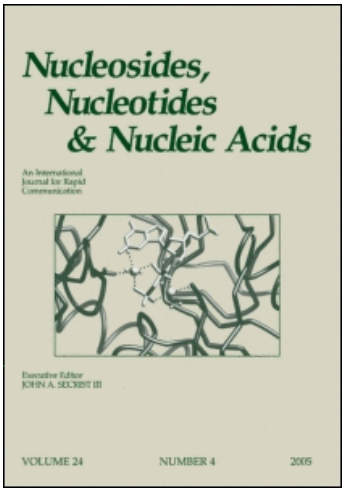

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APPROACHES TO THE SYNTHESIS OF SINEFUNGIN VIA NITROALDOL REACTIONS

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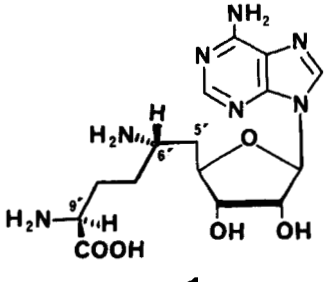
Abstract: The nucleoside antibiotic sinefungin (1) and its 6'-epimer have been synthesised by a reaction sequence involving as a key step the nitro-aldol reaction between N^6-benzoyl-2',3'-O-isopropylideneadenosine-5'-aldehyde (12) and diphenylmethyl-N-t-butyloxycarbonyl-δ-nitro-L-norvaline (11), catalysed by tetrabutylammonium fluoride.

INTRODUCTION

Sinefungin 1, a nucleoside antibiotic isolated from Streptomyces griseolus cultures in 1971,[1] displays a wide range of biological effects, probably as a consequence of the close structural similarity to the important metabolites S-adenosylmethionine and S-adenosylhomocysteine. Sinefungin shows activity against several methyltransferase enzymes,[2] and inhibits the growth of fungi[1,3] in addition to having antiviral,[4] antitumour,[5] and antiparasitic activity.[6] Antibiotic A9145 E (2), occurs together with sinefungin;[3,7] the two are readily interconverted.[8]



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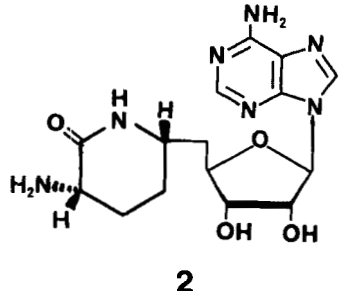

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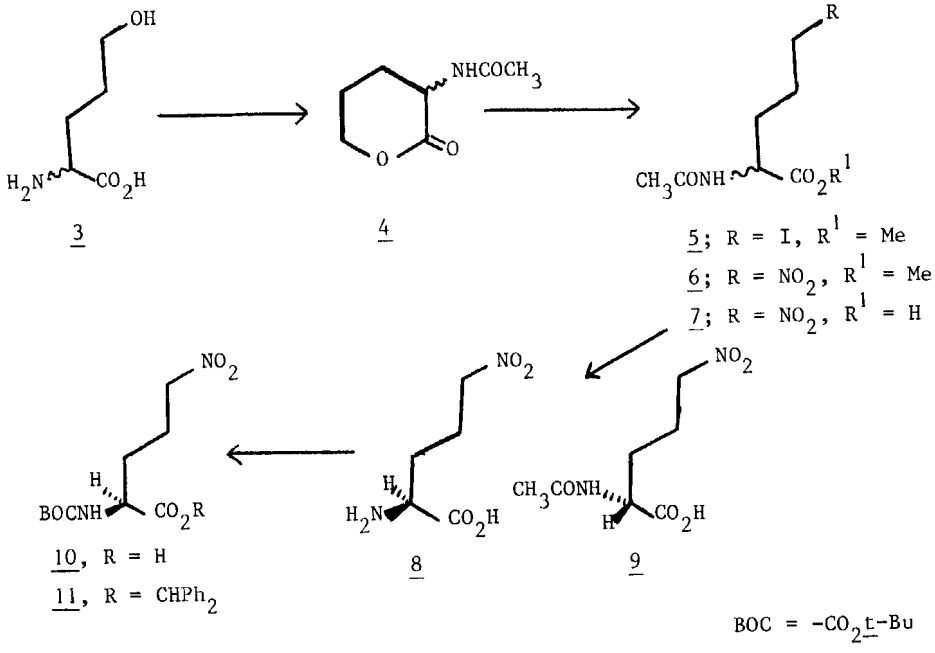
In considering the synthesis of sinefungin (1), 5'-6' bond formation at a late stage via nitroaldol methodology would provide a suitably convergent synthetic approach, with some similarities to the proposed biosynthesis.[9] Before this work was completed model studies toward the synthesis of sinefungin were reported[10] in addition to two total syntheses,[11,12] one of which[12] used nitroaldol methods but was less convergent than the present report.

