Synthesis and Phosphodiesterase Activity of Carboxylic Acid Mimetics of Cyclic Guanosine 3′,5′-Monophosphate

Deen Tulshian,* Michael Czarniecki, Ronald J. Doll, and Ho-Sam Ahn

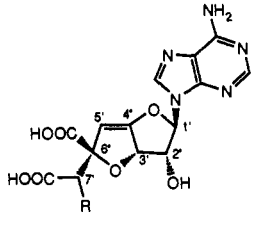
Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033

Received November 19, 1992

Synthetic analogs of the natural product griseolic acid in which a guanine base is substituted for the adenine have been prepared. The best of these compounds inhibits a cyclic guanosine 3′,5′-monophosphate (cGMP) phosphodiesterase preparation with an IC_{50} of 0.34 μM but is a very weak inhibitor of a cyclic adenosine 3′,5′-monophosphate (cAMP) phosphodiesterase. An exploration of stereochemistry indicates that the configuration of the carboxylic acids and the ring fusion in the inhibitors is important for potent cGMP PDE inhibition. PDE inhibition is not sensitive to the presence of the 2′ or 4′ oxygen atoms in the ribose, but inhibition is decreased when the 3′ oxygen is removed. A selected group of analogs in which a monocarboxylic acid is present are poor inhibitors. The structure–activity relationship is consistent with the carboxylic acid functionality acting as a mimetic for the phosphate anion in cGMP. This concept is supported by a conformational analysis of two of the inhibitors.

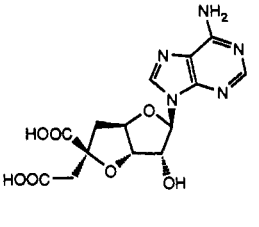
Introduction

Certain natural products from fermentation appear to act as nonhydrolyzable cyclic nucleotide mimetics in which the carboxylic acid functional group plays the role of the 3′–5′ cyclic phosphate. This principle is most elegantly expressed in the naturally occurring griseolic acid series (**1–3**) which are mimics of cyclic adenosine monophosphate (AMP) and are potent competitive (with cAMP) inhibitors of crude cyclic nucleotide phosphodiesterases from several sources.[1] The naturally occurring griseolic acids A (**1**), B (**2**), and C (**3**) have been reported to inhibit a rat brain phosphodiesterase preparation (EC 3.1.4.17) with IC_{50} values of 0.16, 0.16, and 0.12 μM, respectively, when cAMP was the substrate.[2] Using a semisynthetic approach, the discoverers of the griseolic acids have extensively explored structure–activity relationships in this interesting series.[3] The natural limitations of the semisynthetic approach, i.e. a single starting material and a limited array of functional transformations, has left many questions regarding this class of compounds unanswered.



1 R = OH

2 R = H



3

In our study of the phosphodiesterase enzymes that effect the hydrolysis of cyclic guanosine 3′,5′-monophosphate (cGMP), a second messenger which appears to be an increasingly important component of cellular regulation, we were intrigued by the possibility that the principles expressed in the griseolic acids could be translated into totally synthetic inhibitors of good potency and selectivity. Levels of cyclic GMP appear to be involved in the relaxation and the proliferation of vascular smooth muscle cells.[4] The selective inhibition of these enzymes could form the basis for a new class of molecules effective in the treatment of cardiovascular diseases. Furthermore, we felt that a totally synthetic approach would afford the opportunity to develop aspects of the structure–activity relationships unattainable by the existing semisynthetic approaches. Of particular interest to this investigation was an assessment of the role played by the carbohydrate specific oxygen functionality and the stereochemistry of the ring junctions and carboxylate groups.

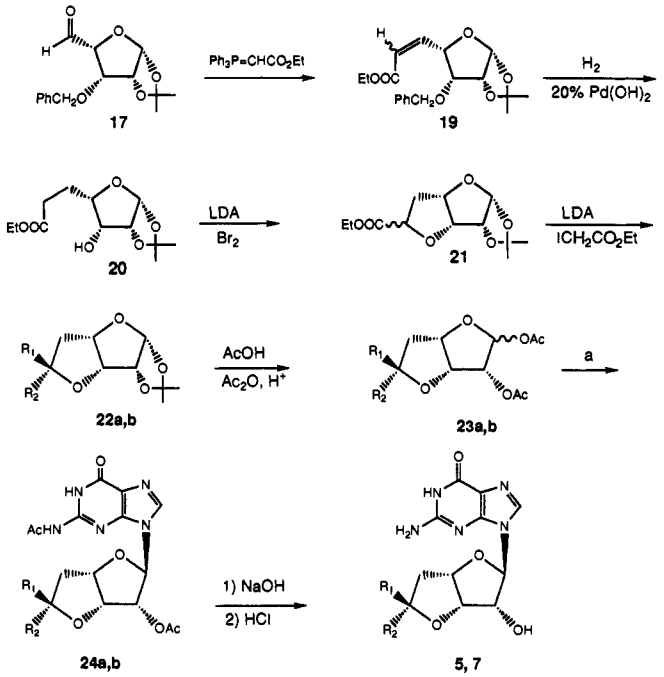
Chemistry

The new compounds prepared can be grouped into three categories. Molecules **4–7** are guanine analogs of griseolic acid C in which the stereochemistry is varied at the 3′, 4′, and 6′ positions, but other functionality remains intact. Compounds **8–12** contain functional group and atomic changes in both the ribose and purine base substructures. Finally, **13–16** represent a limited exploration into monocarboxylic analogs of griseolic acid.

