Synthesis of Kifunensine, an Immunomodulating Substance Isolated from a Microbial Source[1)]

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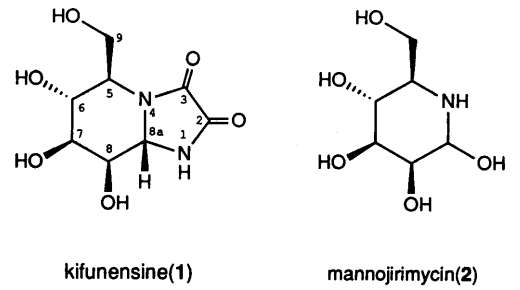
Kifunensine (1), a novel immunomodulator isolated from an actinomycete, was enantiospecifically synthesized from D-mannosamine *via* a double cyclization of the oxamide-aldehyde precursor with ammonia as a key step. The absolute stereochemistry of natural kifunensine was confirmed to be the D form.

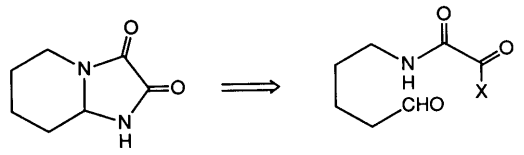
Keywords kifunensine; D-mannosamine; enantiospecific synthesis; double cyclization; polyhydroxylated piperidine; 4,5-dioxoimidazolidine; immunomodulator; α-mannosidase inhibitor

In the preceding papers[2)] we reported the structure of kifunensine (**1**) isolated from *Kitasatosporia kifunense* no. 9482 as a new immunomodulator with α-mannosidase-inhibitory activity.[3)] It induces the expression of Ia antigen on mouse peritoneal macrophages[4)] and restores, in mouse spleen cells, the immune response depressed by immunosuppressive factors in the tumor-bearing mouse serum. Kifunensine has a unique basic framework, an octahydro-2,3-dioxoimidazo[1,2-*a*]pyridine ring system, which to our knowledge has not previously been found in nature, and corresponds structurally to a cyclic oxamide derivative of 1-amino-substituted mannojirimycin[5,6)] (Chart 1).



kifunensine(**1**) mannojirimycin(**2**)

Chart 1



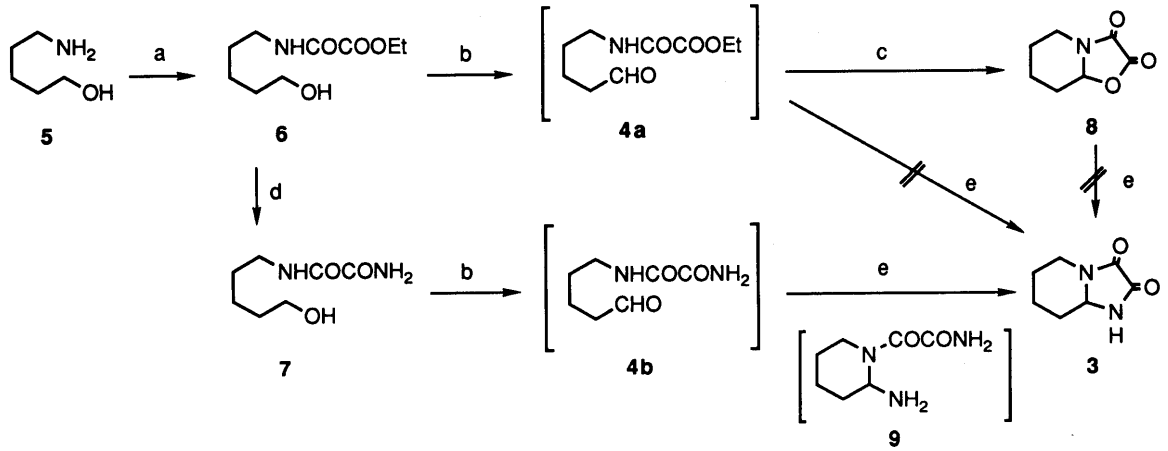
3 **4a** : X=OEt
4b : X=NH_2

Chart 2

This novel structure of **1** and its interesting biological activity prompted us to establish an efficient route for the synthesis of this natural product. Herein we report a highly stereo-controlled synthesis of kifunensine from D-mannosamine, *via* a double cyclization of the oxamide-aldehyde precursor with ammonia as a key step.

The main problem to be solved for the synthesis of this substance was the construction of the bicyclic framework. In order to find a solution to this problem, we initially investigated in a model study preparation of the simplest octahydro-2,3-dioxoimidazo[1,2-*a*]pyridine system **3**, consisting of the basic framework of **1**. We envisioned that this bicyclic structure would be constructed by a double cyclization of oxalylamino-aldehyde precursor **4** with ammonia (Chart 2). At first we chose the ethoxy group as the leaving group X and prepared the precursor **4a** from 1-aminopentanol (**5**) as follows (Chart 3).