RESULTS AND DISCUSSION

Chemistry. The synthesis of the aminoacid portion is outlined in Scheme I. δ-Hydroxy-DL-norvaline (3) was produced from 2,3-dihydrofuran.[13] The synthesis of 8 followed a previously published route[14] with modifications. Lactonisation of 3 followed by acetylation provided α-acetamido-δ-valerolactone (4),[14] which underwent ring opening with boron tribromide-sodium iodide in acetonitrile solution;[15] the intermediate acyloxyborane adduct was treated with methanol to give the δ-iodo amino acid 5 in 74% yield from 4. The iodo function of 5 was converted to the corresponding nitro compound 6 in 76% yield using a polymer supported nitrite reagent,[16]



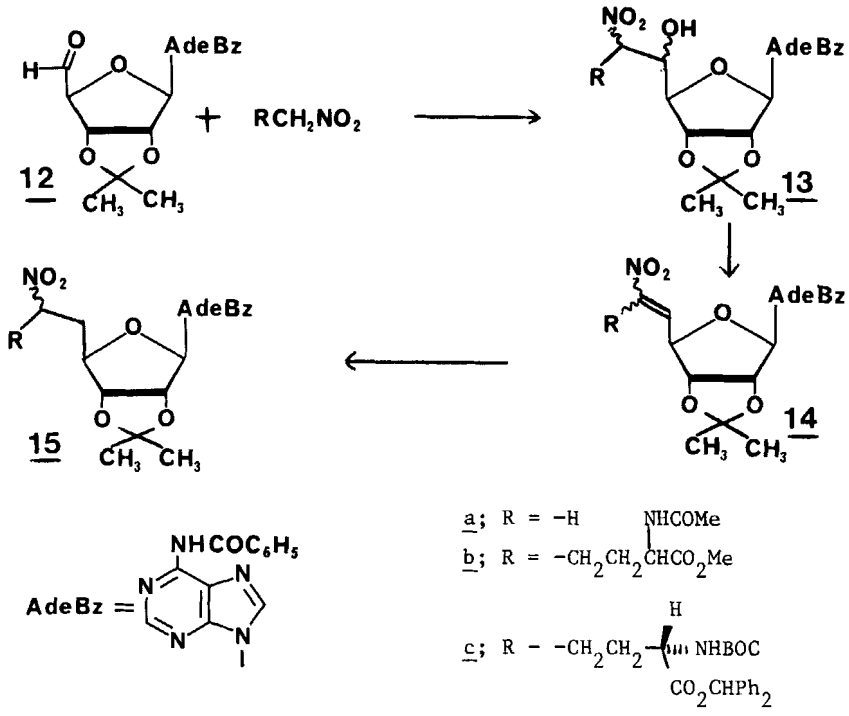
Scheme I

representing a considerable increase in both convenience and yield over the published procedure.[14] Resolution was effected with acylase,[14] and the N-acetyl D-aminoacid (9) could be recycled by racemisation with acetic anhydride. The L-amino acid (8) was converted into its t-butoxycarbonyl derivative (10), and esterification with diphenyldiazomethane gave 11, the aminoacid portion in the overall synthesis. The lipophilic nature of the α-aminoacid protecting groups proved advantageous in subsequent chromatographic operations.

