Structure and Synthesis of Nectrisine, a New Immunomodulator Isolated from a Fungus[1)]

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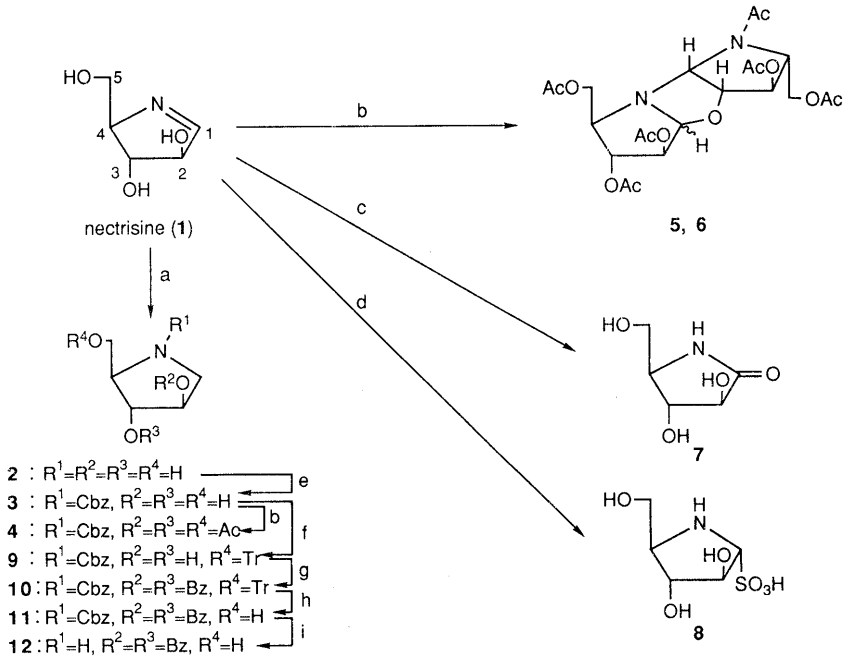
The structure of a novel immunomodulator, nectrisine (1), has been elucidated on the basis of chemical and spectroscopic evidence. Its absolute stereochemistry was predicted on the basis of the dibenzoate chirality rule and finally confirmed by a synthesis from D-glucose.

Keywords nectrisine; *Nectria lucida*; D-glucose; D-arabinose; enantiospecific synthesis; immunomodulator; α-glucosidase inhibitor; α-mannosidase inhibitor; Ia antigen

In our continuing screening program for immunologically active compounds from microorganisms, nectrisine (**1**), which was tentatively designated as WF4490, was isolated as a new type of immunomodulator from a fungus, *Nectria lucida* F-4490.[2)] This natural product induces the expression of Ia antigen[3)] and restores the immune response depressed by immunosuppressive factors of tumors.[2)] It also possesses potent α-glucosidase-inhibitory activity and α-mannosidase-inhibitory activity.[2)] In the previous communication,[1)] we reported the structural elucidation and synthesis of nectrisine. This paper is devoted to a full account of that work.

Nectrisine was isolated as a colorless powder: $[\alpha]_D$ +21.8° (c=0.6, H_2O). The molecular formula ($C_5H_9NO_3$) was established by elemental analysis and fast atom bombardment mass spectrometry (FAB-MS). The infrared (IR) spectrum showed absorption bands ascribed to hydroxyl groups (3330 cm^{-1}) and an imino function (1640 cm^{-1}). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I) showed five signals consisting of one methylene (δ 61.8 (t)), three methines (δ 83.9 (d), 78.8 (d), 77.4 (d)), and one sp^2-carbon (δ 171.0 (d)) assignable to the C-1 imino carbon. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table II), the corresponding imino proton was observed at δ 7.71 (1H, d, J=2 Hz), together with five protons of methylene and methine moieties (δ 4.12—3.14, 5H, m). The chemical shifts of ^{1}H- and ^{13}C-NMR suggested that all carbons bear oxygen or nitrogen atoms.

Catalytic hydrogenation of **1** (H_2 (5 atm), 10% Pd–C, H_2O) provided the dihydro derivative **2** whose ^{13}C-NMR spectrum (Table I) showed a new methylene signal (δ 51.0 (t)), instead of the imino carbon signal of **1**, along with other carbons consisting of one methylene (δ 61.8) and three methines (δ 78.8 (d), 77.2 (d), 66.1 (d)). In the ^{1}H-NMR spectrum of **2** (Table II), the corresponding two methylene protons appeared at δ 3.20 (1H, dd, J=12, 6 Hz) and δ 2.92 (1H, dd, J=12, 4 Hz). A spin-decoupling experiment on **2** clarified the ^{1}H–^{1}H relationships as shown in Fig. 1 to reveal the structure of 3,4-dihydroxy-2-(hydroxymethyl)pyrrolidine for **2**. This structure was corroborated by the fact that treatment of **2** with carbobenzyloxy chloride followed by



(a) H_2, 10% Pd-C, H_2O (b) Ac_2O, Py (c) I_2, NaOH, H_2O (d) SO_2, H_2O (e)CbzCl, NaOH, H_2O
(f) TrCl, Et_3N, DMAP, CH_2Cl_2 (g)BzCl, Py, CH_2Cl_2 (h)TsOH, MeOH (i)H_2, 10% Pd-C, MeOH

Chart 1

TABLE I. ^{13}C-NMR (67.8 MHz, D_2O) Chemical Shifts (in ppm) for **1** and **2**[a]

C	1	2
1	171.0 (d)	51.0 (t)
2	83.9 (d)[b]	77.2 (d)[c]
3	78.8 (d)[b]	78.8 (d)[c]
4	77.4 (d)[b]	66.1 (d)[c]
5	61.8 (t)	61.8 (t)

a) Abbreviations given in parentheses denote signals observed in the off-resonance experiments. *b, c*) Assignments may be interchangeable in each column.