The synthetic routes to compounds **4–7** are outlined in Schemes I and II. We have previously described the experimental details for the preparation of these four compounds, and the reader is referred to this published work.[5] The precursor aldehydes **17** and **18** were derived from diacetone D-glucose using the literature procedures. A Wittig reaction on **17** produced alkene **19**, which was hydrogenated with 20% palladium hydroxide to afford **20**. Treatment of **20** with bromine and 2 equiv of lithium diisopropylamide led directly to bicyclic ester **21**. Alkylation of **21** with ethyl iodoacetate then gave both **22a** and **22b** in a 4:1 ratio. The absolute configuration at C6 in these compounds was established by ^1H NMR studies which revealed an NOE between the C7 methylene and H-3 proton in case of **22a**. No such NOE was observed for compound **22b**. An X-ray crystallographic analysis of **22b** corroborated the structural assignment based on NMR data. Acetolysis of **22** followed by guaninalation of diacetate **23** produced **24**. This guaninalation also produced a small amount of N^7 alkylation (less than 10%), but the desired N^9 product was easily isolated using chromatographic methods. The regioisomers were characterized by NMR methods. Subsequent base hydrolysis of **24** produced **5** and **7**.

0022-2623/93/1836-1210$04.00/0

Scheme I[a]



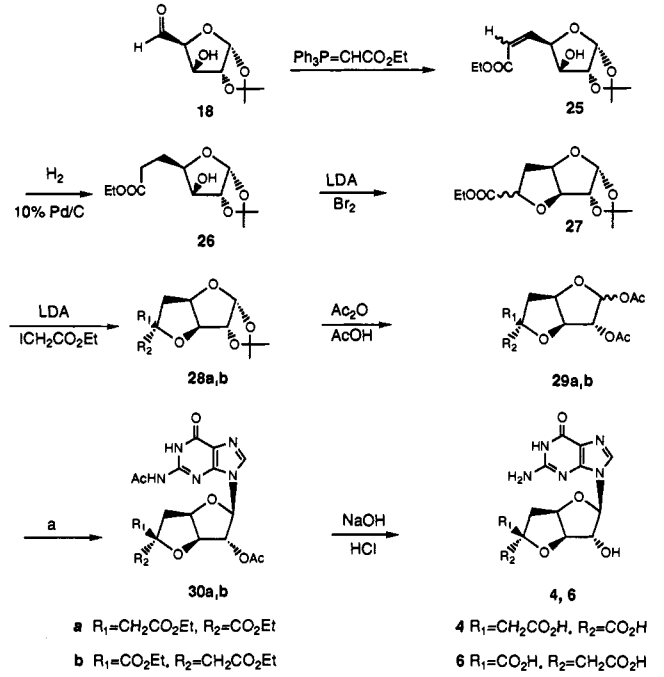
17 19 20 21 22a,b 23a,b 24a,b 5, 7

a R_1=CO_2Et, R_2=CH_2CO_2Et 5 R_1=CH_2CO_2H, R_2=CO_2H

b R_1=CH_2CO_2Et, R_2=CO_2Et 7 R_1=CO_2H, R_2=CH_2CO_2H

[a] (a) Silylated N^2-acetylguanine, TMSOTf, CH_2ClCH_2Cl.

Scheme II[a]



18 25 26 27 28a,b 29a,b 30a,b 4, 6

a R_1=CH_2CO_2Et, R_2=CO_2Et 4 R_1=CH_2CO_2H, R_2=CO_2H

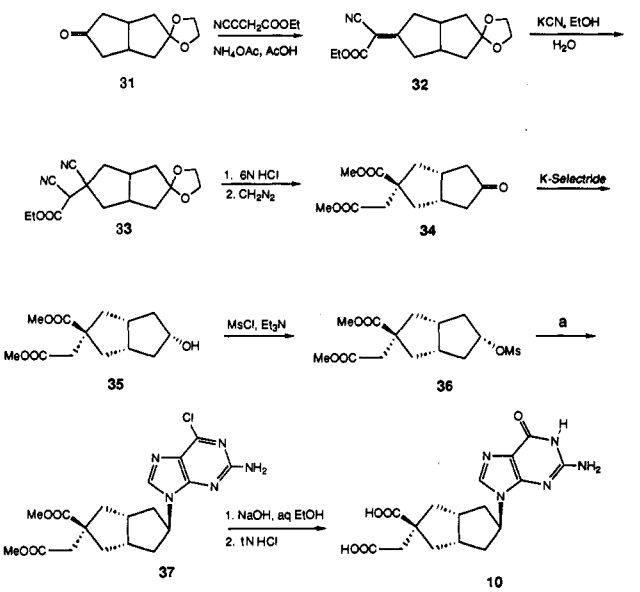
b R_1=CO_2Et, R_2=CH_2CO_2Et 6 R_1=CO_2H, R_2=CH_2CO_2H

[a] (a) Silylated N^2-acetylguanine, TMSOTf, CH_2ClCH_2Cl.

The synthetic sequence for the preparation 4 and 6 starting from aldehyde 18, is shown in Scheme II and is very similar to that depicted in Scheme I except that the alkylation of 27 with ethyl iodoacetate was much less stereoselective than that involving compound 21, the ratio of products being 1.5:1 in favor of 28a. Compound 28 was then converted to 4 and 6 as described earlier.

