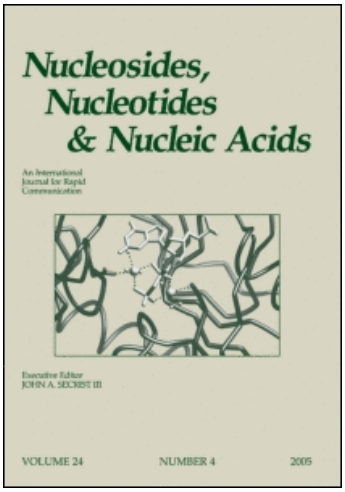

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Studies on Griseolic Acid Derivatives X. Synthesis and Phosphodiesterase Inhibitory Activity of 2-Substituted Derivatives of Griseolic Acid

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STUDIES ON GRISEOLIC ACID DERIVATIVES X[1]. SYNTHESIS AND PHOSPHODIESTERASE INHIBITORY ACTIVITY OF 2-SUBSTITUTED DERIVATIVES OF GRISEOLIC ACID

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ABSTRACT Griseolic acid derivatives which were modified at the 2- and/or 6-positions were first synthesized from griseolic acid by a ring opening - reclosure reaction of the adenine ring. Among these derivatives, the 2-amino-6-deamino-6-hydroxyl (guanine) derivative showed 3.3 and 45 times stronger inhibitory activity against cAMP and cGMP PDE, respectively, than those of griseolic acid. Structure-activity relationships among these derivatives are also discussed.

INTRODUCTION We have reported synthetic procedures for various derivatives of griseolic acids (GA's, e.g. **1**, SCHEME 1) and their inhibitory activities against 3',5'-cyclic nucleotide phosphodiesterases (PDE).[1-5] It was also suggested that a guanine congener of **1** would have a strong inhibitory activity against 3',5' cyclic guanosine monophosphate phosphodiesterase (cGMP PDE) from the results of a previous report.[5] In that report, we tried to synthesize the guanine derivative (**20**, SCHEME 2) by a trans-glycosidation reaction with **1** or by glycosidation of an acylated GA-sugar derivative with silylated guanine. However, both attempts

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This paper is dedicated to the late Professor Tohru Ueda.

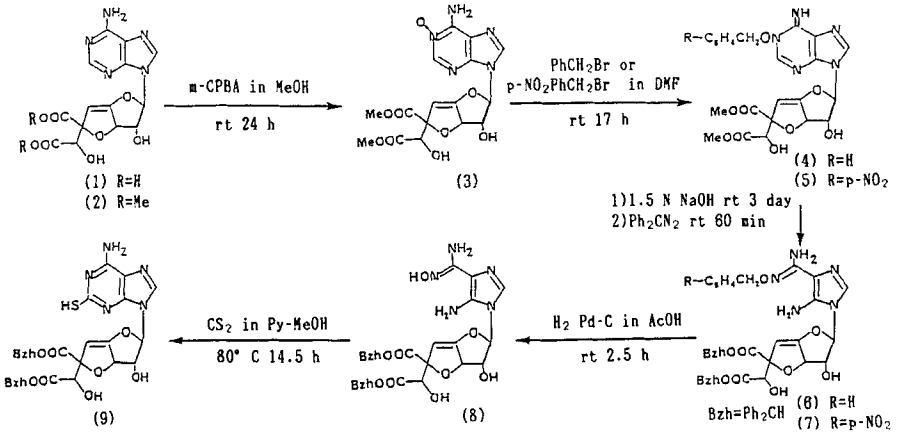
were unsuccessful except for the guanine derivative of a GA in which the double bond at $C_{4'}$ and $C_{5'}$ position was saturated. This paper presents synthetic methodology for guanine derivatives of **1** by ring opening - reclosure of the pyrimidine ring in an N^1-benzyloxide **3**. Structure-activity relationships for GA derivatives which have various sibstituent at the 2 positons of their base moieties are also discussed.

RESULTS AND DISCUSSION

Ring Opening of the Pyrimidine Moiety of GA (1)

It is well-known that 5-aminoimidazole-4-carboxamide (AICA) nucleosides give guanine nucleoside derivatives by a ring closure reaction with an appropriate C_1 unit reagent. Then, first we tried to synthesize AICA type GA derivatives from GA derivatives having hypoxanthine, N^1-alkyladenine and adenine N^1-oxide in place of adenine according to the reported methods.[6-8] However, none of the trials gave good results because of the too severe reaction conditions for the GA derivatives. It has been reported that N^1-benzyloxyadenosine underwent ring-opening at the pyrimidine moiety under relatively mild alkaline conditions.[8] For applying this reaction conditions to a GA derivative, N^1-benzyloxy dimethyl griseolate **4** was synthesized according to a reaction sequence shown in SCHEME 1. Dimethyl griseolate N^1-oxide **3** was obtained in 91% yield by treating a methyl ester **2** with m-chloroperbenzoic acid (m-CPBA) in methanol for 17 h at room temperature (rt).[9] Treatment of **3** with benzyl bromide in dimethylformamide (DMF) at rt for 17 h gave **4** in 58% yield. A *p*-nitrobenzyloxy derivative **5**[10] was also obtained in 77% yield by the reaction of **4** with *p*-nitrobenzyl bromide under the same reaction conditions.[8] The ring opening reaction of **4** with aqueous NaOH solution gave a complex mixture by judging from TLC analysis, because of accompanying partial hydrolysis of the dimethyl ester. Then, after complete hydrolysis of the methyl esters was checked by TLC, the mixture was acidified and then treated with diphenyldiazomethane. As the result, a ring opened compound **6** was obtained in 46% yield by treating **4** with 1.5 N aqueous NaOH at rt for 3 days followed successively by esterification and purification on a silica gel column. In a similar way, **5** gave **7** in 33.4% yield.

The structures of the new compounds in this paper were determined by UV, NMR and IR spectra and elemental analysis unless otherwise noted. Compound **6** gave a hydroxyamidino derivative **8** in 30% yield by catalytic reduction of **6** with 10% palladium-on-carbon



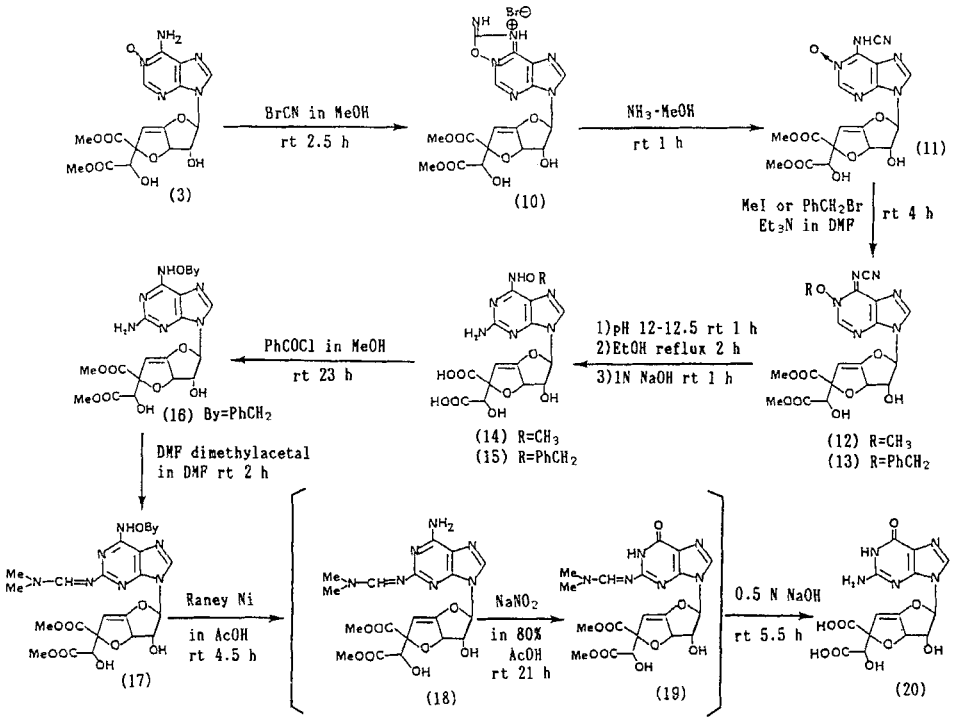
SCHEME 1

at rt for 2.5 h. This hydroxy-amidino derivative **8** gradually colored dark green when its solution was exposed to air at rt. Compound **8** gave a 2-mercapto GA derivative **9** in 23% yield by treating it with carbon disulfide in a mixture of pyridine and methanol at 80 °C for 14.5 h.[11]

