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Isolation and Structure Elucidation of Vicenistatin M, and Importance of the Vicenisamine Aminosugar for Exerting Cytotoxicity of Vicenistatin

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A new analogue of vicenistatin was isolated from the producing strain *Streptomyces* sp. HC-34. A characteristic of the elucidated structure involved the existence of a neutral sugar mycarose instead of an aminosugar vicenisamine of vicenistatin. The absolute stereochemistry of the new analogue (named as vicenistatin M) was determined by the synthesis of D-mycarose and of vicenistatin M itself. Biological testing of vicenistatin M suggested the importance of vicenisamine for exerting the cytotoxicity of vicenistatin.

Vicenistatin (**1**), an antitumor antibiotic isolated from *Streptomyces* sp. HC-34, is unique in its structure including a 20-membered macrocyclic lactam aglycon and a novel aminosugar (vicenisamine).[1] The absolute stereochemistry of the aglycon was determined as shown in Fig. 1.[2] Its significant antitumor activity and unique structure prompted us to launch synthetic and biosynthetic studies for modification in order to clarify the essential chemical features for the biological activities. The whole 20-membered macrolactam ring skeleton was successfully synthesized and the absolute configuration was verified.[3]

In the course of the biosynthetic studies, the fermentation broth of *Streptomyces* sp. HC-34 was further analyzed more closely. Continuous HPLC analysis with an aid of photodiode array (PDA) detection suggested the presence of an analogue of vicenistatin in a crude extract of the fermentation. Similarity of the aglycon structure to that of vicenistatin was easily predicted on the basis of the PDA absorption spectrum.

This new metabolite was shown to be accumulated predominantly in the mycelium of the producing microorganism. Isolation and purification of this compound (**2**) was accomplished by solvent extraction of the filtered mass, followed by a series of chromatography as described in the experimental section.

The new compound **2** was characterized by physicochemical (Table 1) and spectroscopic methods. UV and IR spectra suggested close similarity of **2** to vicenistatin **1**. The aglycon part was in fact determined to be totally identical with that of **1** by comparison of ^{1}H-, ^{13}C-NMR spectra including ^{1}H-^{1}H COSY, HMQC, the data being summarized in Table 2. Significant difference was found in the sugar part; 1) a lack of the *N*-methyl group of **1** (δ_H 2.42 ppm, singlet), 2) a lack of 3′-H of **1** (δ_H 4.39 ppm, ddd), 3) an appearance of a singlet methyl group (δ_H 1.52 ppm). The coupling constants (J_H=1.9, 9.6 Hz) between the anomeric proton (1′-H) and the adjacent methylene protons (2′-H) indicated the glycosidic linkage as β. The odd molecular weight (MW 501) deduced from FAB-MS (M+H$^+$=*m/z* 502) spectra suggested the sugar moiety to be a neutral hexose rather than an aminohexose. The ^{1}H-spin network indicated the sugar being either a mycarose (2,6-dideoxy-3-*C*-methyl-*ribo*-hexose) or a evermicose (2,6-dideoxy-3-*C*-methyl-*arabino*-hexose). This was clarified on the basis of

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Fig. 1. Structure of vicenistatin (**1**) and vicenistatin M (**2**).

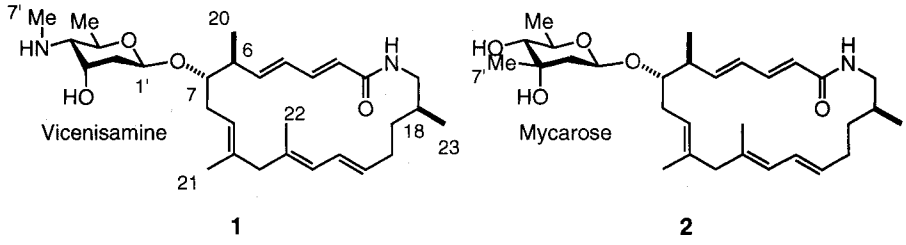


Table 1. Physico-chemical properties of vicenistatin M.

Appearance	White powder
MP	201 ~ 210°C
$[\alpha]_D^{27}$	– 4.2° (*c* 0.05, MeOH)
Molecular formula	$C_{30}H_{47}O_5N$
FAB-MS (*m/z*)	502 $(M+H)^+$
UV λ_{max} (ε) nm	237 (37,200), 241 (38,000),
(in MeOH)	268 (13,650)
IR ν_{max} cm^{-1}	3403, 3334, 2917, 1656, 1627,
(KBr)	1535, 993

significant nuclear Overhauser effect between 4′-H and the 3′-methyl singlet, and the sugar was determined to be a mycarose (Fig. 2). Accordingly, we designated this new analogue **2** as vicenistatin M. In fact, methyl β-mycaroside was degradatively derived from **2**, but only in a small amount. Unfortunately therefore, reliable optical rotation data was not obtained because of the lack of quantity. However, since vicenisamine, which is the original sugar component of **1**, is known as D-sugar,[1] we proposed that mycarose in **2** could also be the same D-configuration. There are some precedence of mycarose found in antibiotics. L-Mycarose is commonly known in such antibiotics as magnamycin, erythromycin C and D, tylosin, and kedarcidin chromophore. D-Mycarose is rarely known, only found in mithramycin[4] and P371 A1[5]. In order to unambiguously determine the absolute stereochemistry of **2**, synthetic approach was undertaken to D-mycarose and **2** itself.

As mentioned above, the aglycon of **1** and **2** is identical. It was also established already that the macrolactam aglycon could be obtained from natural vicenistatin **1**. Therefore, what we had to do here was the synthesis of D-mycarose and its glycosidation with the macrolactam.

