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Studies on Griseolic Acid Derivatives. VI.[1] Synthesis and Phosphodiesterase-Inhibitory Activity of 6- and N^1-Substituted Derivatives of Griseolic Acid[2]

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Twenty kinds of 6-substituted and five kinds of N^1-substituted griseolic acid derivatives were synthesized. The inhibitory activities of these compounds towards adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) phosphodiesterase (PDE) were investigated to clarify the structure–activity relationship. Substitution at the 6-position of griseolic acid caused a change of PDE IC_{50} from 0.16 to 206 μM against cAMP PDE. Substitution at the N^1-position also caused a change of PDE IC_{50} from 0.16 to 127 μM against cAMP PDE.

Keywords——griseolic acid; 6-substituted derivative; N^1-substituted derivative; adenosine 3′,5′-cyclic monophosphate; guanosine 3′,5′-cyclic monophosphate; phosphodiesterase inhibition

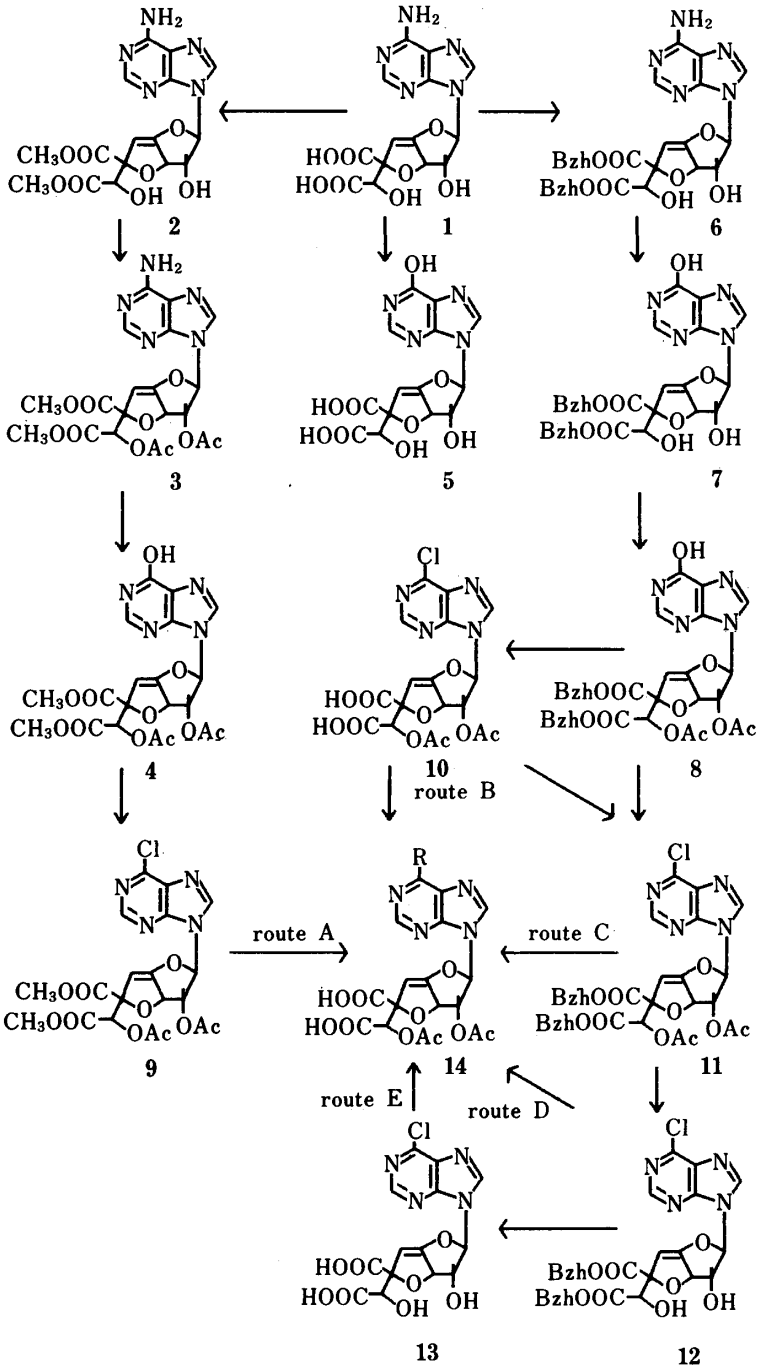
Introduction

In a previous paper,[1] we have reported on the synthesis of acylated derivatives of griseolic acid (**1**) at the N^6-, $O^{2'}$-, and $O^{7'}$-positions. It has become apparent that acylation of the N^6-position strongly reduces the inhibitory activity of griseolic acid against cyclic nucleotide phosphodiesterase (PDE). It would be interesting to clarify how the inhibitory activity of griseolic acid changes if its amino group is replaced by another functional group. In this work, therefore, the authors have synthesized griseolic acid derivatives which have various functional groups at the 6-position. The synthesis of N^1-substituted derivatives of griseolic acid was also studied. Finally, the contribution of the functional groups at the 6-position to the inhibitory activity against cyclic nucleotide PDE is discussed in some detail.

Synthesis of 6-Substituted Griseolic Acid Derivatives

Several methods are known to convert the functional group at the 6-position of purine nucleosides by using the 6-chloro,[3a] 6-trimethylsilyloxy,[3b] 6-alkylsulfonyloxy[3c] and 6-alkylsulfonyl[3d] derivatives as active intermediates. We chose 6-chloro derivatives as the active intermediates because they can give a wide variety of 6-substituted derivatives. Dimethyl griseolate (**2**) was obtained by reacting griseolic acid (**1**) with diazomethane in a mixture of dimethyl sulfoxide and methanol.[4] However, this procedure was accompanied with the formation of the derivative methylated at the 2′-hydroxy group as a by-product. Thus, we prepared **2** *via* a mixed anhydride as the active intermediate.

Dropwise addition of benzoyl chloride to a suspension of **1** in methanol under ice-cooling and continuous stirring at room temperature for 17 h gave the dimethyl ester (**2**) in good yield without producing 2′-*O*-methylated derivatives.[1b] Acetylation of **2** with acetic anhydride in pyridine gave the crystalline diacetate (**3**) in 55% yield. Deamination of **3** with sodium nitrite in 80% aqueous acetic acid[5] gave the 6-deamino-6-hydroxy derivative (**4**) in 94% yield.



route A: **14a**: R = OCH_3
route B: **14b**: R = SH, **14c**: R = SCH_3, **14d**: R = $NHCH_3$, **14e**: R = $NHCH_2C_6H_5$, **14f**: R = $NH(CH_2)_2C_6H_5$, **14g**: R = $NHCH_2$-α-naphthyl, **14h**: R = piperidino, **14i**: R = morpholino, **14j**: R = NHC_6H_5, **14k**: R = $NHCH_2CH_2OH$, **14l**: R = $NHCH_2CH_2NH_2$
route C: **14m**: R = $N(CH_3)_2$
route D: **14n**: R = H
route E: **14o**: R = $NHNH_2$, **14p**: R = N_3, **14q**: R = $NHOCH_3$

Chart 1

Reaction of **4** with phosphorus oxychloride in ethyl acetate in the presence of *N*,*N*-dimethylaniline[6] gave dimethyl $O^{2'}$,$O^{7'}$-diacetyl-6-chloro-6-deaminogriseolate (**9**) in 94% yield. The structure of this compound was determined from the analogy of its ultraviolet (UV) spectrum with that of tri-*O*-acetyl-6-chloropurine riboside[7] in addition to nuclear magnetic

resonance (NMR) spectra and elemental analysis data. Nucleophilic displacement of the chlorine atom at the 6-position of **9** with a methoxy group was achieved by reacting it with sodium methoxide at 0 °C for 2.5 h. Successive deprotection under aqueous alkaline conditions gave 6-deamino-6-methoxygriseolic acid (**14a**) in a yield of 79%. However, it seems likely that this route (route A; from **9** to **14**) is not suitable for the replacement of the chlorine atom with substituted amino groups, because the methoxycarbonyl groups of **9** would give amide functions which are very difficult to convert to the free acid. Thus, we wished to synthesize 6-chloro derivatives of griseolic acid whose carboxy groups are free or protected with acid-labile groups. The dibenzhydryl ester (**6**) of griseolic acid was obtained by reacting it with diphenyldiazomethane.[1] Deamination of **6** was performed by the same method as described previously to give **7** in good yield. Acetylation of **7** with acetic anhydride in pyridine gave the diacetate **8**. The chlorination reaction of **8** with phosphorus oxychloride in the presence of a small amount of ethyl acetate and *N*,*N*-diethylaniline at refluxing temperature for 20 min produced **10** in 63% yield. Meanwhile, the chlorination of **8** by using a 1 : 1 mixture of phosphorus oxychloride and ethyl acetate in the presence of *N*,*N*-diethylaniline at refluxing temperature for 3 h gave the 6-chlorodibenzhydryl drivative (**11**) in 81% yield. Selective deacetylation of **11** was performed by treating it in 20% methanolic ammonia at room temperature for 50 min to give compound **12** in 83% yield. Removal of the benzhydryl groups of **12** in the usual manner with trifluoroacetic acid and anisole gave the 6-chloro derivative (**13**) of griseolic acid in 72% yield. Introduction of various substituents at the 6-position *via* the 6-chloro intermediate was performed as follows, according to the method reported in the case of 6-chloropurine riboside.[8]