The nucleoside portion, N^6-benzoyl-2',3'-O-isopropylidene adenosin-5'-aldehyde (12), was prepared by known procedures;[17] the preparation contained 15-20% of the corresponding hydrate.

Formation of the 5'-6' bond was investigated using the aldehyde 12 and excess nitromethane as a model system. Construction of 5'-6' bonds *via* nitroaldol reactions in adenosine derivatives has previously been described.[18] The conditions necessary were strongly basic and low yielding. This, coupled with the knowledge that *ribo*-nucleosides with a carbonyl group in the 5'-position are rather unstable compounds with respect to base[17,19] prompted a search for milder methods of catalysing the nitroaldol reaction. The most effective method was found to be tetrabutylammonium fluoride trihydrate (TBAF) in THF. Fluoride ions are well known as bases in organic synthesis[20] and have been used in conjunction with nitroalkanes.[21] *tert*-Amines as catalysts gave minor by-products while the use of other fluoride ion sources[8,21] gave less satisfactory results. The residual hydrate component of 12[17] does not present a problem in these reactions; the hygroscopic properties of the TBAF reagent ensure that the hydrate is dehydrated completely to the aldehyde 13. This results in higher yields of 13 (quantitative in the case of 13a) and as a consequence the chromatographic separations are less complicated. TBAF catalysis gives the further advantages of rapid reaction, straightforward workup procedure and minimal side reactions.

The initial nitroaldol reaction products, presumably a mixture of diastereomers, were not further characterised. The 5'-hydroxyl group of 13a was removed by an acylation-elimination-reduction sequence (Scheme II). Acetylation with acetic anhydride catalysed by 4-dimethylaminopyridine was followed by a slower elimination. The resulting 5'-6' unsaturation (compound 14a) was removed by reduction with ethanolic sodium borohydride. This provided, after chromatography, the 5'-deoxy-5'-nitromethyl derivative 15a in 35% yield from 12. A more appropriate model



a; R = -H NHCOMe
b; R = $-CH_2CH_2\overset{|}{C}HCO_2Me$

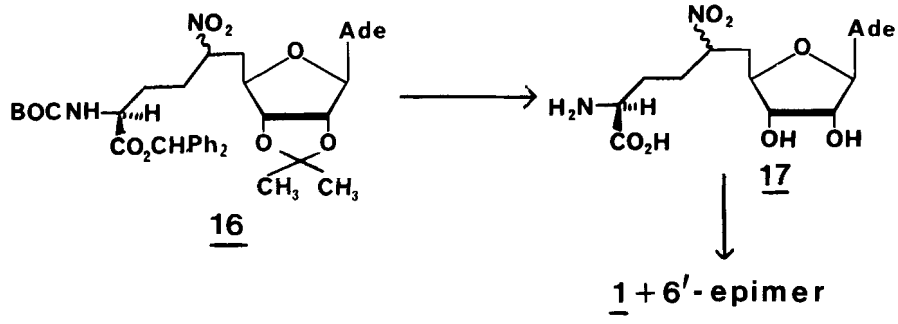
c; R = $-CH_2CH_2-$ H NHBOC CO_2CHPh_2

Scheme II

reaction used the racemic nitro aminoacid 6. When similar conditions were employed the corresponding compound 15b was ultimately produced as a mixture of four diastereoisomers in a yield of 68% based on 6. The complete carbon skeleton of sinefungin 1 is therefore readily accessible in good yield through this one-pot sequence. In all of these compounds n.m.r. and t.l.c. analysis pointed to the retention of the integrity of the stereochemistry at the 4'-position.

Sinefungin 1 possesses L-stereochemistry at the 9'-position.[11] It thus remained to extend the present method to the incorporation of an amino acid with L-stereochemistry in addition to one containing protecting groups that could be removed selectively and under mild conditions. Thus, application of the method to the amino acid derivative 11 gave the four diastereoisomers 13c. Acetylation/elimination and reduction in the usual manner provided 15c as a 1:1 mixture of two 6'-epimers in 70% yield from 11; the product was readily identified by fast atom bombardment mass spectroscopy (FAB MS) analysis with a MH_2^+ peak at 823. The two isomers can readily be separated by chromatography. They undergo equilibration either with silica gel or more rapidly with triethylamine.