Selective N-acylation of **5** was achieved by silylation with bistrimethylsilylacetamide (BSA) followed by acylation with ethyl oxalyl chloride and by subsequent acidic desilylation to give the alcohol **6** in 95% yield. Collins oxidation of **6** afforded the required aldehyde **4a**, which was directly used without further purification because of its instability during chromatography on silica gel or Florisil. We found that by heating in toluene, this aldehyde was transformed into the 4,5-dioxooxazolidine **8**, which corresponds to the 1-oxa

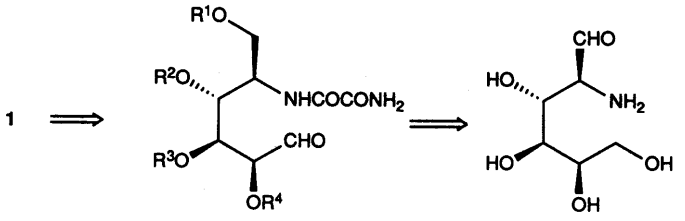


a: 1) BSA, THF; 2) ClCOCOOEt; 3) 1 N aq. AcOH b: $CrO_3 \cdot 2Py$, CH_2Cl_2 c: reflux in toluene d: 2.4 N NH_3–MeOH e: 6 N NH_3–MeOH.

Chart 3

derivative of **3**, probably *via* an intramolecular double cyclization, in 30% yield from **6**. Encouraged by this result, we examined treatment of **4a** and **8** with ammonia in MeOH. In both cases, however, an unknown material was mainly produced and only trace amounts of **3** were detected on thin layer chromatography (TLC). It was supposed that polymerization might occur much more quickly than the desired cyclization. We then attempted a double cyclization of the oxamide-aldehyde precursor **4b** whose oxalyl group might be much less reactive than that of **4a**. Compound **4b**, prepared from **6** by ammonolysis to **7** (quantitative yield) followed by Collins oxidation, was also unstable, and was used directly for the next reaction without further purification. After several attempts, we found that the desired cyclization took place in **4b** to afford **3** in 48% yield from **7** on treatment with 6 N ammonia–MeOH at room temperature for 48 h. Since this cyclization did not occur in the case of treatment with tertiary amines such as Et_3N and diisopropylethylamine, it was presumed that **3** arose *via* the intermediary amine **9**.

With these results in hand, we devised a synthetic route for kifunensine. Though the absolute stereochemistry of **1** was unknown, it was presumed to be the D form because **1** showed α-mannosidase-inhibitory activity. In our strategy, the piperidine portion of **1** was retrosynthetically related to D-mannosamine (**11**), which could be converted into the precursor **10** for **1** *via* interchange of its C-1 aldehyde and C-6 hydroxymethyl groups: reduction of C-1 to hydroxymethyl and oxidation of C-6 to aldehyde (Chart 4). This starting material could provide four of the five asymmetric centers in **1**. For protection of the four hydroxyl functions in **10**, we chose the acetonide groups in the expectation that the cyclization would proceed stereoselectively as a result of restricting the flexibility of the molecule.

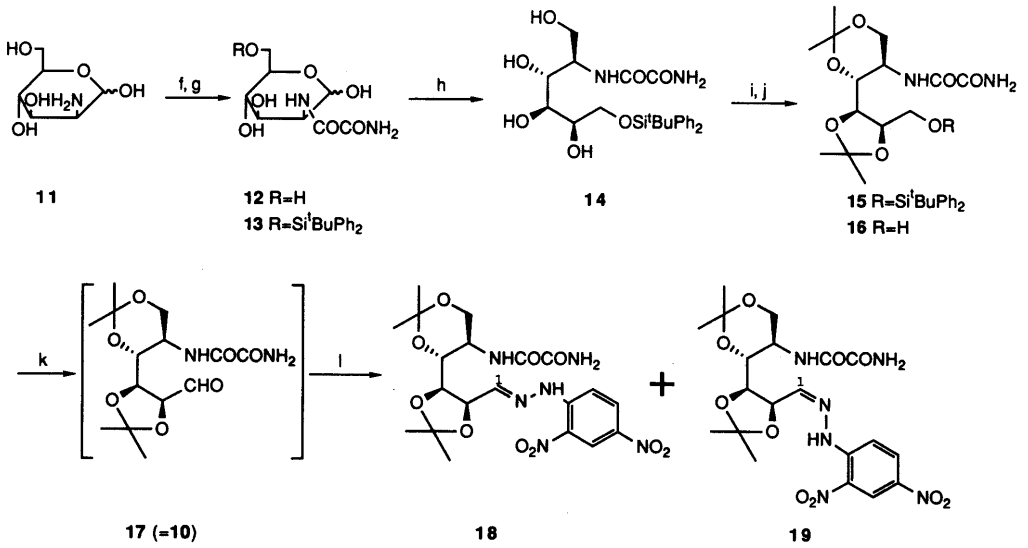


10 **11**

Chart 4

The requisite intermediate **17** (**10**) was prepared from D-mannosamine (**11**) as follows (Chart 5). Selective N-acylation of **11** with oxamic acid, using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) in dimethylformamide (DMF), and subsequent silylation of the primary alcohol gave, *via* **12**, an anomeric mixture (*ca.* 7:3) of **13** in 66% yield from **11**. Compound **13** was subjected to $NaBH_4$ reduction to furnish the tetrol **14** in 92% yield. Diacetonization of the four hydroxyl groups in **14** was achieved successfully with acetone–$BF_3 \cdot Et_2O$ to give the diacetonide **15** in 86% yield, and this was desilylated with *n*-Bu_4NF in tetrahydrofuran (THF) to afford the alcohol **16** quantitatively. Collins oxidation of **16** provided the desired oxamide-aldehyde precursor **17** as a crude oil, which was directly subjected to the key cyclization reaction because of its instability. The structure of this key intermediate was supported by infrared (IR, $CHCl_3$, 1720 cm^{-1}) and proton nuclear magnetic resonance (^{1}H-NMR, $CDCl_3$, δ 9.60, 1H s) data, and confirmed by derivatization to the dinitrophenylhydrazone derivatives, **18** (*anti*) and **19** (*syn*). The geometries of these hydrazones were presumed on the basis of comparison of the chemical shifts of the C-1 protons,[7] δ 8.05 for **18** and δ 6.96 for **19**, in their ^{1}H-NMR spectra.