6-Deamino-6-mercaptogriseolic acid (**14b**) was obtained by reacting **10** with sodium hydrosulfide in dimethylformamide at room temperature for 17 h under a nitrogen atmosphere in a yield of 57% after removal of acetyl groups with 1 N aqueous NaOH and purification by reverse-phase column chromatography [route B (from **10** to **14**)]. Methylation of **14b** was achieved by reaction with methyl iodide in 1 N aqueous NaOH at room temperature for 17 h to give **14c** in 87% yield. N^6-Methylgriseolic acid (**14d**) was obtained in 71% yield by amination of **10** with methylamine in methanol at room temperature for 5 h followed by deprotection of the acetyl groups with 1 N aqueous NaOH. The compounds which have benzylamino, phenethylamino, α-naphthylmethylamino, piperidino, morpholino, phenylamino, 2-hydroxyethylamino and 2-aminoethylamino groups at the 6-position of griseolic acid were obtained by route B through almost the same procedure as in the case of **14d** except that the methylamine was replaced by appropriate amines to give **14e—l**.

N^6,N^6-Dimethylgriseolic acid was produced by route C (from **11** to **14m**). Compound **11** was reacted with 40% aqueous dimethylamine in acetone for 17 h at room temperature to give the 6-dimethylamino derivative. Deprotection of the acetyl and benzhydryl groups of the crude product was performed by treating it with 2 N aqueous NaOH in methanol at room temperature for 17 h to give **14m** in 65% yield. Introduction of a hydrogen atom at the 6-position was accomplished by reacting **12** with zinc powder in 80% aqueous acetic acid solution at room temperature for 4 h followed by deprotection of the benzhydryl groups to produce **14n** in 56% yield (route D). Reaction of **13** with hydrazine hydrate at room temperature for 20 h gave the 6-hydrazino derivative (**14o**) in moderate yield (route E). For the preparation of the 6-azido derivative (**14p**), direct introduction of an azido group at the 6-position of **13** was attempted; however, treatment of **13** with sodium azide proceeded with decomposition, and the desired compound could not be isolated. Therefore, the 6-hydrazino derivative (**14o**) was treated with nitrous acid at 0 °C according to the method reported by Johnson *et al.*[9] to give a compound having UV λ_{max} at 251, 259, 287, and 300 (sh) nm at neutral pH. This UV spectrum was very similar to that reported for the 6-azide drivative. N^6-Methoxygriseolic acid (**14q**) was obtained by reacting **13** with methoxyamine in methanol at

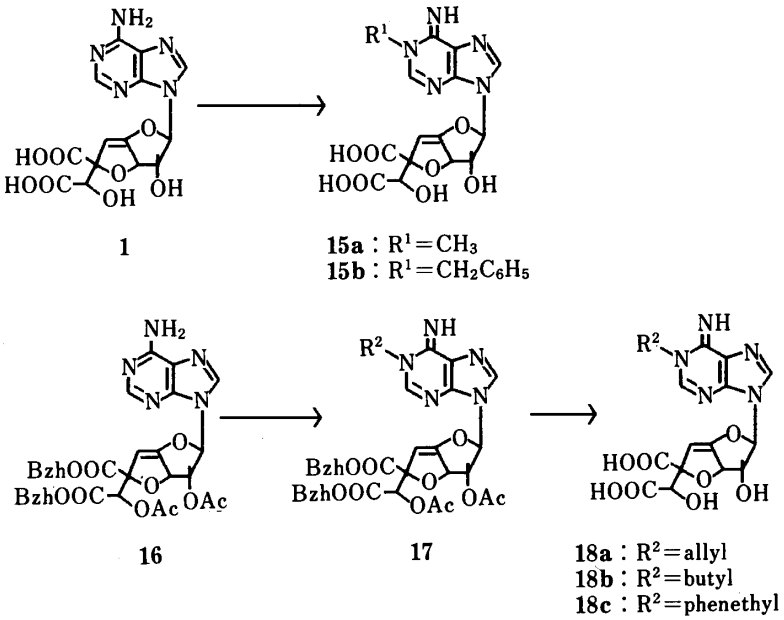


Chart 2

60—80 °C for 22 h.

Synthesis of N^1-Substituted Griseolic Acid Derivatives

N^1-Methyl and N^1-benzylgriseolic acid derivatives were synthesized by a slightly modified version of the method reported by Jones *et al.*[10] for N^1-methyladenosine. Griseolic acid was reacted with methyl iodide in dimethylformamide at room temperature for 2 d and then the reaction mixture was treated with a mixture of methanol and concentrated aqueous ammonia at room temperature for 4 h. The reaction mixture was purified by reverse-phase chromatography to give N^1-methyl griseolic acid (**15a**) in 50% yield. Protected N^1-allyl, butyl and phenethyl derivatives were obtained by alkylation of the protected derivative (**16**) at 70 °C for 48 h in good yield after purification by silica gel chromatography. The acetyl groups were removed by ammonia in methanol and the benzhydryl groups were removed by trifluoroacetic acid according to the usual method to give **18a**, **18b**, and **18c** in good yields.

PDE-Inhibitory Activity of the 6- or N^1-Substituted Griseolic Acid Derivatives

The PDE-inhibitory activities of the 6-substituted derivatives are shown in Table I. Among these 20 derivatives, the strongest inhibitor was natural griseolic acid (**1**). However, the deaminated derivative (**5**) showed activity of the same order as **1**. Concerning the relationship between the inhibitory activity and the size of the substitutent at the 6-position, it seems likely that the derivatives which have small substituents at the 6-position show relatively strong activity, except the 6-benzylamino derivative (**14l**).

Furthermore, the derivatives which have a secondary amino or aromatic amino group at the 6-position showed weak activity. As seen in the cases of **14k** and **14l**, alkyl substituents of the 6-amino group which have an amino or hydroxy function at the end of the alkyl chain showed decreased activity compared to the 6-methylamino derivative (**14d**). In addition, it is very interesting from the point of view of hydrogen bonding ability, that the 6-chloro (**13**) or 6-hydrogen (**14n**) derivatives showed moderate activity, whereas the 6-mercapto derivative (**14b**) showed much weaker activity than the 6-hydroxy derivative (**5**). It is noteworthy that the orders of potency of the inhibitory activities against 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) PDE were not the same. In the case of the 6-hydrogen compound (**14n**), the cAMP/cGMP ratio showed an extremely high value, 11.66.

In contrast to the 6-substituted drivatives, increasing size of N^1-substituents tends to

TABLE I. PDE-Inhibitory Activity of 6-Substituted Derivatives

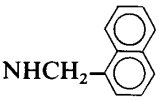
Compound No.	6-Substituent	PDE IC_{50} (μM)		
		cAMP	cGMP	cAMP/cGMP
1	NH_2	0.16	0.63	0.25
5	OH	0.32	0.13	2.46
14d	$NHCH_3$	2.00	14.00	0.14
14q	$NHOCH_3$	2.40	14.40	0.17
13	Cl	2.60	1.30	2.00
14o	$NHNH_2$	5.40	4.80	1.13
14e	$NHCH_2C_6H_5$	7.40	9.70	0.76
14c	SCH_3	8.30	8.50	0.98
14p	N_3	9.90	16.80	0.59
14a	OCH_3	10.00	4.90	2.04
14n	H	14.00	1.20	11.66
14f	$NHCH_2CH_2C_6H_5$	18.20	3.40	5.35
14b	SH	19.00	9.10	2.09
14j	NHC_6H_5	25.00	20.00	1.25
14m	$N(CH_3)_2$	31.00	39.00	0.79
14k	$NHCH_2CH_2OH$	41.00	172.00	0.24
14h		43.00	19.00	2.26
14g		84.00	244.00	0.34
14i		187.00	312.00	0.60
14l	$NHCH_2CH_2NH_2$	206.00	272.00	0.76

