An approach to the total synthesis of sinefungin¹

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ABSTRACT

Condensation of N⁶-benzoyl-2',3'-O-isopropylideneadenosine-5'-aldehyde with nitromethane followed by acid catalyzed acetylation and borohydride reduction leads to N⁶-benzoyl-9-(5,6-dideoxy-2,3-O-isopropylidene-6-nitro-β-D-ribo-hexofuranosyl)adenine (4). A second nitroaldol condensation between 4 and N-benzylloxycarbonyl-L-aspartic acid-β-semialdehyde α-benzyl ester (5) followed by acetylation and borohydride reduction leads to a fully protected 6'-nitro modification of sinefungin and its C₆'-epimer (7). Hydrolysis of the acetonide followed by sequential reduction of the benzyl derived protecting groups and the nitro group and debenzoylation leads to a modest yield of a 3:1 mixture of sinefungin (1) and 6'-episinefungin which can only be separated by analytical ion exchange chromatography.

INTRODUCTION

The isolation of a wide range of biologically active nucleoside analogs containing unusual carbohydrate moieties³ has provided a continuing synthetic challenge. As part of our prolonged program on the synthesis of carbohydrate modified nucleosides⁴ we have previously reported syntheses of some members of this group, including nucleocidin⁵, angustmycin A⁶, showdomycin⁷, cordycepin⁸ and the nucleoside skeleton of the polyoxin group⁹.

The nucleoside analog sinefungin (1) was isolated in 1973 from *Streptomyces griseolus* by a group at Eli Lilly¹⁰ and shown to possess potent antifungal, and in particular anticandidal, properties. Subsequently, sinefungin was also found to exhibit antiviral¹¹⁻¹³ and antiparasitic^{14,15} properties, all of these being attributable to its ability to mimic S-adenosylhomocysteine and inhibit S-adenosylmethionine dependent methyltransferases¹⁶. While both the biosynthetic incorporation of L-ornithine¹⁷ and the degradation of sinefungin by L-amino acid oxidase¹⁸ strongly support the S-configuration for the amino bearing C₉', the configuration at C₆' remains unknown.

DISCUSSION

The basic synthetic strategy selected for this project is outlined in Scheme I and has, as its major feature, the introduction of the ornithine side chain via two nitroaldol condensations. A somewhat similar use of the nitroaldol reaction for elaboration of carbohydrates related to the side chain of tunicamycin has recently been described^{19,20}.

The nucleoside starting material for this synthesis is the readily available hydrate of N⁶-benzoyl-2',3'-O-isopropylideneadenosine-5'-aldehyde (2) prepared via dimethylsulfoxide-dicyclohexylcarbodiimide oxidation of the

Scheme I

corresponding alcohol, as previously described²¹. Condensations of 2 with nitromethane were carried out in the presence of a variety of bases. The use of potassium t-butoxide in a mixture of t-butanol and nitromethane proved to be particularly facile and consistently gave a mixture of the 6-deoxy-6-nitro- β -D-allo and α -L-talofuranosyl nucleosides (3a) in greater than 90% yield. Reactions using sodium methoxide or potassium fluoride²² were slower and led to the generation of some polar by-products. It should be noted that condensations of nitromethane with unprotected adenosine-5'-aldehyde²³ and its 2',3'-O-isopropylidene derivative²⁴ have previously been described. In both cases it was possible to chromatographically isolate low yields of the individual β -D-allo- and α -L-talofuranosyl isomers. In each case the more polar allo epimer predominated in a ratio of roughly 3:1 and the configurations were assigned by degradation to the known hexofuranosylhypoxanthines. The ease of preparing pure 2 and the excellent yield of the mixed epimers (3a) make the present route attractive. While it is possible to separate the C_5 -epimers (roughly 4:1) by preparative TLC, this is unnecessary in the present project since the asymmetry at C_5 , will be removed in subsequent steps.