The key double cyclization was carried out by treating **17** with 6 N NH_3–MeOH at room temperature for 6 h to afford the objective kifunensine diacetonide **20** in 76% yield from **16** along with its **8a**-epimer **21** (4.0% yield) (Chart 6). This diacetonide **20** was identical with an authentic sample derived from the natural product. The remarkable stereoselectivity might be explained by the relative ther-



11 **12** R=H, **13** R=Si^tBuPh_2 **14** **15** R=Si^tBuPh_2, **16** R=H

17 (=**10**) **18** **19**

Si^tBuPh_2 = *tert*-butyldiphenylsilyl

f: $H_2NCOCOOH$, DCC, HOBT, DMF g: $^tBuPh_2SiCl$, imidazole, DMF, 0 °C h: $NaBH_4$, MeOH i: acetone, $BF_3 \cdot OEt_2$, −20 °C j: *n*-Bu_4NF, THF, −20 °C k: $CrO_3 \cdot 2Py$, CH_2Cl_2 l: 2,4-DNP, H_3PO_4, EtOH.

Chart 5

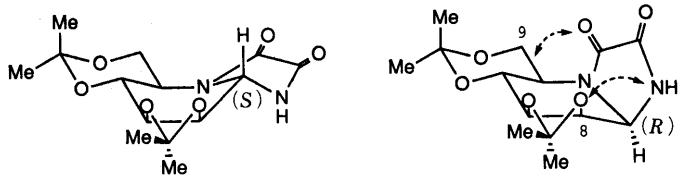
modynamic stability of the desired (**8a**-*S*)epimer **20** and its (**8a**-*R*)epimer **21**. In our study using molecular models, it seemed that **20** is much more stable than **21** because, in the latter compound, the dioxoimidazolidine ring is hindered by the methylene (C-9) or/and the oxygen on C-8 (Fig. 1). Probably the direction of ring closure was regulated by this difference of thermodynamic stability between **20** and **21**.

All other cyclization methods examined under alkaline conditions (NaH/THF, NaOMe/MeOH, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU)/MeOH, *etc.*), acidic conditions (camphorsulfonic acid/THF, $BF_3 \cdot Et_2O$/THF, *etc.*), and other conditions (heating, pyridinium *p*-toluenesulfonate/CH_2Cl_2, trimethylsilyl chloride–pyridine/THF, *etc.*) were unsuccessful. On the other hand, treatment of **17** with saturated aqueous NH_4HCO_3 or $(NH_4)_2CO_3$ afforded the desired cyclization product **20** in a stereoselective manner, but in lower yield. On the basis of these results, we speculated that this reaction proceeded through the intermediacy of the amine **B**, formed by a condensation of the aldehyde **A** with ammonia (Chart 7). This speculation is supported by the fact that similar treatment of the oxamide-aldehyde **17** with 30% $MeNH_2$–MeOH in a similar manner afforded ^1N-methylkifunensine diacetonide **22**, identical with an authentic sample derived from the natural product, in 81% yield from the alcohol **16**. In this cyclization, the (**8a**-*R*)epimer was not obtained.

Removal of the acetonide protecting groups in **20** with aqueous trifluoroacetic acid (TFA) furnished kifunensine (**1**) which was identical with an authentic sample, confirming the absolute stereochemistry of **1** to be the D form, as presumed. Similar treatment of **21** and **22** also afforded **8a**-*epi*-kifunensine (**23**) and ^1N-methylkifunensine (**24**), respectively.

The basic framework **3**, its 1-oxa derivative **8**, ^1N-methyl derivative **24** and **8a**-epimer **23** did not inhibit α-mannosidase and had no effect on Ia antigen expression. These facts might suggest that the hydroxyl groups,amide NH and the stereochemistry of kifunensine (**1**) are important for its biological activities.[8]

In conclusion, we have developed a double cyclization method to construct the octahydro-2,3-dioxoimidazo[1,2-*a*]pyridine ring system and by adopting it as the key step, we have established an efficient route for the synthesis of kifunensine (**1**). This synthetic route is capable of providing sufficient amounts for detailed biological evaluation and may also be applicable to the preparation of analogous compounds.



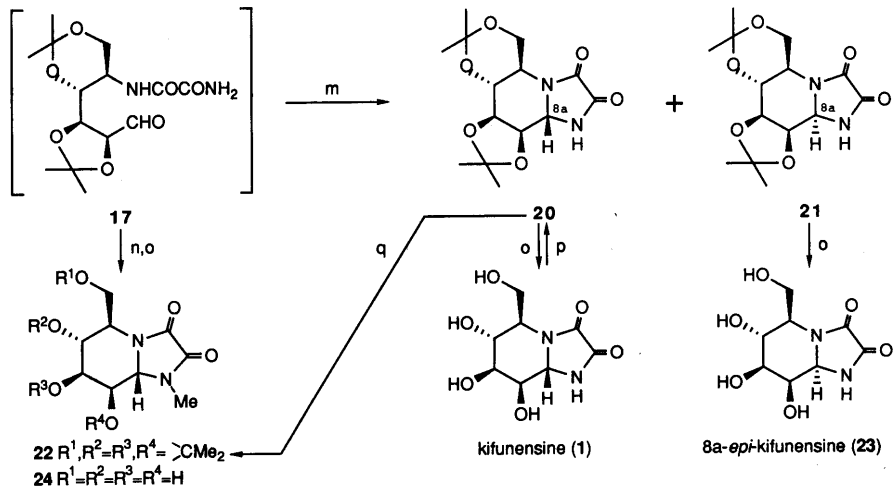
20 (8a-*S*) **21** (8a-*R*)