TABLE II. PDE-Inhibitory Activity of N^1-Substituted Derivatives

Compound No.	N^1-Substituent	PDE IC_{50} (μM)		
		cAMP	cGMP	cAMP/cGMP
1	—	0.16	0.63	0.25
15b	$CH_2C_6H_5$	0.60	1.20	0.50
18c	$CH_2CH_2C_6H_5$	6.00	5.80	1.03
18b	$CH_2CH_2CH_2CH_3$	17.00	14.00	1.21
18a	$CH_2CH=CH_2$	26.00	15.00	1.73
15a	CH_3	127.00	183.00	0.69

increase inhibitory activity. In fact, it is surprising that the N^1-methyl derivative showed about 800 times weaker activity than griseolic acid itself. In addition, the order of potency of inhibitory activity against cAMP PDE was the same as that against cGMP PDE. We have already reported that acylation or substitution of hydroxy groups of griseolic acid had little effect on the PDE-inhibitory activity. Meanwhile, acylation of the 6-amino group greatly reduced the activity.[1a] In this work, it became clear that substitution of the 6-amino group or alkylation at the N^1-position caused a great change of the inhibitory activity of PDE. This fact suggests that the 6-amino group and the N^1-position of griseolic acid play an important role in binding with PDE, whereas the hydroxy groups of the sugar moiety may not bind with PDE.[1b] This conclusion is consistent with that reported by Severin *et al.*[11]

Experimental

General——Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. NMR spectra were obtained with a Varian EM-390 spectrometer (90 MHz) and the chemical shifts are expressed in ppm from tetramethylsilane as internal standard; s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; br, broad. UV spectra were obtained on a Hitachi 200-20 spectrophotometer. Thin-layer chromatographies (TLC) were carried out on Merck Silica gel F_{254} pre-coated TLC plates, layer thickness 0.25 mm, and 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). Unprotected griseolic acid derivatives were obtained in the ordinary manner as a white powder after lyophilization of the desired peak, which was purified by reverse-phase column chromatography.

Dimethyl Griseolate (2)——Benzoyl chloride (43.2 ml, 371 mmol) was added to a suspension of 35.7 g (94.2 mmol) of griseolic acid (**1**) in 930 ml of methanol under ice-cooling with stirring. After 10 min of stirring at room temperature, the suspension became a clear solution. The reaction solution was stirred for 24 h at room temperature, then, 1 l of ethyl acetate was added and the whole was concentrated to about 500 ml. Another 1 l of ethyl acetate was added, and the solution was concentrated to about 500 ml. The, 1.5 l of ether was added and the resulting insoluble material was filtered off to give 40.3 g of a white solid. This was dissolved in 175 ml of saturated aqueous sodium bicarbonate solution and 700 ml of ethyl acetate. The aqueous layer was saturated with sodium chloride and was extracted with 300 ml of ethyl acetate 3 times. Each organic layer was washed with 200 ml of aqueous saturated sodium chloride solution successively, then the layers were combined and dried over magnesium sulfate. The ethyl acetate was removed under reduced pressure. The residue was recrystallized with 200 ml of ethanol to give 29.0 g (76%) of white fine needles, mp 210—212 °C (dec.). *Anal.* Calcd for $C_{16}H_{17}N_5O_8 \cdot H_2O$: C, 46.16; H, 4.36; N, 16.82. Found: C, 46.37; H, 4.53; N, 16.66. UV (methanol) λ_{max} nm (ε): 258 (15600). NMR (d_6-DMSO) δ ppm: 3.67 and 3.73 (each 3H, s, CH_3), 4.60 (1H, d, J=6.0 Hz. 2′-H), 4.66 (1H, s, 7′-H), 5.12 (1H, d, J=3.0 Hz, 5′-H), 6.06 (1H, dd, J= 3.0 and 6.0 Hz, 3′-H), 6.53 (1H, s, 1′-H), 7.35 (2H, br s, NH_2), 8.33 and 8.37 (each 1H, s, 2- and 8-H).

Dimethyl $O^{2'}$,$O^{7'}$-Diacetylgriseolate (3)——Compound **2** (4.07 g, 10 mmol) was dissolved in 50 ml of pyridine, then 3.06 g (30 mmol) of acetic anhydride was added under ice-cooling and the solution was stirred for 3 h at room temperature. Then 10 ml of water was added at 0—5 °C. The solvent was removed under reduced pressure. Addition and evaporation of 30 ml of ethanol were repeated 3 times. The residue was dissolved in 100 ml of methylene chloride and was washed with 30 ml each of 0.2 N aqueous HCl, saturated aqueous sodium chloride, and a 5% aqueous solution of sodium bicarbonate successively. The organic layer was dried over magnesium sulfate and was concentrated to about 30 ml. This solution was allowed to stand in a refrigerator for 17 h. The resulting crystals were collected by filtration to give 2.73 g (56%) of **3**, mp 123—5 °C. *Anal.* Calcd for $C_{20}H_{21}N_5O_{10} \cdot 5/2H_2O$: C, 44.77; H, 4.88; N, 13.05. Found: C, 44.87; H, 4.59; N, 12.95. UV (methanol) λ_{max} nm (ε): 257 (17100). NMR (d_6-DMSO) δ ppm: 2.19 (6H, s, CH_3CO–), 3.69 and 3.78 (each 3H, s, CH_3O–), 5.17 (1H, d, J=3.0 Hz, 5′-H), 5.66 (1H, d, J= 6.0 Hz, 2′-H), 5.73 (1H, s, 7′-H), 6.31 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.89 (1H, s, 1′-H), 7.41 (2H, br s, NH_2), 8.23 and 8.36 (each 1H, s, 2- and 8-H).

Dimethyl $O^{2'}$,$O^{7'}$-Diacetyl-6-deamino-6-hydroxygriseolate (4)——Compound **3** (1.82 g, 3.7 mmol) was dissolved in an 80% (v/v) aqueous solution of acetic acid. Sodium nitrite (2.55 g, 37 mmol) was added to the resulting solution under ice-cooling, and the mixture was left standing for 16 h in a tightly stoppered vessel. At this stage, some of the starting material was shown to be still present by TLC, and therefore a further 1 g (14.5 mmol) of sodium nitrite was added and the mixture was left standing for 3 h. The residue obtained by distilling off the solvent under reduced pressure was dissolved in acetone; toluene was added to this solution and then distilled off. This process was repeated 3 times. The residue was dissolved in a mixture of water and chloroform. The organic layer was washed with an aqueous solution of sodium bicarbonate and then with a saturated aqueous solution of sodium chloride and dried over anhydrous magnesium sulfate. The solvent was distilled off to yield a pale brown glass-like substance. This substance was purified by silica gel column chromatography (5% methanol in methylene chloride) and dissolved in a small quantity of acetone; benzene was then added, and the mixture was left standing. The resulting white crystals were collected by filtration to yield 1.28 g (70%) of **4** in the form of white crystals, mp 252—252.5 °C (dec.). *Anal.* Calcd for $C_{20}H_{20}N_4O_{11}$: C, 48.78; H, 4.09; N, 11.38. Found: C, 48.91; H, 3.91; N, 11.35. UV [50% (v/v) aqueous methanol] λ_{max} nm (ε): 243 (12700), 248 shoulder (12500), 270 shoulder (4300). NMR (d_6-DMSO) δ ppm: 2.19 (6H, s, CH_3), 5.22 (1H, d, J=3.0 Hz, 5′-H), 5.62 (1H, d, J=6.0 Hz, 2′-H), 5.73 (1H, s, 7′-H), 6.13 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.88 (1H, s, 1′-H), 8.18 and 8.34 (each 1H, s, 2- and 8-H).

6-Deamino-6-hydroxygriseolic Acid (5)——Griseolic acid (**1**) (5.31 g, 14.0 mmol) was dissolved with heating in an 80% (v/v) aqueous solution of acetic acid, and the solution was then cooled to room temperature. Sodium nitrite (9.60 g, 139 mmol) was added. The air in the vessel containing the solution was replaced by nitrogen and the vessel was tightly stoppered and left standing for 16 h. The solvent was distilled off under reduced pressure to yield a residue, to which ethanol was added and then distilled off; this process was repeated until the mixture no longer smelled of acetic acid. The residue was dissolved in 50 ml of water and the pH of the solution was adjusted to a value of 1.0 with concentrated hydrochloric acid under ice-cooling. The solution was left standing for 16 h in a refrigerator and the

precipitate was collected by filtration, washed with a small quantity of water, and then recrystallized from aqueous acetone to yield 1.66 g (31%) of **5**. On concentration of the mother liquor, 2.20 g (41%) of crude crystals were obtained. These crude crystals were recrystallized from aqueous acetone to yield a further 1.2 g (23%) of **5**, mp 256—258 °C. *Anal.* Calcd for $C_{14}H_{12}N_4O_9 \cdot 1/2H_2O$: C, 43.19; H, 3.45; N, 14.39. Found: C, 43.21; H, 3.26; N, 14.42. UV λ_{max} nm (ε): (H_2O): 247 (11800), 270 shoulder (3700), (0.1 N HCl): 248 (12300), 270 shoulder (3900), (0.1 N NaOH): 253 (13000), 274 shoulder (5300). NMR (d_6-DMSO) δ ppm: 4.50 (1H, s, 7′-H), 4.57 (1H, d, J=6.0 Hz, 2′-H), 5.12 (1H, d, J=3.0 Hz, 5′-H), 5.88 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.50 (1H, s, 1′-H), 8.17 and 8.33 (each 1H, s, 2- and 8-H).