Compound 10 was prepared from *cis*-bicyclo[3.3.0]-octane-3,7-dione monoketal[6] 31 as outlined in Scheme III. A Knoevenagel reaction on 31 with cyanoethyl acetate produced 32, which on Michael reaction with potassium

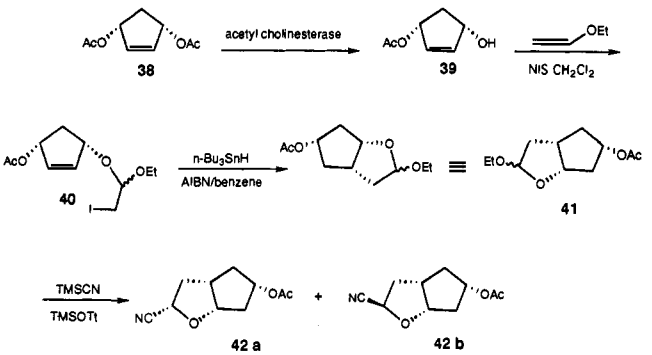
Scheme III[a]



31 32 33 34 35 36 37 10

[a] (a) 2-Amino-6-chloropurine, DMF, K_2CO_3.

Scheme IV

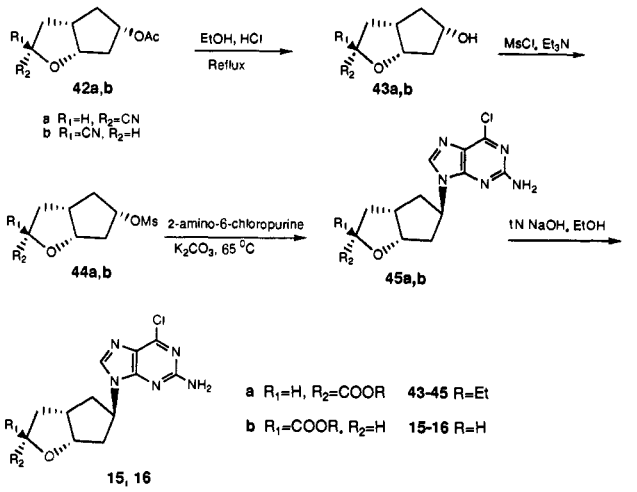


38 39 40 41 42 a 42 b

cyanide gave 33. An acid hydrolysis of 33 in refluxing 6 N hydrochloric acid gave the diacid, which on treatment with diazomethane produced a single compound 34. This four-step conversion of 31 to 34 was carried out without purification of intermediates and went in 55% overall yield. The ketone 34 was reduced stereoselectively with K-Selectride (potassium tri-*sec*-butylborohydride) (Aldrich). The stereochemistry of the hydroxy group was established using ^1H NMR experiments which showed an NOE between H-5 proton and two ring junction protons H-3a and H-6a. Mesylation of 35 under standard conditions gave 36. The displacement of mesylate of 36 with 2-amino-6-chloropurine and K_2CO_3 in DMF at 60 °C produced 37 in only 45% yield. Various attempts to improve the yield of this displacement failed. Treatment of 37 with 1 N sodium hydroxide in aqueous ethanol at room temperature produced the diacid derivative which produced 10 upon refluxing in 1 N hydrochloric acid.

Synthesis of compounds 9, 11, 12, 15, and 16 was accomplished from a common intermediate 42 (Scheme IV). This intermediate was prepared from *cis*-3,5-diacetoxycyclopentene[7] 38. The enzymatic hydrolysis[8] of 38 with commercially available acetylcholinesterase (from electric eel, Sigma) in a buffered media gave 39 in high optical (>99%) and chemical yield. Treatment of 39 with *N*-iodosuccinimide and ethyl vinyl ether gave iodoacetal 40 in 90% yield. The iodoacetal was cyclized[9] to acetal 41 under homolytic conditions, using catalytic amount of azobisisobutyronitrile (AIBN) in refluxing benzene with

Scheme V



dropwise addition of tributyltin hydride (1.0 equiv, 0.25 M in benzene). Treatment of **41** with trimethylsilyl cyanide in the presence of (trimethylsilyl)trifluoromethanesulfonate gave an easily separable mixture of two anomers in an 8:1 ratio. The stereochemistry of these anomers was established by ^{1}H NMR experiments in which the major anomer **42a** exhibited a NOE between H-2 proton and the two ring protons H-3a and H-6a. No such NOE was observed in the minor anomer **42b**.

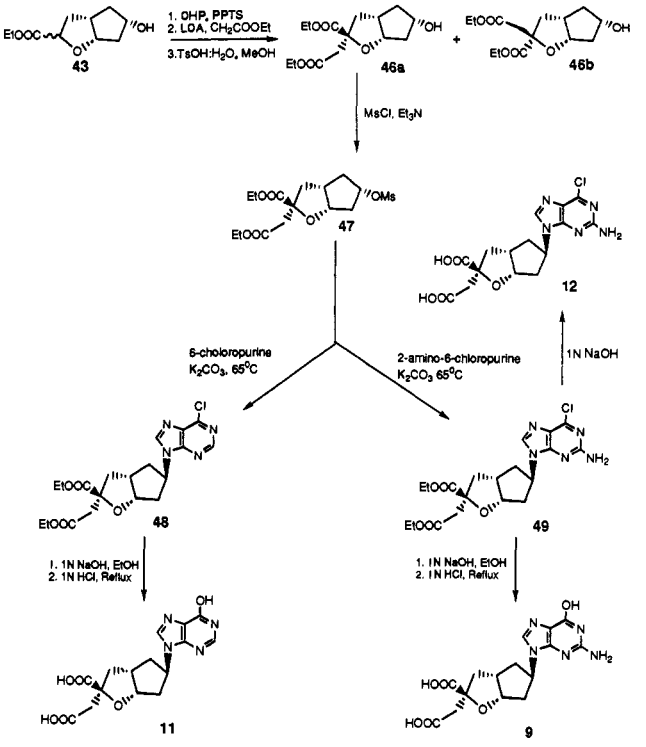
Conversion of **42** to the compounds **15** and **16** was straightforward and is outlined in Scheme V. Accordingly, the cyano group of each isomer was converted to the corresponding ester **43** in refluxing ethanol saturated with hydrogen chloride gas. The mesylation of the hydroxyl group of **43** under standard conditions gave **44**, which was displaced with 2-amino-6-chloropurine in the presence of potassium carbonate in *N*,*N*-dimethylformamide at 60 °C to produce **45**, again in a moderate yield of 45%. Hydrolysis of **45** to **15** and **16** occurred with 1 N sodium hydroxide in aqueous ethanol.