Reduction of the nitro group of 15c under a variety of conditions could not be achieved satisfactorily.[22] The compound was therefore deprotected in two steps. Firstly, treatment with methanolic zinc bromide[23] cleaved the N^6- benzoyl group to give 16; secondly, 80% trifluoroacetic acid removed the three remaining protecting groups to give 17, which was treated directly with ammonium formate-Raney nickel in methanol.[24] This provided two components in 38% yield, the major of which was identical to sinefungin 1 by TLC. FAB MS analysis and biological testing confirmed its structure.



Biological evaluation. The nitro compound 17 was inactive against Candida Albicans in vitro. The synthetic sinefungin mixture was found to be approximately as active as sinefungin 1 in the same assay.

EXPERIMENTAL

General Methods. N.m.r. spectra were recorded on JEOL JNM-MH-100 and Bruker WP 200 NMR spectrometers. Infrared spectra were recorded on a Perkin-Elmer 257 instrument. Ultraviolet spectra were recorded on a Shimadzu UV-240 UV-visible recording spectrophotometer. Specific rotations were measured on a NPL Automatic Polarimeter 143D. A VG Micromass 3D8 mass spectrometer was used for MS analysis. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Chromatography columns utilised (Merck) 7736 Kieselgel 60M or 7734 Kieselgel 60. T.l.c. plates contained a precoated layer of Merck Kieselgel $60F_{254}$ of 0.2 mm thickness. Preparative t.l.c. plates were prepared with 7736 Kieselgel 60H.

Methyl-N-acetyl-δ-iodo-DL-norvaline (5). To a stirred solution of α-acetamido-δ-valerolactone (4)[14] (146 mg, 0.93 mmol) and anhydrous sodium iodide (209 mg, 1.4 mmol) in dry acetonitrile (0.9 ml) was added boron

tribromide in acetonitrile (2.32 ml of 0.4 M solution, 0.928 mmol) with cooling. After 20 hr dry methanol (5.0 ml) was added and after a further 3 hr the solvents were evaporated and the crystalline residue partitioned between dichloromethane (5 ml) and water (15 ml). The organic phase was washed with aqueous sodium bicarbonate, sodium thiosulphate and finally water. The aqueous phase was washed with dichloromethane. The combined organic phase was dried ($MgSO_4$) and evaporated to give a pale brown crystalline residue of 5 (206.8 mg, 74.4%). Recrystallisation from methanol-water gave material of m.p. 81.0° (lit.,[14] 81-82°); δ_H(100 MHz, $CDCl_3$) 1.83(m, 4H, H-3,4), 2.05(s, 3H, CH_3CO), 3.16(m, 2H, H-5), 3.72(s, 3H, CH_3O), 4.56(d, 1H, J 8Hz, H-2), 7.06(d, 1H, NH); ν_{max}(KBr) 1740, 1635, 1540 cm^{-1}; m/z 299(M^+, 2), 198(100), 172 (47). Found 172.094 ± .003. Calc. for $C_8H_{14}NO_3[M-I]^+$ 172.097.

Methyl-N-acetyl-δ-nitro-DL-norvaline (6). Iodide 5 (200 mg, 0.670 mmol) in dry benzene (13.4 ml) containing Amberlite IR-900 resin (NO_2^- form,[16] 0.58 g, 0.70 mmol nitrite) was stirred at R.T. for 72 hr. The resin was placed in a chromatography column and washed with ethanol (600 ml). The eluate was concentrated in vacuo and the residue chromatographed on SiO_2 eluting with 0-4% methanol in dichloromethane to give (6) (111.4 mg, 76.3%), m.p. 57.5-58.5° (lit.,[14] 58°); δ_H(100 MHz, $CDCl_3$) 1.98(m, 4H, H-3,4), 2.06 (s, 3H, CH_3CO), 3.82(s, 3H, CH_3O), 4.50(t, 2H, J 6Hz, H-5), 4.74(m, 1H, H-2), 6.66(d, 1H, J 8Hz, NH); ν_{max}($CHCl_3$), 1735, 1665, 1530 cm^{-1}; m/z 219(M^+, 1) 159(49), 117(100).

