° in N_2 atmosphere with stirring for 4 hr. The mixture was diluted with H_2O and extracted with ether. The extract was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crystalline mass which was recrystallized from MeOH to give 3.64 g of **10** as needles of mp 71—72°, $[\alpha]_D^{20}$ −36.9° (c=2.9, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 2100 (N_3). *Anal.* Calcd. for $C_{23}H_{27}O_5N_3$: C, 64.92; H, 6.40; N, 9.88. Found: C, 64.83; H, 6.50; N, 9.97.

5-Amino-5-deoxy-D-glucose (Nojirimycin) (2)——To an ice-cold solution of 1.00 g of **8** in 25 ml of MeCN, 3 ml of trifluoroacetic anhydride was added with stirring, and the resulting mixture was stirred at room temperature for 30 min. The mixture was diluted with 30 ml of 0.1N HCl solution and 5 ml of MeCN, and the mixture was kept at 70—80° for 2 hr. After treated with activated carbon (Darco G-60), the solution was adjusted to pH 7—8 by adding Dowex 1×4 (OH^-). After stirring for a few min., the resin was filtered off and the aqueous layer was washed with 50 ml of $CHCl_3$ and freeze-dried, giving 0.5 g of **2** as a colorless powder. An analytical sample was prepared as follows: 100 mg of crude **2** was placed on 20 ml of Dowex 1×2 (OH^-) (100—200 mesh), and eluted with H_2O. The fractions (5 ml in each tube), showing activity against *Sarcina lutea*, were collected and freeze-dried, giving 55 mg of pure **2** as a powder of mp 95—115° (decomp.), $[\alpha]_D^{20}$ +89° (1 min) → +63.0° (c=1.2, H_2O, equilibrium) (reported mp 115° (decomp.), $[\alpha]_D^{31}$ +49°,[4a]; mp 126—130° (decomp.), $[\alpha]_D^{24}$ +100°→+73.5° (equilibrium)[4c]. *Anal.* Calcd. for $C_6H_{13}O_5N$: C, 40.22; H, 7.31; N, 7.82. Found: C, 40.82; H, 6.97; N, 7.86.

The sample of **2** thus obtained was identified with the natural nojirimycin by thin-layer and paper chromatographies and infrared spectrometry.

In another run, the intermediate, trifluoroacetate (**12**) of **8** was obtained as follows: To a cooled solution of 0.36 g of **8** in 10 ml of MeCN, was added 1.5 ml of trifluoroacetic anhydride, and the mixture was stirred for 15 min at room temperature. Evaporation of the mixture *in vacuo* below 40° left 0.93 g of **12** as a syrup. IR $\nu_{max}^{liq.}$ cm^{-1}: 3400 (NH), 1800 (-OCOCF$_3$), 1740, 1650 (-NHCOCF$_3$), no OH. The sample was identical with the product, which was prepared by the same procedure in the presence of sodium trifluoroacetate, by thin-layer chromatography (MeOH–benzene (1:9, v/v)) and gas chromatography (1.5% SE-30 on Chromosorb W, 4 mm×1.5 m, at 145°, using a Shimadzu Model GC-1B).

Deoxynojirimycin (13)——The synthesized **2** (200 mg) was hydrogenated in a mixture of 10 ml of H_2O and 10 ml of EtOH over 100 mg of Pt (Adams). After one equivalent of H_2 was consumed in 1 hr, the absorption almost ceased. After filtering the catalyst off, the mixture was evaporated *in vacuo* to give a syrup which was redissolved in H_2O, and after addition of EtOH to turbidity, cooled. The crystals obtained (172 mg) was recrystallized from H_2O–EtOH to give **13** as prisms of mp 195°, $[\alpha]_D^{20}$ +43.1° (c=1.4, H_2O) (reported mp 196°, $[\alpha]_D^{21}$ +47°[4a]). *Anal.* Calcd. for $C_6H_{13}O_4N$: C, 44.16; H, 8.03; N, 8.58. Found: C, 43.84; H, 8.08; N, 8.49.

The sample was also identified with an authentic sample[4c] obtained from the natural product by mixed mp, and infrared spectrometry.

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