Preparation of compounds **9**, **11**, and **12** from **43** is shown in Scheme VI. The hydroxyl group of **43** was protected as a tetrahydropyranyl ether. The alkylation of this THP derivative with ethyl iodoacetate and lithium diisopropylamide, followed by removal of the THP group gave a mixture of two compounds in a 4:1 ratio. The overall yield of this three-step alkylation sequence was 60%. These isomers were separated by flash column chromatography, and the stereochemistry was established using NOE experiments as described earlier. The major isomer **46a** was mesylated to produce **47**. The displacement of the mesylate of **47** with 6-chloropurine gave **48** in 60% isolated yield whereas a similar displacement with 2-amino-6-chloropurine produced **49** in only 45% yield. Both compounds **48** and **49** were converted to **11** and **9** using the earlier described hydrolysis conditions. A simple hydrolysis of **49** with 1 N NaOH produced **12**.

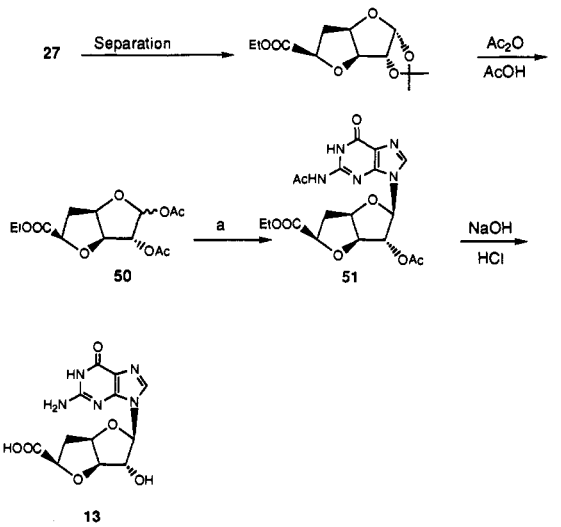
Compounds **13** and **14** were prepared from monoester derivatives **27** and **21**, respectively. The major isomers were first separated using flash column chromatography, and the pure compounds were treated with the reaction sequence described for the conversion of compound **22** to **5** (see Schemes VII and VIII) to produce **13** and **14** in 60% overall yield.

Compound **8** was prepared from lactone derivative **62**[10] as depicted in Scheme IX. The sodium borohydride reduction of **62** and subsequent protection with benzoyl

Scheme VI



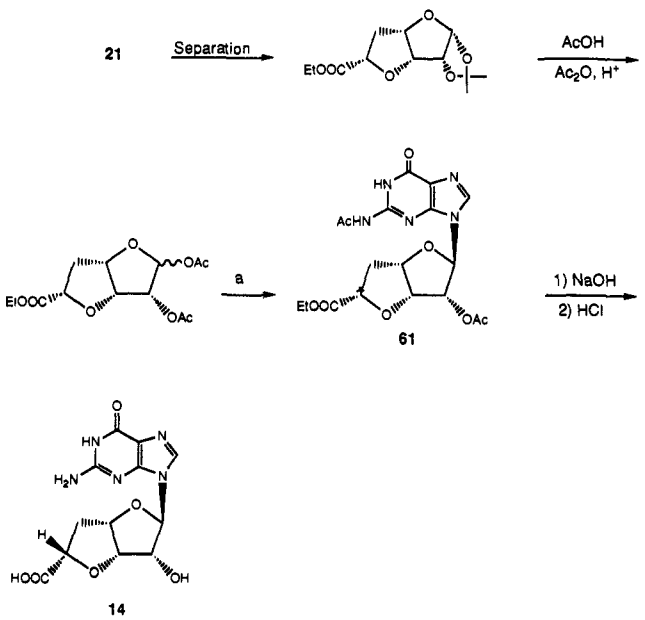
Scheme VII[a]



[a] (a) Silylated N^2-acetylguanine, TMSOTf, CH_2ClCH_2Cl.