The synthetic route for D-mycarose, being shown in Scheme 1, was mainly adopted from the FLAHERTY's[6] and THIEM's[7] method with modifications. Methyl α-D-mannopyranoside **3** was transformed to the key deoxyulose derivative **4** according to the literature procedure.[8,9] The 3-*C*-methyl group was stereoselectively introduced with MeMgI. The resulting methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-methyl-α-D-*ribo*-hexopyranoside **5** was oxidatively converted with *N*-bromosuccinimide (NBS) to 4-*O*-benzoyl-6-bromo-6-deoxy-derivative **6**, which was subsequently treated reductively with $LiAlH_4$ to give methyl α-D-mycaroside **7** in good yield. All the spectroscopic data of **7** were identical with those reported in the literatures.[6,7]

The next stage of our work was glycosidation. It should be noted that, once it finely crystallizes, the free aglycon of **1** is extremely insoluble in any solvents. Therefore, it is necessary to suitably protect the 7-OH group of the aglycon, which should be directly used for glycosidation. The *O*-TMS (trimethylsilyl) ether was ultimately selected as a glycosyl acceptor, which was in fact obtained in good yield by methanolysis of natural **1**, followed by the standard TMS protection. Naturally, 1-*O*-acetyl sugar was chosen as a glycosyl donor, because of its ease of preparation and its usefulness in glycosidation with *O*-TMS-alcohol.[10] At first, both of the hydroxy groups of methyl α-D-mycaroside **7** were protected as a cyclic carbonate with *N*, *N*′-carbonyl-diimidazole (CDI) to give **8**.[11] After hydrolysis of **8** in aqueous acetic acid, the resulting free sugar **9** was acetylated to give 1-*O*-acetyl-3,4-*O*-carbonyl-α-D-mycarose **10**. Glycosidation of **10** with the *O*-TMS-aglycon acceptor **11** was performed in the presence of $SnCl_4$-$AgClO_4$[12] to give a inseparable anomeric mixture in 35% yield. This

Table 2. 125 MHz ^{13}C-NMR and 500 MHz ^{1}H-NMR spectral data of vicenistatin M (**2**) and vicenistatin (**1**).[a]

Position	**2**		**1**	
	δ_C	δ_H	δ_C	δ_H
1	166.3		166.4	
2	124.6	6.25 (d, 15.0[b])	124.7	6.26 (d, 15.0)
3	140.3	7.59 (dd, 11.0, 15.0)	140.2	7.59 (dd, 11.5, 15.0)
4	128.4	6.22 (dd, 11.0, 15.1)	128.4	6.20 (dd, 11.5, 15.5)
5	143.3	5.88 (dd, 9.5, 15.0)	143.2	5.86 (dd, 10.0, 15.5)
6	46.3	2.41[c]	46.1	2.40[c]
7	85.9	3.40 (dd, 8.3, 9.1)	85.9	3.36 (ddd, 2.8, 9.0, 9.0)
8	36.5	2.30 (ddd, 8.0, 8.4, 13.9), 3.08[c]	36.5	2.27[c], 3.08 (ddd, 2.8, 7.5, 9.0)
9	122.1	5.23 (dd, 7.6, 7.8)	122.3	5.20 (dd, 7.5, 7.5)
10	135.0		134.8	
11	49.3	2.64 (d, 15.0), 2.75 (d, 15.0)	49.5	2.62 (d, 15.0), 2.74 (d, 15.0)
12	134.1		134.1	
13	128.0	5.96 (d, 11.0)	127.9	5.95 (d, 11.5)
14	128.4	6.78 (dd, 11.0, 15.0)	128.4	6.79 (dd, 11.5, 15.0)
15	132.6	5.69 (ddd, 5.5, 9.4, 15.0)	132.6	5.68 (ddd, 5.8, 6.0, 15.0)
16	27.7	2.08 (m), 2.43[c]	27.9	2.07 (m), 2.39[c]
17	32.8	1.46 (m), 1.58 (m)	33.0	1.48[c], 1.57[c]
18	33.5	1.85[c]	33.6	1.86[c]
19	43.2	3.05 (ddd, 3.1, 4.5, 13.5), 4.00 (ddd, 9.8, 9.8, 13.4)	43.5	3.03 (ddd, 3.0, 5.0, 13.5), 4.00 (m)
NH		8.37 (dd, 2.5, 9.0)		8.47 (br d, 6.2)
20	18.7	1.11 (d, 6.6)	18.7	1.08 (d, 6.5)
21	17.9	1.70 (s)	18.0	1.68 (s)
22	17.3	1.95 (s)	17.3	1.94 (s)
23	17.7	0.85 (d, 6.9)	17.8	0.84 (d, 6.5)
1'	101.5	5.34 (dd, 1.9, 9.5)	100.7	5.29 (dd, 3.0, 9.5)
2'	45.1	1.90 (dd, 9.7, 13.3), 2.36 (dd, 1.8, 13.3)	39.5	1.90 (ddd, 2.8, 9.5, 14.5), 2.43[c]
3'	70.9		63.4	4.39 (ddd, 2.8, 3.0, 4.2)
4'	77.4	3.36 (m)	65.3	2.24 (dd, 3.0, 9.8)
5'	71.4	4.20 (dq, 6.2, 9.2)	71.5	4.02 (dq, 6.5, 9.8)
6'	19.1	1.61 (d, 6.2)	19.6	1.52 (d, 6.5)
7'	27.9	1.52 (s)	34.0	2.42 (s)

[a] in pyridine-d_5.
[b] coupling constants (J, Hz).
[c] obscured by overlapping.

mixture was subsequently deprotected by hydrolysis to give β-glycoside (1'-H: dd, J=1.9, 9.5 Hz) and α-glycoside (1'-H: d, J=3.8 Hz), which were ultimately separated by flash silica gel chromatography. Unfortunately, the desired β-glycoside was a minor product (β: α=28 : 72), but the spectroscopic properties of the obtained β-glycoside were completely identical with those of natural **2** (Fig. 3). In conclusion, the absolute stereochemistry of the sugar part in vicenistatin M was determined to be D-mycarose.

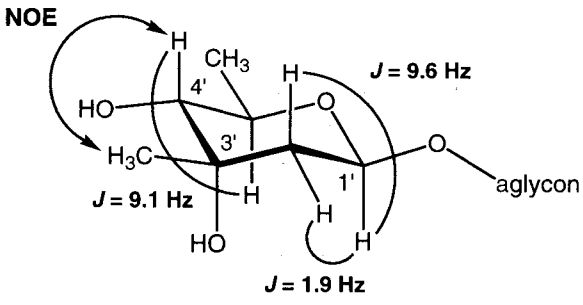
Since the sugar moiety of **2** was determined to be different from **1**, we anticipated that biological comparison between them could shed light on the role of the sugar moiety of this class of antibiotics.