Fig. 1

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our preceding paper.[2b]

Ethyl *N*-(5-Hydroxypentyl)oxamate (6) BSA (20 ml) was added dropwise to a stirred anhydrous solution of 5-amino-1-pentanol (**5**, 2.06 g) in freshly distilled THF (100 ml) at room temperature over a period of 20 min under an N_2 atmosphere and the mixture was stirred for 1 h. The



m: 6 N NH_3–MeOH n: 30% $MeNH_2$–MeOH o: 75% aq. TFA p: 2,2-dimethoxypropane, TsOH, DMF, 60 °C q: MeI, K_2CO_3, acetone, reflux.

Chart 6

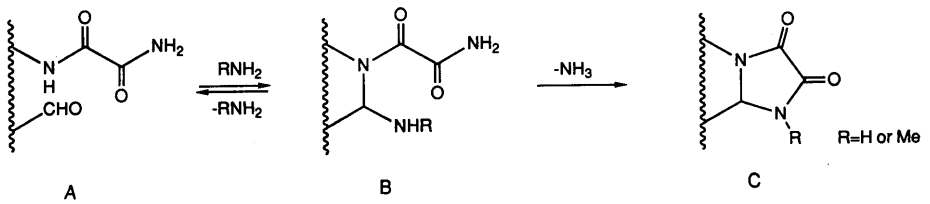


Chart 7

reaction mixture was cooled in an ice-water bath and a solution of ethyl oxalyl chloride (2.5 ml) in freshly distilled THF (7.5 ml) was added dropwise at 7—14 °C over a period of 5 min. The mixture was stirred for 1 h, then 1 N aqueous AcOH (20 ml) was added dropwise at 6—14 °C over a period of 5 min. The mixture was washed with brine and the aqueous layer was extracted with AcOEt twice. The combined organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and evaporated *in vacuo* to give a pale yellow oil (4.15 g), which was purified by column chromatography (SiO_2 200 g, CH_2Cl_2 : EtOH = 20 : 1) to afford **6** (3.85 g, 95%). **6**: a colorless oil. IR (neat): 3300 (br), 2940, 1732, 1682, 1532 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.20 (1H, br s), 4.36 (2H, q, J = 7 Hz), 3.68 (2H, t, J = 6 Hz), 3.37 (2H, q, J = 7 Hz), 1.70—1.40 (6H, m), 1.40 (3H, t, J = 7 Hz), Fast atom bombardment mass spectra (FAB-MS) m/z: 204 $(M+H)^+$. High-resolution FAB-MS Calcd for $C_9H_{18}NO_4$ $(M+H)^+$: 204.124. Found: 204.123.

***N*-(5-Hydroxypentyl)oxamide (7)** An anhydrous solution of **6** (1.50 g) in MeOH (10 ml) was treated with 6 N NH_3–MeOH (5 ml) at room temperature for 10 min under an N_2 atmosphere. Removal of the solvent under reduced pressure afforded **7** (1.28 g, quant.). **7**: colorless fine crystals, mp 168—170 °C (MeOH). *Anal.* Calcd for $C_7H_{14}N_2O_3$: C, 48.26; H, 8.10; N, 16.08. Found: C, 47.97; H, 7.85; N, 16.12. IR (Nujol): 3380, 3305, 1652, 1540 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 8.66 (1H, t, J = 6 Hz, D_2O-exchangeable), 8.03, 7.75 (each 1H, s, D_2O-exchangeable), 4.36 (1H, t, J = 5 Hz, D_2O-exchangeable), 3.37 (2H, q, J = 5 Hz), 3.16 (2H, q, J = 6 Hz), 1.55—1.15 (6H, m). FAB-MS m/z: 175 $(M+H)^+$.

Octahydro-2,3-dioxoimidazo[1,2-*a*]pyridine (3) A stirred anhydrous solution of pyridine (1.0 ml) in CH_2Cl_2 (25 ml) was treated with CrO_3 (615 mg) at room temperature under an N_2 atmosphere. The mixture was stirred for 15 min, then a suspension of **7** (100 mg) in anhydrous pyridine (3.5 ml) was added and the mixture was stirred for an additional 30 min followed by vacuum filtration through cellulose powder. The insoluble material was washed with CH_2Cl_2 (25 ml). The filtrate and washings were combined and evaporated *in vacuo* to give *N*-(4-formylbutyl)oxamide (**4b**, 825 mg) as a crude oil. ^{1}H-NMR (DMSO-d_6) δ: 9.68, 1H, br s. This crude aldehyde was directly treated with 6 N NH_3–MeOH (5.0 ml) for 48 h at room temperature under an N_2 atmosphere. After vacuum filtration through cellulose powder, the filtrate was evaporated *in vacuo* and the residue was purified by preparative TLC (CH_2Cl_2 : MeOH = 9 : 1) to furnish **3** (42 mg, 48% from **7**). **3**: colorless fine crystals, mp 164—165 °C (AcOEt). *Anal.* Calcd for $C_7H_{10}N_2O_2$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.25; H, 6.33; N, 17.90. IR (Nujol): 3220, 1748, 1718, 1698 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 9.92 (1H, br s, D_2O-exchangeable), 4.72 (1H, dd, J = 10, 4 Hz), 4.06 (1H, dd, J = 12, 5 Hz), 2.91 (1H, td, J = 12, 4 Hz), 2.20—1.00 (6H, m). FAB-MS m/z: 155 $(M+H)^+$.

