Synthesis of Kifunensine, an Immunomodulating Substance Isolated from a Microbial Source

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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-alddehyde 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-dioxoimidazolidinone; immunomodulator; D-mannosidase inhibitor

In the preceding papers, we reported the structure of kifunensine (1) isolated from Kitasatospora kifunense no. 9482 as a new immunomodulator with D-mannosidase-inhibitory activity. It induces the expression of Ia antigen on mouse peritoneal macrophages 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 (Chart 1).

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-alddehyde 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 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 oxalamido-alddehyde 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-acetylation of 5 was achieved by silylation with bistrimethylsilylacacetamide (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

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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 6N ammonia−MeOH at room temperature for 48h. Since this cyclization did not occur in the case of treatment with tertiary amines such as Et3N and diisopropylethylamine, it was presumed that 3 arose via the intermediary amine 9.

With these results in hand, we devised a synthetic route for kufunensine. 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 β-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 acetamide groups in the expectation that the cyclization would proceed stereoselectively as a result of restricting the flexibility of the molecule.

The requisite intermediate 17 (10) was prepared from β-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 anomic mixture (ca. 7:3) of 13 in 66% yield from 11. Compound 13 was subjected to NaBH4 reduction to furnish the tetrot 14 in 92% yield. Diacetonization of the four hydroxyl groups in 14 was achieved successfully with acetone−BF3·Et2O to give the diacetone 15 in 86% yield, and this was desilylated with n-Bu4NF 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, CHCl3, 1720 cm−1) and proton nuclear magnetic resonance (1H-NMR, CDCl3, δ 9.60, 1H s) data, and confirmed by derivatization to the dinitrophenylhydrazone derivatives, 18 (anii) 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 1H-NMR spectra.

The key double cyclization was carried out by treating 17 with 6N NH3−MeOH at room temperature for 6h to afford the objective kufunsine diacetone 20 in 76% yield from 16 along with its 8α-epimer 21 (4.0% yield) (Chart 6). This diacetone 20 was identical with an authentic sample derived from the natural product. The remarkable stereoselectivity might be explained by the relative ther-
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 dioximidazolidine 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, BF3·Et2O/THF, etc.), and other conditions (heating, pyridinium p-toluenesulfonate/CH2Cl2, trimethylsilyl chloride-pyridine/THF, etc.) were unsuccessful. On the other hand, treatment of 17 with saturated aqueous NH4HCO3 or (NH4)2CO3 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% MeNH2-MeOH in a similar manner afforded 1′-N-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 1′-N-methylkifunensine (24), respectively.

The basic framework 3, its 1-oxa derivative 8, 1′-N-methyl derivative 24 and 8a-epimer 23 did not inhibit α-mannosidase and had no effect on 1a 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-dioximidazoo[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.

**Experimental**

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

Ethyl 1-(5-Hydroxypentyl)oxamates (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 N2 atmosphere and the mixture was stirred for 1 h. The

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**Chart 6**

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**Chart 7**

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**Chart 8**

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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 1.0 ml of ethyl alcohol was added over a period of 5 min. The mixture was heated to 50 °C for 6—14 h over a period of 5 min. The mixture was then stirred with brine and the aqueous mixture was extracted with AcOEt twice. The combined organic layer was washed with saturated aqueous NaHCO₃, dried over MgSO₄, and evaporated in vacuo to give a pale yellow oil (4.15 g), which was purified by column chromatography (SiO₂ 200 g, CH₂Cl₂:EtOH = 20:1) to afford 6 (3.85 g, 85%), 6a (2.55 g, 65%), and 6b (1.1 g, 25%). IR (KBr): 3360, 1732, 1682, 1532 cm⁻¹. 1H-NMR (200 MHz, CDCl₃) δ: 7.20 (1H, brs), 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 Calcul for C₉H₁₄NO₂ (M+H)⁺: 204.1234. Found: 204.1232.