TABLE II. ^{1}H-NMR Chemical Shifts (D_2O, in ppm), Multiplicities, and Coupling Constants (in Hz, in parentheses) for **1**, **2**, and **7**

H	1[a]	2[a]	7[b]
1-H	7.71 d (2)	3.20 dd (12, 6) 2.92 dd (12, 4)	
2-H	4.12—3.14, 5H m	4.18 dt (6, 4)	4.08 d (8)
3-H		3.87 dd (5, 4)	3.77 t (8)
4-H		3.09 dt (6, 5)	3.21 ddd (8, 5, 4)
5-H		3.78 dd (12, 5) 3.69 dd (12, 6)	3.56 dd (12, 4) 3.48 dd (12, 5)

a) 270 MHz. *b*) 200 MHz.

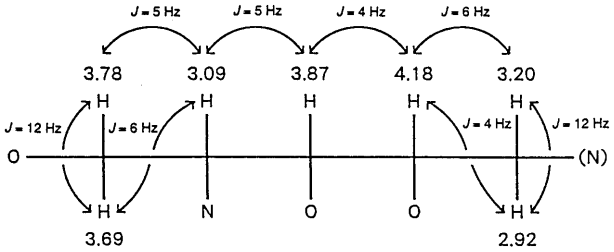


Fig. 1

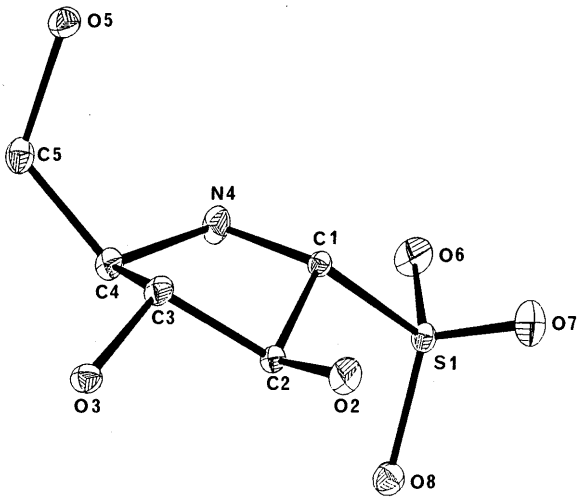


Fig. 2

TABLE III. Atomic Coordinates with e.s.d.'s in Parentheses and Thermal Parameters (Å^2)

Atom	x	y	z	B_{eq}
C1	0.399 (1)	0.842 (1)	0.703 (2)	1.2
C2	0.364 (1)	0.880 (1)	0.949 (2)	1.1
C3	0.179 (1)	0.803 (1)	0.908 (2)	1.3
C4	0.217 (1)	0.665 (1)	0.821 (2)	1.3
C5	0.048 (2)	0.580 (1)	0.674 (2)	1.7
N4	0.336 (1)	0.697 (1)	0.654 (2)	1.7
S1	0.6420 (3)	0.8544 (4)	0.7223 (4)	1.3
O2	0.358 (1)	1.0178 (9)	0.991 (1)	1.9
O3	0.142 (1)	0.7955 (9)	1.137 (1)	1.6
O5	−0.064 (1)	0.6405 (9)	0.432 (1)	1.7
O6	0.651 (1)	0.7589 (9)	0.526 (2)	2.2
O7	0.668 (1)	0.992 (1)	0.670 (2)	2.4
O8	0.757 (1)	0.8127 (9)	0.980 (1)	2.0

TABLE IV. Bond Lengths (Å) and Angles (°) with Their e.s.d.'s in Parentheses

Bond lengths (Å)			
C1–C2	1.55 (2)	C1–N4	1.52 (2)
C1–S1	1.80 (1)	C2–C3	1.53 (2)
C2–O2	1.41 (1)	C3–C4	1.53 (2)
C3–O3	1.42 (1)	C4–N4	1.55 (2)
C4–C5	1.51 (2)	S1–O6	1.48 (1)
S1–O7	1.44 (1)	S1–O8	1.46 (1)
C5–O5	1.45 (2)		
Bond angles (°)			
C2–C1–N4	104.8 (9)	C2–C1–S1	115.5 (9)
N4–C1–S1	108.9 (8)	C1–C2–C3	100.4 (9)
C1–C2–O2	114.7 (9)	C3–C2–O2	115.8 (9)
C2–C3–C4	103.8 (9)	C2–C3–O3	111.0 (9)
C4–C3–O3	111.0 (10)	C3–C4–N4	102.6 (9)
C3–C4–C5	118.1 (10)	N4–C4–C5	109.9 (10)
C1–N4–C4	107.5 (9)	C1–S1–O6	103.4 (6)
C1–S1–O7	105.5 (6)	C1–S1–O8	105.4 (6)
O6–S1–O7	115.0 (6)	O6–S1–O8	112.6 (6)
O7–S1–O8	113.7 (6)	C4–C5–O5	111.6 (10)

acetylation with acetic anhydride gave the *N*-benzyloxycarbonyl-tri-*O*-acetyl derivative **4**, *via* **3**. Hence the structure of nectrisine was deduced to be **1**, without the stereochemistry.