Octahydro-2,3-dioxooxazo[3,2-*a*]pyridine (8) A stirred anhydrous solution of pyridine (5.0 ml) in CH_2Cl_2 (125 ml) was treated with CrO_3 (3.08 g) at room temperature under an N_2 atmosphere. The mixture was stirred for 15 min, then a solution of **6** (613 mg) in anhydrous CH_2Cl_2 (5 ml) was added and the whole was stirred for an additional 30 min. The reaction mixture was diluted with Et_2O (125 ml), filtered through cellulose powder, and evaporated *in vacuo* to give a residue, which was extracted with Et_2O (100 ml). The extract was evaporated *in vacuo* to give ethyl *N*-(4-formylbutyl)oxamate (**4a**, 601 mg) as a crude oil. ^{1}H-NMR ($CDCl_3$) δ: 9.81 (1H, t, J = 2 Hz). This crude aldehyde was directly heated under reflux in anhydrous toluene (12 ml) for 1 h under an N_2 atmosphere. The reaction mixture was evaporated *in vacuo* and the residue was purified by column chromatography (SiO_2 10 g, CH_2Cl_2 : MeOH = 30 : 1) to afford **8** (140 mg, 30% from **6**). **8**: colorless fine crystals, mp 72—73 °C (isopropyl ether). *Anal.* Calcd for $C_7H_9NO_3$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.08; H, 5.79; N, 9.00. IR ($CHCl_3$): 2950, 2930, 1818, 1732 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 5.51 (1H, dd, J = 10, 5 Hz), 4.06 (1H, ddd, J = 12, 4, 2 Hz), 3.01 (1H, td, J = 12, 4 Hz), 2.23 (1H, m), 1.97—1.22 (5H, m). FAB-MS m/z: 156 $(M+H)^+$.

6-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-2-oxamoylamino-D-mannose (13) DCC (4.95 g), HOBT (3.24 g) and Et_3N (2.8 ml) were added to a stirred ice-cold suspension of D-mannosamine hydrochloride (**11**, 4.31 g) and oxamic acid (2.14 g) in DMF (40 ml). The mixture was stirred at room temperature for 15 h under an N_2 atmosphere, then DCC (1.24 g) and oxamic acid (534 mg) were added and the whole was stirred for an additional 5 h. The insoluble material was removed by vacuum filtration and washed with water (200 ml). The filtrate and washings were combined, washed five times with CH_2Cl_2 and evaporated *in vacuo* to give 2-deoxy-2-oxamoylamino-D-mannose (**12**) as a crude white amorphous solid (9.53 g), which was directly subjected to silylation without further purification. *tert*-Butyldiphenylsilyl chloride (7.8 ml) was added dropwise to an ice-cold solution of this residue (9.51 g) and imidazole (2.04 g) in DMF (80 ml). The mixture was stirred in an ice-water bath for 3 h under an N_2 atmosphere, then *tert*-butyldiphenylsilyl chloride (2.6 ml) and imidazole (1.36 g) were added and the whole was sirred for an additional 3 h. The reaction mixture was poured into water (400 ml) and extracted with AcOEt (300 ml). The organic layer was washed with 1 N aqueous HCl 3 times, brine, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2 300 g, CH_2Cl_2 : MeOH = 40 : 1—15 : 1) to afford **13** (6.47 g, 66% from **11**). **13**: amorphous solid, $[\alpha]_D$ +12.9° (c = 0.6, MeOH). *Anal.* Calcd for $C_{24}H_{32}N_2O_7Si$: C, 59.00; H, 6.60; N, 5.73. Found: C, 58.72; H, 6.62; N, 5.72. IR ($CHCl_3$): 3580, 3500, 3450, 3390, 2930, 1680, 1530, 1110 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6–D_2O) δ: 7.78—7.60 (4H, m), 7.52—7.36 (6H, m), 5.08 (0.7H, br s), 4.86 (0.3H, br s), 4.18—3.22 (6H, m), 1.01 (9H, s). FAB-MS m/z: 511 $(M+Na)^+$.

6-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-2-oxamoylamino-D-mannitol (14) $NaBH_4$ (2.13 g) was added to a stirred ice-cold solution of **16** (5.51 g) in MeOH (120 ml) and the mixture was stirred at room temperature for 30 min. After an acidic treatment with 0.1 N HCl (500 ml) in an ice-water bath, the reaction mixture was extracted with AcOEt twice. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo* to give **14** (5.12 g, 92%). **14**: colorless fine crystals, mp 174—175 °C (AcOEt), $[\alpha]_D$ −16.9° (c = 0.7, MeOH). *Anal.* Calcd for $C_{24}H_{34}N_2O_7Si$: C, 58.75; H, 6.98; N, 5.71. Found: C, 58.42; H, 7.01; N, 5.67. IR (Nujol): 3380, 3315, 1660, 1537, 1112 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6), δ: 8.33 (1H, d, J = 7 Hz, D_2O-exchangeable), 8.08 (1H, br s, D_2O-exchangeable), 7.86 (1H, br s, D_2O-exchangeable), 7.75—7.60 (4H, m), 7.50—7.35 (6H, m), 4.77 (1H, t, J = 4 Hz, D_2O-exchangeable), 4.68 (1H, d, J = 4 Hz, D_2O-exchangeable), 4.51 (1H, d, J = 6 Hz, D_2O-exchangeable), 4.29 (1H, d, J = 6 Hz, D_2O-exchangeable), 3.96—3.30 (8H, m), 0.99 (9H, s). FAB-MS m/z: 513 $(M+Na)^+$, 491 $(M+H)^+$.