Dibenzhydryl Griseolate (6)——Griseolic acid (**1**) (10 g, 26.4 mmol) was suspended in a mixture of 400 ml of acetone and 50 ml of water. To this was added a solution of 15.4 g (79.3 mmol) of diphenyldiazomethane in 100 ml of acetone, and the mixture was stirred for 16 h at room temperature. The reaction product was added dropwise to 2 l of hexane. The resulting powdery substance was collected by filtration and then washed with 500 ml of hexane and dried at 55—65 °C for 10 h under a pressure of 1—2 mmHg (100—250 Pa) to yield 17.95 g (96%) of **6** as a white powder. *Anal.* Calcd for $C_{40}H_{33}N_5O_8 \cdot 1/2H_2O$: C, 66.66; H, 4.75; N, 9.72. Found: C, 66.67; H, 4.60; N, 9.59. UV (methanol) λ_{max} nm (ε): 258 (11200). NMR (d_6-DMSO) δ ppm: 4.68 (1H, d, J=6.0 Hz, 2′-H), 4.93 (1H, s, 7′-H), 5.27 (1H, d, J=3.0 Hz, 5′-H), 6.35 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.58 (1H, s, 1′-H), 6.79 and 6.73 (each 1H, s, CH), 7.1—7.6 (br m, 20H, phenyl-H), 8.18 and 8.37 (each 1H, s, 2- and 8-H).

Dibenzhydryl 6-Desamino-6-hydroxygriseolate (7)——Compound **6** (28.5 g, 40 mmol) was dissolved with slight heating in 560 ml of acetic acid, and 140 ml of water was added. The air in the reaction vessel was replaced by nitrogen under ice-cooling and sodium nitrite (55 g, 800 mmol) was added bit by bit without stirring. The mixture was left standing overnight, tightly stoppered. Water was then added to the reaction product and the solvent was distilled off. This process was repeated and the precipitate was collected by filtration then thoroughly washed with water to yield 28.5 g (100%) of **7**, mp 167—170 °C. *Anal.* Calcd for $C_{40}H_{32}N_4O_9 \cdot 5/2H_2O$: C, 63.40; H, 4.92; N, 7.39. Found: C, 63.65; H, 4.62; N, 7.08. UV (methanol) λ_{max} nm (ε): 242 (13500), 248 shoulder (12700), 270 shoulder (5200). NMR (d_6-DMSO) δ ppm: 4.60 (1H, d, J=6.0 Hz, 2′-H), 4.90 (1H, s, 7′-H), 5.31 (1H, d, J=3.0 Hz, 5′-H), 6.06 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.53 (1H, s, 1′-H), 6.71 and 6.79 (each 1H, s, CH), 7.1—7.5 (20H, br m, phenyl-H), 8.06 and 8.28 (each 1H, s, 2- and 8-H).

Dibenzhydryl $O^{2'}$,$O^{7'}$-Diacetyl-6-deamino-6-hydroxygriseolate (8)——The title compound (80%) was synthesized as white crystals according to the conventional method of acetylation used in the synthesis of compound **3**. *Anal.* Calcd for $C_{44}H_{36}N_4O_{11} \cdot H_2O$: C, 64.86; H, 4.70; N, 6.88. Found: C, 64.97; H, 4.50; N, 6.96. UV (methanol) λ_{max} nm (ε): 242 (12800), 250 (11500), 270 shoulder (4800). NMR (d_6-DMSO) δ ppm: 2.03, 2.17 (each 3H, s, CH_3), 5.35 (1H, d, J=3.0 Hz, 5′-H), 5.66 (1H, d, J=6.0 Hz, 2′-H), 5.97 (1H, s, 7′-H), 6.32 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.79 (1H, s, 1′-H), 6.87 and 6.79 (each 1H, s, CH), 7.1—7.5 (20H, br m, phenyl-H), 8.00 and 8.29 (each 1H, s, 2- and 8-H).

Dimethyl $O^{2'}$,$O^{7'}$-Diacetyl-6-chloro-6-deaminogriseolate (9)——Compound **4** (492 mg, 1 mmol) was suspended in 10 ml of ethyl acetate. Phosphorus oxychloride (10 ml, 107 mmol) was added to the suspension, followed by 0.24 ml (1.5 mmol) of *N*,*N*-diethylaniline. The mixture was refluxed for 1.5 h under protection from moisture. The solvent was distilled off under reduced pressure and ethyl acetate was added to the residue and then distilled off; this process was repeated 3 times. The residue was dissolved in 20 ml of ethyl acetate and the solution was washed with a saturated aqueous solution of sodium bicarbonate and with a saturated aqueous solution of sodium chloride. The organic layer was dried over anhydrous magnesium sulfate and the solvent was distilled off. The resulting residue was purified by silica gel chromatography (2 : 1 solution of ethyl acetate and methylene chloride) to yield 482 mg (94%) of **9** as a white powder. *Anal.* Calcd for $C_{20}H_{19}ClN_4O_{10} \cdot 1/2H_2O$: C, 46.21; H, 3.88; Cl, 6.82; N, 10.78. Found: C, 46.34; H, 3.76; Cl, 6.90; N, 10.61. UV (methanol) λ_{max} nm (ε): 247 (7500), 262 (8700). NMR (d_6-DMSO) δ ppm: 2.21 (6H, s, CH_3CO–), 4.70, 4.81 (each 3H, s, CH_3O–), 5.28 (1H, d, J=3.0 Hz, 5′-H), 5.73 (1H, d, J=6.0 Hz, 2′-H), 5.76 (1H, s, 7′-H), 6.21 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 7.06 (1H, s, 1′-H), 8.92 (2H, s, 2- and 8-H).

$O^{2'}$,$O^{7'}$-Diacetyl-6-chloro-6-deaminogriseolic Acid (10)——Ethyl acetate (1.8 ml), phosphorus oxychloride (180 ml, 1.93 mol) and *N*,*N*-diethylaniline (5.4 ml, 33.7 mmol) were added to compound **8** (18 g, 22.6 mmol), and the mixture was refluxed for 20 min. The solvent was then distilled off and ethyl acetate and water were added to the residue. The ethyl acetate layer was separated and extracted twice, each time with a 10% (w/v) aqueous solution of sodium bicarbonate. The pH of the aqueous layer was adjusted to 2, and the resulting precipitate was collected by filtration to yield 6.9 g (63%) of **10** as a white powder after lyophilization from benzene. Sodium chloride was added to saturate the filtrate, which was extracted twice with ethyl acetate to yield a further 2.4 g (22%) of **10**. *Anal.* Calcd for $C_{18}H_{15}ClN_4O_{10} \cdot H_2O$: C, 43.17; H, 3.42; Cl, 7.08; N, 11.19. Found: C, 43.45; H, 3.41; Cl, 7.15; N, 10.89. UV λ_{max} nm (ε): (50% aqueous methanol); 246.5 (7400), 262 (8700), [0.2 N HCl : methanol (1 : 1)]; 246.5 (7700), 262 (8900), [0.2 N NaOH : methanol (1 : 1)]; 248 (11300). NMR (d_6-DMSO) δ ppm: 2.17 and 2.19 (each 3H, s, CH_3), 5.23 (1H, d, J=3.0 Hz, 5′-H), 5.68 (1H, s, ′-H), 5.72 (1H, d, J=6.0 Hz, 2′-H), 6.17 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 7.01 (1H, s, 1′-H), 8.91 (2H, s, 2- and 8-H).

Dibenzhydryl $O^{2'}$,$O^{7'}$-Diacetyl-6-chloro-6-deaminogriseolate (11)——Compound **8** (12.2 g, 15.3 mmol) was dissolved in 120 ml of ethyl acetate (dried with a molecular sieve). Phosphorus oxychloride (120 ml, 1.29 mol) and 3.66 ml (23 mmol) of *N*,*N*-diethylaniline were added to the solution, and the mixture was refluxed for 3 h. The solvent was distilled off and the residue was dissolved in a mixture of ethyl acetate and water. The ethyl acetate layer was

separated and washed first with dilute hydrochloric acid and then with a saturated aqueous solution of sodium chloride. The solution was dried over anhydrous magnesium sulfate. The solvent was distilled off and the residue was purified by silica gel column chromatography (2% methanol in methylene chloride) to yield 10.2 g (82%) of **11** as a white powder after lyophilization from benzene. *Anal.* Calcd for $C_{44}H_{35}ClN_4O_{10}$: C, 64.83; H, 4.33; Cl, 4.35; N, 6.87. Found: C, 64.59; H, 4.50; Cl, 4.30; N, 6.82. UV (methanol) λ_{max} nm (ε): 247 (7700), 258 shoulder (8400), 263 (9000). NMR (d_6-DMSO) δ ppm: 2.07, 2.19 (each 3H, s, CH_3), 5.40 (1H, d, J=3.0 Hz, 5′-H), 5.76 (1H, d, J=6.0 Hz, 2′-H), 6.01 (1H, s, 7′-H), 6.41 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.79 and 6.83 (each 1H, s, CH), 7.07 (1H, s, 1′-H), 7.1—7.6 (20H, br m, phenyl-H), 8.72 and 8.87 (each 1H, s, 2- and 8-H).