N-tert-Butyloxycarbonyl-δ-nitro-L-norvaline (10). To a solution of δ-nitro-L-norvaline (8) (70.9 mg, 0.438 mmol) in dioxane-water [2:1 (5 ml)] was added sodium hydroxide (35 mg, 0.88 mmol) followed by di-tert-butyl-dicarbonate (105 mg, 0.48 mmol) in dioxane-water [2:1 (1.5 ml)]. After 2 hr the solvent was evaporated and the residue in water (10 ml) was acidified to pH 3.5 with solid citric acid. The aqueous phase was extracted with ethyl acetate (5 x 10 ml) and the organic extracts dried ($MgSO_4$) and evaporated to give 10 (102.3 mg, 92.6%) as an oil, $[\alpha]_D$ -7.4° (c 1.0 in CH_3OH) (lit.,[14] -7.6°, c 1.08 in CH_3OH); δ_H(CD_3OD, 100 MHz) 1.38(s, 9H, CMe_3), 1.5-2.05 (m, 4H, H-3,4), 4.08(m, 1H, H-2), 4.48(t, 2H, J 7Hz, H-5); ν_{max}(film) 3650-2300 br, 1700, 1545, 1380 cm^{-1}.

Diphenylmethyl N-tert-butyloxycarbonyl-δ-nitro-L-norvaline (11). A solution of diphenyldiazomethane (0.66 g, 3.4 mmol) in CH_2Cl_2 (20 ml) was added to acid (10) (0.74 g, 2.83 mmol) in CH_2Cl_2 (10 ml); vigorous gas evolution occurred. After 5 hr at room temperature the solvent was evaporated. Excess diphenyldiazomethane was removed by slow elution with

CH_2Cl_2 through a 4 cm pad of SiO_2 in a sintered glass funnel. When all of the colour had disappeared the SiO_2 was washed (with suction) with 5% CH_3OH in CH_2Cl_2 (1500 ml). After concentration in vacuo the residue was chromatographed on SiO_2 eluting with CH_2Cl_2 to give 11 (0.935 g, 77.3%), m.p. 61-62.5° $[\alpha]_D$-11.9° (c 1.27 in $CHCl_3$); δ_H(100 MHz, $CDCl_3$) 1.4(s, 9H, CMe_3), 1.86(m, 4H, H-3,4), 4.16(m, 3H, H-2,5), 5.36(d, 1H, J 8Hz, NH), 6.84(s, 1H, $CHAr_2$), 7.24(m, 10H, Ar); ν_{max}($CHCl_3$) 1740, 1710, 1550, 1370 cm^{-1}; m/z 372(0.01), 167(100), 117(63). (Found C, 64.43; H, 6.54; N, 6.38; $C_{23}H_{28}N_2O_6$ requires C, 64.47; H, 6.59; N, 6.54%).