6-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-1,3 : 4,5-di-*O*-isopropylidene-2-oxamoylamino-D-mannitol (15) $BF_3 \cdot OEt_2$ (1.40 ml) was added dropwise to a stirred solution of **14** (3.80 g) in acetone (70 ml) at −25 °C under an N_2 atmosphere. After being stirred for 4 h at −25—−18 °C, the reaction mixture was poured into stirred, ice-cold saturated aqueous $NaHCO_3$ and extracted twice with AcOEt. The extracts were combined, washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2 150 g, CH_2Cl_2 : AcOEt = 1 : 1, then CH_2Cl_2 : MeOH = 8 : 1) to afford **15** (3.82 g, 86%). **15**: amorphous solid, $[\alpha]_D$ −39.0° (c = 0.6, MeOH). *Anal.* Calcd for $C_{30}H_{42}N_2O_7Si$: C, 63.13; H, 7.42; N, 4.91. Found: C, 62.87; H, 7.51; N, 4.78. IR ($CHCl_3$): 3520, 3400, 2945, 1692, 1530, 1381, 1116 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 8.60 (1H, d, J = 7 Hz, D_2O-exchangeable), 8.10 (1H, br s, D_2O-exchangeable), 7.82 (1H, br s, D_2O-exchangeable), 7.74—7.58 (4H, m), 7.55—7.35 (6H, m), 4.42 (1H, td, J = 6, 2 Hz), 4.10—3.20 (7H, m), 1.41, 1.28, 1.08, 1.03 (each 3H, s), 1.02 (9H, s). FAB-MS m/z: 593 $(M+Na)^+$, 571 $(M+H)^+$.

2-Deoxy-1,3 : 4,5-di-*O*-isopropylidene-2-oxamoylamino-D-mannitol (16) A solution of *n*-$Bu_4NF \cdot 3H_2O$ (789 mg) in THF (15 ml) was added dropwise to a stirred solution of **15** (571 mg) in THF (10 ml) at −17—−14 °C over a period of 20 min. The mixture was stirred at −17—−21 °C for 1.5 h, then the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2 28 g, CH_2Cl_2 : AcOEt : MeOH = 50 : 50 : 2) to furnish the primary alcohol **16** (332 mg, quant.). **16**, amorphous solid, $[\alpha]_D$ −77.5° (c = 0.5, MeOH). *Anal.* Calcd for $C_{14}H_{24}N_2O_7$: C, 50.59; H, 7.28; N, 8.43. Found: C, 50.87; H, 7.08; N, 8.10. IR ($CHCl_3$): 3525, 3475, 3402, 3325, 3002, 1687, 1532, 1381, 1162 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 8.68 (1H, d, J = 7 Hz, D_2O-exchangeable), 8.12 (1H, br s), 7.83 (1H, br s), 4.81 (1H, t, J = 4 Hz), 4.28—3.80 (4H, m), 3.78—3.50 (4H, m), 1.44, 1.40, 1.28, 1.24 (each 3H, s). FAB-MS m/z: 355 $(M+Na)^+$, 333 $(M+H)^+$.

5-Deoxy-2,3 : 4,6-di-*O*-isopropylidene-5-oxamoylamino-D-mannose (17) A stirred anhydrous solution of pyridine (3.6 ml) in CH_2Cl_2 (90 ml) was treated with CrO_3 (2.22 g) at room temperature under an N_2 atmosphere. The mixture was stirred for 15 min, then a solution of **16** (730 mg) in anhydrous CH_2Cl_2 (90 ml) was added dropwise over a period of 5 min. The mixture was stirred for an additional 30 min, diluted with Et_2O (200 ml) and filtered through cellulose powder. Removal of the solvent under reduced pressure gave a residue, which was extracted with Et_2O (250 ml). The combined extracts were evaporated *in vacuo* to afford the aldehyde **17** (846 mg) as a crude oil. (IR ($CHCl_3$): 1720 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.60 (1H, s)).

2,4-Dinitrophenylhydrazones of 17 (18, 19) 2,4-Dinitrophenylhydrazine (90 mg) and H_3PO_4 (0.02 ml) were added to a stirred solution of **17** (43 mg)