Chemical evidence supporting this presumed structure was obtained as follows. Acetylation of **1** with acetic anhydride in pyridine afforded the two epimeric dimeric hexaacetates **5** (18%) and **6** (30%).[4] The C-1′ configurations of the dimers were could not be clarified because the two epimers showed similar $J_{1',2'}$ values, 5 Hz for **5** and 4 Hz for **6**, in their ^{1}H-NMR spectra. The dimers were isomerized by treatment with 0.5% TsOH in $CHCl_3$ at room temperature to give a mixture of **5** and **6** (*ca.* 1 : 1). Further evidence in support of the structure **1** was provided by oxidation of **1** with iodine (NaOH, H_2O) to give the lactam **7**, whose IR spectrum showed an absorption band ascribed to an amide function at 1658 cm^{-1}. The ^{1}H-NMR spectrum of **7** (Table II) showed five signals at δ 4.08 (1H, d, J=8 Hz), 3.77 (1H, t, J=8 Hz), 3.56 (1H, dd, J=12, 4 Hz) and 3.48 (1H, dd, J=12, 5 Hz), and 3.21 (1H, ddd, J=8, 5, 4 Hz), which were assigned to 2-H, 3-H, 5-H$_2$ and 4-H, respectively. Thse data indicate that nectrisine has the structure **1**.

In order to gain information on the stereochemistry of nectrisine, we examined several reactions for obtaining the acetonide of **2** (*e.g.*, 2,2-dimethoxypropane, TsOH). These attempts were all unsuccessful, and accordingly the two hydroxyl groups and the hydroxymethyl function are presumed to be all *trans*. This presumed structure was confirmed by X-ray crystallographic analysis of the bisulfite adduct **8**, which was prepared by treatment of **1** with SO_2 in H_2O (Fig. 2).

The absolute stereochemistry was deduced to be the D-form by applying the dibenzoate chirality rule[5] to the 2,3-di-*O*-benzoyl derivative **12**, which was prepared as follows. The *N*-benzyloxycarbonyl derivative **3** was transformed into **10** *via* **9** by selective protection of the primary alcohol with trityl chloride and subsequent acylation of the two hydroxyl groups with benzoyl chloride. Treatment of **10** with TsOH and subsequent hydrogenolysis of the resulting **11** over 10% Pd–C afforded the dibenzoate **12**. A negative sign of the first Cotton effect ($[\theta]_{234}$ −67000) was observed in the circular dichroism (CD) spectrum of **12**, indicating that the configurations of C-2 and C-3 are both *R* and hence, that of C-4 is *R*. Since nectrisine showed potent α-glucosidase inhibitory activity, this deduction on the stereochemistry of C-2, C-3, and C-4 is reasonable by analogy with that of the corresponding C-3, C-4, and C-5 in nojirimycin (**13**), a representative α-glucosidase inhibitor[6] (Fig. 3).

Finally the presumed structure was confirmed by a synthesis from D-glucose, whose three asymmetric carbons, C-3, C-4, and C-5, correspond to C-2, C-3, and C-4 of nectrisine, respectively. We devised a synthetic route for **1** that includes oxidative cleavage between C-1 and C-2 of the appropriately protected 5-amino-5-deoxy-D-glucose (**14**) (Chart 2). The requisite intermediate **14** could be obtained from 5-amino-5-deoxy-3-*O*-benzyl-1,2-*O*-isopropylidene-6-*O*-trityl-α-D-glucofuranose (**15**), prepared by Niida *et al.* for their synthesis of nojirimycin.[6] We chose a trifluoroacetyl group for protection of the amino function in **15** because we expected that it would be stable under acidic or oxidative conditions and against hydrogenolysis, and it is easily removed by mild alkaline hydrolysis in the last step. *N*-Acylation of **15** with trifluoroacetic anhydride and subsequent acidic hydrolysis of the resulting **16** with 75% aqueous trifluoroacetic acid (TFA) afforded the triol **17**. The vicinal diol function was oxidatively cleaved with $NaIO_4$ to give the pyranose **18**. In this reaction the C-1 carbon of **17** remained as the *O*-formyl group at the 3-position of **18**. Although the 2-*O*-benzyl group resisted the usual hydrogenolysis (H_2 (5 atm), 10% Pd–C), deprotection was achieved by hydrogenolysis using Pd black in 4.4% HCOOH–MeOH to afford **19** in 98% yield. The two acyl groups in **19** were finally hydrolyzed with a slight excess of 0.5 N aqueous NaOH to furnish **1** in 96% yield. This product was identical with the natural product. The total yield from **15** was 56%. The structure of nectrisine was thus established to be **1**.

It is notable that nectrisine exists as the imino form, as judged from the ^1H- and ^{13}C-NMR spectral data, while the corresponding L-xylo (**21**)[4a] and L-lyxo (**22**)[4b] stereoisomers were reported to adopt mainly the dimeric forms **21c** and **22c**, respectively (Fig. 4). This might be explained by steric factors. It seems that **1** is much more stable than **21b** and **22b** because the three substituents on the pyrroline ring of