N^6-Benzoyl-2',3'-O-isopropylidene-5'-deoxy-5'-nitromethyladenosine (15a). To the aldehyde 12 (180 mg of 80% purity, 0.353 mmol) in THF (0.8 ml) was added nitromethane (0.6 ml, 1.1 mmol) followed by tetrabutylammonium fluoride trihydrate (14 mg, 0.044 mmol). The reaction was stirred at R.T. for 18 hr, and then evaporated to give two components (13a) in an approximate ratio of 2:3. The residue was dissolved in dry CH_2Cl_2 to which was added acetic anhydride (0.055 ml, 0.59 mmol) and 4-dimethylaminopyridine (5.4 mg, 0.044 mmol). The reaction was stirred at room temperature for 24 hr, then evaporated, and the residue was dissolved in cold ethanol (0.5 ml). Sodium borohydride (35 mg, 0.92 mmol) in ethanol (1.5 ml) was added over 3 min with cooling. After 1 hr at room temperature the solvent was evaporated and the residue partitioned between ethylacetate (10ml) and water (10 ml). The organic phase was washed with water (2 x 5 ml), dried (Na_2SO_4) and evaporated to give an amorphous solid (160 mg). Chromatography on SiO_2 using 0-1% CH_3OH in CH_2Cl_2 gave 15a (55.7 mg, 35%), $[\alpha]_D$ + 96° (c 0.84 in $CHCl_3$), + 12.5° (c 0.8 in CH_3OH) (lit.,[12] + 12.73° (c 0.8 in CH_3OH)]; δ_H(360 MHz, $CDCl_3$). 1.38 and 1.59 (each s, 3H, CMe_2), 2.48(q, 2H, J 6.8Hz, H-5'), 4.25(dt, 1H, J 6.7, 4.0Hz, H-4'), 4.38(t, 2H, J 6.8Hz, H-6'), 5.02 (dd, 1H, J 4.0, 6.4Hz, H-3'), 5.50(dd, 1H, J 6.4, 2.1Hz, H-2'), 6.07 (d, 1H, J 2.1Hz, H-1'), 7.4-7.6(m, 3H, Ar), 7.9-8.1(m, 3H, 2Ar + H-2), 8.77(s, 1H, H-8), 9.16(s, 1H, NH); IR($CHCl_3$), 1707, 1585, 1375 cm^{-1}; m/z 454(M^+, 1.8), 439(1.5), 425(15), 105(100).

N^6-Benzoyl-9-[O-methyl-9'(R,S)-acetamido-6'(R,S)-nitro-5',6',7',8',9'-pentadeoxy-2',3'-O-isopropylidene-β-D-ribodecafuranosyluronate]adenine(15b). To a solution of aldehyde 12 (103.2 mg, 0.253 mmol) and nitro compound 6 (46.0 mg, 0.211 mmol) in THF (0.3 ml) was added TBAF (13.3 mg, 42 μmol). After 18 hr stirring at room temperature the solvent was evaporated in vacuo. Purification by preparative TLC eluting with 5% CH_3OH in CH_2Cl_2 gave 13b as a white amorphous solid (120 mg, 91%).

To 13b (147.7 mg, 0.236 mmol) prepared as above, in CH_2Cl_2 (0.48 ml) was added acetic anhydride (0.04 ml, 0.30 mmol) and 4-dimethylamino-pyridine (2.9 mg, 24 μmol). The mixture was stirred at room temperature for 16 hr, and evaporated to give a residue (154 mg) which was treated with a solution of sodium borohydride in ethanol (1M, 0.47 ml) with cooling. After 75 min at room temperature the solvent was evaporated and the residue partitioned between CH_2Cl_2 and water. The organic phase was washed with water, dried ($MgSO_4$) and evaporated. Isolation by preparative TLC eluting with 5% CH_3OH in CH_2Cl_2 gave 15b as a white amorphous solid (97.1 mg, 67.7%), $[\alpha]_D$ + 6.7° (c 1.35 in CH_3OH); δ_H(200 MHz, $CDCl_3$) 1.34, 1.39, 1.54, 1.55, 1.62(s, 6H, CMe_2), 1.6-2.4(m, 9H, CH_3CO, H-7',8',5'), 3.75 (2s, 3H, CH_3O), 4.3-4.6(m, 3H, H-4',6',9'), 4.95(m, 1H, H-3'), 5.3(m, 0.5H, NH) 5.5(m, 1H, H-2'), 6.05, 6.16 (2brs, 1H, H-1'). 7.4-7.6(m, 3H, Ar), 7.95-8.2(m, 3H, 2Ar, H-2), 8.73, 8.75(2s, 1H, H-8), 9.30(s, 1H, NH); ν_{max}(KBr) 1735, 1707, 1667, 1610, 1550, 1375 cm^{-1}; m/z(FAB, thiodiglycol) 634($[M+Na]^+$, 1.31), 612($[MH^+$, 9.75), 105(100); λ_{max}(EtOH), 278(ε16, 200), 259(ε10, 800), 231 nm (ε10,700). (Found: C, 55.8; H, 5.3; N, 15.7. $C_{28}H_{33}N_7O_9$ requires C, 56.0; H, 5.4; N, 16.0%).