While it became possible to synthesize **9** the yield was too low and the purification procedures of the intermediates **6-7** were not appropriate for further investigation. Then, we turned to study another synthetic pathway to get the 2-substituted GA derivatives.

Guanine Derivative of GA (**1**)

In 1978, Ueda *et al.*[12] reported the transformation reaction of adenosine to guanosine. In this method, 6-thioguanosine was obtained by the reaction of the key intermediate 2-amino-N^6-methoxyadenosine, which was provided from the reaction product of adenosine N^1-oxide and cyanogen bromide through three reaction processes, with hydrogen sulfide in aqueous pyridine. Oxidative hydrolysis of 6-thioguanosine gave guanosine in fair yield. Then, first we attempted to synthesize the 2-amino-N^6-methoxy derivative of GA. Reaction of N^1-oxide **3** with cyanogen bromide in methanol at rt for 2.5 h gave oxadiazole **10** in quantitative yield as a crystalline powder. When **10** was treated with methanolic ammonia at rt for 1 h, rearrangement of the oxazolidine ring occurred and an



SCHEME 2

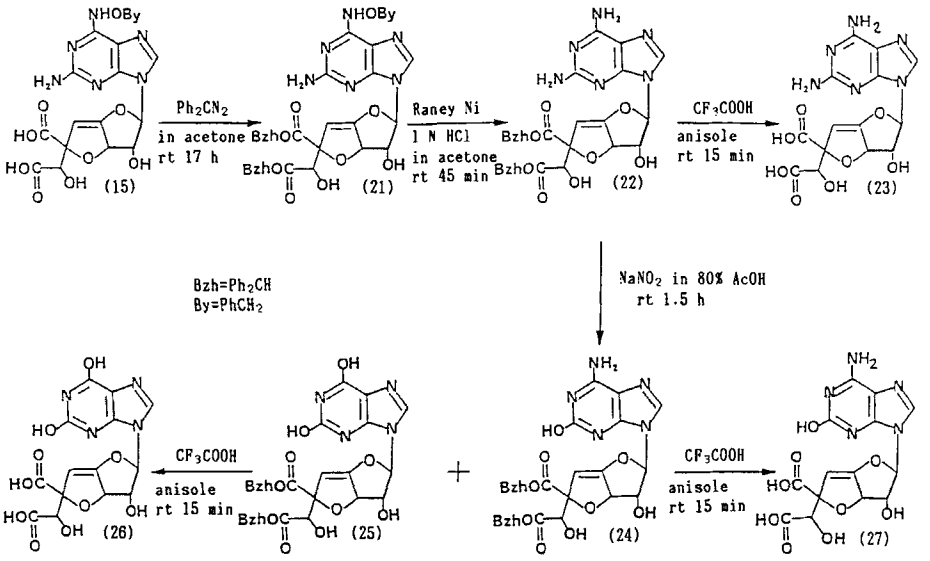
N^6-cyano-N^1-oxide **11** was obtained in 94% yield. Methylation of **11** with methyl iodide in DMF provided the N^6-cyano-N^1-methoxy derivative **12** in 80% yield. On treating the methanol solution of **12** at pH 12-12.5 by adding 1 N aqueous NaOH at rt for 1 h, followed by refluxing a 50% aqueous ethanol solution of the neutralized reaction mixture for 1 h, a ring opening-reclosure reaction of the pyrimidine ring of **12** occurred between the amino group at the 5 position of the imidazole ring and the cyano group to form a 2-amino-N^6-methoxy GA **14**. During the course of this reaction, methyl esters of the carboxyl group were partially hydrolyzed and the reaction mixture showed a complex pattern on TLC. After complete hydrolysis of the ester moiety with 1 N aqueous NaOH, the desired compound **14** was obtained in 76% yield as a semicrystalline powder. In spite of examining the reaction conditions,the thiation reaction of the methoxyamino group of **14** was unsuccessful due to the presence of the double bond at the sugar moiety. Then we chose the

benzyl group instead of the methyl group since the former would be removed by catalytic reduction.

A 2-amino-N^6-benzyloxy derivative **15** was obtained in reasonable yield by the use of procedures similar to those for the synthesis of **14** from **11**. To protect the carboxylic groups of **15**, it was allowed to react with benzoyl chloride in methanol at rt for 23 h to give **16** in 90% yield. In preference to removal of the benzyloxy group in **16**, its amino group at the 2 position was protected with dimethylaminomethylidene group.[13] Compound **16** was allowed to react with freshly distilled DMF dimethylacetal in DMF at rt for 2 h to give **17** in 90% yield. Subsequently, the benzyloxy group of **17** was removed by reduction with Raney Ni in acetic acid at rt for 4.5 h. The desired compound **18** was obtained in good yield after passing the reaction mixture through a column of high porous resin (CHP-20P, Mitsubishi Chemical Industries Ltd.) to remove Ni ion. Without further purification of **18**, the deamination reaction was performed with sodium nitrite in 80% aqueous acetic acid at rt for 21 h to give **19** as a white powder after purification by silica gel column chromatography. The protecting groups of **19** were finally removed by treating it with 0.5 N aqueous NaOH at rt for 5.5 h. The reaction mixture was purified on a reversed phase prepacked column to yield the guanine derivative of GA **20** in 52% yield from **17**.

2-Substituted GA Derivatives

Next, compound **14** was reacted with diphenyldiazomethane in acetone at rt for 17 h to synthesize 2-substituted GA derivatives (SCHEME 3). Unexpectedly, the main product of this reaction was a tribenzhydrylated compound and the desired compound **21** was obtained in only 25% yield after purification by silica gel column chromatography. The benzyloxy group of **21** was removed by treatment with Raney Ni in acetone in the presence of 1 N aqueous HCl at rt for 45 min to afford a 2,6-diamino derivative **22** in 71% yield. This compound was treated with trifluoroacetic acid in the usual way to give 2-aminogriseolic acid **23** in 62% yield. Deamination of **22** with sodium nitrite in 80% aqueous acetic acid at rt for 1.5 h afforded a 2-hydroxy GA derivative **24** as the main product and xanthine derivative **25** as a minor product. Both compounds gave unprotected derivatives **26** and **27** in reasonable yields by treating them with trifluoroacetic acid. It is noteworthy that no guanine derivative was detected in the deamination reaction.



SCHEME 3

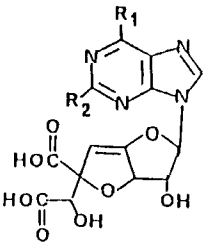
PDE Inhibitory Activity

The tests were carried out essentially according to the method reported by Pichard and Cheung.[14] The details were reported previously.[2,3] The PDE IC_{50} values of the 2-substituted GA derivatives are shown in Table 1.