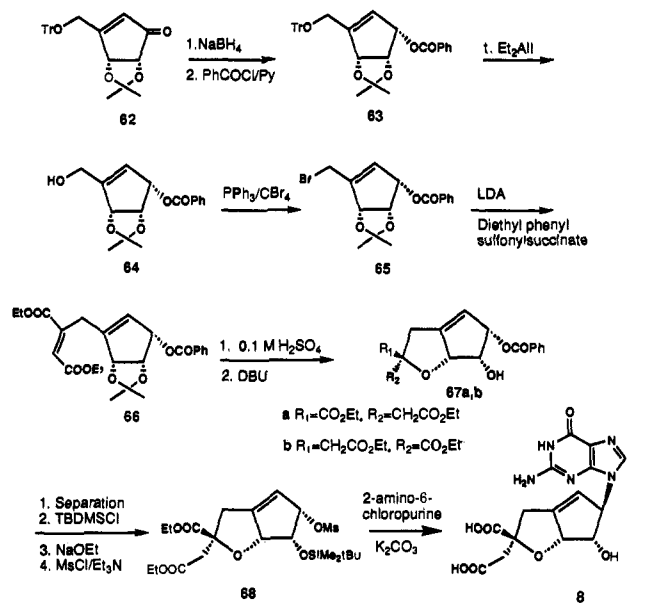
chloride produced **63** in 80% yield. The trityl group was then removed with dimethylaluminum iodide, and bromination of the resulting alcohol with triphenylphosphine/carbon tetrabromide produced bromide **65**. The displacement of this bromide with the anion of diethyl (phenylsulfonyl)succinate and elimination in refluxing benzene in the presence of pyridine gave compound **66**. The isopropylidene group was then hydrolyzed with mild acid, and the subsequent Michael reaction occurred with DBU to produce **67**. The free hydroxyl group of **67** was protected as a silyl ether, and the benzoate group was exchanged with mesylate using standard reaction conditions. The mesylate was then displaced with 2-amino-6-chloropurine as described earlier. The esters were hydrolyzed with 1 N sodium hydroxide at room temperature. The diacid derivative was then refluxed in 1 N hydrochloric acid to hydrolyze the 6-chloro group of purine. Under these

Scheme VIII[a]



[a] (a) Silylated N^2-acetylguanine, TMSOTf, CH_2ClCH_2Cl.

Scheme IX

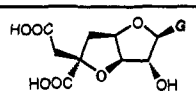
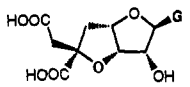
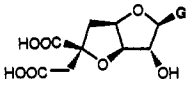
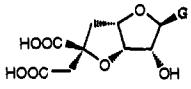
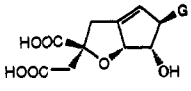
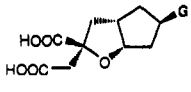
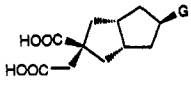
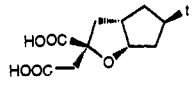
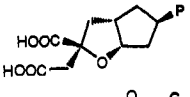
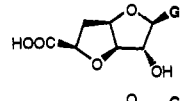
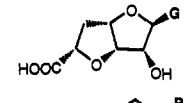
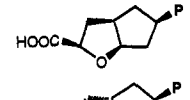
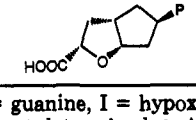


conditions the silyl group was also removed to produce compound 8.

Results and Discussion

The goal of this study was to isolate various substructures within the nucleotide mimetics and to vary stereochemistry and functionality. The compounds were evaluated as inhibitors of the cGMP PDE enzyme and for selected compounds against a cAMP hydrolyzing PDE to understand selectivity in this series (Table I). In general these purine analogs are much more potent inhibitors of the cGMP phosphodiesterase than the cAMP phosphodiesterase. This is not unexpected, since unlike the griseolic acids (with an adenine base) these guanine, inosine, and 6-chloropurine derivatives more closely represent cGMP and were designed to elicit this selectivity. The very weak inhibition of cAMP PDE observed in all compounds does not permit an examination of the structure–activity relationships. When the most active PDE inhibitors were

Table I. Inhibition of Phosphodiesterases by Cyclic Nucleotide Mimetics

compd	structure[a]	cGMP PDE (IC_{50} or % inhibition)	cAMP PDE[b] (IC_{50} or % inhibition)
4		3% at 100 μM	ND
5		42% at 100 μM	ND
6		20 μM	13% at 100 μM[c]
7		2 μM	22% at 100 μM[c] 62% at 100 μM[d]
8		1 μM	66% at 100 μM[d]
9		0.34 μM	94 μM[d]
10		57% at 100 μM	20% at 100 μM[d]
11		9 μM	66% at 100 μM[d]
12		1.2 μM	ND
13		20% at 100 μM	ND
14		18% at 100 μM	ND
15		29 μM	78% at 100 μM[d]
16		42 μM	33% at 100 μM[d]

[a] G = guanine, I = hypoxanthine, P = 2-amino-6-chloropurine. [b] ND = not determined. [c] cAMP PDE (see Experimental Section). [d] cGi PDE (see Experimental Section).

assessed for their ability to relax precontracted smooth muscle strips they showed little or no activity. This suggests that transport across the plasma membrane to the intracellular pools of cGMP PDE may have a limiting effect on activity in this preparation. Modifications of the target structures to reduce polarity or the choice of an appropriate prodrug might overcome this limitation.

Compounds 4–7 were synthesized in order to examine the importance of ring geometry and diacid configuration on enzyme inhibition. The results suggest a specific interaction of the carboxylic acid charges with a complementary binding site on the cGMP PDE. The β configuration of the ring fusion is preferred over the α, as in 4 compared to 5 and 6 compared to 7. Compounds in which the carboxymethylene group is in an anti configuration relative to the guanine base (4 vs 6 and 5 vs 7) are more potent inhibitors than their syn counterparts.

The function of the 4′ and 2′ oxygen functions is evaluated in the activity of compounds 8 and 9, respectively. Clearly no important binding interactions occurs at these positions. In fact, the apparent increase in the activity of 9, resulting in the most active inhibitor in this series, may be due to an increased hydrophobic effect of the carbocyclic ring system. The dramatic decrease in inhibition observed with compound 10 when compared to 9 shows the oxygen function which links the 3′ position to the 6′ position forming the fused tetrahydrofuran ring must have an important interaction with the enzyme active site, perhaps as a hydrogen bond acceptor. The repositioning of the 3′ oxygen could account for, at least in part, the difference in cGMP PDE inhibition between 6 and 7.