N^6-Benzoyl-9-[diphenylmethyl-9'(S)-tert-butyloxycarbonylamino-6'-(R)-nitro-5',6',7',8',9'-pentadeoxy-2',3'-O-isopropylidene-β-D-ribo-decafuranosyluronate] adenine and the 6'(S)-isomer (15c).- To a solution of aldehyde 12 (310 mg, 0.759 mmol) in dry THF (1.0 ml) was added amino acid 11 (240 mg, 0.563 mmol), and TBAF (35 mg, 0.113 mmol). The reaction was stirred for 18 hr at room temperature. Acetic anhydride (0.07 ml, 0.704 mmol) and 4-dimethylamino pyridine (6.9 mg, 0.06 mmol) was added and the reaction stirred for a further 24 hr. The solvent was evaporated and the residue dissolved in ethanol (5 ml). An ethanolic solution of sodium borohydride (45 mg in 1.0 ml) was added over a 10 min period with cooling. After 1 hr at room temperature the solvent was evaporated. The residue was partitioned between ethyl acetate and water, washed with water, dried ($MgSO_4$) and evaporated in vacuo. Chromatography on SiO_2 using 0-3% CH_3OH in CH_2Cl_2 gave an approximately 1:1 mixture of the 6'-epimers of 15c as an amorphous solid (322 mg, 70% based on 11). Greater than 90% separation was achieved by chromatography on SiO_2(Merck 7736 [1:100 loading]) eluting with acetone/petrol (1:2) under pressure. m/z (mixed isomers, FAB, thiodiglycol), 845 ($[M+Na]^+$, 0.46), 823 ($[MH_2]^+$, 2.62), 240(29.18), 167(100), 105(32.24).

Physical data for more mobile epimer: $[\alpha]_D$ + 10.8° (c 2.4 in $CHCl_3$), -3.0° (c 1.48 in CH_3OH). δ_H(200MHz, $CDCl_3$) 1.36(s, 9H, CMe_3), 1.38, 1.57 (each s, 3H, CMe_2), 1.6-2.45(m, 6H, H-5',7',8'), 4.11(m, 1H, H-4'), 4.42 (m, 1H, H-6') 4.53(m, 1H, H-9'), 4.93(dd, 1H, J 6,4Hz, H-3'), 5.23(d, 1H, J 8HZ, NH), 5.45(br d, 1H, J 6Hz, H-2'), 6.06(d, 1H, J 2Hz,H-1'), 6.82 (s, 1H, $CHAr_2$), 7.2-7.4(m, 10H, 2Ar), 7.4-7.7(m, 3H, PhCO), 8.0-8.1 (m, 3H, 2 PhCO + H-2), 8.75(s, 1H, H-8), 9.27(bs, 1H, NH); ν_{max}($CHCl_3$) 1710, 1610, 1550, 1380 cm^{-1}; λ_{max}(EtOH) 280(ε 24, 000), 260 nm(ε 15, 500). (Found: C, 62.59;H, 5.75; N, 11.12. $C_{43}H_{47}N_7O_{10}$ requires C, 62.84; H, 5.76; N, 11.93%.

Physical data for less mobile epimer: $[\alpha]_D$ + 5.0° (c 0.62 in $CHCl_3$); δ_H(100MHz, $CDCl_3$) 1.40 (s, 9H, CMe_3) 1.39 and 1.60 (each s, 3H, CMe_2), 1.5-2.6(m, 7H, H-5',7',8',NH), 4.2(m, 1H, H-4'), 4.3-4.5(m, 2H, H-6',9'), 4.95 (dd, 1H, J 3.7, 7.2Hz, H-3'), 5.08(d, 1H, J 7.6Hz, NH), 5.48(dd, 1H, J 7.2, 1.7Hz, H-2'), 6.05(d, 1H, J 1.7Hz, H-1'),6.87(s, 1H, $CHPh_2$), 7.3(m, 10H, $CHPh_2$), 7.45-7.65(m, 3H, PhCO), 8.01(m, 2H, PhCO), 8.04 and 8.79 (each s, 1H, H-2,8), 9.16(bs, 1H, NH); ν_{max}($CHCl_3$) 1707, 1609, 1550, 1363 cm^{-1}; λ_{max}(EtOH) 279(ε 26, 000), 260 nm(ε 17, 200). (Found: C, 62.70; H, 5.70, N, 11.25. $C_{43}H_{47}N_7O_{10}$ requires C, 62.84; H 5.76; N, 11.93%).