Vicenistatin M (**2**) was subjected to the bioassay for *in vitro* cytotoxicity, but essentially no activity was observed against several cell lines including human lung carcinoma: H661, H727, and H1299; human prostate carcinoma: DU145 and CRL1740, mouse leukemia P388 at a dose

of 20 μg/ml. In addition, the α-glycoside isomer of vicenistatin M has no activity either. It appears therefore, that the aminosugar vicenisamine plays an important role in expressing the potent biological activities of vicenistatin **1**.

In summary, vicenistatin M, a new D-mycarosyl analogue of vicenistatin antitumor antibiotic, was isolated and its structure was elucidated. The synthesis of vicenistatin M was also performed to determine its absolute stereochemistry. The bioassay of vicenistatin M implied that the vicenisamine aminosugar is crucial for the cytotoxic and antitumor activity of vicenistatin.*

Fig. 2. Selected NOE correlations and J_H values of the sugar part of **2**.



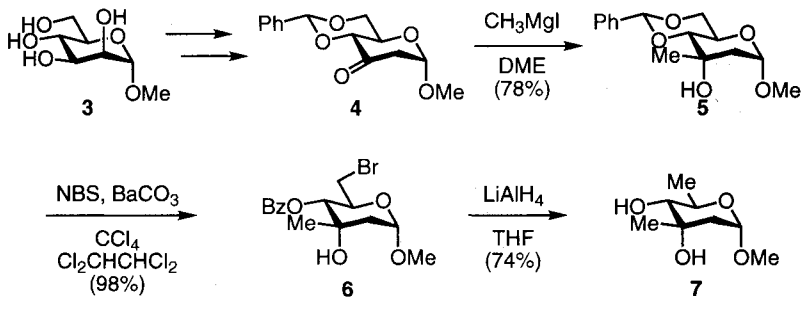
Experimental

All melting points are uncorrected. NMR spectra were recorded on a Jeol LA-300, LA-400, or a Bruker DRX-500 spectrometer. ^{1}H-NMR and ^{13}C-NMR chemical shifts were reported in δ value based on internal TMS (δ_H=0), or solvent signal ($CDCl_3$ δ_C=77.0) as reference. IR spectra were recorded on a Horiba FT-710 Fourier-transform infrared spectrometer. Optical rotations were measured on a Jasco DIP-360 polarimeter. Mass spectra were measured on a JEOL AX-505HA mass spectrometer. Column chromatography was carried out with Merck Kieselgel 60, Art. Nr. 7734. Preparative thin layer chromatography was carried out with Merck Kieselgel 60 F_{254}, Art. Nr. 5744.

Isolation and Purification

The fermentation was conducted under the same conditions as previously published.[1] Fermentation broth (4.5 liters) was centrifuged and the resulting mycelium cake (wet weight, 253 g) was allowed to stand overnight in acetone (1 liter) for extraction. The whole was filtered and the acetone extract was concentrated *in vacuo* to give an aqueous solution. This solution was adjusted to pH 10 and further extracted with EtOAc (1.2 liters). The organic extract was evaporated and the residue was chromatographed over silica gel with $CHCl_3$/MeOH (100 : 1 to 5 : 1). The appropriate fractions were combined and further

Scheme 1. Synthesis of D-mycarose.



* Based on the suggestion of a referee, we synthesized a β-L-mycarosyl analog of vicenistatin M. Protected L-mycarose, which was degradatively derived from commercially available tylosin tartrate, was reacted with the *O*-TMS ether of the aglycon in a similar mannar to give the desired analog and its ^{1}H-NMR spectral data were clearly different from those of natural vicenistatin M. ^{1}H-NMR spectral data of the β-L-mycarosyl analog (pyridine-d_5): δ 0.84 (d, 3H, J=6.9 Hz, 23-H), 1.31 (d, 3H, J=6.6 Hz, 20-H), 1.47 (m, 1H, 17-H), 1.52 (m, 1H, 17-H), 1.53 (s, 3H, 7'-H), 1.58 (s, 3H, 21-H), 1.63 (d, 3H, J=6.7 Hz, 6'-H), 1.83 (m, 1H, 18-H), 1.90 (s, 3H, 22-H), 1.94 (dd, 1H, J=9.6, 13.2 Hz, 2'-H_{ax}), 2.10 (m, 1H, 16-H), 2.26 (ddd, 1H, J=8.1, 8.6, 14.7 Hz, 8-H), 2.32 (dd, 1H, J=1.8, 13.3 Hz, 2'-H_{eq}), 2.41 (m, 1H, 16-H), 2.50 (m, 1H, 6-H), 2.51 (m, 1H, 8-H), 2.57 (d, 1H, J=14.8 Hz, 11H), 2.69 (d, 1H, J=14.8 Hz, 11 H), 3.08 (ddd, 1 H, J=3.4, 4.6, 13.4 Hz, 19-H), 3.39 (d, 1H, J=9.2 Hz, 4'-H), 3.80 (dd, 1H, J=8.3, 8.8 Hz, 7-H), 3.96 (ddd, 1H, J=9.4, 9.4, 13.5 Hz, 19-H), 4.23 (dq, 1H, J=6.2, 9.2 Hz, 5'-H), 5.13 (m, 1H, 9-H), 5.51 (dd, 1H, J=1.8, 9.5 Hz, 1'-H), 5.68 (ddd, 1H, J=5.8, 8.8, 14.9 Hz, 15-H), 5.95 (d, 1H, J=11.3 Hz, 13-H), 5.96 (dd, 1H, J=9.5, 14.8 Hz, 5-H), 6.24 (dd, 1H, J=11.2, 14.9 Hz, 4-H), 6.25 (d, 1H, J=15.1 Hz, 2-H), 6.78 (dd, 1H, J=11.1, 14.9 Hz, 14-H), 7.59 (dd, 1H, J=11.2, 15.0 Hz, 3-H), 8.47 (dd, 1H, J=2.4, 8.5 Hz, NH).