As can be seen from the Table 1, it has become apparent that **i)** the guanine derivative **20** showed the strongest inhibitory activity against not only cGMP•PDE but also cAMP•PDE; **ii)** the derivatives having a substituent at the 2 position had a weaker inhibitory activity against both of cGMP•PDE and cAMP•PDE than **1** except **20**; **iii)** the derivatives having the NH_2 or OH group at the 2 position tend to showing a stronger activity against cGMP•PDE than cAMP•PDE except **14** and **23**. We expected that the inhibitory activity of **20** against cGMP•PDE would be stronger than that of **1**, whereas, the inhibitory activity against cAMP•PDE might be weaker than that of **1**. In the case of cGMP•PDE,the strength of the inhibitory effect of **20** was in fact 45 times stronger than that of **1**. However, **20** also showed 3.3 times stronger activity against cAMP•PDE than that of **1** contrary to our expectation. Consequently,

Table 1

PDE IC_{50} of 2 substituted Griseolic Acid derivatives



Compound No	R_1	R_2	PDE IC_{50} (μMol)	
			c-AMP	c-GMP
14	$NHOCH_2C_6H_5$	NH_2	7.4	10.2
15	$NHOCH_3$	NH_2	6.1	3.1
20	OH	NH_2	0.049	0.014
23	NH_2	NH_2	0.19	0.33
26	OH	OH	3.3	0.91
27	NH_2	OH	14.7	5.6
1	NH_2	H	0.16	0.63
deaminated GA	OH	H	0.32	0.13

it is obvious that the amino group of **20** would play an important role for the inhibitory effect against cGMP•PDE because the inhibitory effect of **20** is still 9.3 times stronger than that of a deaminated GA[4] whose structure is very similar to that of **20** except the amino group at the 2-position. On the contrary, in the case of **23**, the inhibitory effect against cAMP•PDE is almost equal to that of **1** despite having an amino group at the 2-position. Accordingly, it is unclear why **20** showed stronger inhibitory activity against cAMP•PDE than GA.

We have reported that GA pivaloyloxymethyl esters markedly increased the cellular cAMP levels and reduced the intraocular pressure in rabbits by topical application.[15] Thus, some of

griseolic acid derivatives are expected to be used as an ocular hypotensive drug. We are anticipating that some of the guanine derivatives of GA, whose permeabilities across the cell membrane are improved, would have a different pharmacological effect, since **20** has very strong inhibitory activity against cGMP•PDE. The studies along this line are in progress.

Experimental

General – Melting pionts were determined using a Yanagimoto melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained with Varian EM-390 (90 MHz) and JEOL GX-400 (400 MHz) spectrometers, and the chemical shifts are expressed in ppm from tetramethylsilane as the internal standard; s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; br, broad. UV spectra were obtained using a Hitachi 200-20 spectrophotometer. Thin-layer chromatography (TLC) was carried out using Merck silica gel F_{254} pre-coated TLC plates, with a layer thickness 0.25 mm. Spots were visualized by UV irradiation or by spraying with 30% aqueous sulfuric acid followed by heating. Ordinary chromatography was performed by the rapid chromatography method using Merck silica gel (Kieselgel 60 Art. 9385).

Dimethyl griseolate N^1-oxide (3). Dimethyl griseolate (**2**)[4] (8.14 g, 20 mmol) was suspended in methanol (180 ml). *m*-Chloroperbenzoic acid (6.90 g, 40 mmol) was added, and the mixture was stirred at room temperature for 24 h. The solvent was then distilled off under reduced pressure. When the solvent was almost removed, diethyl ether (300 ml) was added and lumps in the solution were pulverized with a spatula until they disappeared. The mixture was filtered, and then the residue was washed with diethyl ether (100 ml) and dried to give a white powdery substance (7.81 g). This powder was dissolved in a mixture of 200 ml of methanol and 300 ml of methylene chloride as thoroughly as possible, with heating, and then the solvent was concentrated to about 50 ml under reduced pressure with an aspirator. The resulting crystals were filtered off, to give 6.31 g of **3** as white powdery crystals: mp 245°C (dec., colored at 225°C). UV λmax in MeOH nm(ε) 262.5 (8800), 300 (2700); ^{1}H-NMR (DMSO-d_6+D_2O) δ 3.68 and 3.75 (3H each, s, OCH_3), 4.64 (1H, d, H2' J = 5.0 Hz), 4.67 (1H, s, H7'), 5.19 (1H, d, H5', J = 2.2 Hz), 5.94 (1H, dd, H3', J = 2.2, 5.0 Hz), 6.55 (1H, s, H1'), 8.51

and 8.68 (1H each, s, H2 or 8). Anal. Calcd for $C_{16}H_{17}N_5O_9 \cdot 1/2H_2O$: C, 44.45; H, 4.20; N, 16.20. Found: C, 44.70; H, 4.05; N, 16.24.

Dimethyl N^1-benzyloxygriseolate (4). Compound **3** (5.35 g, 12.6 mmol) and benzyl bromide (7.6 ml, 64 mmol) were allowed to react overnight in 90 ml of dimethylformamide at room temperature. The solvent was distilled off under reduced pressure, and the residue was crystallized by the addition of diethyl ether. The mixture was then filtered, and the residue was dissolved in a mixture of ethyl acetate and a 10% (w/v) aqueous solution of sodium bicarbonate. The organic layer was washed with water and dried over anhydrous magnesium sulphate. The drying agent was filtered off, and the solvent was removed. The residue was dissolved in a mixture of acetone (20 ml) and methanol (20 ml). The resulting solution was poured into 1 *l* of hexane and 0.5 *l* of diethyl ether with stirring. The resulting powdery substance was filtered to give 5.4 g (83.5%) of **4** as a crude powder. This powder could be used in the next reaction without further purification. The analytical sample was obtained as a foam by purification with silica gel column chromatography eluted with methylene chloride containing 3% (v/v) methanol. UV λmax in MeOH nm (ε) 258 (14600), 263 sh (14100), NMR (DMSO-d_6+D_2O) δ 3.64 and 3.71 (3H each, s, OCH_3), 4.54 (1H, d, H2', J = 5.0 Hz), 4.63 (1H, s, H7'), 5.17 (1H, d, H5', J = 2.2 Hz), 5.33 (2H, s, CH_2), 5.83 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.46 (1H, s, H1'), 7.3-7.7 (5H, m, $PhCH_2$), 8.31 and 8.39 (1H each, s, H2 or 8). Anal. Calcd for $C_{23}H_{23}N_5O_9 \cdot 1/2H_2O$: C, 52.87; H, 4.44; N, 13.40. Found: C, 53.12; H, 4.34; N, 13.10.

Dimethyl N^1-*p*-nitrobenzyloxygriseolate (5) Dimethylformamide (35 ml) was added to 1.48 g (3.49 mmol) of **3** and 2.27 g (10.5 mmol) of *p*-nitrobenzyl bromide, and the mixture was stirred at room temperature overnight. The solvent was distilled off under reduced pressure, and the residue was crystallized by the addition of diethyl ether. The mixture was then filtered, and the residue was dissolved in a mixture of ethyl acetate and a 10% (w/v) aqueous sodium bicarbonate. The organic layer was washed with water and dried over anhydrous magnesium sulphate. The drying agent was filtered off, and the solvent was removed. The residue was then purified by silica gel column chromatography eluted with methylene chloride containing 3% (v/v) methanol to give 1.5 g (77.0%) of **5** as a foam. NMR (DMSO-d_6) δ 3.65 and 3.73 (3H each, s, OCH_3), 4.53 (1H,

d, H2', J = 5.0 Hz), 4.63 (1H, s, H7'), 5.17 (1H, d, H5', J = 2.2 Hz), 5.45 (2H, s, CH_2), 5.87 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.45 (1H, s, H1'), 7.8-8.5 (6H, m, aromatic protons). Anal. Calcd for $C_{23}H_{22}N_6O_{11}\cdot H_2O$: C, 47.92; H, 4.20; N, 14.58. Found: C, 47.62; H, 4.00; N, 14.88.