Removal of the C_5 -hydroxyl group was accomplished via acetylation and treatment with sodium borohydride^{22,25,26}. The acetylation step could be carried out using acetic anhydride in the presence of a catalytic amount of 4-dimethylaminopyridine²⁷ but was accompanied by the formation of some polar impurities. Much better results were achieved using acetic anhydride in ethyl acetate in the presence of a catalytic amount of perchloric acid, a method that consistently gave essentially quantitative yields of the 5'-O-acetate (3b). This compound was then treated with an excess of sodium borohydride in ethanol-tetrahydrofuran at 0° giving pure N⁶-benzoyl-9-(5,6-dideoxy-2,3-O-isopropylidene-6-nitro- β -D-ribo-hexofuranosyl)adenine (4) in 55% yield following chromatography on silica gel. Without purification of intermediates 4 could be obtained in a 51% overall yield from the aldehyde hydrate 2.

It should be noted that the TLC mobility of 4 is only slightly less than that of 3b and that its formation is accompanied by the generation of a number of minor polar impurities. These by-products are in substantially greater amounts if methanol is used rather than ethanol during the borohydride reduction, but the use of 2-propanol offers no advantage. We were initially concerned that intramolecular conjugate addition of N³ of the purine ring to the nitroolefin, 10, that is the presumed intermediate in the reductive step²⁶, might give the N³,5'-cyclonucleoside, 11, or a ring opened derivative⁵, as a

by-product. We have not, however, screened the minor products for this type of compound, and the spectral properties of 4 assure the correctness of its structure.

The 6'-nitro group in 4 now provides both a structural precursor for the 6'-amino function in sinefungin and a source of activation for attachment of the remaining side chain via a second nitroaldol condensation. The aldehyde component for this condensation would be a suitably protected derivative of L-aspartic acid β -semialdehyde (5) derived by oxidation of the corresponding L-homoserine derivative. To this end, N-benzoyloxycarbonyl-L-homoserine benzyl ester²⁸ was oxidized using the dimethylsulfoxide-dicyclohexylcarbodiimide method²⁹. This particular oxidation has been done previously using the racemic starting material³⁰, and 5 has also been prepared using chromium trioxide-pyridine as the oxidant²⁸. In our hands the latter method did not give good yields, while the former gave crystalline 5 in 72% yield. The use of the frequently advantageous dichloroacetic acid as the proton source in this reaction led to the formation of appreciable amounts of N¹-dichloroacetyl-N¹,N³-dicyclohexylurea³¹ which had a TLC mobility very similar to that of the product. By use of methylphosphonic acid as the proton source, this side reaction could be avoided without the necessity of handling hygroscopic anhydrous phosphoric acid²⁹.

Once again, various conditions were investigated for the nitroaldol condensation of 4 and 5, and the use of potassium t-butoxide in a mixture of t-butanol and tetrahydrofuran at room temperature seemed optimal. Following chromatography on silica gel the mixed diastereomers of the nitroalcohol, 6a, were obtained in 69% yield³². While the product gave the desired elemental analysis, its ¹H-NMR spectrum was expectedly complex and showed the presence of at least three isomers by the presence of singlets at 8.55, 8.57 and 8.59 corresponding to C₂H or C₈H. Since the asymmetry at C₇, would be removed in the following steps, no efforts were made to further resolve this mixture.

Acetylation of 6a was readily accomplished by treatment with acetic anhydride and a catalytic amount of 4-dimethylaminopyridine. Without purification, the isomeric acetates were reacted with sodium borohydride in ethanol-tetrahydrofuran at 0° giving, after chromatography on silica gel, the analytically pure mixed C₆'-diastereomers of 7 in an overall yield of 77% from the nitroalcohol 6a. The mixture could be partially separated into the individual C₆'-epimers by multiple developments on a Harrison Research Chromatotron. By this technique fractions of each isomer that were essentially homogeneous by careful TLC analysis were obtained in addition to a substantial amount of unresolved mixture. On deprotection of the individual isomers as described later, however, the same mixture of sinefungin and its C₆'-epimer was obtained. Presumably epimerization of the nitro group accompanied acidic hydrolysis of the 2',3'-O-isopropylidene function and rendered a separation of isomers at this stage useless.