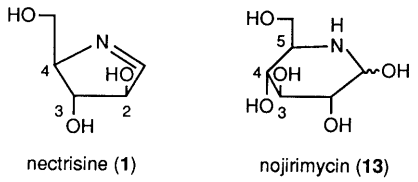


Fig. 3

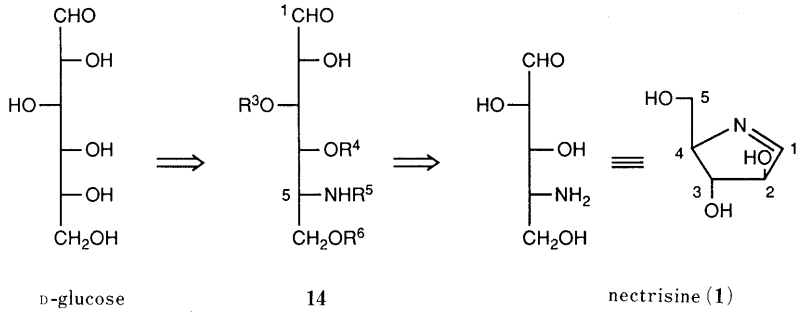
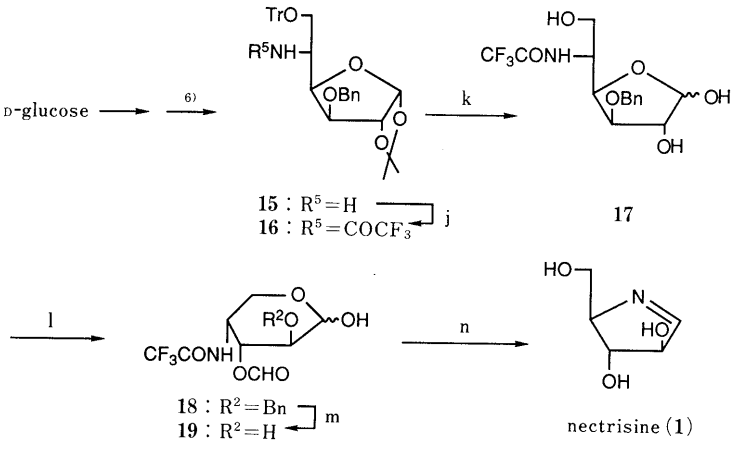
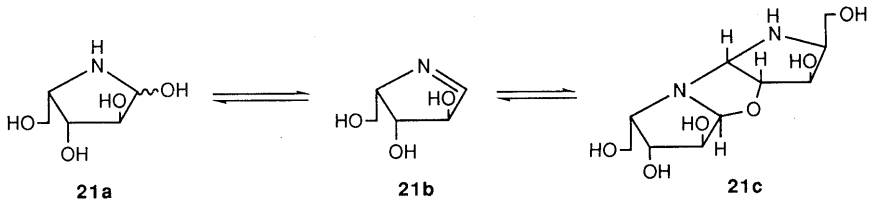


Chart 2



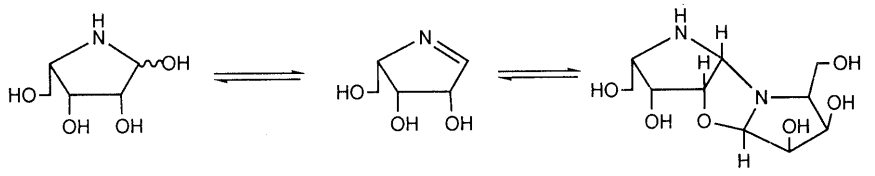
(j) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2 (k) 75% aq. TFA (l) $NaIO_4$, aq. THF (m) Pd-black, 4.4% HCOOH–MeOH (n) 0.5 N aq. NaOH

Chart 3



21a 21b 21c

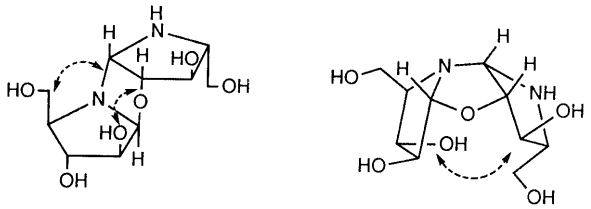
21 (L-xylo)



22a 22b 22c

22 (L-lyxo)

Fig. 4



23 24

Fig. 5

TABLE V. Biological Activities of Nectrisine Derivatives

Compound	α-Glucosidase IC_{50} (μg/ml)	α-Mannosidase IC_{50} (μg/ml)	Ia induction EC_{70} (μg/ml)	Restoration of immune response MEC (μg/ml)
Nectrisine	0.05	6.5	0.04	0.08
2	0.2	310	1.6	2.0
3	>33	>33	>12.5	>500
7	>33	>33	>12.5	N.T.
1-Deoxynojirimycin	0.03	>33	>25	N.T.
Swainsonine	>33	0.12	0.02	0.02

1 are in all *trans*, and not eclipsed, relationships, while **21b** has one pair and **22b** has two pairs of substituents which are in *cis*, and eclipsed, relationships. On the other hand, **23** and **24**, dimeric forms of **1**, are likely to be less stable than **21c** and **22c** because of steric hindrance (Fig. 5).

Biological activities[7] of nectrisine (**1**), **2**, **3** and **7** are shown in Table V. A representative α-glucosidase inhibitor, 1-deoxynojirimycin,[8] and a representative α-mannosidase inhibitor, swainsonine,[9] were also tested. It appears that the imino function and the basic nitrogen atom of nectrisine (**1**) are important for its biological activities. These results also seem to suggest that the immunomodulating activities, *i.e.*, induction of Ia antigen and restoration of immune response, are correlated with each other and that α-mannosidase-inhibitory activity, but not α-glucosidase-inhibitory activity, might contribute to the immunomodulating activities.

In conclusion, we established the structure of nectrisine to be 4-amino-4-deoxy-D-arabinose (**1**), and developed an efficient synthetic route from D-glucose which is capable of providing sufficient amounts for detailed biological evaluation.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our preceding paper[10] except for the following. A JEOL FX-270 spectrometer was also used to take ^1H (270 MHz) and ^{13}C (67.8 MHz)-NMR spectra. CD spectra were measured with a JASCO J-20 automatic recording spectropolarimeter.

Nectrisine (1) A colorless amorphous powder, $[\alpha]_D$ +21.8° (c=0.6, H_2O). *Anal.* Calcd for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68. Found: C, 45.08; H, 6.57; N, 10.16. IR (KBr): 3330, 2900, 1640, 1500, 1400, 1240, 1200, 1040 cm^{-1}. ^1H- and ^{13}C-NMR: see Tables I and II. FAB-MS m/z: 132 $(M+H)^+$.