Dibenzhydryl 1'-deadenino-1'-β-(5-amino-4-(N^2-benzyloxyamidino)imidazol-1-yl)griseolate (6). Compound **4** (5.4 g) was dissolved in 53 ml of a 1.5 N aqueous NaOH, and the mixture was stirred at room temperature for 3 days. The reaction mixture was then acidified with concentrated hydrochloric acid, and then 15 g of diphenyldiazomethane and 100 ml of acetone were added. The mixture was stirred to esterify the acid at room temperature for 60 min, after which time acetone was distilled off and 30 ml of methylene chloride were added. The organic layer was washed successively with a 10% (w/v) aqueous sodium bicarbonate and a saturated aqueous sodium chloride. The solvent was distilled off and the residue was dissolved in 2 ml of acetone. The resulting solution was poured into 300 ml of hexane with stirring. The powdery substance thus obtained was purified by silica gel column chromatography eluted with methylene chloride containing 1% (v/v) methanol to give 3.9 g of **6** as a foam. NMR (DMSO-d_6+D_2O) δ 4.54 (1H, d, H2', J = 5.0 Hz), 4.90 (1H, s, H7'), 4.94 (2H, s, CH_2), 5.26 (1H, d, H5', J = 2.2 Hz), 5.59 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.20 (1H, s, H1'), 6.69, 6.76 (1H each, s, Ph_2-C<u>H</u>), 7.1-7.5 (26H, m, aromatic protons). Anal. Calcd for $C_{46}H_{41}N_5O_{19}\cdot 1/4H_2O$: C, 68.01; H, 5.15; N, 8.62. Found: C, 68.08; H, 5.04; N, 8.51.

Dibenzhydryl 1'-deadenino-1'-β-(5-amino-4-(N^2-p-nitrobenzyloxyamidino)imidazol-1-yl)griseolate (7)
Compound **5** (0.7 g, 1.25 mmol) was dissolved in 6.2 ml (9.3 mmol) of a 1.5 N aqueous sodium hydroxide, and the mixture was stirred at room temperature for 3 days. The reaction mixture was then acidified with a 3 N aqueous HCl, and 20 ml of acetone and 2.5 g (12.87 mmol) of diphenyldiazomethane were added. The mixture was stirred to esterify the acid at room temperature for 60 min, after which time acetone was distilled off and 30 ml of methylene chloride were added. The organic layer was washed successively with a 10% (w/v) aqueous sodium bicarbonate and a saturated aqueous sodium chloride. The solvent was distilled off and the residue was dissolved in 2 ml of acetone. The resulting solution was poured

into 300 ml of hexane with stirring. The powdery substance thus obtained was purified by silica gel column chromatography eluted with a mixture of cyclohexane and ethyl acetate (1:2, v/v) to give 0.35 g (29.4%) of **7** as a foam. NMR (DMSO-d_6+D_2O) δ 4.51 (1H, d, H2', J = 5.0 Hz), 4.87 (1H, m, H7'), 5.07 (2H, s, CH_2), 5.25 (1H, br s, H5'), 5.56 (1H, br s, H3'), 6.15 (1H, s, H1'), 6.66, 6.72 (1H each, s, PhC<u>H</u>), 7.15-7.40 (21H, m, H2, two <u>Ph</u>$_2$ CH's), 7.66 & 8.20 (2H each, d, *p*-NO_2 <u>Ph</u>, J = 7.0 Hz). Anal. Calcd for $C_{46}H_{40}N_6O_9 \cdot 1/2H_2O$: C, 64.11; H, 4.68; N, 9.75. Found: C, 64.08; H, 4.41; N, 9.63.

Dibenzhydryl 1'-deadenino-1'-β-(5-amino-4-(N^2-hydroxyamidino)imidazol-1-yl)griseolate (8) Compound **6** (1.0 g, 1.2 mmol) was dissolved in 20 ml of acetic acid, and then 0.6 g of 10% (w/w) palladium-on-carbon was added, after replacing the air in a container with nitrogen. The mixture was then stirred at room temperature in a stream of hydrogen for 2 h. The palladium-on-carbon was filtered off, and the solvent was distilled from the filtrate. The residue was dissolved in a mixture of 30 ml of ethyl acetate and 20 ml of a 10% (w/v) aqueous sodium bicarbonate. The organic layer which separated was washed with water and dried over anhydrous magnesium sulphate. The drying agent was filtered off, and then the solvent was distilled off, and the residue was purified by silica gel column chromatography eluted with methylene chloride containing 5% (v/v) methanol to give 0.26 g (29%) of **8** as a foam. UV λmax in MeOH nm (ε) 252.5 (11300), 257.5 (11500). NMR (DMSO-d_6) δ 4.54 (1H, d, H2', J = 5.0 Hz), 4.88 (1H, d, H7', J = 9.9 Hz), 5.2-5.7 (5H, m, H3', NH_2, OH, H5'), 6.22 (1H, s, H1'), 6.72, 6.78 (1H each, s, Ph_2C<u>H</u>), 7.2-7.6 (23H, H2, m, NH_2, two <u>Ph</u>$_2$CH's), 8.95 (1H, s, NOH). Anal. Calcd for $C_{39}H_{35}N_5O_9 \cdot 1/2H_2O$: C, 64.46; H, 4.99; N, 9.64. Found: C, 64.34; H, 4.77; N, 9.32.

Dibenzhydryl 2-mercaptogriseolate (9). Compound **8** (0.25 g, 0.4 mmol) was dissolved in a mixture of 2 ml of methanol, 2 ml of pyridine and 1 ml of carbon disulphide. The mixture was allowed to react at 80°C in a steel cylinder for 14.5 h. The solvent was then distilled off. The pyridine was removed by repeated co-evaporation with toluene. The residue was purified by silica gel column chromatography eluted with methylene chloride containing 4% (v/v) methanol to give 0.062 g (43%) of **9** as a foam. UV λmax in MeOH nm(ε) 283 (12300). NMR (DMSO-d_6+D_2O) δ 4.63 (1H, d, H2', J = 5.0

Hz), 4.88 (1H, s, H7'), 5.26 (1H, d, H5', J = 2.2 Hz), 6.03 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.39 (1H, s, H1'), 6.69, 6.74 (1H each, s, Ph_2CH), 6.9-7.5 (20H, m, two Ph_2CH's), 8.24 (1H, s, H8). $[\alpha]^{20}$ +5.4° (c 0.3, DMSO). Anal. Calcd for $C_{40}H_{33}N_5O_8S_4 \cdot H_2O$: C, 58.89; H, 5.07; N, 8.58; S, 3.93. Found: C, 58.58; H, 4.78; N, 8.27; S, 3.81.