Dibenzhydryl 6-Chloro-6-deaminogriseolate (12)——A 20% (v/v) methanolic solution of ammonia (150 ml) was added to 5 g (6.13 mmol) of compound **11** and the mixture was stirred for 50 min at room temperature. The solvent was distilled off at a low temperature and the residue was purified by column chromatography (3% methanol in methylene chloride) to yield 3.7 g (83%) of **12** as a white powder after lyophilization from benzene. *Anal.* Calcd for $C_{40}H_{31}ClN_4O_8 \cdot 1/2H_2O$: C, 64.90; H, 4.36; Cl, 4.85; N, 7.57. Found: C, 65.19; H, 4.35; Cl, 4.77; N, 7.38. UV (methanol) λ_{max} nm (ε): 247 shoulder (8100), 258 shoulder (9300), 263 (9800). NMR (d_6-DMSO) δ ppm: 4.72 (1H, dd, J=4.8 and 6.0 Hz, 2′-H), 4.94 (1H, d, J=9.0 Hz, 7′-H), 5.38 (1H, d, J=3.0 Hz, 5′-H), 5.69 (1H, d, J=9.0 Hz, 7′-OH), 6.17 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.37 (1H, d, J=4.8 Hz, 2′-OH), 6.72 and 6.75 (each 1H, s, CH), 6.81 (1H, s, 1′-H), 7.1—7.6 (20H, br m, phenyl-H), 8.80 and 8.91 (each 1H, s, 2- and 8-H).

6-Chloro-6-deaminogriseolic Acid (13)——Compound **12** (3.7 g, 5.06 mmol) was dissolved in 25 ml of anisole, and 25 ml of trifluoroacetic acid was added under ice-cooling. The mixture was left standing at room temperature for 15 min. The solvent was distilled off. A mixture of acetone and toluene was added to the residue and distilled off; this process was repeated. The residue was dissolved in a small quantity of acetone and this solution was poured into 300 ml of hexane to yield a powdery substance. This substance was dissolved in a 10% (w/v) aqueous solution of sodium bicarbonate and the pH of the resulting solution was adjusted to 2 with 3 N aqueous HCl. The solution was purified by reverse-phase chromatography using a Merck Rp-8 prepacked column (15% acetonitrile and 0.02% acetic acid in H_2O) to yield 1.46 g (72%) of **13** as a white powder after lyophilization. *Anal.* Calcd for $C_{14}H_{11}ClN_4O_8 \cdot 3/2H_2O$: C, 39.50; H, 3.31; Cl, 8.33; N, 13.16. Found: C, 39.74; H, 3.43; Cl, 8.30; N, 13.10. UV λ_{max} nm (ε): (H_2O); 250 shoulder (7200), 263 (9200), (0.1 N HCl); 249 shoulder (7500), 263 (9500), (0.1 N NaOH); 264 (9700). NMR (d_6-DMSO) δ ppm: 4.21 (1H, s, 7′-H), 4.59 (1H, d, J=6.0 Hz, 2′-H), 4.96 (1H, d, J=3.0 Hz, 5′-H), 5.87 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.58 (1H, s, 1′-H), 8.88 (2H, s, 2- and 8-H). $[\alpha]_D^{25}$ −5° (c=0.5, DMSO).

6-Deamino-6-methoxygriseolic Acid (14a)——Compound **9** (408 mg, 0.80 mmol) was suspended in 16 ml of anhydrous methanol and the mixture was cooled to between −20 and −10 °C. A 1 N methanolic solution of sodium methoxide (4 ml, 4 mmol) was added and the mixture was stirred while keeping the temperature below 0 °C for 2—2.5 h. The solvent was then distilled off and 4 ml of water was added. The mixture was stirred for 3 h at room temperature and its pH was adjusted to 2 with 3 N aqueous HCl. The precipitated crystals were collected by filtration to yield 251 mg (80%) of **14a**. mp 209—212 °C (dec). *Anal.* Calcd for $C_{15}H_{14}N_4O_9 \cdot 1/2H_2O$: C, 44.67; H, 3.75; N, 13.89. Found: C, 44.41; H, 3.55; N, 13.79. UV λ_{max} nm (ε): (H_2O); 246 (12700), (0.1 N HCl); 246 (12600), (0.1 N NaOH); 248 (13100). NMR (d_6-DMSO) δ ppm: 4.14 (3H, s, CH_3), 4.53 (1H, s, 7′-H), 4.63 (1H, d, J=6.0 Hz, 2′-H), 5.13 (1H, d, J=3.0 Hz, 5′-H), 6.03 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.60 (1H, s, 1′-H), 8.61 and 8.63 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ +17.4° (c=0.5, DMSO).

6-Deamino-6-mercaptogriseolic Acid (14b)——Compound **10** (1.0 g, 2.07 mmol) was dissolved in 30 ml of dimethylformamide. The air in the reaction vessel was replaced by nitrogen and 1.5 g (26.7 mmol) of sodium hydrosulfide was added to the solution. The mixture was stirred overnight at room temperature. The mixture was acidified with concentrated hydrochloric acid under a stream of nitrogen gas and the solvent was distilled off. A 1 N aqueous NaOH solution (20 ml, 20 mmol) was added to the residue and the mixture was kept standing overnight at room temperature. The pH of the solution was adjusted to 2 with concentrated hydrochloric acid and the mixture was purified by reverse-phase chromatography (30% acetonitrile in H_2O) through an Rp-8 prepacked column (Merck) to yield 0.44 g (54%) of **14b** as a white powder after lyophilization. *Anal.* Calcd for $C_{14}H_{12}N_4O_8S \cdot 6/5H_2O$: C, 40.23; H, 3.47; N, 13.41; S, 7.67. Found: C, 40.13; H, 3.62; N, 13.13; S, 7.97. UV λ_{max} nm (ε): (H_2O); 321 (20400), (0.1 N HCl); 322 (19000), (0.1 N NaOH); 310 (19200). NMR (d_6-DMSO) δ ppm: 4.51 (1H, s, 7′-H), 4.58 (1H, d, J=6.0 Hz, 2′-H), 5.14 (1H, d, J=3.0 Hz, 5′-H), 5.81 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.51 (1H, s, 1′-H), 8.30 and 8.50 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −15.5° (c=0.2, DMSO).

6-Deamino-6-methylmercaptogriseolic Acid (14c)——Compound **14b** (0.44 g, 1.11 mmol) was dissolved in 20 ml of a 1 N aqueous NaOH solution, and 0.4 ml (6.4 mmol) of methyl iodide was added to the resulting solution. The mixture was left standing overnight at room temperature. The excess methyl iodide was distilled off and the pH of the residue was adjusted to 2.5. The resulting solution was purified by reverse-phase chromatography (12% acetonitrile, 0.02% acetic acid in H_2O) through an Rp-8 prepacked column (Merck), to yield 0.4 g (88%) of **14c** as a white powder after lyophilization. *Anal.* Calcd for $C_{15}H_{14}N_4O_8S \cdot 3/2H_2O$: C, 41.19; H, 3.92; N, 12.81; S, 7.33. Found: C, 41.37; H, 3.66; N, 12.60; S, 7.61. UV λ_{max} nm (ε): (H_2O); 299 (20500), (0.1 N HCl); 287 shoulder (17800), 292 (18700), (0.1 N NaOH); 287 (21100), 291 (21000). NMR (d_6-DMSO) δ ppm: 2.69 (3H, s, CH_3), 4.53 (1H, s, 7′-H), 4.64 (1H, d, J=6.0 Hz, 2′-H), 5.16 (1H, d, J=3.0 Hz, 5′-H), 6.03 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.62 (1H, s, 1′-H), 8.68 and 8.83

(each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ +0.6° (c=0.5, DMSO).

N^6-Methylgriseolic Acid (14d)——Compound **10** (2.5 g, 5.18 mmol) was dissolved in 20 ml of methanol. A 40% (w/v) methanolic solution of methylamine (4 ml, 3.52 mmol) was added to the resulting solution and the mixture was stirred for 5 h at room temperature. The solvent was distilled off and 20 ml of a 1 N aqueous solution of sodium hydroxide was added to the residue. The mixture was left standing overnight at room temperature. The pH of the mixture was adjusted to 2 with concentrated hydrochloric acid and the mixture was left standing overnight in a cool place. The precipitated crystals were collected by filtration to yield 1.45 g (71%) of **14d**. mp 214—220 °C (dec.). *Anal.* Calcd for $C_{15}H_{15}N_5O_8$: C, 45.81; H, 3.84; N, 17.80. Found: C, 45.83; H, 4.86; N, 17.84. UV λ_{max} nm (ε): (H_2O); 265 (17200), (0.1 N HCl); 261 (19200), (0.1 N NaOH); 265 (17800). NMR (d_6-DMSO) δ ppm: 3.02 (3H, br d, J=4.5 Hz, CH_3), 4.52 (1H, s, 7′-H), 4.60 (1H, d, J=6.0 Hz, 2′-H), 5.10 (1H, d, J=3.0 Hz, 5′-H), 6.08 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.53 (1H, s, 1′-H), 7.78 (1H, br d, J=4.5 Hz, NH), 8.31 and 8.35 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −3.6° (c=0.5, DMSO).