in EtOH (1 ml). After being stirred for 1 h, the reaction mixture was diluted with CH_2Cl_2 (5 ml) and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2–MeOH (4 : 1) 3 times. The organic layers were combined, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by preparative TLC (CH_2Cl_2 : MeOH = 20 : 1) to afford the *anti*-isomer **18** (32 mg, 41% from **16**) and the *syn*-isomer **19** (16 mg, 21% from **16**). **18**: yellow amorphous solid, $[\alpha]_D$ +185.5° (c = 0.5, $CHCl_3$). IR ($CHCl_3$): 3520, 3400, 3310, 1692, 1618, 1596, 1338 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.86 (1H, d, J = 1 Hz), 8.72 (1H, d, J = 10 Hz, D_2O-exchangeable), 8.39 (1H, dd, J = 10, 1 Hz), 8.09 (1H, br s, D_2O-exchangeable), 8.05 (1H, d, J = 8 Hz), 7.90 (1H, d, J = 10 Hz), 7.81 (1H, br s, D_2O-exchangeable), 4.83 (1H, t, J = 8 Hz), 4.32 (1H, d, J = 8 Hz), 4.07 (1H, d, J = 11 Hz), 3.97 (1H, m), 3.66 (1H, t, J = 11 Hz), 3.58 (1H, dd, J = 11, 7 Hz), 1.49 (3H, s), 1.35 (6H, s), 1.32 (3H, s). FAB-MS m/z: 533 $(M+Na)^+$. High-resolution FAB-MS Calcd for $C_{20}H_{26}N_6NaO_{10}$ $(M+Na)^+$: 533.161. Found: 533.159. **19**: yellow amorphous solid, $[\alpha]_D$ +175.2° (c = 0.5, $CHCl_3$). IR ($CHCl_3$): 3520, 3400, 3280, 1690, 1618, 1592, 1338 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.90 (1H, d, J = 1 Hz), 8.68 (1H, d, J = 10 Hz, D_2O-exchangeable), 8.42 (1H, dd, J = 10, 1 Hz), 8.11 (1H, br s, D_2O-exchangeable), 7.96 (1H, d, J = 10 Hz), 7.83 (1H, br s, D_2O-exchangeable), 6.96 (1H, d, J = 3 Hz), 5.30 (1H, dd, J = 7, 3 Hz), 4.48 (1H, d, J = 7 Hz), 4.02 (1H, d, J = 10 Hz), 3.97 (1H, m), 3.66 (1H, t, J = 11 Hz), 3.60 (1H, dd, J = 11, 6 Hz), 1.67, 1.37, 1.20, 0.96 (each 3H, s). FAB-MS m/z: 533 $(M+Na)^+$. High-resolution FAB-MS Calcd for $C_{20}H_{26}N_6NaO_{10}$ $(M+Na)^+$: 533.161. Found: 533.161.

Double Cyclization of 17 The crude aldehyde **17** (460 mg), obtained as described above, was treated with 6 N NH_3–MeOH (20 ml) at room temperature for 20 h under an N_2 atmosphere. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (SiO_2 25 g, CH_2Cl_2 : MeOH = 50 : 1) to afford kifunensine diacetonide **20** (284 mg, 76% from **16**) along with its **8a**-epimer **21** (15 mg, 4.0% from **16**). Recrystallization of **20** from *n*-hexane–AcOEt provided colorless fine crystals. This product was identical with an authentic sample derived from the natural product as judged from mixed melting-point determination and direct TLC comparison (CH_2Cl_2 : MeOH = 9 : 1, Rf = 0.55; AcOEt, Rf = 0.50), $[\alpha]_D$, IR ($CHCl_3$), and ^{1}H-NMR ($CDCl_3$).[2] **21**: colorless fine crystals, mp >280 °C (MeOH), $[\alpha]_D$ −15.8° (c = 0.1, DMSO). *Anal.* Calcd for $C_{14}H_{20}N_2O_6 \cdot H_2O$: C, 50.90; H, 6.71; N, 8.48. Found: C, 50.92; H, 6.43; N, 8.45. IR (Nujol): 3130, 1740, 1722, 1708, 1220, 1200, 1160, 1024 cm^{-1}. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 10.03 (1H, s, D_2O-exchangeable), 5.25 (1H, d, J = 3 Hz), 4.70 (1H, t, J = 11 Hz), 4.38 (1H, dd, J = 4, 3 Hz), 4.29 (1H, dd, J = 11, 5 Hz), 4.15 (1H, dd, J = 8, 4 Hz), 3.78 (1H, dd, J = 10, 8 Hz), 3.25 (1H, td, J = 11, 5 Hz), FAB-MS m/z: 335 $(M+Na)^+$, 313 $(M+H)^+$.

Kifunensine (1) Compound **20** (219 mg) was treated with 75% aqueous TFA (5.0 ml) at room temperature for 5 h. The precipitate was collected by vacuum filtration and washed with water (2.0 ml) to give **1** as a white powder (134 mg, 82%). Recrystallization from water furnished colorless prisms. This product was identical with an authentic sample as judged from mixed melting-point determination and direct TLC comparison ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1, Rf = 0.31; isopropyl alcohol : H_2O = 9 : 1, Rf = 0.37), $[\alpha]_D$, IR (KBr), and ^{1}H-NMR (400 MHz, D_2O).[2]

8a-*epi*-Kifunensine (23) Compound **21** (10 mg) was treated with 75% aqueous TFA (1 ml) at room temperature for 3 h. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to afford **8a**-*epi*-kifunensine (**23**, 7 mg, 94%). **23**: amorphous solid, $[\alpha]_D$ −38.7° (c = 0.1, H_2O). IR (KBr): 3350, 3290, 3190, 1720, 1700, 1680, 1400, 1095, 1080, 1056, 1036 cm^{-1}. ^{1}H-NMR (400 MHz, D_2O) δ: 5.16 (1H, d, J = 2 Hz), 4.38 (1H, dd, J = 13, 2 Hz), 4.30 (1H, t, J = 2 Hz), 4.27 (1H, dd, J = 13, 5 Hz), 3.87—3.83 (2H, m), 3.45 (1H, ddd, J = 8, 5, 2 Hz). FAB-MS m/z: 255 $(M+Na)^+$. High-resolution FAB-MS Calcd for $C_8H_{12}N_2NaO_6$ $(M+Na)^+$: 255.059. Found: 255.058.