Dimethyl 1'-deadenino-1'-β-[2-imino-(1,2,4)oxadiazolo-(3,2,f)purin-7-yl]griseolate (10). Compound **3** (8.46 g, 20 mmol) was suspended in 400 ml of methanol. Cyanogen bromide (2.52 g, 23.8 mmol) was added and the mixture was stirred at room temperature for 1.5 h. An additional 630 mg (5.6 mmol) of cyanogen bromide was added, and then the mixture was stirred for a further 1 h. The mixture was concentrated to about 100 ml under reduced pressure. Ethyl acetate (100 ml) was added and the distillation under reduced pressure was continued until the liquid volume reached 100 ml. Ethyl acetate (1000 ml) was gradually added to the residual solution with stirring. The suspension containing crystals, was placed in a refrigerator overnight. The crystals were filtered off to give 9.77 g (92%) of **10**. NMR (DMSO-d_6) δ 3.67 and 3.73 (3H each, s, OCH_3), 4.67 (1H, d, H2', J = 5.0 Hz), 4.66 (1H, s, H7'), 5.26 (1H, d, H5', J = 2.2 Hz), 5.84 (1H, dd, H2', J = 2.2 & 5.0 Hz), 6.76 (1H, s, H1'), 9.07 and 10.23 (1H each, s, H8 or 2). Anal. Calcd for $C_{17}H_{17}N_6O_9Br \cdot 1/2H_2O$: C, 37.93; H, 3.37; N, 15.61; Br, 14.84. Found: C, 37.71; H, 3.33; N, 15.60; Br, 14.96.

Dimethyl N^6-cyanogriseolate N^1-oxide (11). Compound **10** (9.77 g, 18.5 mmol) was dissolved in 50 ml of methanol. A 20% (v/v) ammonia solution in methanol (50 ml) was added to the mixture, which was then allowed to stand at room temperature for 60 min. Ethyl acetate (200 ml) was added to the reaction mixture and the solvent was distilled off under reduced pressure with an aspirator. When the liquid volume became about 100 ml, 200 ml of ethyl acetate was added and then distilled off under reduced pressure. When the liquid volume reached 200 ml, the resulting mixture was placed in a refrigerator overnight. The crystals thus produced in the solution were filtered off to give 8.53 g (95%) of **11** as yellowish white fine crystals. NMR (DMSO-d_6) δ 3.67 and 3.74 (3H each, s, OCH_3), 4.63 (1H, d, H2', J = 5.0 Hz), 4.66 (1H, s, H7'), 5.16 (1H, d, H5', J = 2.2 Hz), 5.93 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.50 (1H, s, H1'), 8.33 and 8.49 (1H each, s, H8 or 2). $[\alpha]_D^{20}$ -8.6° (c 0.5, DMSO). Anal. Calcd for $C_{17}H_{16}N_6O_9 \cdot 2H_2O$: C, 42.15; H, 4.16; N, 17.35. Found: C, 42.37; H, 4.31; N, 17.64.

Dimethyl N^6-cyano-N^1-methoxygriseolate (12). Triethylamine (8.3 ml, 60 mmol) and methyl iodide (3.9 ml, 62.6 mmol) were added to 40 ml of dimethylformamide containing compound **11** (4.5 g, 10 mmol) with ice-cooling. The mixture was stirred at room temperature for 4.5 h. The solvent was then distilled off, and 100 ml of diethyl ether was added to the residue to yield a powder after pulverizing the mixture with a spatula. The insoluble substance was filtered off, washed with 30 ml of diethyl ether and partitioned between 80 ml of ethyl acetate and 20 ml of water. The aqueous solution was extracted repeatedly with ethyl acetate. The ethyl acetate layers were combined and dried over anhydrous magnesium sulphate. After filtering off the drying agent, the solvent was distilled off to give 3 g (64.9%) of **12**. This powder could be used to the next reaction without further purification. The analytical sample was obtained by purification with silica gel column eluted with methylene chloride containing 3% (v/v) methanol and lyophilization from benzene. UV λmax in MeOH nm (ε) 283.5 (19500). $[\alpha]_D^{20}$ +5.4° (c 0.5, DMSO). NMR (DMSO-d_6) δ 3.66 and 3.73 (3H each, s, OCH_3), 4.14 (3H, s, OCH_3), 4.5-4.7 (2H, m, H7', H2'), 5.20 (1H, d, H5', J = 2.2 Hz), 5.83 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.55 (1H, s, H1'), 8.59 and 9.02 (1H each, s, H8 or 2). Anal. Calcd for $C_{18}H_{18}N_6O_9 \cdot 1/4C_6H_6$: C, 48.60; H, 4.08; N, 17.44. Found: C, 48.44; H, 4.28; N, 17.08.

Dimethyl N^1-benzyloxy-N^6-cyanogriseolate (13). Compound **11** (8.53 g, 17.6 mmol) was dissolved in 100 ml of dimethylformamide. Benzyl bromide (10 ml, 84.2 mmol) and triethylamine (10 ml, 72.1 mmol) were added and the mixture was stirred at room temperature for 4 h. The solvent was distilled off under reduced pressure. Ethanol (30 ml) and toluene (30 ml) were added to the residue and then distilled off. This procedure was repeated 4 times. The oily substance thus obtained was mixed with 500 ml of diethyl ether, subjected to ultrasonic treatment with stirring with a spatula to give a pale yellow powdery substance. This substance was filtered off and dissolved in a mixture of water (300 ml) and ethyl acetate (500 ml). The organic layer which separated was washed successively with 100 ml each of a saturated aqueous solution of sodium chloride, a 0.2 N aqueous HCl, a 10% (w/v) aqueous sodium bicarbonate and a saturated aqueous sodium chloride, in that order, and dried over anhydrous magnesium sulphate. After treatment of the solution with active carbon, the solvent was distilled off under

reduced pressure to give 8.2 g (89%) of **13** as a yellow powder after lyophilization from benzene. This powder could be used in the next reaction without further purification. The analytical sample was obtained by purification with silica gel column eluted with methylene chloride containing 3% (v/v) methanol and lyophilization from benzene. UV λmax in MeOH nm (ε) 284.5 (18400). NMR (DMSO-d_6) δ 3.68 and 3.76 (3H each, s, OCH_3), 4.61 (1H, d, H2', J = 5.0 Hz), 4.67 (1H, s, H7'), 5.26 (1H, d, H5', J = 2.2 Hz), 5.37 (2H, s, $PhC\underline{H}_2$), 5.83 (1H, dd, H3', J = 2 .2 & 5.0 Hz), 6.57 (1H, s, H1'), 7.2-7.8 (6H, m, $PhCH_2$ and $1/6C_6H_6$), 8.56 and 8.81 (1H each, s, H8 or 2). Anal. Calcd for $C_{24}H_{22}N_6O_9 \cdot 1/6C_6H_6$: C, 54.40; H, 4.20; N, 15.22. Found: C, 54.46; H, 4.17; N, 14.90.

2-Amino-N^6-methoxygriseolic acid (14). A 0.2 N aqueous NaOH (50 ml) was added to methanol (70 ml) containing 3 g (6.5 mmol) of compound **13**. The resulting mixture was stirred at room temperature for 1.5 h and then its pH was adjusted to 11.7 with 2 ml of a 1 N aqueous NaOH, and the mixture was stirred for a further 30 min. The solution was adjusted to pH 7.0 with concentrated hydrochloric acid and methanol was distilled off. The remaining solution was mixed with 70 ml of ethanol and heated for 1.5 h under reflux. The solvent was distilled off until about 50 ml of the solution remained, and the resulting solution was mixed with 50 ml of a 1 N aqueous NaOH and stirred at room temperature for 60 min. The resulting mixture was adjusted to pH 1 with concentrated hydrochloric acid and then washed with ethyl acetate. The aqueous layer was adjusted to pH 2.3 with a 10% (w/v) aqueous sodium bicarbonate. It was then purified by column chromatography using an RP-8 reverse phase prepacked column (Merck) eluted with an aqueous solution containing 5% (v/v) acetonitrile and 0.02% (v/v) acetic acid. The main peak was collected and lyophilized from water to give 1.91 g (45%) of **14** as a white powder. UV λmax in 0.1 N aqueous HCl nm (ε) 256 (11600), 295 (12100); in H_2O, 260 sh (10900), 281 (14100); in 0.1 N aqueous NaOH, 287 (14900). $[\alpha]_D^{20}$ -6.4° (c 0.5, DMSO). NMR (DMSO-d_6) δ 3.76 (3H, s, OCH_3), 4.46 (1H, s, H7'), 4.51 (1H, d, H2', J = 5.0 Hz), 5.04 (1H, d, H5', J = 2.2 Hz), 5.77 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.23 (1H, s, H1'), 7.76 (1H, s, H8). Anal. Calcd for $C_{15}H_{16}N_6O_9 \cdot H_2O$: C, 40.73; H, 4.10; N, 19.00. Found: C, 40.98; H, 4.08; N, 19.29.