Accordingly, 7 was directly treated with 90% trifluoroacetic acid giving a quantitative recovery of the epimeric nitrodiols, 8, following chromatography on silica gel. Unfortunately, separation of the C₆'-epimers of 8 could not be achieved by TLC techniques. The mixture was therefore directly reduced using hydrogen and palladium on charcoal catalyst in order to hydrogenolyze both the benzyloxycarbonyl and benzyl ester protecting groups. This stage of the sequence could be followed by either TLC or reverse phase HPLC, which showed the formation of a major component (presumably 9) and several lesser impurities. By preparative reverse phase HPLC it was possible to separate small amounts of the presumed C₆'-epimers from the leading and trailing edges of the major peak. Upon repeated lyophilization of these substances to remove ammonium acetate present in the eluant, however, either component reverted to a common mixture, once again pointing out the ease of racemization of the nitro group.

The crude palladium reduction product (9) was therefore directly subjected to further hydrogenation in order to reduce the nitro group. A variety of palladium and platinum catalysts were examined under differing conditions. In general, palladium catalysts did not effect further reduction, while the use of an equal weight of platinum oxide at 50 psi for up to 70 hr led to a complex mixture of at least five components by HPLC analysis. The major, but still impure, component was isolated by preparative HPLC and debenzoylated by treatment with methanolic ammonium hydroxide. Preparative reverse phase HPLC allowed the isolation of the most polar component of the mixture in a yield of 22% from 8. This material showed a single peak that was identical to auth-

entic sinefungin³³ upon analytical HPLC analysis as well as by TLC and paper electrophoresis. Upon examination using a Beckman Model 119C amino acid analyzer and employing the 3 hr single column procedure³⁴, however, the synthetic product was cleanly separated into two peaks with retention times close to those of tryptophan and arginine. The second of these peaks, which comprised 73% of the mixture, behaved identically to authentic sinefungin upon independent and mixed analysis. We have not as yet been successful in achieving preparative separation of sinefungin from its C₆'-epimer by ion exchange techniques. The mixture, which showed the expected ultraviolet spectrum and analytical data, gave a ¹H-NMR spectrum that was essentially similar to that previously reported for sinefungin³⁵. Upon antifungal screening³⁶ it showed a spectrum of activity similar to that of authentic sinefungin, with particular effectiveness towards several strains of *Candida albicans*. As expected, the minimum effective doses were one dilution higher than those with the standard, suggesting that 6'-episinefungin is probably biologically inactive.

While the presently reported synthetic route provides a fairly effective method for assembly of the sinefungin skeleton, the poor yield in the final deprotection step is disappointing. This is presumably a consequence of partial reduction of the nitro group with concomitant side reactions. An adequate resolution of this problem must, however, await further studies.

EXPERIMENTAL

General Methods

¹H- and ¹³C-nuclear magnetic resonance (NMR) spectra were obtained using Varian HA-100 and Bruker WH-90 spectrometers and are reported in ppm downfield of an internal standard of tetramethylsilane. Analytical and preparative high performance liquid chromatography (HPLC) were done using a Chromatronix Model 3500 instrument fitted with Merck Lichroprep RP-18 (25-40μ) columns. For preparative use, a 2 x 50 cm stainless steel column was used. Thin layer chromatography (TLC) was done on a 250μ layer of silica gel GF from Analtech Inc. Elemental analyses and spectral measurements were obtained by the staff of the Analytical Laboratories of Syntex Research, to whom we extend our thanks. N⁶-Benzoyl-9-(6-deoxy-2,3-O-isopropylidene-β-D-allo- and α-L-talofuranosyl)-adenines (3a)

Dry nitromethane (35 mL) was added dropwise over 15 min at room temperature to a stirred solution of potassium *t*-butoxide (from 1.01 g, 25.9 mmol of potassium) in *t*-butanol (70 mL). The solution was then cooled in an ice bath and solid **2** (11.0 g, 25.8 mmol) was added portionwise over 6 min. The mixture