Scheme 2. Synthesis of vicenistatin M.

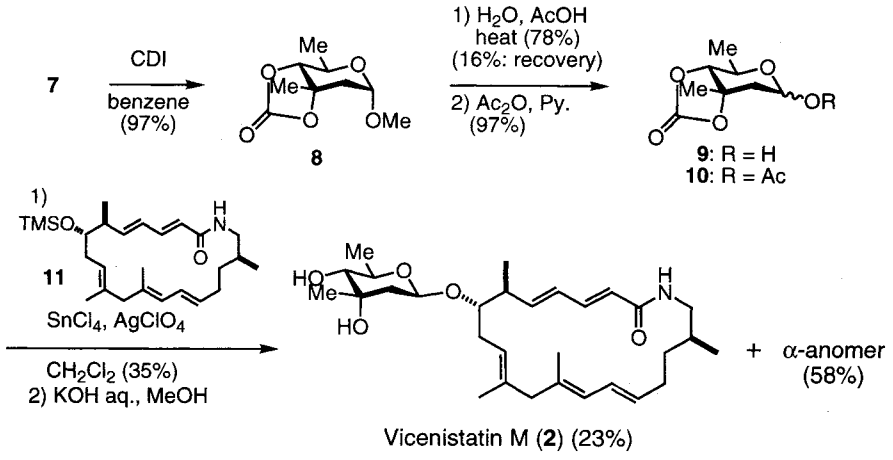
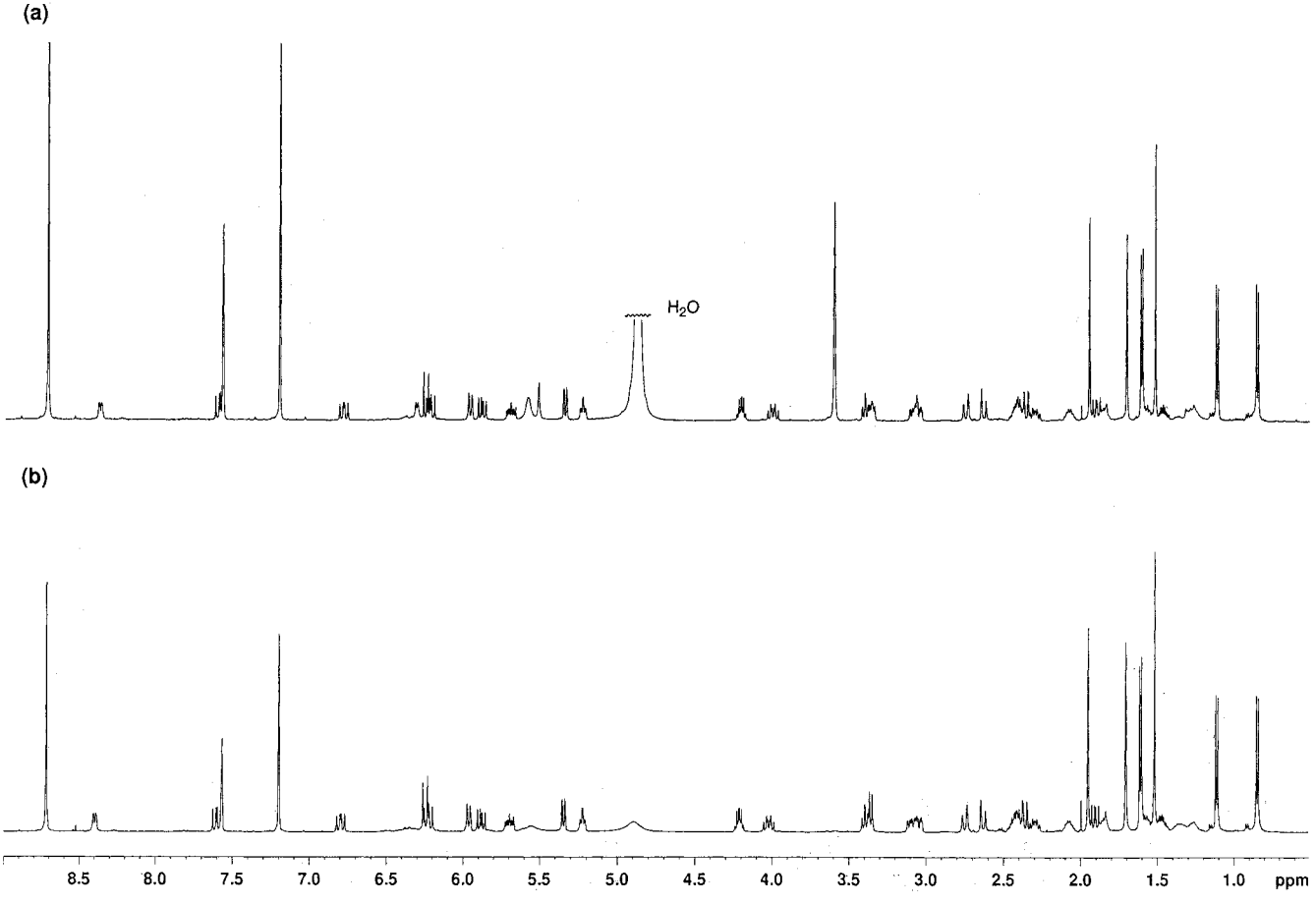


Fig. 3. ^{1}H-NMR spectra of vicenistatin M (500 MHz, pyridine-d_5).

(a) natural, (b) synthetic.



subjected to Sephadex LH-20 chromatography ($CHCl_3$/MeOH, 1 : 1) affording compound **2**. Further purification was carried out by preparative HPLC using a Senshu Pak ODS column (ODS-4251-N, 10 mm×25 cm) maintained at 50°C with a solvent of MeOH/H_2O (3 : 1) at a flow rate of 5 ml/minute, monitoring the UV absorbance by a photodiode array (PDA) detector Waters Model 600E system. The final purification was achieved by preparative TLC ($CHCl_3$/MeOH, 14 : 1) to give pure vicenistatin M (**2**) (5.9 mg).