2-Amino-N^6-benzyloxygriseolic acid (15). A 0.2 N aqueous NaOH (100 ml) was added to methanol (400 ml) containing 8.8 g (16.8 mmol) of compound **12** with stirring. The reaction solution was adjusted to a pH 12-12.5 by the addition of a 1 N aqueous NaOH, and it was then allowed to stand at room temperature for 60 min. The solvent was distilled off until the remaining liquid volume reached about 100 ml. The resulting solution was mixed with 50 ml of water and 150 ml of ethanol and heated under reflux for 2 h. The solvent was then distilled off again under reduced pressure until the liquid volume was reduced to about 100 ml. A 0.2 N aqueous NaOH (100 ml) was added, and the resulting solution was allowed to stand at room temperature for 60 min. Ethyl acetate (200 ml) was added to the solution, and the pH was adjusted to 0.5 with concentrated hydrochloric acid with stirring. The aqueous and organic layers were separated, and the organic layer was washed with 50 ml of a 0.1 N aqueous HCl. The aqueous layers were combined and treated with active carbon. The pH of the solution was adjusted to 2.3 by the addition of solid sodium bicarbonate with vigorous stirring. The resulting powdery substance was filtered off, washed with water after storage in a refrigerator overnight and dried to give 5.29 g (63%) of **15** as a yellowish white powder. mp 300°C (dec., colored at 160°C). $[\alpha]_D^{20}$ -3.8° (c 0.5, DMSO). UV λmax in 0.1 N aqueous HCl-MeOH nm (ε) 256 (12700), 295 (13400); in H_2O-MeOH 280 (12900); in 0.1 N aqueous NaOH-MeOH 289 (15800). NMR (DMSO-d_6) δ 4.54 (1H, s, H7'), 4.55 (1H, d, H2', J = 5.0 Hz), 5.06 (2H, s, CH_2), 5.08 (1H, d, H5', J = 2.2 Hz), 5.83 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.27 (1H, s, H1'), 7.2-7.6 (5H, m, PhCH), 7.79 (1H, s, H8). Anal. Calcd for $C_{21}H_{20}N_6O_9 \cdot 2H_2O$: C, 47.02; H, 4.51; N, 15.67. Found: C, 46.77; H, 4.25; N, 15.37.

Dimethyl 2-amino-N^6-benzyloxygriseolate (16). Compound **14** (7.3 g) was suspended in methanol (146 ml) and to it was added benzoyl chloride (5 equivalent) under ice-cooling. The reaction solution was stirred for 23 h at rt and 150 ml of ethyl acetate was added. The solvent was removed under reduced pressure and to the residue was added 100 ml of ethyl acetate. The solution was condensed to a half volume under reduced pressure. To the solution was added 450 ml of ether and the mixture was allowed to stand for 17 h in a refrigerator. The resulting precipitate was collected by filtration and was partitioned between 300 ml of ethyl acetate and 200 ml of saturated sodium bicarbonate solution. The organic layer

was washed with saturated aqueous sodium chloride and was dried with magnesium sulfate. The solvent was distilled off and the residue was purified by silica gel column chromatography eluted with methylene chloride containing 5% (v/v) methanol to give 4.8 g of **16** as a foam. UV λmax in MeOH nm (ε) 282.5 (16400). NMR (DMSO-d_6+D_2O) δ 3.63 and 3.69 (3H each, s, OCH_3), 4.51 (1H, d, H2', J = 5.0 Hz), 4.62 (1H, s, H7'), 5.09 (1H, d, H5', J = 2.2 Hz), 5.83 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.23 (1H, s, H1'), 7.2-7.6 (5H, m, Ph), 7.70 (1H, s, H8). Anal. Calcd for $C_{23}H_{24}N_4O_9 \cdot 1/2H_2O$: C, 51.40; H, 4.69; N, 15.64. Found: C, 51.30; H, 4.53; N, 15.38.

Dimethyl N^6-benzyloxy-2-(*N'*,*N'*-dimethylaminomethylene)-aminogriseolate (17). Compound **16** (1.0 g, 1.9 mmol) was dissolved in dimethylformamide (19 ml). Dimethylformamide dimethylacetal (0.4 ml) was added, and the mixture was allowed to stand at room temperature for 2 h. After the disappearance of **16** had been confirmed by TLC, the solvent was distilled off under reduced pressure. The residue was partitioned between 80 ml of methylene chloride and 80 ml of water, and the organic layer was washed with 30 ml of a saturated aqueous sodium chloride. All the aqueous layers were combined and extracted with 40 ml of methylene chloride. The organic layers were combined, dried over anhydrous magnesium sulphate and the solvent was distilled off under reduced pressure. The residue was subjected to silica gel column chromatography and eluted with methylene chloride containing 3% (v/v) methanol to give 1.07 g (97%) of **17** as a white powder. UV λmax in MeOH nm (ε) 270 (14900), 329 (3400). NMR (DMSO-d_6+D_2O) δ 3.04 and 3.19 (3H each, s, NCH_3), 3.67 and 3.72 (3H each, s, OCH_3), 4.47 (1H, d, H2', J = 5.0 Hz), 4.63 (1H, s, H7'), 5.01 (2H, s, CH_2), 5.12 (1H, d, J = 2.2 Hz), 6.00 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.38 (1H, s, H1'), 7.2-7.6 (5H, m, Ph), 7.83 (1H, s, H8), 8.50 (1H, s, -N-CH=N-). Anal. Calcd for $C_{26}H_{29}N_7O_9 \cdot 1/3H_2O$: C, 52.91; H, 5.07; N, 16.63. Found: C, 53.01; H, 5.03; N, 16.34.

Dimethyl 2-(*N'*,*N'*-dimethylaminomethylene)amino-griseolate (18). Compound **17** (584 mg, 1 mmol) was dissolved in acetic acid (16 ml) and water (4 ml). Raney nickel W-2 (2 ml) suspended in water was added, and the resulting mixture was stirred at room temperature for 4.5 h. The Raney nickel was removed by filtration and the filtrate was condensed by evaporation under reduced pressure. When the acetone was almost completely removed,

the resulting mixture was mixed with 200 ml of methylene chloride and neutralized with an aqueous sodium bicarbonate solution, and the resulting insoluble substance was removed by filtration. After separation of the organic layer, the aqueous layer was extracted 3 times with 50 ml of methylene chloride. The organic layers were combined and dried over anhydrous magnesium sulphate. The solvent was distilled off under reduced pressure and the residue (460 mg) was subjected to the next reaction. NMR (DMSO-d_6+D_2O) δ 3.04 and 3.15 (3H each, s, NCH_3), 3.67 and 3.73 (3H each, s, OCH_3), 4.53 (1H, d, H2', J = 5.0 Hz), 4.64 (1H, s, H7'), 5.13 (1H, d, H5', J = 2.2 Hz), 6.06 (1H, dd, H3', J=2.2 & 5.0 Hz), 6.47 (1H, s, H1'), 7.33 (1H, s, H8).