1,4-Dideoxy-1,4-imino-D-arabinitol (2) A solution of **1** (120 mg) in H_2O (5.0 ml) was treated with 10% Pd–C (30 mg) under hydrogen (4 atm) at room temperature for 4 h. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to give a brownish oil (125 mg), which was purified by carbon treatment in H_2O to afford **2** (116 mg, 95%). **2**: A viscous colorless oil, $[\alpha]_D$ +15.7° (c=0.4, H_2O). *Anal.* Calcd for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52. Found: C, 44.82; H, 8.60; N, 10.37. IR (neat): 3350, 2940, 1540, 1420, 1052 cm^{-1}. ^1H- and ^{13}C-NMR: see Tables I and II. FAB-MS m/z: 134 $(M+H)^+$.

***N*-Benzyloxycarbonyl-1,4-dideoxy-1,4-imino-D-arabinitol (3)** Carbobenzyloxy chloride (1.0 ml) and 1 N aqueous NaOH (0.7 ml) were added to a stirred ice-cold solution of **2** (665 mg) in H_2O (10 ml) at room temperature over a period of 10 min and the mixture was stirred for 1 h. After removal of the solvent under reduced pressure, the residue was extracted with CH_2Cl_2–MeOH (4:1, 20 ml). The extract was combined, evaporated *in vacuo*, and purified by column chromatography (SiO_2 20 g, CH_2Cl_2–MeOH = 20:1) to afford **3** (1.18 g, 88%). **3**: Colorless fine crystals, mp 126—128 °C (Et_2O), $[\alpha]_D$ −28.9° (c=1.9, MeOH). *Anal.* Calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.10; H, 6.41; N, 5.15. IR (Nujol): 3340, 1664, 1420, 1352, 1190, 1118, 1072, 1050, 1010 cm^{-1}. ^1H-NMR (D_2O) δ: 7.49 (5H, s), 5.23 (2H, s), 4.30—4.18 (2H, m), 4.01—3.73 (4H, m), 3.39 (1H, m). FAB-MS m/z: 268 $(M+H)^+$.

2,3,5-Tri-*O*-acetyl-*N*-benzyloxycarbonyl-1,4-dideoxy-1,4-imino-D-arabinitol (4) Compound **3** (100 mg) was treated with acetic anhydride (1.0 ml) and pyridine (2.0 ml) at room temperature for 5 h. After concentration *in vacuo*, the residue was dissolved in Et_2O (5 ml) and washed with 1 N aqueous HCl, brine, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to afford **4** (140 mg, 95%). **4**: A colorless viscous oil, $[\alpha]_D$ −22.7° (c=0.6, MeOH). *Anal.* Calcd for $C_{19}H_{23}NO_8$: C, 58.01; H, 5.89; N, 3.56. Found: C, 57.78;

H, 5.84; N, 3.52. IR ($CHCl_3$): 1736, 1696, 1408, 1350, 1202 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.38 (5H, br s), 5.25—5.10 (4H, m), 4.43—3.84 (4H, m), 3.53 (1H, m), 2.11 (3H, s), 2.08 (6H, s). FAB-MS *m/z*: 394 $(M+H)^+$.

Acetylation of Nectrisine (1) A solution of **1** (1.2 g) in pyridine (20 ml) was treated with acetic anhydride (10 ml) at room temperature for 12 h. After concentration *in vacuo*, the residue was purified by column chromatography (SiO_2 100 g, *n*-hexane : AcOEt = 1 : 1—1 : 2) to afford two epimers of dimeric hexaacetates **5**, the faster-eluted one, (424 mg, 18%) and **6**, the later-eluted one, (716 mg, 30%). **5**: A colorless viscous oil, $[\alpha]_D$ +37.3° (*c* = 0.7, MeOH). IR ($CHCl_3$): 2990, 2950, 1738, 1660, 1366, 1224, 1204, 1040 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5:33 (1H, d, *J* = 5 Hz), 5.24 (1H, t, *J* = 5 Hz), 5.13 (1H, br s), 5.03 (1H, m), 4.78 (1H, d, *J* = 2 Hz), 4.57 (1H, d, *J* = 5 Hz), 4.37—4.28 (3H, m), 4.12—4.00 (2H, m), 3.37 (1H, m). FAB-MS *m/z*: 515 $(M+H)^+$. High-resolution FAB-MS Calcd for $C_{22}H_{31}N_2O_{12}$ $(M+H)^+$: 515.188. Found: 515.188. **6**: A colorless viscous oil, $[\alpha]_D$ −39.2° (*c* = 0.6, MeOH). IR ($CHCl_3$): 2990, 2940, 1738, 1654, 1364, 1220, 1202, 1042 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.77 (1H, d, *J* = 4 Hz), 5.20 (1H, dd, *J* = 6, 4 Hz), 5.18—5.11 (2H, m), 5.04 (1H, br s), 4.40 (1H, d, *J* = 4 Hz), 4.30—4.22 (2H, m), 4.19 (1H, dd, *J* = 10, 5 Hz), 4.06 (1H, m), 3.98 (1H, dd, *J* = 10, 8 Hz), 3.66 (1H, m). FAB-MS *m/z*: 515 $(M+H)^+$. High-resolution FAB-MS Calcd for $C_{22}H_{31}N_2O_{12}$ $(M+H)^+$: 515.188. Found: 515.189.

Isomerization of 5 and 6 Compound **5** (50 mg) was treated with a 0.5% solution of TsOH·H_2O in $CHCl_3$ (5.0 ml) at room temperature for 30 min. After being washed with saturated aqueous $NaHCO_3$ and brine, the organic layer was dried over $MgSO_4$, evaporated *in vacuo*, and subjected to column chromatography (SiO_2 5.0 g, *n*-hexane : AcOEt = 1 : 1) to give **5** (19 mg, 38%) and **6** (20 mg, 40%) which were found to be identical with authentic samples by direct comparison: TLC (AcOEt, *Rf* = 0.50 for **5** and 0.40 for **6**; *n*-hexane : acetone = 1 : 1, *Rf* = 0.46 for **5** and 0.39 for **6**; CH_2Cl_2 : MeOH = 19 : 1, *Rf* = 0.47 for **5** and 0.45 for **6**) and ^{1}H-NMR (DMSO-d_6). The same treatment of **6** (50 mg) afforded **5** (21 mg, 42%) and **6** (23 mg, 46%).