Methanolysis of Vicenistatin M (**2**)

To an ice-cooled dry MeOH (3 ml) was added AcCl (280 μl) under Ar. After stirring at room temperature for 30 minutes, vicenistatin M **2** (3.1 mg) in dry MeOH (1.5 ml) was added to the solution. The resulting solution was stirred for 1 hour and the whole was evaporated to dryness. The semicrystalline residue was triturated with ether and the insoluble free aglycon was removed by filtration. The filtrate containing the sugar part was concentrated *in vacuo*. The residue was purified by flash column chromatography ($CHCl_3$/MeOH 14 : 1) to afford 0.81 mg of methyl β-mycaroside (**3**) (yield 74%).

^{1}H-NMR ($CDCl_3$): δ 1.29 (s, 3H, 7-Me), 1.33 (d, 3H, J=6.2 Hz, 6-Me), 1.58 (dd, 1H, J=9.9, 13.8 Hz, 2-H_{ax}), 2.00 (dd, 1H, J=2.1, 13.8 Hz, 2-H_{eq}), 3.07 (dd, 1H, J=6.6, 8.6 Hz, 4-H), 3.48 (s, 3H, OMe), 3.62 (dq, 1H, J=6.3, 9.4 Hz, 5-H), 4.67 (dd, 1H, J=2.1, 9.6 Hz, 1-H)

Methyl 4,6-*O*-Benzylidene-2-deoxy-3-*C*-methyl-α-D-*ribo*-hexopyranoside (**5**)

To a suspension of magnesium turnings (1.25 g, 51.5 mmol) in dry ether (10 ml) was added dropwise methyl iodide (3.21 ml, 51.5 mmol) in dry ether (40 ml) at a rate of addition to maintain gentle reflux under Ar. After refluxing ceased, ether was evaporated and the resultant Grignard reagent (MeMgI) was resuspended in dry dimethoxyethane (70 ml). To the suspension was added dropwise ketone **4**[8,9] (5.44 g, 20.6 mmol) in dry dimethoxyethane (100 ml) at room temperature. The reaction mixture was stirred at room temperature for 14 hours. The reaction was quenched by the addition of water at 0°C, and the solvent was evaporated *in vacuo*. The residual aqueous mixture was extracted with EtOAc (100 ml×3). The combined extract was dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 150 g; hexane/Et_2O, 2 : 1 to 1 : 2) to afford 4.52 g of **5** (yield 78%); An analytical sample was obtained as colorless needles by recrystallization from ether-hexane. mp 121.5~122.8°C (lit.[6] mp 125.5~126°C, lit.[7] mp 121~123°C); $[\alpha]_D^{26}$ +116° (c=1.01, EtOH) {lit.[6] $[\alpha]_D^{20}$ +121° (c=0.2, EtOH), lit.[7] $[\alpha]_D^{20}$ +134° (c=1.0, EtOH)}

IR (KBr): ν_{max} 3518, 1122, 1103, 1043, 758, 708 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.31 (s, 3H, 7-Me), 1.88 (dd, 1H, J=4.1, 14.6 Hz, 2-H_{ax}), 2.07 (d, 1H, J=14.6 Hz, 2-H_{eq}), 3.40 (s, 3H, OMe), 3.42 (d, 1H, J=10.0 Hz, 4-H), 3.77 (d, 1H, J=10.2 Hz, one of 6-H), 4.11 (dt, 1H, J=5.1, 10.0 Hz, 5-H), 4.32 (dd, 1H, J=5.1, 10.2 Hz, one of 6-H), 4.79 (br d, 1H, J=4.1 Hz, 1-H), 5.60 (s, 1H, PhC*H*), 7.25~7.53 (m, 5H, Ph).

^{13}C-NMR ($CDCl_3$): δ 25.3, 41.6, 55.3, 59.6, 68.4, 69.2, 83.2, 98.8, 101.9, 126.2, 128.1, 128.9, 137.5.

Anal Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19.
Found: C, 64.49; H, 7.28.

Methyl 4-*O*-Benzoyl-6-bromo-2,6-dideoxy-3-*C*-methyl-α-D-*ribo*-hexopyranoside (**6**)

A mixture of compound **5** (4.11 g, 14.8 mmol), *N*-bromosuccinimide (3.16 g, 17.8 mmol) and $BaCO_3$ (1.46 g, 7.40 mmol) in carbon tetrachloride (220 ml), and tetrachloroethane (13 ml) was refluxed for 0.5 hour under Ar. The reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 100 g; hexane/EtOAc, 5 : 1 to 4 : 1) to afford 5.17 g of **6** (yield 98%); (lit.[7] mp 71°C), $[\alpha]_D^{26}$ +106° (c=1.77, $CHCl_3$), $[\alpha]_D^{29}$ +103° (c=1.77, CH_2Cl_2) {lit.[7] $[\alpha]_D^{20}$ +22.2° (c=4.5, CH_2Cl_2)}.

IR ($CHCl_3$): ν_{max} 3504, 1720, 1267, 1126, 1041, 712 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.19 (s, 3H, 7-Me), 1.98 (dd, 1H, J=3.7, 14.6 Hz, 2-H_{ax}), 2.08 (d, 1H, J=14.6 Hz, 2-H_{eq}), 3.47 (dd, 1H, J=7.6, 11.0 Hz, one of 6-H), 3.49 (s, 3H, OMe), 3.54 (dd, 1H, J=2.7, 11.0 Hz, one of 6-H), 4.30 (ddd, 1H, J=2.7, 7.6, 10.0 Hz, 5-H), 4.94 (br d, 1H, J=3.4 Hz, 1-H), 4.99 (d, 1H, J=10.0 Hz, 4-H), 7.44~8.13 (m, 5H, Ph)

^{13}C-NMR ($CDCl_3$): δ 25.8, 32.7, 41.2, 55.5, 67.1, 70.2, 74.1, 74.9, 98.6, 128.5, 130.0, 133.5, 166.0.

Anal Calcd for $C_{15}H_{19}O_5Br$: C, 50.15; H, 5.33.
Found: C, 50.41; H, 5.45.