Dimethyl 6-desamino-2-(*N*',*N*'-dimethylaminomethylene)-amino-6-hydroxygriseolate (19). Compound **18** (460 mg, 0.96 mmol) was dissolved in 80% (v/v) aqueous acetic acid (50 ml). Sodium nitrite (1.34 g, 19 mmol) was added under ice-cooling, and the mixture was allowed to stand at room temperature for 17 h. After the disappearance of **18** had been confirmed by TLC, the solvent was distilled off under reduced pressure. The acetic acid was removed by repeated co-evaporation with ethanol. The residue was dissolved in a mixture of 50 ml of methylene chloride, 20 ml of water and 5 ml of a 5% (w/v) aqueous sodium bicarbonate. The organic layer was separated and extracted 3 times with 30 ml of methylene chloride, and the extracts were combined. The solvent was distilled off under reduced pressure. The residue (350 mg) was used in the next reaction without further purification. NMR (DMSO-d_6) δ 3.06 and 3.21 (3H each, s, NCH_3), 3.63 and 3.71 (3H each, s, OCH_3), 4.53 (1H, d, H2', J = 4.4 Hz), 4.61 (1H, s, H7'), 5.15 (1H, d, H5', J = 2.0 Hz), 5.85 (1H, dd, H3', J = 2.4 & 4.9 Hz), 6.42 (1H, s, H1'), 8.16 (1H, s, H8).

2-Amino-6-desamino-6-hydroxygriseolic acid (20). Compound **19** (350 mg, 0.73 mmol) was dissolved in 6 ml of 0.5 N aqueous NaOH, and the mixture was allowed to stand at room temperature for 5.5 h. The resulting solution was adjusted to pH 1.3 and then subjected to chromatography using an RP-8 prepacked column (Merck), which was washed with water and eluted with water containing 5% (v/v) acetonitrile to give 207 mg (52% from **17**) of **20** as a white powder. UV λmax in 0.1 N aqueous HCl nm (ε) 256 (13000), 281 sh (8300); in H_2O 252 (14300), 275 sh (9400); in 0.1 N

aqueous NaOH 264 (12500). NMR (DMSO-d_6+D_2O) δ 4.53 (1H, d, H2', J = 4.9 Hz), 4.48 (1H, s, H7'), 5.07 (1H, d, H5', J = 2.4 Hz), 5.79 (1H, dd, H3', J = 2.4 & 4.9 Hz), 6.25 (1H, s, H1'), 7.87 (1H, s, H8). Anal. Calcd for $C_{14}H_{13}N_5O_9 \cdot 5/2H_2O$: C, 38.19; H, 4.12; N, 15.90. Found: C, 38.21; H, 3.84; N, 15.55.

Dibenzhydryl 2-amino-N^6-benzyloxygriseolate (21). Compound **14** (1.0 g, 2.0 mmol) was suspended in a mixture of acetone (100 ml) and water (100 ml). Diphenyldiazomethane[16] was added until no further disappearance of its red colour was observed. The reaction mixture was then stirred during the addition of 4 ml of 1 N aqueous HCl and diphenyldiazomethane was added again until no further disappearance of its red colour was observed. The mixture was then stirred for 60 min. Acetone was removed by distillation under reduced pressure and water was removed by decantation. The residue was partitioned between 50 ml of ethyl acetate and 50 ml of water, and the organic layer was washed successively with 30 ml of 5% (w/v) aqueous sodium bicarbonate and 30 ml of saturated aqueous sodium chloride and then dried over anhydrous magnesium sulphate. The solvent was distilled off under reduced pressure and the residue was dissolved in 30 ml of ethyl acetate, and the resulting solution was poured into 500 ml of hexane with stirring. The resulting insoluble substance was collected by filtration and purified using a silica gel prepacked column (Merck) and an eluent consisting of methylene chloride containing 2.5% (v/v) methanol. Of the two main fractions, the fraction eluted later was collected, evaporated to dryness to give 430 mg (25%) of **21** as a white powder. UV λ max in MeOH nm (ε) 282 (17300). NMR (DMSO-d_6+D_2O) δ 4.63 (1H, d, H2', J = 5.0 Hz), 4.99 (1H, s, H7'), 5.07 (2H, s, CH_2), 5.31 (1H, d, H5', J = 2.2 Hz), 5.97 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.33 (1H, s, H1'), 6.75 and 6.81 (1H each, s, $Ph_2C\underline{H}$), 7.0 - 7.7 (25H, s, aromatic protons), 7.74 (1H, s, H8). Anal. Calcd for $C_{47}H_{39}N_5O_9 \cdot 1/2H_2O$: C, 67.15; H, 4.79; N, 9.99. Found: C, 67.41; H, 4.99; N, 9.82.

Dibenzhydryl 2-aminogriseolate (22). Compound **21** (83 mg, 0.1 mmol) was dissolved in a mixture of 20 ml of acetone and 10 ml of a 1 N aqueous HCl. Raney nickel W-2 (1 ml) was added to the mixture, which was then stirred vigorously at room temperature for 45 min. The Raney nickel was removed by filtration, and acetone was distilled off under reduced pressure. The resulting

mixture was mixed with 30 ml of ethyl acetate. The organic layer was separated and washed successively with 20 ml of 10% (w/v) aqueous sodium bicarbonate and 20 ml of saturated aqueous sodium chloride, and dried over anhydrous magnesium sulphate. The solvent was distilled off under reduced pressure, and the residue was purified using a prepacked silica gel column (Merck) and an eluent consisting of methylene chloride containing 5% v/v methanol. The main fraction was collected to give 73 mg (99%) of **22** as a white powder. UV λmax in MeOH nm (ε) 258 (11400), 281 (10700). NMR (DMSO-d_6+D_2O) δ 4.65 (1H, d, H2', J = 5.0 Hz), 4.91 (1H, s, H7'), 5.25 (1H, d, H5', J = 2.2 Hz), 6.06 (1H, dd, H3', J=2.2 & 5.0 Hz), 6.36 (1H, s, H1'), 6.68 and 6.74 (1H each, s, Ph_2CH), 7.0-7.6 (20H, s, two Ph_2CH's), 7.92 (1H, s, H8). Anal. Calcd for $C_{40}H_{34}N_6O_8I/2H_2O$: C, 65.30; H, 4.79; N, 11.42. Found: C, 65.60; H, 4.74; N, 11.18.