^{1}*N*-Methylkifunensine Diacetonide (22) Compound **17** (130 mg) was treated with 30% $MeNH_2$–MeOH (5 ml) at room temperature for 1 h under an N_2 atmosphere. The reaction mixture was evaporated *in vacuo* and the residue was purified by column chromatography (SiO_2 7 g, CH_2Cl_2 : MeOH = 200 : 1—50 : 1) to afford **22** (88 mg, 80% from **16**). This compound was also prepared from the natural product (**1**) *via* the diacetonide **20** as follows. A mixture of **20** (100 mg), MeI (114 mg), K_2CO_3 (45 mg), and acetone (5 ml) was heated under reflux for 1.5 h. The reaction mixture was cooled to room temperature, filtered, and evaporated *in vacuo*. The residue was purified by preparative TLC to give authentic **22** (65 mg, 62%). Synthetic **22** was identical with this authentic sample as judged from mixed melting-point determination and direct TLC comparison (CH_2Cl_2 : MeOH = 9 : 1, Rf = 0.68; AcOEt, Rf = 0.57) and ^{1}H-NMR (200 MHz, $CDCl_3$). **22**: colorless fine crystals, mp 245—246 °C (AcOEt), $[\alpha]_D$ −64.8° (c = 0.5, MeOH). *Anal.* Calcd for $C_{15}H_{22}N_2O_6$: C, 55.21; H, 6.79; N, 8.58. Found: C, 55.21; H, 6.75; N, 8.67. IR ($CHCl_3$): 2990, 1752, 1420, 1377, 1090, 1068 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 4.73 (1H, dd, J = 10, 4 Hz), 4.70 (1H, d, J = 8 Hz), 4.38 (1H, t, J = 8 Hz), 4.19 (1H, dd, J = 10, 8 Hz), 4.02 (1H, t, J = 8 Hz), 3.77 (1H, t, J = 10 Hz), 3.59 (1H, td, J = 10, 4 Hz), 3.19, 1.59, 1.57, 1.50, 1.39 (each 3H, s). FAB-MS m/z: 327 $(M+H)^+$.

^{1}*N*-Methylkifunensine (24) Compound **22** (65 mg) was treated with 75% aqueous TFA (2 ml) in the same way as described for deprotection of **20** to afford **24** (41 mg, 84%). **24**: colorless fine crystals, mp 283—285 °C (dec., MeOH), $[\alpha]_D$ +66.0° (c = 0.4, H_2O). *Anal.* Calcd for $C_9H_{14}N_2O_6$: C, 43.90; H, 5.73; N, 11.38. Found: C, 43.68; H, 5.68; N, 11.25. IR (KBr): 3405, 3310, 3190, 2910, 1720, 1706, 1692, 1430, 1232, 1110, 1061, 1050, 1000 cm^{-1}. ^{1}H-NMR (200 MHz, D_2O) δ: 5.08 (1H, d, J = 10 Hz), 4.43 (1H, dd, J = 10, 4 Hz), 4.18 (1H, d, J = 3 Hz), 4.07 (1H, t, J = 3 Hz), 3.98 (1H, d, J = 10 Hz), 3.92—3.78 (2H, m), 3.30 (3H, s). FAB—MS m/z: 247 $(M+H)^+$.

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References and Notes

1) A preliminary report of this work: H. Kayakiri, C. Kasahara, T. Oku, and M. Hashimoto, *Tetrahedron Lett.*, **31**, 225 (1990).
2) *a*) H. Kayakiri, S. Takase, T. Shibata, M. Okamoto, H. Terano, M. Hashimoto, T. Tada, and S. Koda, *J. Org. Chem.*, **54**, 4015 (1989); *b*) *Idem*, *Chem. Pharm. Bull.*, **39**, 1378 (1991).
3) M. Iwami, O. Nakayama, H. Terano, M. Kohsaka, H. Aoki, and H. Imanaka, *J. Antibiot.*, **40**, 612 (1987).
4) EC_{70} = 0.02 μg/ml: S. Izumi, T. Kaizu, M. Yamashita, M. Okamoto, and M. Okuhara, "Novel Microbial Products for Medicine and Agriculture," ed. by A. L. Demain, G. A. Somkuti, J. C. Hunter-Cevera, and H. W. Rossmoore, Elsevier Science Publishers, Amsterdam, in press.
5) Mannojirimycin (nojirimycin B): T. Niwa, T. Tsuruoka, H. Goi, Y. Kodama, J. Itoh, S. Inoue, Y. Yamada, T. Niida, M. Nobe, and Y. Ogawa, *J. Antibiot.*, **37**, 1579 (1984).
6) For a review of 1,5-iminopyranoses and 1,4-iminofuranoses, see G. W. J. Fleet, *Spec. Publ. Royal Chem. Soc.*, **65**, 149 (1988).
7) G. J. Karabatsos, R. A. Taller, and F. M. Vane, *J. Am. Chem. Soc.*, **85**, 2326 (1963).
8) Compounds **3**, **8**, **23**, **24** and kifunensine (**1**) did not inhibit α-glucosidase (rabbit intestine).