Methyl 2,6-Dideoxy-3-*C*-methyl-α-D-*ribo*-hexopyranoside (methyl α-D-mycaroside) (**7**)

To an ice-cooled solution of **6** (3.59 g, 10.0 mmol) in dry tetrahydrofuran (80 ml) was added lithium aluminum hydride (1.52 g, 40.0 mmol). After being refluxed for 2.5 hours, the reaction mixture was ice-cooled, to which were added dropwise water (1.52 ml), 15% $NaHCO_3$ aq. (1.52 ml), and water (4.56 ml) successively, and the whole was then stirred at room temperature for 0.5 hour. Celite and $MgSO_4$ was added to the mixture. After filtration, the

filtered cake was washed several times with hot tetrahydrofuran. The combined filtrate and washings were concentrated *in vacuo* and the residue was purified by column chromatography (silica gel, 120 g; hexane/EtOAc, 6 : 1 to 2 : 1) to afford 1.30 g of **7**; (yield 74%); mp 56.0~57.5°C {methyl α-D-mycaroside: lit.[6] mp 56~57°C, lit.[7] mp 56~58°C; methyl α-L-mycaroside: lit.[11] mp 57~58.5°C, lit.[13] mp 55~57°C, lit.[14] mp 60.5~61.0°C, lit.[15] mp 60.5~61°C}; $[\alpha]_D^{26}$ +139° (c=1.16, $CHCl_3$) {methyl α-D-mycaroside: lit.[6] $[\alpha]_D$ +136° (c=1.0, $CHCl_3$), lit.[7] $[\alpha]_D$ +138.8° (c=0.45, CH_2Cl_2); methyl α-L-mycaroside: lit.[11] $[\alpha]_D^{20}$ −138° (c=0.2, CH_2Cl_2), lit.[13] $[\alpha]_D$ −143° (c=0.7, EtOH), lit.[14] $[\alpha]_D$ −134° (c=0.39, $CHCl_3$), lit.[15] $[\alpha]_D^{25}$ −141° (c=1, $CHCl_3$)}.

IR (KBr): ν_{max} 3417, 1126, 1063, 1045 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.23 (s, 3H, 7-Me), 1.33 (d, 3H, J=6.3 Hz, 6-Me), 1.82 (dd, 1H, J=3.7, 14.6 Hz, 2-H_{ax}), 2.03 (dd, 1H, J=1.0, 14.6 Hz, 2-H_{eq}), 2.40 (d, 1H, J=11.0, 4-OH), 2.97 (dd, 1H, J=9.5, 11.0 Hz, 4-H), 3.38 (s, 3H, OMe), 3.61 (dq, 1H, J=6.3, 9.5 Hz, 5-H), 3.87 (s, 1H, 3-OH), 4.76 (br d, 1H, J=3.4 Hz, 1-H).

^{13}C-NMR ($CDCl_3$): δ 17.9, 25.7, 40.7, 55.0, 65.5, 69.9, 76.4, 98.4.

Anal Calcd for $C_8H_{16}O_4$: C, 54.53; H, 9.15.
Found: C, 54.24; H, 9.45.

Methyl 3,4-*O*-Carbonyl-2,6-dideoxy-3-*C*-methyl-α-D-*ribo*-hexopyranoside (**8**)

To a solution of **7** (845 mg, 4.79 mmol) in dry benzene (39 ml) was added *N*,*N*′-carbonyldiimidazole (3.11 g, 19.2 mmol) and the reaction mixture was stirred at 50°C for 30 minutes. After cooling, water was added to the solution and the mixture was extracted with EtOAc (50 ml×3). The combined extract was dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 40 g; hexane/EtOAc, 5 : 1 to 3 : 1) to afford 932 mg of **8** (yield 97%); mp 79.5~80.5°C {lit.[11] (L-form) mp 78~79°C}, $[\alpha]_D^{27}$ +159° (c=1.14, CH_2Cl_2) {lit.[7] $[\alpha]_D^{20}$ +157.4° (c=0.78, CH_2Cl_2), lit.[11] (L-form) $[\alpha]_D^{20}$ −158.5° (c=0.6, CH_2Cl_2)}.

IR ($CHCl_3$): ν_{max} 1795, 1068, 1051 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.36 (d, 3H, J=6.1 Hz, 6-H), 1.54 (s, 3H, 3-Me), 2.14 (dd, 1H, J=6.1, 14.8 Hz, 2-H_{ax}), 2.19 (dd, 1H, J=5.9, 14.8 Hz, 2-H_{eq}), 3.37 (s, 3H, OMe), 3.89 (d, 1H, J=8.7 Hz, 4-H), 3.94 (dq, 1H, J=6.1, 8.7 Hz, 5-H), 4.70 (dd, 1H, J=5.9, 6.1 Hz, 1-H).

^{13}C-NMR ($CDCl_3$): δ 18.8, 26.5, 36.7, 55.2, 64.6, 80.3, 82.9, 96.8, 153.6.

Anal Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98.
Found: C, 53.61; H, 7.04.

3,4-*O*-Carbonyl-2,6-dideoxy-3-*C*-methyl-D-*ribo*-hexopyranose (**9**)

A solution of **8** (547 mg, 2.72 mmol) in AcOH (30 ml) and water (6 ml) was heated at around 90°C for 21 hours. The reaction mixture was evaporated *in vacuo* with an aid of azeotropic distillation with toluene. The residue was purified by column chromatography (silica gel, 50 g; hexane/EtOAc, 5 : 1 to 1 : 1) to afford 400 mg of **9** (yield 78%); mp 108.5~110.5°C, $[\alpha]_D^{27}$ +82.4° (c=0.765, $CHCl_3$), with recovery of starting material **8** (84 mg, 16%).