(3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-hydroxymethyl-2-pyrrolidone (7) Aqueous I_2 (0.1 N, 35 ml) and NaOH (52.5 ml) were added to a stirred solution of **1** (183 mg) in H_2O (7.5 ml) simultaneously over 12 min. After being stirred for 3 h, the reaction mixture was neutralized with 1 N HCl under ice-cooling and evaporated *in vacuo* to give a residue, which was extracted with $CHCl_3$: MeOH : H_2O = 6 : 4 : 1 (30 ml). The extract was evaporated *in vacuo*, and this residue was purified by cation exchange resin column chromatography (Dowex 50W × 8 (H^+ form) 15 ml, H_2O) and subsequent anion exchange resin column chromatography (Amberlite IRA-45 (OH^- form) 15 ml, H_2O) to afford **7** (55 mg, 27%). **7**: Colorless fine crystals, mp 136—137 °C (EtOH), $[\alpha]_D$ +15.6° (*c* = 0.5, H_2O). *Anal.* Calcd for $C_5H_9NO_4$: C, 40.82; H, 6.17; N, 9.52. Found: C, 40.52; H, 6.09; N, 9.22. IR (KBr): 3200, 2910, 2850, 1658, 1340, 1314, 1278, 1090, 1058 cm^{-1}. ^{1}H-NMR (D_2O) δ: 4.08 (1H, d, *J* = 8 Hz), 3.77 (1H, t, *J* = 8 Hz), 3.56 (1H, dd, *J* = 12, 4 Hz), 3.48 (1H, dd, *J* = 12, 5 Hz), 3.21 (1H, ddd, *J* = 8, 5, 4 Hz). FAB-MS *m/z*: 148 $(M+H)^+$.

Bisulfite Adduct of 1 (8) SO_2 gas was introduced into a stirred ice-cold solution of **1** (730 mg) in H_2O (0.73 ml) for 30 min. The reaction mixture was left to stand at room temperature for 2 d. MeOH (7.3 ml) was added thereto under ice-cooling and the mixture was stirred at the same temperature for 30 min. The precipitate was collected by vacuum filtration and washed with MeOH to afford **8** (836 mg, 72%). **8**: Colorless needles, mp > 250 °C (6% aqueous SO_2–EtOH), $[\alpha]_D$ +42.7° (*c* = 1.0, H_2O). *Anal.* Calcd for $C_5H_{11}NO_6S$: C, 28.17; H, 5.20; N, 6.57; S, 15.04. Found: C, 28.14; H, 5.03; N, 6.39; S, 14.72. IR (Nujol): 3370, 3300, 3150, 1568, 1247, 1234, 1213, 1200, 1160 cm^{-1}. ^{1}H-NMR (400 MHz, D_2O) δ: 4.46 (1H, dd, *J* = 7, 7 Hz), 4.38 (1H, d, *J* = 7 Hz), 4.14 (1H, dd, *J* = 10, 7 Hz), 3.96 (1H, dd, *J* = 12, 5 Hz), 3.91 (1H, dd, *J* = 12, 4 Hz), 3.69 (1H, ddd, *J* = 10, 5, 4 Hz). FAB-MS *m/z*: 425 $(2M-H)^+$, 212 $(M-H)^+$.

X-Ray Analysis of 8 The crystals were obtained by recrystallization from 6% aqueous SO_2–EtOH: $C_5H_{11}NO_6S$, monoclinic, space group $P2_1$, *a* = 7.545(1), *b* = 10.040(1), *c* = 5.627(1) Å, β = 111.28(1)°, *V* = 397.1(1) Å^3, *Z* = 2, D_x = 1.783 g/cm^3, μ = 36.4 cm^{-1}. The X-ray intensity data from a selected crystal (0.20 × 0.10 × 0.05 mm) were obtained on a Rigaku AFC-5 diffractometer equipped with a rotating anode X-ray generator (40 kV-100 mA), using graphite-monochromated CuK_α radiation (λ = 1.54178 Å). A total of 715 independent reflections $2\theta < 130°$ were collected with the $2\theta/\omega$ scan mode. The structure was solved by the direct method using MULTAN 84 (Main *et al.*, 1984). The refinement was carried out by the block-diagonal least-squares method with anisotropic thermal parameters for non H atoms. The *R* factor was reduced to 0.068 using 713 reflections with $F_o > 3\sigma(F_o)$. The atomic parameters, bond lengths and bond angles are given in Tables III and IV.

***N*-Benzyloxycarbonyl-1,4-dideoxy-1,4-imino-5-*O*-triphenylmethyl-D-arabinitol (9)** Triphenylmethyl chloride (463 mg) and 4-dimethylaminopyridine (10 mg) were added to a stirred anhydrous solution of **3** (423 mg) and Et_3N (0.24 ml) in CH_2Cl_2 (8.5 ml) at room temperature. After being stirred for 12 h, the reaction mixture was washed twice with water, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2 30 g, *n*-hexane : AcOEt = 1 : 1) to afford **9** (644 mg, 84%). **9**: Amorphous, $[\alpha]_D$ −42.8° (*c* = 0.5, MeOH). *Anal.* Calcd for $C_{32}H_{31}NO_5$: C, 75.42; H, 6.13; N, 2.75. Found: C, 75.14; H, 6.28; N, 2.72. IR ($CHCl_3$): 3400, 1688, 1410, 1348, 1074 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.50—7.10 (20H, m), 5.20—4.90 (2H, m), 4.18—3.30 (7H, m). FAB-MS *m/z*: 267 $(M-Tr+H)^+$, 251 $(M-TrO+H)^+$.