was then stirred at room temperature for 20 min and, after addition of ammonium chloride (4.0 g, 75 mmol), the solvent was removed in vacuo. Solutions of the residue were evaporated twice with carbon tetrachloride (100 mL) and the final residue was partitioned between dichloromethane (1 L) and brine (1 L). The aqueous phase was back-extracted twice with dichloromethane (700 mL) and then with ethyl acetate (2 x 500 mL). The combined organic layers were dried (MgSO_4) and evaporated leaving 11.0 g (91%) of 3a as a white foam that showed only two very close moving spots (4:1 in favor of the more polar spot) upon TLC using dichloromethane-acetone (3:1). This material was entirely satisfactory for direct use, and an analytical sample of the mixed isomers was isolated by preparative TLC as above: $[\alpha]_D^{25}$ 50.0° (c 1, MeOH); λ_{max} (MeOH) 230 nm (ϵ 13,600), 280 (20,800); $^1\text{H-NMR}$ (CDCl_3) 1.33, 1.57 ppm (s, 3, CMe_2), 4.30 (m, 1, C_4H), 4.49 (m, 2, C_6H), 4.69 (m, 1, C_5H), 5.13 (dd, 1, $J_{2',3'}=5$ Hz, $J_{3',4'}=1.5$ Hz, C_3H), 5.23 (dd, 1, $J_{1',2'}=3$ Hz, C_2H), 6.02 (d, 1, C_1H), 7.58 and 7.98 (m, total 5, Ar), 8.09 and 8.64 (s, 1, C_2H , C_8H), 9.40 (br s, 1, NH). The only clearly resolved signal for the minor isomer was a small singlet at 8.14 ppm; ν_{max} (CHCl_3) 1550 cm^{-1} (NO_2). Anal. Calcd. for $\text{C}_{21}\text{H}_{22}\text{N}_6\text{O}_7$ (470.45): C, 53.61; H, 4.68; N, 17.87. Found: C, 53.49; H, 4.80; N, 17.83.

N^6 -Benzoyl-9-(5-O-acetyl-6-deoxy-2,3-O-isopropylidene- β -D-allo- and α -L-talo-furanosyl)adenines (3b)

A cold solution of 70% perchloric acid (2.25 mL) in a mixture of ethyl acetate (85 mL) and acetic anhydride (85 mL) was added dropwise over 10 min to an ice-cooled, stirred solution of 3a (11.0 g, 23.4 mmol) in ethyl acetate (110 mL). The mixture was kept for an additional 1 hr at 0° and then for 1 hr at room temperature before being added to ice cold, saturated aqueous sodium bicarbonate solution (450 mL) and stirred for 1.5 hr. The aqueous layer was extracted with ethyl acetate (2 x 250 mL) and the combined organic phases were washed with saturated brine and dried (Na_2SO_4). Evaporation of the solvent left 12.0 g (100%) of 3b as pale yellow foam that showed a single spot less polar than 3a upon TLC in dichloromethane-acetone (3:1). This material was used directly in the next step and an analytical sample was prepared by preparative TLC as above: λ_{max} (MeOH) 230 nm (ϵ 10,100), 280 (18,600); $^1\text{H-NMR}$ (CDCl_3) complex and showing the presence of roughly equal amounts of two C_6 -epimers, particularly through singlets at 1.85 and 1.94 (OAc), 8.33 and 8.44 (C_2H , C_8H) and 8.90, 8.92 (C_2H , C_8H); mass spectrum (70 eV) m/e 511 (M^+-H), 496 (m/e 511- CH_3), 482 (M^+-NO_2), 468 (m/e 511- CH_3CO), 426 (m/e 468- CH_2CO). Anal. Calcd. for $\text{C}_{23}\text{H}_{24}\text{N}_6\text{O}_8$ (512.47): C, 53.90; H, 4.72; N, 16.40. Found: C, 53.93; H, 4.57; N, 16.53.

N⁶-Benzoyl-9-(5,6-dideoxy-2,3-O-isopropylidene-6-nitro-β-D-ribo-hexofuranosyl)-adenine (4)