N-[5-Hexynylidene]amide (7) An anhydrous solution of 6 (1.50 g) in MeOH (10 ml) was treated with 6N H₂SO₄ (5 ml) at room temperature for 10 min under an N₂ atmosphere. Removal of the solvent under reduced pressure afforded 7 (1.28 g, quant.). 7: colorless fine crystals, mp 168—170 °C (MeOH). Anal. Calcld for C₇H₁₆N₂O₂: C, 48.26; H, 8.10; N, 11.12. Found: C, 47.97; H, 7.85; N, 11.12. IR (Nujol): 3380, 3305, 1652, 1540 cm⁻¹. 1H-NMR (200 MHz, DMSO-d₆) δ: 8.66 (1H, t, J = 6 Hz, D₂O-exchangeable), 8.03, 7.75 (each 1H, d, J = 6 Hz, D₂O-exchangeable), 4.36 (1H, t, J = 5 Hz, D₂O-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-dioxindolizine[1,2-b]pyridine (3) A stirred anhydrous solution of 7 (200 mg) in CH₂Cl₂ (25 ml) was treated with Cr₂O₇(2) (100 mg, 0.65 ml) at room temperature under an N₂ atmosphere, which was extracted with ether (20 ml) 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 CH₂Cl₂ (25 ml). The filtrate and washings were concentrated in vacuo to give N-(4-formylbutyl)oxazide (4b, 825 mg) as a crude oil. 1H-NMR (DMSO-d₆) δ: 6.98, 1H, brs. This crude aldehyde was directly treated with 6N H₂SO₄ (5 ml) for 48 h at room temperature under an N₂ atmosphere. After vacuum filtration through cellulose powder, the filtrate was evaporated in vacuo and the residue was purified by preparative TLC (CH₂Cl₂:MeOH = 9:1) to furnish 3 (624 mg, 48% from 7). 3: colorless fine crystals, mp 164—165 °C (AcOEt). Anal. Calcld for C₂₁H₁₆N₂O₄-C, 54.54, H, 5.64; N, 18.17. Found: C, 54.25; H, 6.33; N, 17.90. IR (Nujol): 3220, 1748, 1718, 1698 cm⁻¹. 1H-NMR (200 MHz, DMSO-d₆) δ: 9.92 (1H, brs, D₂O-exchangeable), 7.42 (1H, dd, J = 10, 4 Hz), 4.06 (1H, dd, J = 12, 5 Hz), 2.91 (1H, td, J = 12, 4 Hz), 2.16—0.88 (4H, m). FAB-MS m/z: 269 (M⁺H)⁺.

Octahydro-2,3-dioxoafoxazole[3,2-e]pyridine (8) A stirred anhydrous solution of pyridine (5.0 ml) in CH₂Cl₂ (125 ml) was treated with Cr₂O₇(2) (3.08 g) at room temperature under an N₂ atmosphere. The mixture was stirred for 15 min, then a solution of 6 (613 mg in anhydrous CH₂Cl₂ (5 ml) was added and the whole was stirred for an additional 30 min. The reaction mixture was diluted with Et₂O (40 ml), filtered through cellulose powder, and evaporated in vacuo to give a residue. A solution of 8 (400 mg) in Et₂O (100 ml). The extract was evaporated in vacuo to give ethyl N-(4-formylbutyl)oxazide (4a, 601 mg) as a crude oil. 1H-NMR (CDCl₃) 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 N₂ atmosphere. The reaction mixture was evaporated in vacuo and the residue was purified by column chromatography (SiO₂ 10 g, CH₂Cl₂:MeOH = 30:1) to afford 8 (140 mg, 30% from 6). 8: colorless fine crystals, mp 72—73 °C (isopropyl ether). Anal. Calcld for C₂₁H₁₆N₂O₄: C, 54.19; H, 5.85; N, 9.00. Found: C, 54.08; H, 5.79; N, 9.00. IR (CHCl₃): 2950, 2930, 1818, 1732 cm⁻¹. 1H-NMR (200 MHz, DMSO-d₆) δ: 8.51 (1H, dd, J = 10, 5 Hz), 4.06 (1H, dd, J = 12, 4 Hz), 3.01 (1H, td, J = 12, 4 Hz), 2.21 (3H, m), 1.97—1.22 (5H, m). FAB-MS m/z: 356 (M⁺H)⁺.