Synthesis of 14e—l——The procedure described for the synthesis of N^6-methylgriseolic acid (**14d**) was repeated, except that methylamine was replaced by appropriate amines, to prepare **14e—l**.

N^6-Benzylgriseolic Acid (14e)——mp 198—200 °C (dec.). *Anal.* Calcd for $C_{21}H_{19}N_5O_8 \cdot 3/2H_2O$: C, 50.81; H, 4.26; N, 14.11. Found: C, 50.52; H, 4.52; N, 13.92. UV λ_{max} nm (ε): [50% (v/v) aqueous methanol]; 267 (22000), [0.2 N HCl : methanol (1 : 1)]; 264 (22800), 270 shoulder (21100), [0.2 N NaOH : methanol (1 : 1)]; 267 (22700). NMR (d_6-DMSO) δ ppm: 4.53 (1H, s, 7′-H), 4.61 (1H, d, J=6.0 Hz, 2′-H), 4.83 (2H, br m, CH_2), 5.10 (1H, d, J=3.0 Hz, 5′-H), 6.07 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.54 (1H, s, 1′-H), 7.2—7.5 (5H, br m, phenyl-H), 8.30 and 8.40 (each 1H, s, 2- and 8-H), 8.46 (1H, br s, NH). $[\alpha]_D^{25}$ +0.6° (c=0.5, DMSO).

N^6-Phenethylgriseolic Acid (14f)——mp 187—190 °C (dec.). *Anal.* Calcd for $C_{22}H_{21}N_5O_8 \cdot H_2O$: C, 52.70; H, 4.62; N, 13.97. Found: C, 52.47; H, 4.61; N, 13.94. UV λ_{max} nm (ε): [50% (v/v) aqueous methanol]; 268 (18400), [0.2 N HCl : methanol (1 : 1)]; 263 (18400), [0.2 N NaOH : methanol (1 : 1)]; 268 (19600). NMR (d_6-DMSO) δ ppm: 2.8—4.1 (4H, m, CH_2–CH_2), 4.55 (1H, s, 7′-H), 4.59 (1H, d, J=6.0 Hz, 2′-H), 5.10 (1H, d, J=3.0 Hz, 5′-H), 6.04 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.50 (1H, s, 1′-H), 7.29 (5H, s, phenyl-H), 7.86 (1H, br m, NH), 8.29 and 8.33 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ +0.6° (c=0.5, DMSO).

N^6-(α-Naphthyl)methylgriseolic Acid (14g)——mp 193—195 °C (dec.). *Anal.* Calcd for $C_{25}H_{21}N_5O_8 \cdot 5/3H_2O$: C, 54.65; H, 4.46; N, 12.74. Found: C, 54.46; H, 4.19; N, 12.44. UV λ_{max} nm (ε): [50% (v/v) aqueous methanol]; 271 (24000), 280 (23300), 293 shoulder (13000), [0.2 N HCl : methanol (1 : 1)]; 264 shoulder (21700), 271 (22800), 281 (22100), [0.2 N NaOH : methanol (1 : 1)]; 271 (23300), 281 (22800), 293 shoulder (12800). NMR (d_6-DMSO) δ ppm: 4.44 (1H, s, 7′-H), 4.59 (1H, d, J=6.0 Hz, 2′-H), 5.05 (1H, d, J=3.0 Hz, 5′-H), 5.24 (2H, m, CH_2), 6.03 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.51 (1H, s, 1′-H), 7.3—8.6 (8H, br m, phenyl-H and NH), 8.30 and 8.38 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −9.6° (c=0.5, DMSO).

6-Deamino-6-piperidinogriseolic Acid (14h)——A white powder after lyophilization. *Anal.* Calcd for $C_{19}H_{21}N_5O_8 \cdot H_2O$: C, 49.03; H, 4.98; N, 15.05. Found: C, 49.32; H, 4.69; N, 15.27. UV λ_{max} nm (ε): (H_2O); 280 (21100), (0.1 N HCl); 271 (20800), (0.1 N NaOH); 282 (21600). NMR (d_6-DMSO) δ ppm: 1.3—1.9, 3.9—4.3 (10H, each br s, piperidino), 4.52 (1H, s, 7′-H), 4.58 (1H, d, J=6.0 Hz, 2′-H), 5.11 (1H, d, J=3.0 Hz, 5′-H), 6.02 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.52 (1H, s, 1′-H), 8.29 and 8.36 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −5° (c=0.5, DMSO).

6-Deamino-6-morpholinogriseolic Acid (14i)——A white powder after lyophilization. *Anal.* Calcd for $C_{18}H_{19}N_5O_9 \cdot H_2O$: C, 46.26; H, 4.53; N, 14.98. Found: C, 45.98; H, 4.23; N, 14.78. UV λ_{max} nm (ε): (H_2O); 279 (21500)., (0.1 N HCl); 273 (19800), (0.1 N NaOH); 279 (21800). NMR (d_6-DMSO) δ ppm: 3.6—4.4 (8H, m, morpholino), 4.51 (1H, s, 7′-H), 4.57 (1H, d, J=6.0 Hz, 2′-H), 5.11 (1H, d, J=3.0 Hz, 5′-H), 6.01 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.53 (1H, s, 1′-H), 8.33 and 8.40 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −3.4° (c=0.5, DMSO).

N^6-Phenylgriseolic Acid (14j)——mp 205—208 °C (dec.). *Anal.* Calcd for $C_{20}H_{17}N_5O_8 \cdot 2H_2O$: C, 48.88; H, 4.31; N, 14.25. Found: C, 48.59; H, 4.38; N, 14.05. UV λ_{max} nm (ε): [50% (v/v) aqueous methanol]; 293 (23200), [0.2 N HCl : methanol (1 : 1)]; 275 (18900), [0.2 N NaOH : methanol (1 : 1)]; 293 (24400). NMR (d_6-DMSO) δ ppm: 4.54 (1H, s, 7′-H), 4.66 (1H, d, J=6.0 Hz, 2′-H), 5.14 (1H, d, J=3.0 Hz, 5′-H), 6.10 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.59 (1H, s, 1′-H), 6.9—8.1 (5H, m, phenyl-H), 8.46 and 8.56 (each 1H, s, 2- and 8-H), 9.98 (1H, s, NH). $[\alpha]_D^{25}$ −0.4° (c=0.5, DMSO).

N^6-(2-Hydroxyethyl)griseolic Acid (14k)——A white powder after lyophilization. *Anal.* Calcd for $C_{16}H_{17}N_5O_9 \cdot 8/5H_2O$: C, 42.50; H, 4.50; N, 15.49. Found: C, 42.35; H, 4.37; N, 15.53. UV λ_{max} nm (ε): (H_2O); 265 (18400), (0.1 N HCl); 263 (19200), (0.1 N NaOH); 277 (19000). NMR (d_6-DMSO) δ ppm: 3.59 (4H, br s, CH_2–CH_2), 4.48 (1H, s, 7′-H), 4.57 (1H, d, J=6.0 Hz, 2′-H), 5.08 (1H, d, J=3.0 Hz, 5′-H), 6.04 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.51 (1H, s, 1′-H), 7.69 (1H, br s, NH), 8.28 and 8.36 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −0.2° (c=0.5, DMSO).

N^6-(2-Aminoethyl)griseolic Acid (14l)——A white powder after lyophilization. *Anal.* Calcd for $C_{16}H_{18}N_6O_8 \cdot 3H_2O$: C, 40.34; H, 5.08; N, 17.64. Found: C, 40.53; H, 4.85; N, 17.40. UV λ_{max} nm (ε): (H_2O); 264 (18700), (0.1 N HCl); 262 (17300), (0.1 N NaOH); 267 (19300). NMR (d_6-DMSO) δ ppm: 3.0—4.4 (4H, m, CH_2–CH_2), 4.15 (1H, s, 7′-H), 4.48 (1H, d, J=6.0 Hz, 2′-H), 4.86 (1H, d, J=3.0 Hz, 5′-H), 5.89 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.45 (1H, s, 1′-H), 8.0 (1H, br s, NH), 8.32 and 8.39 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −3.2° (c=0.5, 0.1 N HCl).