Sodium borohydride (1.9 g, 50 mmol) was added gradually over 20 min to a stirred, ice-cooled solution of 3b (12.0 g, 23.4 mmol) in a mixture of anhydrous ethanol (100 mL) and tetrahydrofuran (100 mL). After an additional 40 min the mixture was added to ice cold 1M hydrochloric acid (200 mL) and extracted with dichloromethane (3 x 200 mL). The organic phases were washed with aqueous sodium bicarbonate and with saturated aqueous sodium chloride, dried (Na₂SO₄) and evaporated leaving 9 g of a yellow foam. This was chromatographed on a column of silica gel (700 g) using ethyl acetate-hexane (85:15) giving 5.9 g (55%, 51% overall from 2) of 4 that was homogeneous by TLC using dichloromethane-acetone (3:1) and NMR: $[\alpha]_D^{25}$ 12.73° (c 0.8, MeOH); $\lambda_{\max}$ (MeOH) 230 nm (ϵ 12,400), 280 (18,600); $\nu_{\max}$ (CHCl₃) 1550 cm⁻¹ (NO₂); ¹H-NMR (CDCl₃) 1.39, 1.60 ppm (s, 3, CMe₂), 2.47 (dt, 2, J_{4',5'}=J_{5',6'}=6.5 Hz, C₅H₂), 4.2-4.3 (m, 1, C₄H), 4.39 (t, 2, C₆H₂), 5.03 (dd, 1, J_{2',3'}=6 Hz, J_{3',4'}=3.5 Hz, C₃H), 5.52 (dd, 1, J_{1',2'}=2 Hz, C₂H), 6.08 (d, 1, C₁H), 7.4-7.65 (m, 3, Ar), 7.95-8.1 (m, 3, Ar and C₂H or C₈H), 8.80 (s, 1, C₂H or C₈H), 9.1 (br s, 1, NH); ¹³C-NMR (CDCl₃) 25.4 and 27.2 ppm (CMe₂), 30.65 (C₅), 71.91 (C₆), 84.04 (C₂, C₃, C₄), 90.70 (C₁), 115.21 (CMe₂), 123.99 (C₅), 142.78 (C₈), 150.19, 151.20 (C₄, C₆), 152.89 (C₂), 164.98 (CO) plus aryl signals; mass spectrum (70 eV) m/e 454 (M⁺), 439 (M⁺-CH₃), 408 (M⁺-NO₂). Anal. Calcd. for C₂₁H₂₂N₆O₆ (454.45): C, 55.50; H, 4.88; N, 18.50. Found: C, 55.48; H, 4.97; N, 17.92.

N-Benzyloxycarbonyl-L-aspartic acid-β-semialdehyde α-Benzyl Ester (5)

Methylphosphonic acid (400 mg, 3.6 mmol) was added to an ice-cooled solution of N-benzyloxycarbonyl-L-homoserine benzyl ester (2.5 g, 7.2 mmol)²⁸ and dicyclohexylcarbodiimide (4.5 g, 22 mmol) in anhydrous dimethylsulfoxide (16 mL). The mixture was then stirred at room temperature for 2 hr, cooled to 0° and carefully treated with a solution of oxalic acid dihydrate (1.8 g, 15 mmol) in methanol (7.5 mL). The mixture was filtered and the solids carefully washed with ether (7 x 15 mL). The combined filtrates were washed with saturated aqueous sodium chloride and the washings back-extracted with ether. Evaporation of the combined and dried (Na₂SO₄) organic phases left 2.7 g of slightly impure 5 that was chromatographed on a column of silicic acid (66 g). Elution with dichloromethane gave a less polar impurity, while acetone gave 1.8 g (72%) of crystalline 5 with mp 76-77.5° from ether-hexane (reported mp 76-78°²⁸): $[\alpha]_D^{25}$ 14.4° (c 1, CHCl₃) (reported²⁸ 13.54°); ¹H-NMR (CDCl₃) 2.99 ppm (d, 1, J_{2,3}=5 Hz, C₃H₂), 4.60 (m, 1, C₂H), 5.00, 5.07 (s, 2, ArCH₂), 5.65

(br d, 1, $J=7$ Hz, NH), 7.2 (s, 10, Ar), 9.40 (s, 1, CHO).