Modifications at the purine base had significant effects in the inhibitors examined. Removal of the amino group in the guanine to form an inosine resulted in more than a 1 order of magnitude loss in inhibition, e.g. compounds 9 and 11. However, the effect of substitution of a 2-amino-6-chloropurine for the guanine, compounds 12 and 9, was much smaller.

A selected group of monocarboxylic acids were synthesized and examined as mimics for the phosphate anion of cGMP. In contrast to the best examples of the diacid series these materials are significantly less active as inhibitors of cGMP phosphodiesterase. Compounds 13 and 14 place a single negative charge above and below the bicyclic ring system. Both are very weak inhibitors. The chloropurine/cyclopentane analogs (15 and 16) show only a modest improvement in activity, perhaps due to the dominance of an increased hydrophobic contribution to binding relative to the weak polar interactions supplied by the carboxylic acids. The significantly poorer inhibition exhibited by these few examples discouraged a further investigation in the complete structure activity relationship of the monocarboxylic acids.

An analysis of the structure–activity relationships in these cGMP mimetics points to the importance of the orientation of the carboxylic acids as a component of enzyme inhibition. It is not unreasonable to suggest that the negative charge provided by the carboxylate acts as a mimic for the delocalized negative charge of the ionized phosphate in cGMP. It has been shown that simple ester derivatives of griseolic acid are inactive as enzyme inhibitors.[1] We chose to probe this possibility using molecular modeling techniques. We compared cGMP with two close mimetics which retained the full functionality of the ribose ring present in cGMP but differed dramatically in the orientation of the carboxylate groups. Compounds 4 and 7 represent opposite stereochemistries at the ring fusion and the orientation of the carboxylate/acetate pair. These isomers are, respectively, inactive and very active as cGMP PDE inhibitors. Thus they represent extrema within the structure–activity relationship. Conformational analysis of these systems is complicated by the fact that the bioactive conformation of cGMP at the enzyme active site is not known. The inhibitors have not only the glycosidic rotation in common with cGMP but also considerable flexibility in the pendant acetic acid functionality. We chose the technique of vector mapping to overcome these analysis problems.[11] This method allows one to visualize the range of conformations available to these systems.

SYBYL (Tripos Associates, St. Louis, MO) was used as the tool for conformational analysis. The three molecules were built in SYBYL as the ionized species and minimized

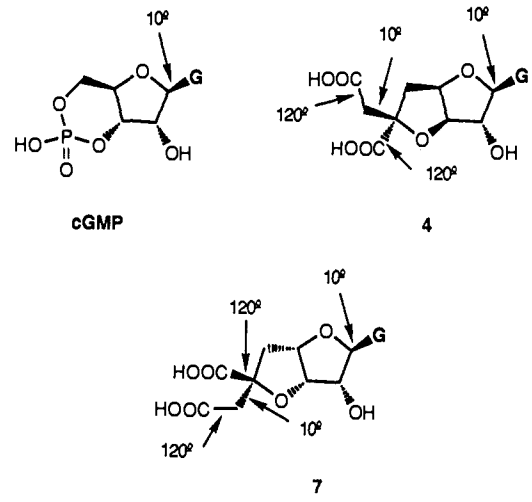


Figure 1. Angle resolution parameters used in SEARCH conformer generation.

(converging to a $\Delta E < 0.05$ kcal/mol per iteration) using MAXIMIN2 and the Tripos molecular mechanics force field. The minimization was done in vacuo without the inclusion of electrostatic terms. Inclusion of these terms produced only very minor changes in conformation. The molecules were then placed in a common orientation using the least squares FIT routine at three points, two from the common guanine heterocycle and the glycosidic carbon (C1′). SEARCH was used to generate the conformations of each molecule. Rotatable torsions were examined through a full 360° range with the angle resolution chosen as shown in Figure 1. The anchor atom was the guanine nitrogen attached to the ribose ring. Searching at higher or lower resolution did not qualitatively change the interpretation of the data. The angle increments used represent the optimum for visualization. Sterically unreasonable conformations were excluded using the van der Waals scaling factors within SEARCH (0.950 general, 0.870 1–4, and 0.650 H-bonding). To facilitate vector mapping, a dummy atom (chargeless and dimensionless) was defined as the centroid (nominally, the center of delocalized charge) of either the O–P–O triad or the O–C–O triad. A vector was then defined at each available conformation connecting the dummy atom and the central P or C atom. This technique thus allows a qualitative visualization of the space defined by the negative charges through the range of conformers generated. The sweep of the vectors maps out the space occupied by the charge centers of the phosphate and carboxylate groups as the torsions are driven among the rotatable bonds.

Using the procedures described, 17, 276, and 600 available conformers were generated for cGMP, compound 4, and compound 7, respectively. These conformations are visualized in Figure 2 in panels A, B, and C with a colored vector drawn at each available conformation superimposed on a single conformation of the molecule used to generate the map. As can be clearly seen a wide range of space available to the charged groups is described by the vectors. This space is compared in panel D which shows an overlay of all three maps, now for clarity without the molecules. The vector space defining the phosphate charge of cGMP is sandwiched tightly within the space defined by the two negative charges of the potent inhibitor 7, whereas the conformations of 4 fall outside this region of space. This comparison, however, does not have sufficient precision to identify if the interaction of one