2,3-Di-*O*-benzoyl-*N*-benzyloxycarbonyl-1,4-dideoxy-1,4-imino-5-*O*-triphenylmethyl-D-arabinitol (10) Benzoyl chloride (0.26 ml) was added dropwise to a stirred anhydrous solution of **9** (387 mg) and pyridine (0.2 ml) in CH_2Cl_2 (4 ml) at room temperature. After being stirred for 12 h, the reaction mixture was washed with 1 N aqueous HCl, brine, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2 30 g, *n*-hexane : AcOEt = 9 : 1—5 : 1) to afford **10** (489 mg, 90%). **10**: Amorphous, $[\alpha]_D$ −32.8° (*c* = 0.6, MeOH). *Anal.* Calcd for $C_{46}H_{39}NO_7$: C, 76.97; H, 5.48; N, 1.95. Found: C, 76.65; H, 5.55; N, 1.88. IR ($CHCl_3$): 1716, 1700, 1444, 1410, 1258, 1100, 1088 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (2H, dd, *J* = 7, 1 Hz), 7.96—7.05 (28H, m), 5.95 (1H, br s), 5.53 (1H, d like), 5.13—4.98 (2H, m), 4.40—4.13 (2H, m), 3.85—3.60 (2H, m), 3.32 (1H, m). FAB-MS *m/z*: 458 $(M-TrO)^+$.

2,3-Di-*O*-benzoyl-*N*-benzyloxycarbonyl-1,4-dideoxy-1,4-imino-D-arabinitol (11) A solution of **10** (421 mg) and TsOH·H_2O (100 mg) in MeOH (10 ml) was stirred at room temperature for 20 h. The reaction mixture was diluted with CH_2Cl_2 (40 ml), washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2 100 g, *n*-hexane : AcOEt = 3 : 1) to afford **11** (269 mg, 96%). **11**: Amorphous, $[\alpha]_D$ −47.0° (*c* = 0.8, MeOH). *Anal.* Calcd for $C_{27}H_{25}NO_7$: C, 68.20; H, 5.30; N, 2.95. Found: C, 67.95; H, 5.32; N, 2.87. IR ($CHCl_3$): 3400, 1716, 1700, 1680, 1412, 1258, 1102 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 8.08—8.00 (4H, m), 7.68—7.30 (11H, m), 5.70—5.48 (2H, m), 5.17 (2H, s), 4.35—3.70 (5H, m). FAB-MS *m/z*: 476 $(M+H)^+$.

2,3-Di-*O*-benzoyl-1,4-dideoxy-1,4-imino-D-arabinitol (12) Compound **11** (179 mg) was treated with 10% Pd–C (36 mg) in MeOH (4 ml) under hydrogen (4 atm) at room temperature for 24 h. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2 5 g, CH_2Cl_2 : MeOH = 50 : 1—100 : 3) to afford **12** (107 mg, 83%). **12**: Colorless oil, $[\alpha]_D$ −121.0° (*c* = 0.6, MeOH). *Anal.* Calcd for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10. Found: C, 66.58; H, 5.68; N, 4.06. IR ($CHCl_3$): 3520, 3350, 1712, 1262, 1106 cm^{-1}. CD ($c = 1.75 \times 10^{-2}$, MeOH) $[\theta]^{25}$ (nm): −67000 (234) (negative maximum), +25000 (220) (positive maximum). ^{1}H-NMR (CD_3OD) δ: 8.18—8.08 (4H, m), 7.72—7.46 (6H, m). 5.77 (1H, m), 5.56 (1H, br s), 4.18—3.92 (4H, m), 3.80 (1H, d, *J* = 12 Hz). FAB-MS *m/z*: 342 $(M+H)^+$.

3-*O*-Benzyl-5-deoxy-1,2-*O*-isopropylidene-5-trifluoroacetamido-6-*O*-triphenylmethyl-α-D-glucofuranose (16) A solution of trifluoroacetic anhydride (2.25 ml) in CH_2Cl_2 (80 ml) was added dropwise to a stirred anhydrous solution of 5-amino-3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-α-D-glucofuranose (**15**,[6] 8.0 g) and Et_3N (2.5 ml) in CH_2Cl_2 (240 ml) in an ice–H_2O bath under an N_2 atmosphere over 40 min. After being stirred for 20 min, the reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2 250 g, *n*-hexane : AcOEt = 8 : 1) to afford **16** (9.35 g, quant.). **16**: Colorless fine crystals, mp 72—74 °C (*n*-heptane), $[\alpha]_D$ −51.3° (*c* = 0.5, $CHCl_3$). *Anal.* Calcd for $C_{37}H_{36}F_3NO_6$: C, 68.61; H, 5.60; N, 2.16. Found: C, 68.40; H, 5.65; N, 2.18. IR ($CHCl_3$): 3400, 2995, 2940, 1722, 1534, 1452, 1378, 1282, 1162, 1074 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.55—7.20 (21H, m), 7.10—7.00 (2H, m), 5.93 (1H, d, *J* = 4 Hz), 4.82 (1H, m), 4.58 (1H, dd, *J* = 6, 4 Hz), 4.56 (1H, d, *J* = 4 Hz), 4.42 (1H, d, *J* = 11 Hz), 3.97 (1H, d, *J* = 11 Hz), 3.78 (1H, d, *J* = 4 Hz), 3.52 (1H, dd, *J* = 10, 5 Hz), 2.94 (1H, t, *J* = 10 Hz), 1.53, 1.33 (each, 3H, s). FAB-MS *m/z*: 686 $(M+K)^+$, 670 $(M+Na)^+$.