N⁶-Benzoyl-5'-(4S-benzoyloxycarbonyl-4-benzoyloxycarbonylamino-2 ξ -hydroxy-1 ξ -nitrobutyl)-5'-deoxy-2',3'-O-isopropylidene)adenosine (6a)³²

A solution of potassium *t*-butoxide (from 30 mg, 0.77 mmol of potassium) in *t*-butanol (17 mL) was added at room temperature to a solution of **4** (3.0 g, 6.6 mmol) in tetrahydrofuran (20 mL). After 10 min **5** (4.3 g, 12.5 mmol) was added to the yellow solution and the mixture stirred for 18 hr. Ammonium chloride (1.5 g) was then added and the solvent was removed *in vacuo*. The residue was partitioned between water and dichloromethane and the organic phase was washed with saturated aqueous sodium chloride, dried (Na₂SO₄) and evaporated leaving a yellow foam that contained a major product somewhat less polar than **4** upon TLC using ethyl acetate. This was chromatographed on a column of silica gel (800 g) using ethyl acetate-hexane (85:15) which first eluted 2.47 g of **5** and then 3.61 g (69%) of **6a** followed by 160 mg of unreacted **4**. The product gave a single spot upon TLC analysis (*R_f* 0.57 in ethyl acetate): $[\alpha]_D^{25}$ 8.5° (c 0.7, MeOH); $\lambda_{\max}$ (MeOH) 233 nm (sh, ϵ 13,100), 279 (18,900); ¹H-NMR (CDCl₃) showed all the expected functionality but was clearly a mixture of at least three isomers with three singlets at 8.55, 8.57 and 8.59 for C₂H or C₈H. Anal. Calcd. for C₄₀H₄₁N₇O₁₁ (795.79): C, 60.37; H, 5.19; N, 12.32. Found: C, 60.50; H, 5.43; N, 12.59.

N⁶-Benzoyl-5'-(4S-benzoyloxycarbonyl-4-benzoyloxycarbonylamino-1 ξ -nitrobutyl)-5'-deoxy-2',3'-O-isopropylideneadenosine (7)

A solution of **6a** (3.0 g, 3.8 mmol) in tetrahydrofuran was reacted for 30 min at room temperature with acetic anhydride (3.5 mL, 3.8 mmol) and 4-dimethylaminopyridine (30 mg, 0.27 mmol). The mixture was then added to ice-water and extracted with dichloromethane (3 x 60 mL). The extracts were washed with aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried (Na₂SO₄) and evaporated leaving 3.3 g of **6b** as a foam that was essentially homogeneous and slightly less polar than **6a** by TLC using dichloromethane-acetone (3:1). A portion of this material (660 mg, 0.79 mmol) was dissolved in ethanol (6 mL) and tetrahydrofuran (6 mL) and reacted for 20 min at 0° with sodium borohydride (61 mg, 1.6 mmol). The mixture was then added to ice cold 1M hydrochloric acid (15 mL) and extracted with dichloromethane (3 x 20 mL). The extracts were washed with saturated aqueous sodium bicarbonate and brine, dried (MgSO₄) and evaporated to dryness leaving a yellow oil (670 mg) that was chromatographed on a column of silica gel (80 g). Elution with ethyl acetate-hexane (85:15) gave 470 mg (77% from **6a**) of **7** as a white

foam that contained two very close moving spots upon TLC using dichloromethane-ethyl acetate-methanol (90:10:3): $[\alpha]_D^{25}$ 27.4° (c 1, CHCl₃); $\lambda_{\max}$ (MeOH) 230 nm (sh, ϵ 13,200), 280 (18,900); the ¹H-NMR spectrum showed the product to be the expected mixture of diastereomers. Anal. Calcd. for C₄₀H₄₀N₇O₁₀ (778.78): C, 61.69; H, 5.18; N, 12.59. Found: C, 61.60; H, 5.28; N, 12.40.

The mixture could be partially separated into relatively pure epimers by multiple developments on a Harrison Research Chromatotron using dichloromethane-ethyl acetate-methanol (90:10:1). ¹H-NMR (CDCl₃) of more polar isomer: 4.85 ppm (dd, 1, J_{1',2'}=2 Hz, J_{2',3'}=6 Hz, C_{2'}H), 6.01 (d, 1, C_{1'}H); less polar isomer: 5.38 (dd, 1, J_{1',2'}=2 Hz, J_{2',3'}=6 Hz, C_{2'}H), 5.90 (d, 1, C_{1'}H). N⁶-Benzoyl-5'-(4S-benzylloxycarbonyl-4-benzylloxycarbonylamino-1ξ-nitrobutyl)-5'-deoxyadenosine (8)