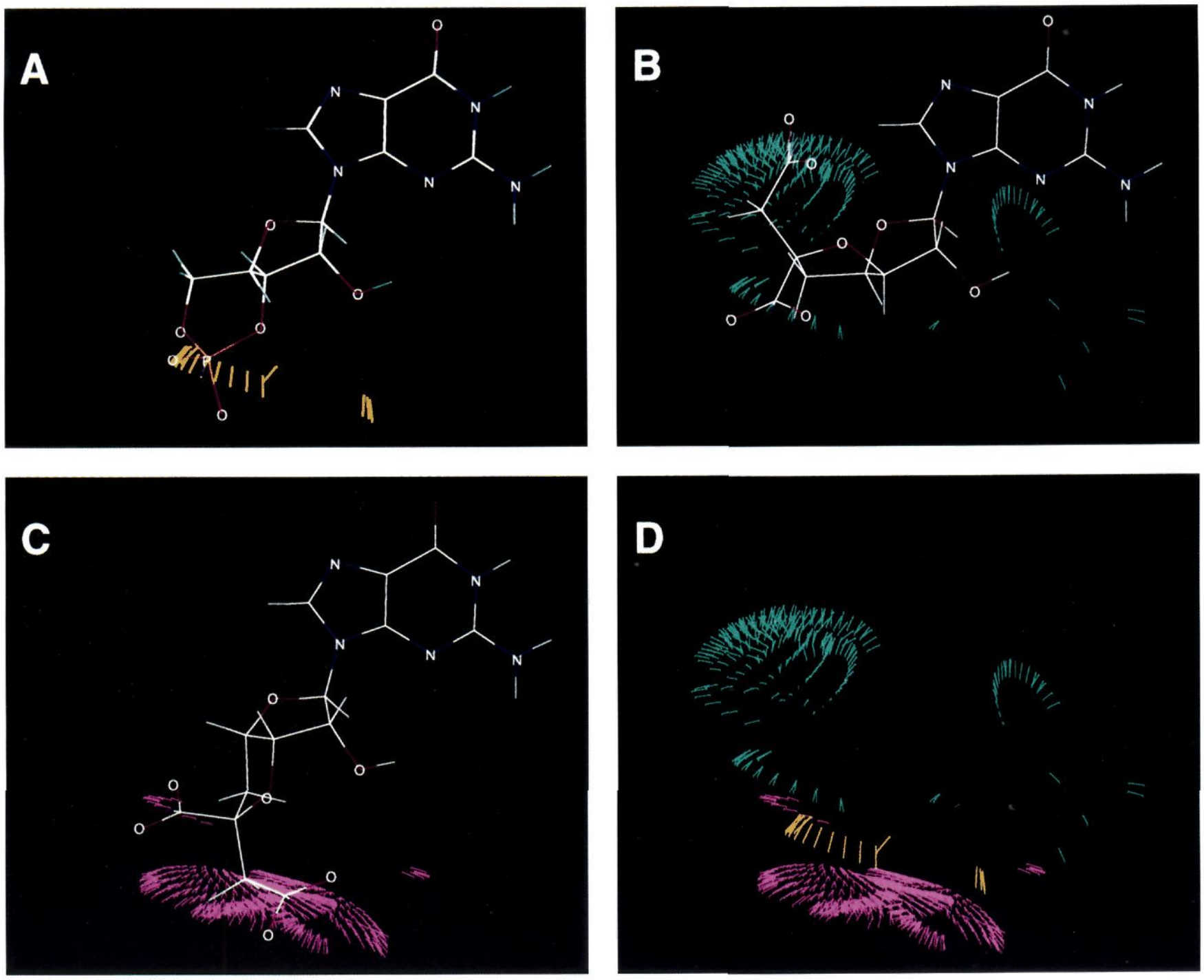


Figure 2. Wireframe and vector map representations of cGMP (panel A), compound **4** (panel B), and compound **7** (panel C). Vector maps were generated as described in the text. Graphical views are from identical orientations of cGMP and the PDE inhibitors. Panel D is a composite of the vector maps in A, B, and C with the wireframe models removed for visualization of the overlap of the vector maps. Orientation in panel D is identical to the other panels.

carboxylate dominates over the other. In addition, it is unlikely that more subtle differences in biological activity between molecules of intermediate activity would be detected with good accuracy. Such a subtle effect may be in play with the 3′ oxygen effect of biological activity, which is not readily explained by this analysis which addresses only the position of the negative charges. Nevertheless, it clearly shows that the orientation of the carboxylic acid negative charges, when taken as a group, differs among the inhibitors examined and cGMP, even when a range of conformations are compared. Furthermore, these differences can be correlated in a rational way with observed cGMP PDE enzyme inhibition in compounds with a significant difference in biological activity.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded at 200 or 400 MHz using tetramethylsilane as the internal standard; chemical shifts are recorded in parts per million (ppm). High-resolution mass spectra (HRMS) were obtained using the fast atom bombardment (FAB) ionization method from a thiogycerol matrix and are recorded when a satisfactory combustion analysis was not obtained. The progress of reactions was monitored by TLC using Analtech silica gel GF plates. The chromatograms were viewed under an ultraviolet light, and/or sprayed with concentrated H_2SO_4 and briefly heated on hot plate. All column chromatography was done using silica gel (Baker silica gel, 40 μm).

General Procedures. (a) **Mesylation.** A solution of an alcohol (1.0 mmol) and trimethylamine (1.0 mmol) in CH_2Cl_2 (20 mL) was cooled to −20 °C. MsCl (1.0 mmol) was then added, and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was then diluted with CH_2Cl_2 (30 mL), washed with water, dried over $MgSO_4$, and evaporated on a rotary evaporator. Crude product was then passed through a short column of silica gel.

(b) **Guaninalation.** A solution of 2-amino-6-chloropurine (1.5 mmol), K_2CO_3 (1.5 mmol), and mesylate (1.0 mmol) in DMF (2 mL) was kept at 60 °C for 12–16 h. EtOAc was then added to the reaction mixture, which was washed with water and $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated. Crude product was then purified on a silica gel column.