Sinefungin (1) and its 6'-(S)-isomer. - A solution of 15c (80 mg, 0.097 mmol) in methanolic zinc bromide solution (4.0 ml of 1 M) was stirred at room temperature for 20 hr, evaporated to dryness, and the residue partitioned between ethyl acetate and water. Aqueous sodium chloride solution was added to disperse an emulsion. The washed, dried organic layer was evaporated to give an oil (70 mg), containing some methyl benzoate.

Chromatography at this stage on silica, eluting with CH_2Cl_2 and then CH_2Cl_2-MeOH (98:2) gave 16 (61.5 mg, 88%) as an oil $[\alpha]_D$ + 13° (c 0.56 in $CHCl_3$); δ_H(200MHz, $CDCl_3$) 1.42(s, 9H, CMe_3), 1.46, 1.53, 1.56 1.58 (each s, total 6H, CMe_2), 1.4-2.6(m, 6H, H-5',-7',-8'), 4.1-4.6 (m, 3H, H-4',6',9'), 4.9-5.3(m, 3H, H-2',-3', NH), 5.8(s, 2H, NH_2), 5.93 and 5.98(m, 1H, H-1'), 6.84 and 6.88(s, 1H, $CHPh_2$), 7.30(m, 10H, $CHPh_2$), 7.82 and 7.86(s, 1H, H-2), 8.29 and 8.32(s, 1H, H-8); m/z(FAB, thiodiglycol) 719(MH_2^+); λ_{max}(EtOH) 258 nm(ε 18000).

The crude product (70 mg) in trifluoroacetic acid-water (4:1, 0.9 ml) was left to stand for 45 min, and evaporated. The residue was partitioned between ethyl acetate (10 ml) and water (10 ml). The aqueous phase was applied to a column of Dowex 50 (H^+) (10 g) which was washed with ether and water. The product was eluted with dilute ammonia. Evapora-

tion gave 17 (34 mg, 84% from 15c) as an amorphous solid; δ_H(200 MHz, CF_3CO_2H-D_2O) δ 1.6-2.1(m, 4H, H-5',-7'), 2.2 (m, 2H, H-8'), 3.7(m, 2H, H-4',-9'), 4.2(m, 2H, H-3',-6'), 4.67(m, 1H, H-2'), 5.88(d, 1H, J 4.2Hz, H-1'), 8.22(s, 1H, H-2), 8.27(s, 1H, H-8); λ_{max}(EtOH) 260nm (ε 10300); (in acid) 257 nm(ε 10200); (in alkali) 254(ε 15000), 300nm(ε 2500).

To a portion of this material (10.0 mg, 24 μmol) in dry methanol (0.1 ml) containing freshly prepared Raney nickel T-4 catalyst (10 mg) was added anhydrous ammonium formate (12 mg, 0.24 mmol). The mixture was stirred for 18 hr at room temperature, diluted with methanol (2 ml), and filtered through celite. The celite was washed well with methanol and the combined filtrates evaporated, finally under high vacuum, to give a residue (4.2 mg), which was applied in water to a column of Dowex 50 (H^+) resin. Elution with dilute aqueous ammonia and evaporation gave a yellowish solid (3.5 mg, 32% from 15c) consisting of two components with R_f in CH_3OH-$CHCl_3$-NH_3 aq. (3:1:1) 0.56 and 0.60 (Sinefungin R_f 0.56); m/z (FAB, thiodiglycol) 426 ($[M+HCO_2]^+$, 1.03), 404($[M+Na]^+$, 2.62), 382(MH^+, 1.64).

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