A solution of **7** (310 mg, 0.4 mmol) in 90% trifluoroacetic acid (2 mL) was kept at room temperature for 20 min, diluted with water (10 mL) and evaporated to dryness. The residue was co-evaporated with methanol twice and the residue chromatographed on a column of silica gel (20 g) using dichloromethane-methanol (19:1) giving 300 mg (100%) of **8** as a TLC homogeneous white foam: $[\alpha]_D^{25}$ 12.6° (c 0.8, MeOH); $\lambda_{\max}$ (MeOH) 232 nm (ϵ 15,100), 279 (19,800); ¹H-NMR (CDCl₃) showed a poorly resolved mixture of C₆-epimers. Anal. Calcd. for C₃₇H₃₆N₇O₁₀·H₂O (756.75): C, 58.72; H, 5.06; N, 12.96. Found: C, 58.39; H, 5.04; N, 12.39.

5'-(1ξ,4S-1,4-Diamino-4-carboxybutyl)-5'-deoxyadenosine (Sinefungin, 1)

A solution of **8** (150 mg, 0.2 mmol) in methanol (7 mL) was shaken for 20 hr in a Parr hydrogenator under 50 psi of hydrogen in the presence of fresh 10% palladium on charcoal catalyst (160 mg). At this point TLC using dichloromethane-methanol-acetic acid (90:10:1) showed complete conversion of **8** (Rf 0.6) to material staying on the origin. Engelhardt platinum oxide catalyst (160 mg) was then added and the mixture was hydrogenated as above for a further 18 hr. The mixture was filtered through Celite and the residue was repeatedly washed with alternating 10 mL portions of methanol and water. The combined filtrates were evaporated in vacuo leaving 72 mg of an oil that contained at least five components upon HPLC analysis. This was subjected to preparative reverse phase HPLC on a 2 x 50 cm column containing 50 g of Merck Lichroprep RP-18 (25-40μ) using water-acetonitrile (9:1) containing 0.03M ammonium acetate (pH 4.5) at a flow rate of 5 mL per min. This partially resolved three major peaks, the first of which was evaporated to dryness and repeatedly lyophilized to remove ammonium acetate leaving 46 mg of a foam that contained 1100 optical density units at $\lambda_{\max}$ 280 nm. This material (30 mg) was treated for 18 hr at

room temperature with conc. ammonium hydroxide-methanol (1:1) and evaporated to dryness. The residue was subjected to preparative reverse phase HPLC as above using water-acetonitrile (99:1) containing 0.03M ammonium acetate (pH 4.5). Four major peaks were collected, the first of which corresponded to authentic sinefungin by analytical reverse phase HPLC using water-acetonitrile (95:5) made 0.03M with ammonium acetate. Repeated lyophilization of this peak with water left 11 mg (22% from 8) of product that was identical to authentic sinefungin by reverse phase HPLC and by TLC on silica gel using 2-propanol-ammonium hydroxide-water (12:3:1, R_f 0.29). Analysis on a Beckman Model 119C amino acid analyzer using the 3 hr single column procedure³⁴, however, clearly shows the product to be a 73:27 mixture of sinefungin (156 min) and 6'-epi-sinefungin (147 min): $\lambda_{\max}$ (H₂O) 260 nm (ϵ 14,700); the ¹H-NMR spectrum (D₂O) was essentially similar to that of an authentic sample of sinefungin as described previously³⁵. The presence of two diastereomers was reflected in the appearance of the C₁H signal as two overlapping doublets at 6.13 ppm. Anal. Calcd. for C₁₅H₂₃N₇O₅ (381.39): C, 47.23; H, 6.08. Found: C, 47.89; H, 6.26.

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32. The nomenclature used in the Experimental section treats compounds such as 1, 6, 7 and 8 as functionalized C₅-butyl derivatives of adenosine. While not strictly correct, these names seem more readily descriptive than those based upon pentadeoxydecofuranuronic acid, particularly since the designation of stereochemistry in the latter is confused by the unknown configuration at C₆'.
33. We are indebted to Dr. R.J. Suhadolnik of Temple University for a generous sample of authentic sinefungin.
34. Instruction Manual, Beckman Instruments Model 119C Amino Acid Analyzer, Sect. 7, September 1975.
35. Belgian Pat. App. 08295A, Rhone-Poullenc Industries, July 26, 1977.
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