3-*O*-Benzyl-5-deoxy-5-trifluoroacetamido-D-glucose (17) Compound **16** (585 mg) was treated with 75% aqueous TFA (2 ml) at room temperature for 40 min. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (SiO_2 30 g, *n*-hexane : AcOEt = 1 : 1—AcOEt only) to afford **17** (270 mg, 82%). **17**: A hygroscopic amorphous powder, $[\alpha]_D$ −14.4° (*c* = 0.5, MeOH). IR (Nujol): 3495,

3330, 3180, 1728, 1662, 1552, 1220, 1200 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.10 (1H, d, J=9.5 Hz, D_2O-exchangeable), 6.23 (0.2H, d, J=8 Hz, D_2O-exchangeable), 6.18 (0.8H, d, J=9 Hz, D_2O-exchangeable), 5.42 (0.2H, d, J=5 Hz, D_2O-exchangeable), 5.24 (0.8H, dd, J=9, 4 Hz), 5.22 (0.8H, d, J=5 Hz, D_2O-exchangeable), 4.78 (1H, t, J=5 Hz, D_2O-exchangeable), 4.57 (1H, d, J=11 Hz), 4.35 (1H, d, J=11 Hz), 4.32—3.20 (6H, m). FAB-MS m/z: 404 $(M+K)^+$, 388 $(M+Na)^+$. High-resolution FAB-MS Calcd for $C_{15}H_{18}F_3NNaO_6$ $(M+Na)^+$: 388.098. Found: 388.095.

2-*O*-Benzyl-4-deoxy-3-*O*-formyl-4-trifluoroacetamido-D-arabinose (18) A solution of **17** (731 mg) in tetrahydrofuran containing 2.5% H_2O (24 ml) was added to a stirred solution of sodium metaperiodate (856 mg) in H_2O (24 ml) at 5—6 °C over a period of 35 min. Stirring was continued for 25 min under ice-cooling, then the insoluble material was removed by filtration and the filtrate was extracted with AcOEt. The extract was dried over $MgSO_4$, evaporated *in vacuo* and purified by column chromatography (SiO_2 10 g, *n*-hexane : AcOEt = 2 : 3) to afford **18** (531 mg, 73%). **18**: A colorless viscous oil, $[\alpha]_D$ −13.1° (c=0.5, MeOH). IR (Nujol): 3430, 3300, 1730, 1714, 1702, 1539 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 8.09 (1H, s), 7.48—7.22 (5H, m), 5.10 (0.5H, d, J=2 Hz), 4.85—4.58 (1.5H, m), 4.37—4.26 (1H, m), 4.16—3.85 (2H, m), 3.72—3.43 (3H, m). FAB-MS m/z: 386 $(M+Na)^+$. High-resolution FAB-MS Calcd for $C_{15}H_{16}F_3NNaO_6$ $(M+Na)^+$: 386.083. Found: 386.086.

4-Deoxy-3-*O*-formyl-4-trifluoroacetamido-D-arabinose (19) A mixture of **18** (225 mg), Pd black (300 mg) and 4.4% HCOOH–MeOH (40 ml) was stirred at room temperature for 1.5 h under an N_2 atmosphere. After removal of the catalyst by filtration, the filtrate was evaporated *in vacuo* and the residue was purified by column chromatography (SiO_2 10 g, AcOEt) to afford **19** (188 mg, 98%). **19**: An amorphous solid, $[\alpha]_D$ −64.3° (c=0.5, MeOH). *Anal.* Calcd for $C_8H_{10}F_3NO_6$: C, 35.18; H, 3.69; N, 5.13. Found: C, 35.27; H, 3.95; N, 5.05. IR (Nujol): 3420, 3300, 1716, 1700, 1558, 1160 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.58 (0.5H, d, J=9 Hz, D_2O-exchangeable), 9.51 (0.5H, d, J=9 Hz, D_2O-exchangeable), 8.23 (1H, s), 6.92 (0.5H, d, J=5 Hz, D_2O-exchangeable), 6.63 (0.5H, d, J=5 Hz, D_2O-exchangeable), 5.47 (0.5H, d, J=5 Hz, D_2O-exchangeable), 5.11 (0.5H, d, J=5 Hz, D_2O-exchangeable), 5.09—4.96 (1H, m), 4.89 (0.5H, dd, J=9, 5 Hz, 4.52—4.28 (1.5H, m), 4.02 (0.5H, dd, J=13, 3 Hz), 3.92—3.54 (2H, m), 3.48 (0.5H, dd, J=13, 4 Hz). FAB-MS m/z: 296 $(M+Na)^+$.

Synthesis of Nectrisine (1) Compound **19** (134 mg) was treated with 0.5 N aqueous NaOH (3.0 ml) at room temperature for 30 min. The mixture was acidified to pH 4 with acetic acid under ice-cooling, diluted with H_2O (100 ml) and subjected to column chromatography (CM-Sephadex (NH_4^+ form) 100 ml, eluted with H_2O 400 ml, then 2% aqueous NH_3 200 ml). The aqueous NH_3 fractions containing the objective compound were collected and evaporated *in vacuo*. The residue was taken up in H_2O (5 ml) and lyophilized to afford **1** (62 mg, 96%), which was identical with an authentic sample by direct comparison: TLC ($CHCl_3$: MeOH : 28% aqueous NH_3 = 5 : 3 : 1, Rf=0.35; *n*-BuOH : AcOH : H_2O = 4 : 1 : 2, Rf=0.21; isopropyl alcohol : H_2O = 7 : 3, Rf=0.27), $[\alpha]_D$ +21.0° (c=0.6, H_2O), IR (KBr), ^{1}H-NMR (D_2O), and ^{13}C-NMR (D_2O) (Tables I and II).

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