2-Aminogriseolic acid (23). Compound **22** (0.56 g, 0.76 mmol) was suspended in 5 ml of anisole and solubilized by adding 5 ml of trifluoroacetic acid under ice-cooling, and the mixture was allowed to stand at room temperature for 10-15 min. Toluene (15 ml) was added to the reaction mixture and then the solvent was distilled off. The procedure comprising the addition of a mixed solvent of 5 ml of acetone and 15 ml of toluene and its removal by distillation was repeated twice and the residue was suspended in 2 ml of acetone. The resulting suspension was poured into 200 ml of hexane with stirring. The resulting powdery substance was filtered off and dissolved in a 10% (w/v) aqueous sodium bicarbonate. The pH of the resulting solution was adjusted to 0.6 with concentrated hydrochloric acid, and the solution was treated with active carbon. The pH of the resulting mixture was adjusted to 2 with 10% (w/v) aqueous sodium bicarbonate, and the mixture was allowed to stand at 5 °C overnight. The deposited crystals were filtered off, washed with water and dried to give 0.15 g of **23**. UV λmax in 0.1 N aqueous HCl nm (ε) 252 (12200), 291 (10000); in H_2O 253 (15700), 286 (13000); in 0.1 N aqueous NaOH 256 (9600), 279 (10600). NMR (DMSO-d_6+D_2O) δ 4.55 (1H, d, H2', J = 5.1 Hz), 4.45 (1H, s, H7'), 4.55 (1H, d, H5', J = 5.1 Hz), 5.87 (1H, dd, H3', J = 2.2 & 5.1 Hz), 6.28 (1H, s, H1'), 7.89 (1H, s, H8). Anal. Calcd for $C_{14}H_{14}N_6O_8 \cdot H_2O$: C, 40.78; H, 3.91; N, 20.38. Found: C, 41.08; H, 3.88; N, 20.44.

Dibenzhydryl 2-hydroxygriseolate (24) and dibenzhydryl 6-desamino-2,6-dihydroxygriseolate (25). Compound **22** (0.60 g, 0.83 mmol) was dissolved in 80% (v/v) aqueous acetic acid (50 ml).

After replacing the air in a container with nitrogen, 1 g (14.5 mmol) of sodium nitrite was added to the mixture under ice-cooling, and the mixture was allowed to react at room temperature for 1.5 h. The solvent was distilled off, and the residue was mixed with water and the water was distilled off again. The deposited substance was suspended in water and collected by filtration. The residue was dissolved in 15 ml of acetone and the pH of the solution was adjusted to 9-10 by the addition of concentrated aqueous ammonia. The resulting solution was allowed to stand at room temperature for 20 min and then the acetone was distilled off. The residue was mixed with a mixture of ethyl acetate (50 ml) and water (50 ml) and stirred thoroughly. The resulting precipitate was filtered off and chromatographed on a silica gel column, which was eluted first with 10% (v/v) methanol in methylene chloride to give 0.35 g (60.1%) of **24** and then with methanol to give 0.06 g (10%) of **25** as a foam. The separated ethyl acetate layer was washed successively with a 10% (w/v) aqueous sodium bicarbonate and a saturated aqueous sodium chloride and dried over anhydrous magnesium sulphate. The drying agent was removed by filtration and the solvent was distilled off. The resulting residue was purified by silica gel column chromatography, using methylene chloride containing 10% (v/v) methanol as the eluent to give an another crop 0.04 g (5.5%, total 65.6%) of **24** as a foam. UV λmax in MeOH nm (ε) 251.5 (11300), 298 (11200). NMR (DMSO-d_6+D_2O) δ 4.59 (1H, d, H2', J = 5.0 Hz), 4.90 (1H, s, H7'), 5.24 (1H, d, H5', J = 2.2 Hz), 6.05 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.30 (1H, s, H1'), 6.67 and 6.73 (1H each, s, Ph_2CH), 7.1-7.5 (20H, s, two Ph_2CH's), 7.93 (1H, s, H8). Anal.Calcd for $C_{40}H_{33}N_5O_9 \cdot 2H_2O$: C, 62.91; H, 4.88; N, 9.17. Found: C, 62.93; H,4.73; N, 9.30.

25. UV λmax in MeOH nm (ε) 258 (12700), 280 sh (9500). NMR (DMSO-d_6+D_2O) δ 4.47 (1H, d, H2', J = 5.0 Hz), 4.85 (1H, s, H7'), 5.19 (1H, d, H5', J = 2.2 Hz), 6.04 (1H, dd, H3', J = 5.0 & 2.2 Hz), 6.22 (1H, s, H1'), 6.65 and 6.73 (1H each, s, Ph_2CH), 7.15-7.45 (20H, s, two Ph_2CH's), 7.56 (1H, s, H8). Anal. Calcd for $C_{40}H_{32}N_4O_{10} \cdot 3H_2O$: C, 61.38; H, 4.89; N, 7.14. Found: C, 61.05; H, 4.60; N, 6.89.

6-Desamino-2,6-dihydroxygriseolic acid (26). Compound **25** (40 mg, 0.05 mmol) was dissolved in 1 ml of anisole. Trifluoroacetic acid (1 ml) was added under ice-cooling, and the

mixture was allowed to stand at room temperature for 10-15 min. The acid was removed by co-evaporation first with toluene and then twice with a mixture of toluene and acetone and the residue was suspended in 0.5 ml of acetone. After the addition of 20 ml of hexane, the deposited substance was collected by filtration. The residue was dissolved in a 10% (w/v) aqueous sodium bicarbonate. The solution was adjusted to pH 0.6 with concentrated hydrochloric acid. The resulting solution was purified by column chromatography using an RP-8 reversed phase prepacked column (Merck) eluted with water. The main peak was collected and lyophilized from water to give 25 mg (100%) of **26** as a white powder. $[\alpha]_D^{20}$ -8.8° (c 0.3, DMSO). UV λmax in 0.1 N aqueous HCl nm (ε) 232 (10200), 260 (10100); in H_2O 232 (9900), 261 (10100); in 0.1 N aqueous NaOH 248 (10400), 276 (10100). NMR (DMSO-d_6+D_2O) δ 4.55 (1H, d, H2', J = 5.0 Hz), 4.47 (1H, s, H7'), 5.11 (1H, d, H5', J = 2.2 Hz), 5.53 (1H, dd, H3', J = 2.2, 5.0 Hz), 6.40 (1H, s, H1'), 7.92 (1H, s, H8). Anal. Calcd for $C_{14}H_{12}N_4O_{10}\cdot 5/2H_2O$: C, 38.10; H, 3.88; N, 12.70. Found: C, 37.67; H, 3.86; N, 12.42.

2-Hydroxygriseolic acid (27). Compound **24** (0.37 g, 0.5 mmol) was dissolved in 3 ml of anisole. Trifluoroacetic acid (3 ml) was added under ice-cooling and the mixture was allowed to stand at room temperature for 10 min. Toluene was then added and the solvent was distilled off. A mixture of acetone and toluene was then added and subsequently distilled off. This procedure was repeated twice and the resulting mixture was suspended in 2 ml of acetone and poured into 100 ml of hexane with stirring. The deposited substance was collected by filtration. The residue was dissolved in 10% (w/v) aqueous sodium bicarbonate and the solution was adjusted to pH 1.2 with concentrated hydrochloric acid. The resulting mixture was purified by column chromatography using an RP-8 prepacked column (Merck) eluted with water. The main peak was collected and lyophilized from water to give 0.17 g (85%) of **27** as a white powder. $[\alpha]_D^{20}$ -1° (c 0.5, DMSO). UV λmax in 0.1 N aqueous HCl nm (ε) 281 (13000); in H_2O 247 (8700), 290 (11100); in 0.1 N aqueous NaOH 252 (6800), 283 (11600). NMR (DMSO-d_6+D_2O) δ 4.47 (1H, d, H2', J = 5.0 Hz), 4.46 (1H, s, H7'), 5.05 (1H, d, H5', J = 2.2 Hz), 5.89 (1H, dd, H3', J = 2.2 & 5.0 Hz), 6.24 (1H, s, H1'), 7.95 (1H, s, H8). Anal. Calcd for $C_{14}H_{13}N_5O_9\cdot 2H_2O$: C, 38.99; H, 3.51; N, 16.24. Found: C, 38.73; H, 3.80; N, 15.99.

PDE inhibitory activity - The test was carried out following essentially the method of Pichard and Cheung.[11] The detailed experiment was reported previously.[2-4]

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