N^6,N^6-Dimethylgriseolic Acid (14m)——Compound **11** (1.63 g, 2 mmol) was dissolved in 10 ml of acetone, and

4.4 ml of 40% aqueous dimethylamine was added to the solution. The mixture was stirred for 17 h at room temperature. The solvent was removed under reduced pressure and the resulting residue was dissolved in 50 ml of ethyl acetate and 40 ml of 0.3 N hydrochloric acid. The organic layer was separated and washed with 40 ml of saturated aqueous sodium chloride. The organic layer was dried over magnesium sulfate and the solvent was distilled off under reduced pressure to give 1.48 g of a caramel-like substance. This was dissolved in 20 ml of methanol and 20 ml of 2 N aqueous NaOH. The solution was stirred at room temperature for 17 h, then the solvent was distilled off under reduced pressure and the residue was dissolved in 30 ml each of methylene chloride and water. The water layer was washed with an additional 30 ml of methylene chloride and the pH value of the solution was adjusted to 2.0 with concentrated hydrochloric acid. This aqueous solution was purified by reverse-phase chromatography through an Rp-8 prepacked column (Merck) eluted with 10% acetonitrile aqueous solution to give 534 mg (66%) of **14m** as a white powder. *Anal.* Calcd for $C_{16}H_{17}N_5O_8 \cdot 3/2H_2O$: C, 44.24; H, 4.63; N, 16.12. Found: C, 44.24; H, 4.62; N, 16.41. UV λ_{max} nm (ε): (H_2O); 273 (19800), (0.1 N HCl); 267 (20100), (0.1 N NaOH); 276 (19900). NMR (d_6-DMSO) δ ppm: 3.46 (6H, s, CH_3), 4.52 (1H, s, 7′-H), 4.58 (1H, d, J=6.0 Hz, 2′-H), 5.11 (1H, d, J=3.0 Hz, 5′-H), 6.02 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.53 (1H, s, 1′-H), 8.30 and 8.38 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −5° (c=0.5, DMSO).

6-Deaminogriseolic Acid (14n)——Compound **12** (1.83 g, 2.50 mmol) was dissolved in an 80% (v/v) aqueous solution of acetic acid, and 5 g (0.0765 atom) of zinc powder was added to the solution. The mixture was stirred at room temperature for 3—4 h. The solvent was distilled off and the residue was dissolved in a mixture of ethyl acetate and water. The ethyl acetate layer was separated and washed with 10% (w/v) aqueous sodium bicarbonate and with saturated aqueous sodium chloride, after which it was dried over anhydrous magnesium sulfate. The solution was filtered and the solvent was distilled off. The residue was purified by silica gel chromatography (5% methanol in methylene chloride) to give 1.2 g (69%) of the dibenzhydryl ester of **14n**. This dibenzhydryl ester (1.2 g, 1.72 mmol) was dissolved in 6 ml of anisole and 6 ml of trifluoroacetic acid under ice-cooling. The mixture was left standing for 15 minutes and the solvent was distilled off. A mixture of acetone and toluene was added to the residue and this solvent was distilled off. This process was repeated, and a small quantity of benzene was added to the solution, after which it was added, with stirring, to 200 ml of hexane to precipitate a powder. The powder was collected by filtration and dissolved in aqueous sodium bicarbonate. The pH of the resulting solution was adjusted to 2, after which the solution was purified by reverse-phase chromatography (4% acetonitrile, 0.02% acetic acid in H_2O) through an Rp-8 prepacked column (Merck) to give 0.5 g (80%) of **14n** as a white powder after lyophilization. *Anal.* Calcd for $C_{14}H_{12}N_4O_8 \cdot 6/5_2O$: C, 43.57; H, 3.76; N, 14.52. Found: C, 43.51; H, 3.66; N, 14.50. UV λ_{max} nm (ε): (H_2O); 262 (7400), (0.1 N HCl); 262 (6900), (0.1 N NaOH); 262 (7900). NMR (d_6-DMSO) δ ppm: 4.53 (1H, s, 7′-H), 4.72 (1H, d, J=6.0 Hz, 2′-H), 5.15 (1H, d, J=3.0 Hz, 5′-H), 6.05 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.66 (1H, s, 1′-H), 8.83, 9.06, and 9.27 (each 1H, s, 2-, 6- and 8-H).

6-Deamino-6-hydrazinogriseolic Acid (14o)——Compound **13** (1.0 g, 2.51 mmol) was dissolved in 120 ml of methanol. Hydrazine hydrate (1.85 ml, 37.8 mmol) was added to the solution and the mixture was stirred at room temperature for 16—20 h. The solvent was then distilled off and the residue was dissolved in 20 ml of water. The pH was adjusted to 2 with 3 N hydrochloric acid. The solution was purified by reverse-phase chromatography (0.5% acetonilrile, 0.02% acetic acid in H_2O) through an Rp-8 prepacked column (Merck), to yield 0.5 g (51%) of **14o** as a white powder after lyophilization. *Anal.* Calcd for $C_{14}H_{14}N_6O_8 \cdot H_2O$: C, 40.78; H, 3.91; N, 20.38. Found: C, 40.85; H, 3.75; N, 20.07. UV λ_{max} nm (ε): (H_2O); 265 (15500), (0.1 N HCl); 260 (17100), (0.1 N NaOH); 265 (12900). NMR (d_6-DMSO) δ ppm: 4.50 (1H, s, 7′-H), 4.59 (1H, d, J=6.0 Hz, 2′-H), 5.09 (1H, d, J=3.0 Hz, 5′-H), 6.05 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.52 (1H, s, 1′-H), 8.34 and 8.37 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −0.8° (c=0.5, DMSO).

6-Azido-6-deaminogriseolic Acid (14p)——Compound **14o** (0.26 g, 0.66 mmol) was dissolved in 4 ml of 5% (v/v) aqueous acetic acid, to which was added 0.052 g (0.75 mmol) of sodium nitrite dissolved in 10 ml of water under ice-cooling and under a stream of nitrogen gas. The mixture was stirred for 2 h under the same conditions. The pH of the mixture was then adjusted to 2.0 with 3 N hydrochloric acid. The mixture was purified by reverse-phase chromatography (2% acetonitrile, 0.02% acetic acid in H_2O) through an Rp-8 prepacked column (Merck) to give 0.18 g (67%) of **14p** as a white powder after lyophilization. *Anal.* Calcd for $C_{14}H_{11}N_7O_8 \cdot 4/5H_2O$: C, 40.07; H, 3.03; N, 23.36. Found: C, 40.37; H, 2.98; N, 23.06. UV λ_{max} nm (ε): (H_2O); 251 (5000), 259 (5200), 287 (8800), 300 shoulder (5100), (0.1 N HCl); 250 (4800), 259 (5000), 287 (8500), 300 shoulder (4900). NMR (d_6-DMSO) δ ppm: 4.53 (1H, s, 7′-H), 4.66 (1H, d, J=6.0 Hz, 2′-H), 5.21 (1H, d, J=3.0 Hz, 5′-H), 5.90 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.75 (1H, s, 1′-H), 8.89 and 10.28 (each 1H, s, 2- and 8-H). IR: 2150 cm^{-1} (N_3). $[\alpha]_D^{25}$ −6.4° (c=0.5, DMSO).

***N*^{6}-Methoxygriseolic Acid (14q)**——Compound **13** (1.0 g, 2.51 mmol) was suspended in methanol, and the air in the reaction vessel containing the suspension was replaced by nitrogen. Methoxyamine (2.3 g, 48.9 mmol) was added to this suspension and the mixture was heated at 60 °C for 7 h, and then a further 1.8 g (38 mmol) of methoxyamine was added. The reaction mixture and the mixture was heated at 80 °C for a further 15 h. The solvent was distilled off and the pH of an aqueous solution of the residue was adjusted to 3.0. The solution was then purified by reverse-phase chromatography (4% acetonitrile, 0.02% acetic acid in H_2O) through an Rp-8 prepacked column (Merck) to give 0.28 g (27%) of **14q** as a white powder after lyophilization. *Anal.* Calcd for $C_{15}H_{15}N_5O_9 \cdot 6/5H_2O$: C, 41.81; H, 4.07; N, 16.25. Found: C, 41.54; H, 3.82; N, 16.17. UV λ_{max} nm (ε): (H_2O); 267 (15400), (0.1 N HCl); 265 (17800), (0.1 N NaOH); 281 (12900). NMR (d_6-DMSO) δ ppm: 3.78 (3H, s, CH_3), 4.25 (1H, s, 7′-H), 4.46 (1H, d, J=6.0 Hz, 2′-H),

4.92 (1H, d, J=3.0 Hz, 5′-H), 5.79 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.37 (1H, s, 1′-H), 7.71 and 8.13 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −7.6° (c=0.5, DMSO).