6-tert-Butyldiphenylsilyl-2-deoxy-2-oxoamylaminono-mannose (13) DCC (4.95 g), HOBT (3.24 g) and Et₃N (2.8 ml) were added to a stirred ice-cold suspension of D-tartaric acid hydrate (11, 4.31 g) and oxamylidene choline (200 mg) at room temperature over a period of 5 min under an N₂ 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 ether (200 ml), CH₂Cl₂, and evaporated in vacuo to give 2-deoxy-2-oxoamylaminono-manganese (12) as a crude white amorphous mass (14.1 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 N₂ atmosphere, then tert-butyldiphenylsilyl chloride (2.6 ml) and imidazole (1.36 g) were added and the whole was stirred for an additional 3 h. The reaction mixture was then concentrated under vacuum and extracted with AcOEt (300 ml). The organic layer was washed with brine, saturated aqueous NaHCO₃, and brine, then dried over MgSO₄, and evaporated in vacuo. The residue was purified by column chromatography (SiO₂ 300 g, CH₂Cl₂:MeOH = 40:1—1:5) to afford 13 (6.47 g, 66% from 11). 13: amorphous solid, [α]D₂ = +129° (c = 0.6, MeOH). Anal. Calcld for C₅₀H₄₇N₂O₂ (546.75): C, 82.29; H, 6.83; N, 2.25. Found: C, 82.57; H, 6.62; N, 5.72. IR (CHCl₃): 3580, 3500, 3450, 3390, 2930, 1680, 1530, 1110 cm⁻¹. 1H-NMR (200 MHz, DMSO-d₆-D₂O) δ: 7.78—7.60 (4H, m), 7.52—7.36 (6H, m), 5.08 (0.71H, brs), 4.86 (0.36H, brs), 4.18—3.62 (2H, m), 1.01 (9H, s). FAB-MS m/z: 511 (M⁺Na⁺).
in EtOH (1 ml). After being stirred for 1 h, the reaction mixture was diluted with CHCl₃ (5 ml) and washed with saturated aqueous NaHCO₃. The aqueous layer was extracted with CHCl₃-MeOH (4:1) 3 times. The organic layers were combined, dried over MgSO₄, and evaporated in vacuo. The residue was purified by preparative TLC (CH₂Cl₂-MeOH = 20:1) to afford the anti-isonorine (32 mg, 41% from 16) and the isomeromerum 19-

**N-Methylkifunisine Diacetate** (22) Compound 17 (130 mg) was treated with 30% MeOH-MeOH (5 ml) under an N₂ atmosphere. The reaction mixture was cooled to room temperature, filtered, and evaporated in vacuo. The residue was purified by preparative TLC (CH₂Cl₂-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 diacetate 22 as follows. A mixture of 20 (100 mg), Mel (114 mg), K₂CO₃ (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 (CH₂Cl₂-MeOH = 9:1). Rf = 0.68, AcOEt, Rf = 0.57 and 1H-NMR (200 MHz, CDCl₃): 22: colorless fine crystals, mp 245—246°C (AcOEt), [α]D = −64.8°C (c = 0.5, MeOH). Anal. Calc. for C₁₄H₁₆N₂O₄: C, 53.21; H, 7.09; N, 8.58. Found: C, 53.21; H, 7.25; N, 8.67. IR (KBr): 2990, 1752, 1420, 1377, 1070, 1068 cm⁻¹. 1H-NMR (200 MHz, CDCl₃): δ 4.73 (1H, dd, J = 10, 4 Hz), 1.70 (1H, J = 8 Hz), 2.23 (1H, J = 4 Hz), 1.02 (1H, J = 10 Hz), 2.23 (1H, J = 4 Hz), 1.70 (1H, J = 8 Hz), 4.73 (1H, dd, J = 10, 4 Hz), 4.73 (1H, dd, J = 10, 4 Hz), 2.23 (1H, J = 4 Hz), 1.70 (1H, J = 8 Hz), 2.23 (1H, J = 4 Hz), 1.70 (1H, J = 8 Hz). FAB-MS m/z: 327 (M+H)⁺.

**N-Methylkifunisine** (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). [α]D = +66.6° (c = 0.4, H₂O). Anal. Calc. for C₁₄H₁₆N₂O₄: C, 53.22; H, 7.09; N, 8.65. Found: C, 53.21; H, 7.25; N, 8.63. IR (KBr): 3100, 2920, 1700, 1692, 1430, 1232, 1110, 1061, 1043 cm⁻¹. 1H-NMR (200 MHz, CDCl₃): δ 5.08 (1H, d, J = 10 Hz), 2.43 (1H, d, J = 10 Hz), 4.18 (1H, d, J = 3 Hz), 4.07 (1H, d, 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**


8) Compounds 3, 8, 23, 24 and kifunisine (I) did not inhibit α-glucosidase (rabbit intestine).