IR (KBr): ν_{max} 3514, 3400, 1778, 1066, 1028 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ (α-anomer) 1.36 (d, 3H, J=6.3 Hz, 3-Me), 1.56 (s, 3H, 6-H), 2.15 (dd, 1H, J=6.8, 14.6 Hz, 2-H_{ax}), 2.26 (dd, 1H, J=5.6, 14.6 Hz, 2-H_{eq}), 3.91 (d, 1H, J=8.8 Hz, 4-H), 4.12 (dq, 1H, J=6.3, 8.8 Hz, 5-H), 5.23 (dd, 1H, J=5.6, 6.8 Hz, 1-H). δ (β-anomer) 1.40 (d, 3H, J=6.3 Hz, 3-H), 1.55 (s, 3H, 3-Me), 1.80 (dd, 1H, J=8.5, 15.1 Hz, 2-H_{ax}), 2.49 (dd, 1H, J=2.7, 15.1 Hz, 2-H_{eq}), 3.60 (dq, 1H, J=6.3, 8.5 Hz, 5-H), 3.92 (d, 1H, J=8.5 Hz, 4-H), 5.08 (dd, 1H, J=2.7, 8.5 Hz, 1-H).

^{13}C-NMR ($CDCl_3$): δ 18.8 (α), 18.9 (β), 26.3 (α), 26.6 (β), 37.4 (α), 38.7 (β), 65.1 (α), 70.7 (β), 80.5 (β), 80.9 (α), 82.7 (β), 82.9 (α), 90.2 (α), 92.2 (β), 153.7 (α, β).

Anal Calcd for $C_8H_{12}O_5$: C, 51.06; H, 6.43.
Found: C, 51.23; H, 6.56.

1-*O*-Acetyl-3,4-*O*-carbonyl-2,6-dideoxy-3-*C*-methyl-D-*ribo*-hexopyranose (**10**)

To a solution of **9** (95 mg, 0.51 mmol) in pyridine (0.3 ml) was added Ac_2O (53 μl, 0.56 mmol) and the mixture was stirred at room temperature, for 3.5 hours. The reaction was quenched by the addition of water and the mixture was extracted with EtOAc (30 ml×3). The combined extract was dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 8 g; hexane/EtOAc, 5 : 1 to 2 : 1) to afford 113 mg of **10** (yield 97%); $[\alpha]_D^{26}$ +70.2° (c=1.36, $CHCl_3$).

IR (film): ν_{max} 1815, 1751, 1219, 1068 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ (α-anomer) 1.38 (d, 3H, J=6.3 Hz, 6-H), 1.60 (s, 3H, 3-Me), 2.10 (s, 3H, Ac), 2.29 (d, 2H, J=6.3 Hz, 2-H), 3.94 (d, 1H, J=8.5 Hz, 4-H), 4.06 (dq, 1H, J=6.3, 8.5 Hz, 5-H), 6.08 (dd, 1H, J=6.3, 6.3 Hz, 1-H). δ (β-anomer) 1.40 (d, 3H, J=6.4 Hz, 3-H), 1.60 (s, 3H, 3-Me), 1.99 (dd, 1H, J=7.8, 15.1 Hz, 2-H_{ax}), 2.10 (s, 3H, Ac), 2.49 (dd, 1H, J=3.4, 15.1 Hz, 2-H_{eq}), 3.76 (dq, 1H, J=6.4, 8.3 Hz, 5-H), 3.96 (d, 1H, J=8.3 Hz, 4-H), 5.99 (dd, 1H, J=3.4, 7.8 Hz, 1-H).

^{13}C-NMR ($CDCl_3$): δ 18.4 (β), 18.6 (α), 20.8 (β), 20.9 (α), 26.0 (α), 26.5 (β), 35.0 (α), 35.7 (β), 66.9 (α), 70.9 (β), 79.6 (α), 80.6 (β), 81.2 (β), 82.1 (α), 89.4 (α), 89.9

(β), 153.2 (α, β), 168.8 (α), 169.5 (β).

Anal Calcd for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13.
Found: C, 51.95; H, 6.27.

(6*S*,7*S*,18*S*)-20-Aza-6,10,12,18-tetramethyl-7-trimethylsiloxy-cycloicosa-2,4,9,12,14-pentaen-1-one (*O*-TMS-Aglycon) (**11**)

To a HCl-MeOH solution, which had been prepared by adding AcCl (9.00 ml, 127 mmol) to an ice-cooled MeOH (160 ml), was added natural vicenistatin **1** (776 mg, mmol) at room temperature and the reaction mixture was stirred at room temperature for 23 hours. After the addition of Et_3N (19.0 ml, 136 mmol) at 0°C, the mixture was concentrated *in vacuo*. To an ice-cooled suspension of the residue, Et_3N (10.0 ml, 71.7 mmol) and DMAP (216 mg, 1.77 mmol) in dry CH_2Cl_2 (160 ml) was added. TMSCl (5.0 ml, 39 mmol) was further added and the reaction mixture was stirred for 1 hour. To the resulting clear solution was added crushed ice and water, and the mixture was extracted with Et_2O (100 ml×3). The extract was washed with water (50 ml) and brine (50 ml) successively, and was then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 80 g; hexane/EtOAc, 5 : 1 to 3 : 1) to afford 467 mg of **11** (yield 70%); mp 120.5~125.5°C, $[\alpha]_D^{28}$ +23.5° (c=1.85, $CHCl_3$).

IR (KBr): ν_{max} 3315, 2956, 1657, 1628, 1541, 1250, 1072, 993, 889, 841 cm^{-1}.

^{1}H-NMR (pyridine-d_5): δ 0.20 (s, 9H, CH_3Si), 0.85 (d, 3H, J=6.9 Hz, 23-CH_3), 1.08 (d, 3H, J=6.7 Hz, 20-CH_3), 1.50 (m, 2H, 17-H), 1.55 (s, 3H, 21-H), 1.85 (m, 1H, 18-H), 1.91 (s, 3H, 22-H), 2.11 (ddd, 1H, J=6.8, 13.6, 13.6 Hz, 16-H), 2.33 (m, 1H, 6-H), 2.36 (m, 2H, 8-H), 2.41 (m, 1H, 16-H), 2.64 (d, 1H, J=14.3 Hz, 11-H), 2.70 (d, 1H, J=14.1 Hz, 11-H), 3.09 (ddd, 1H, J=3.7, 4.4, 13.4 Hz, 19-H), 3.47 (ddd, 1H, J=2.6, 8.0, 8.0 Hz, 7-H), 3.93 (ddd, 1H, J=9.2, 9.2, 13.4 Hz, 19-H), 5.20 (dd, 1H, J=7.3, 7.4 Hz, 9-H), 5.68 (ddd, 1H, J=5.6, 8.8, 15.0 Hz, 15-H), 5.91 (dd, 1H, J=9.2, 15.1 Hz, 4-H), 5.96 (d, 1H, J=11.1 Hz, 13-H), 6.21 (dd, 1H, J=11.2, 15.1 Hz, 4-H), 6.25 (d, 1H, J=14.8 Hz, 2-H), 6.75 (dd, 1H, J=11.0, 14.9 Hz, 14-H), 7.56 (dd, 1H, J=11.1, 15.0 Hz, 3-H), 8.45 (dd, 1H, J=2.3, 8.3 Hz, NH).