N^1-Methylgriseolic Acid (15a)——Griseolic acid (758 mg, 2.0 mmol) was suspended in 20 ml of dimethylformamide. Methyl iodide (2 ml, 32 mmol) was added to the suspension under ice-cooling. The mixture, tightly stoppered, was stirred for 2 d at room temperature. Ethanol (30 ml) was added to the reaction mixture and the solvent was distilled off under reduced pressure. This process was repeated 3 times. The resulting residue was dissolved in 10 ml of methanol and 10 ml of concentrated aqueous ammonia, and the mixture was left standing, tightly stoppered, for 4 h at room temperature. The solvent was distilled off under reduced pressure and the residue was dissolved in a small quantity of water. The pH of the resulting solution was adjusted to 1 with 1 N hydrochloric acid and the mixture was purified by reverse-phase chromatography (H_2O) through an Rp-8 prepacked column (Merck) to give 400 mg (51%) of **15a** in the form of a pale yellow powder after lyophilization. *Anal.* Calcd for $C_{15}H_{15}N_5O_8 \cdot 3H_2O$: C, 40.27; H, 4.73; N, 15.65. Found: C, 40.31; H, 4.43; N, 15.36. UV λ_{max} nm (ε): (H_2O); 256.5 (14900), (0.1 N HCl); 255.5 (19600), (0.1 N NaOH); 259 (16900), 266 shoulder (15200), 300 shoulder (3600). NMR (d_6-DMSO) δ ppm: 3.80 (3H, s, CH_3), 4.15 (1H, s, 7′-H), 4.50 (1H, d, J=6.0 Hz, 2′-H), 4.91, (1H, d, J=3.0 Hz, 5′-H), 5.75 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.49 (1H, s, 1′-H), 8.66 and 8.71 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −2.4° (c=0.5, DMSO).

N^1-Benzylgriseolic Acid (15b)——This compound was obtained in the same manner as described in the synthesis of **15a** except that benzyl bromide was used instead of methyl iodide. White powder after lyophilization. *Anal.* Calcd for $C_{21}H_{19}N_5O_8 \cdot H_2O$: C, 51.76; H, 4.34; N, 14.37. Found: C, 51.92; H, 4.44; N, 14.45. UV λ_{max} nm (ε): [50% (v/v) aqueous methanol]; 259 (13800), [0.2 N HCl : methanol (1 : 1)]; 258 (14100), [0.2 N NaOH : methanol (1 : 1)]; 261 (14200), 268 shoulder (12900), 308 shoulder (2600). NMR (d_6-DMSO) δ ppm: 4.19 (1H, s, 7′-H), 4.50 (1H, d, J= 6.0 Hz, 2′-H), 4.93 (1H, d, J=3.0 Hz, 5′-H), 5.50 (2H, br s, CH_2), 5.74 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.44 (1H, s, 1′-H), 7.37 (5H, s, phenyl-H), 8.57 and 8.77 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ +2° (c=0.5, DMSO).

N^1-Allylgriseolic Acid (18a)——Compound **16** (4.0 g, 5.03 mmol), which was prepared by acetylation of **6** by the same method as used for the synthesis of **3**, was dissolved in dimethylformamide. Allyl iodide (9.4 ml, 103.4 mmol) was added to the solution under ice-cooling. The resulting mixture was reacted at room temperature for 24 h. The solvent was distilled off under reduced pressure and the residue was extracted with a mixture of saturated aqueous sodium bicarbonate and methylene chloride. The organic layer was separated, dried over anhydrous magnesium sulfate and then evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography [methanol : methylene chloride (5 : 95)] to give 3.2 g (76%) of the N^1-allyl derivative with protected hydroxy and carboxy groups. A 20% (v/v) solution of ammonia in methanol was added to 2.5 g (2.99 mmol) of this compound under ice-cooling and the mixture was left standing for 1 h under ice-cooling. The solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography [methanol : methylene chloride (7 : 93)] to give 2.0 g (89%) of the dibenzhydryl ester of **18a**. Trifluoroacetic acid (10 ml) was added to a solution of 1.5 g (2.0 mmol) of this compound in anisole (10 ml) under ice-cooling. The mixture was left standing for 10 min at room temperature and the solvent was distilled off under reduced pressure. The residue was purified by reverse-phase column chromatography through an Rp-8 prepacked column, using a mixture of acetonitrile, acetic acid and water (3 : 0.02 : 96.98 by volume) as the eluent, to give 370 mg (44%) of **18a** as a white powder after lyophilization. *Anal.* Calcd for $C_{17}H_{17}N_5O_8 \cdot 3/2H_2O$: C, 45.74; H, 4.52; N, 15.69. Found: C, 46.03; H, 4.38; N, 15.38. UV λ_{max} nm (ε): [50% (v/v) aqueous methanol]; 258 (14600), [0.2 N HCl; methanol (1 : 1)]; 257 (14400), [0.2 N NaOH : methanol (1 : 1)]; 243 shoulder (12200), 249.5 (14000), 257 shoulder (11600), 295 (4000). NMR (d_6-DMSO) δ ppm: 4.21 (1H, s, 7′-H), 4.54 (1H, d, J=6.0 Hz, 2′-H), 4.97 (1H, d, J=3.0, 5′-H), 4.75—6.42 (5H, m, allyl-H), 5.78 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.53 (1H, s, 1′-H), 8.65 and 8.70 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ −3.2° (c=0.5, DMSO).

N^1-Butylgriseolic Acid (18b)——Compound **16** (4.0 g, 5.03 mmol) was dissolved in dimethylformamide. Butyl iodide (12.8 ml, 111 mmol) was added to the resulting solution under ice-cooling and the mixture was heated at 70 °C for 48 h. The solvent was evaporated off under reduced pressure and the residue was extracted with a mixture of saturated aqueous sodium bicarbonate and methylene chloride. The organic layer was separated, dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography [5% (v/v) methanol in methylene chloride] to give 3.3 g (77%) of the N^1-butyl derivative with protected hydroxy and carboxyl groups. A 20% (w/v) solution of ammonia in methanol was added under ice-cooling to 2.5 g (2.93 mmol) of this compound and the mixture was left standing for 1 h under ice-cooling. The solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography [a 7% (v/v) solution of methanol in methylene chloride] to give 1.7 g (76%) of the dibenzhydryl ester of **18b**. Trifluoroacetic acid was added to a solution of 1.2 g (1.56 mmol) of this compound in anisole under ice-cooling and the mixture was left standing at room temperature for 10 min. The solvent was distilled off under reduced pressure. Toluene was added to the residue and the solvent was evaporated to dryness under reduced pressure. This process was repeated twice. The residue was purified by reverse-phase column chromatography [a mixture of 3% (v/v) acetonitrile, 0.02% (v/v) acetic acid and water] through an Rp-8 prepacked column to give 400 mg (59%) of **18b** as a white powder after lyophilization. *Anal.* Calcd for $C_{18}H_{21}N_5O_8 \cdot 2H_2O$: C, 45.86; H, 5.34; N, 14.86. Found: C, 46.04; H, 5.04; N, 14.65. UV λ_{max} nm (ε): [50% (v/v) aqueous methanol]; 259 (14700), [0.2 N HCl: methanol (1 : 1)]; 258 (14500); [0.2 N

NaOH : methanol (1 : 1)]; 253 shoulder (11800), 260 (13900), 268 shoulder (11500), 300 shoulder (4300). NMR (d_6-DMSO) δ ppm: 0.8—1.95 (7H, m, $CH_2CH_2CH_3$), 4.1—4.45 (2H, m, CH_2), 4.17 (1H, s, 7′-H), 4.50 (1H, d, J=6.0 Hz, 2′-H), 4.91 (1H, d, J=3.0 Hz, 5′-H), 5.80 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.55 (1H, s, 1′-H), 8.63 and 8.70 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ +5° (c=0.5, DMSO).

N^1-Phenethylgriseolic Acid (18c)——This compound was synthesized by the same method as used for compound **18b** except that butyl iodide was replaced by phenethyl iodide. White powder after lyophilization. *Anal.* Calcd for $C_{22}H_{21}N_5O_8 \cdot 2H_2O$: C, 50.86; H, 4.85; N, 13.48. Found: C, 50.76; H, 4.82; N, 13.32. UV λ_{max} nm (ε): (H_2O); 257 (14400), (0.1 N HCl); 257 (14400), (0.1 N NaOH); 252 shoulder (11400), 259 (14100), 267 shoulder (12400), 290 shoulder (4600). NMR (d_6-DMSO) δ ppm: 2.9—3.2 (2H, m, CH_2), 4.15 (1H, s, 7′-H), 4.3—4.7 (3H, m, 2′-H and CH_2), 4.92 (1H, d, J=3.0 Hz, 5′-H), 5.72 (1H, dd, J=3.0 and 6.0 Hz, 3′-H), 6.45 (1H, s, 1′-H), 7.33 (5H, s, phenyl-H), 8.47 and 8.61 (each 1H, s, 2- and 8-H). $[\alpha]_D^{25}$ +5° (c=0.5, DMSO).

PDE-Inhibitory Activity——The assay was carried out by the same method as previously reported.[1a,b]

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