^{13}C-NMR (pyridine-d_5): δ 0.47, 17.2, 17.4, 18.3, 19.2, 28.2, 33.0, 33.5, 38.2, 43.3, 47.4, 49.7, 77.2, 121.9, 124.4, 127.7, 128.3, 128.5, 132.5, 134.0, 134.6, 140.3, 143.2, 166.3.

Anal Calcd for $C_{26}H_{43}O_2NSi$: C, 72.67; H, 10.09; N, 3.26.
Found: C, 72.37; H, 10.36; N, 3.26.

Vicenistatin M {(6*S*,7*S*,18*S*)-20-Aza-6,10,12,18-tetramethyl-7-*O*-(2′,6′-dideoxy-3′-*C*-methyl-β-D-*ribo*-hexopyranosyl)-cycloicosa-2,4,9,12,14-pentaen-1-one} (**2**)

To a suspension of $AgClO_4$ (120 mg, 0.58 mmol) in dry CH_2Cl_2 (10 ml) was added dropwise $SnCl_4$ (58 μl, 1 M in CH_2Cl_2, 0.58 mmol) at room temperature. The mixture was shielded from light and stirred for 1 hour. To this mixture was added dropwise 1-*O*-acetyl sugar **10** (221 mg, 0.96 mmol) and *O*-TMS-aglycon **11** (454 mg, 1.06 mmol) in dry CH_2Cl_2 (9 ml), and the reaction mixture was stirred at room temperature for 24 hour. To the mixture was added sat. $NaHCO_3$ aq. (5 ml) and extracted with Et_2O (15 ml×5). The combined extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 50 g; $CHCl_3$/EtOAc, 10 : 1 to 3 : 1) to afford 180 mg of 3′,4′-*O*-carbonylvicenistatin M (mixture of α and β-anomers: yield 35%). This was used for the next reaction without further purification. To a solution of 3′,4′-*O*-carbonylvicenistatin M (180 mg, 0.34 mmol) in MeOH (30 ml) was added KOH aq. (10 ml, 1 N, 10 mmol) and stirred for 4 hours. The reaction mixture was evaporated, and the resultant residue was extracted with EtOAc (20 ml×3). The combined extract was dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 45 g; CH_2Cl_2/EtOAc, 3 : 1) to afford 39 mg of **2** (β-anomer, yield 23%) and 99 mg of α-anomer (yield 58%).

β-anomer: mp 220.0~222.0°C, $[\alpha]_D^{27}$ −28.5° (c=0.047, MeOH).

Every spectral data were identical with those of natural vicenistatin M.

Anal Calcd for $C_{30}H_{47}O_5N$: C, 71.82; H, 9.44; N, 2.79.
Found: C, 71.60; H, 9.73; N, 2.52.

α-anomer: mp 194.0~197.0°C, $[\alpha]_D^{19}$ +55.4° (c=0.066, MeOH).

IR (KBr): ν_{max} 1657, 1628, 993 cm^{-1}.

^{1}H-NMR (pyridine-d_5): δ 0.86 (d, J=6.9 Hz, 3H), 1.10 (d, J=6.7 Hz, 3H), 1.41 (m, 1H), 1.49 (s, 3H), 1.54 (m, 1H), 1.56 (s, 3H), 1.60 (d, J=6.2 Hz, 3H), 1.85 (m, 1H), 1.87 (s, 3H), 1.97 (dd, J=4.2, 14.4 Hz, 1H), 2.14 (m, 1H), 2.25 (dt, J=8.2, 15.1 Hz, 1H), 2.37 (dd, J=6.6, 14.7 Hz, 1H), 2.43 (dd, J=8.0, 15.2 Hz, 2H), 2.59 (d, J=14.6 Hz, 1H), 2.69 (d, J=14.5 Hz, 1H), 3.22 (dt, J=4.2, 13.5 Hz, 1H), 3.31 (d, J=9.3 Hz, 1H), 3.65 (t, J=8.0 Hz, 1H), 3.79 (dt, J=8.8, 13.4 Hz, 1H), 4.31 (dq, J=6.2, 9.6 Hz, 1H), 5.12 (t, J=7.2 Hz, 1H), 5.25 (d, J=3.8 Hz, 1H), 5.68 (ddd, J=6.3, 8.0, 14.9 Hz, 1H), 5.94 (dd, J=9.2, 15.1 Hz, 1H), 6.18 (d, J=11.1 Hz, 1H), 6.24 (dd, J=15.1 Hz, 1H), 6.72 (dd, J=11.0, 15.0 Hz, 1H), 7.51 (dd, J=11.0, 15.0 Hz, 1H), 8.38 (br d, J=4.2 Hz, 1H);

^{13}C-NMR (pyridine-d_5): δ 17.2, 18.4, 18.7, 26.9, 28.4, 31.4, 33.2, 33.5, 42.3, 43.4, 44.7, 49.6, 66.6, 70.1, 77.6, 80.1, 94.8, 121.5, 124.8, 127.7, 128.4, 128.5, 132.6, 134.0, 135.0, 140.0, 142.7, 166.4.

Anal Calcd for $C_{30}H_{47}O_5N$: C, 71.82; H, 9.44; N, 2.79.
Found: C, 71.52; H, 9.47; N, 2.57.

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