(c) **Hydrolysis.** A solution of chloropurine (1.0 mmol) in aqueous EtOH (2 mL) was treated with 3 mL of 1 N NaOH until TLC showed completion of hydrolysis. Then 3 mL of HCl was added, the solvent was removed, the crude product was dissolved in 1 N HCl (5 mL), and the reaction mixture was refluxed for 6–8 h. The pH of the reaction was adjusted to about 3 with 1 N NaOH, and the volume of the reaction was reduced to half. This residue was put on a CHP^{20}P column (MCI Gel, Mitsubishi Chemical Industries Ltd.) and eluted, first with water to remove NaCl and then with 10–50% acetone in water to elute the compound. All UV-active fractions were combined and evaporated.

1,6-Dihydro-2-amino-9-(3′,6′-anhydro-5′-deoxy-6′(*S*)-carboxy-β-D-glucofuranosyl)-6-hydroxypurine (13). The *R* and *S* isomers of ethyl 3,6-anhydro-5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-heptofuranuronate[5] (**27**) were separated using flash column chromatography eluting with 20% EtOAc–petroleum ether. The *S* isomer (0.4 g, 1.55 mmol) was dissolved in 20 mL of CH_2Cl_2, stirred, and cooled at 0 °C as 20 mL of acetic anhydride and acetic acid (7:10 ratio) and a catalytic amount of H_2SO_4 were added. The reaction mixture was stirred at room temperature for 4 h, diluted with 100 mL of CH_2Cl_2, washed with saturated $NaHCO_3$, water, and brine, and the dried ($MgSO_4$). Flash chromatography with 50% EtOAc–hexane gave compound **50** as a mixture of two anomeric acetates by TLC. The acetate mixture from above was dissolved in 10 mL of 1,2-dichloroethane and cooled to 0 °C. Trisilylated N^2-acetylguanine[12] (0.9 g, 2.0 mmol) was added followed by trimethylsilyl triflate, and the reaction mixture was refluxed for 2 h. It was then diluted with CH_2Cl_2 (50 mL), washed with a cold solution of $NaHCO_3$, dried (Na_2SO_4), and evaporated. The crude product **51** was purified by flash chromatography with 10% MeOH–EtOAc. A small amount of N^7 alkylation product was also isolated. For compound **51** (0.4 g, 60% from **27**): TLC R_f = 0.30 (10% MeOH–EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 (3 H, t, J = 7.10 Hz, OCH_2CH_3), 2.05 and 2.25 (6 H, 2 s, $OCOCH_3$ and $HNCOCH_3$), 2.35 (1 H, ddd, $J_{5',5''}$ = 13.80, $J_{5',6'}$ = 8.50, $J_{4',5'}$ = 4.0 Hz, H-5′), 2.62 (1 H, bd, $J_{5',5''}$ = 13.80, H-5″), 4.88 (1 H, bd, $J_{3',4'}$ = 4.0 Hz, H-3′), 4.90 (1 H, t, H-4′), 6.10 (1 H, d, $J_{1',2'}$ = 5.0 Hz, H-1′), 8.05 (1 H, s, H-8). Compound **51** (0.4 g, 0.92 mmol) was treated with 1 N NaOH (5.0 mmol) in 5 mL of aqueous ethanol for 4 h. The reaction mixture was then neutralized with 1 N HCl, the volume of reaction mixture was reduced to half, and the residue was put on a CHP^{20}P column (MCI gel, Mitsubishi Chemical Industries Ltd.) and eluted first with water and then with 10% acetone–water to produce **13** (a solid, 0.26 g, 90%); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 5.45 (1 H, d, H-2′), 5.90 (1 H, d, $J_{1',2'}$ = 5.1 Hz, H-1′), 6.45 (2 H, bs, NH_2), 7.88 (1 H, s, H-8). Anal. ($C_{12}H_{13}N_5O_6$) C, H, N.

1,6-Dihydro-2-amino-9-(3′,6′-anhydro-5′-deoxy-6′(*R*)-carboxy-β-D-gulofuranosyl)-6-hydroxypurine (14). The mixture *R* and *S* isomers of ethyl 3,6-anhydro-5-deoxy-1,2-*O*-isopropylidene-β-L-*lyxo*-heptofuranuronate[5] (**21**) was separated via flash chromatography, eluting with 45% EtOAc–petroleum ether, and the pure *R* isomer (0.30 g, 1.16 mmol) was converted to crude **61** as described above. The crude material was purified via flash chromatography, eluting with 10% MeOH–CH_2Cl_2 (0.38 g, 75%): TLC R_f = 0.33 (10% MeOH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.35 (3 H, t, OCH_2CH_3), 2.18 (3 H, s, $OCOCH_3$), 2.35 (3 H, s, $HNCOCH_3$), 2.55 (2 H, m, H-5′, H-5″), 4.75 (1 H, dd, $J_{5',6'}$ = 4.0, $J_{5'',6'}$ = 8.0 Hz, H-6′), 4.95 (1 H, m, H-3′), 5.25 (1 H, m, H-4′), 5.92 (1 H, dd, $J_{1',2'}$ = 6.5 Hz, $J_{2',3'}$ = 6.0 Hz, H-2′), 6.25 (1 H, d, H-1′), 7.95 (1 H, s, H-8). Compound **61** was hydrolyzed using the procedure described for **51** to produce compound **14** a solid (0.17 g, 80%): ^{1}H NMR (DMSO, 200 MHz) δ 2.28 (1 H, bd, $J_{5',5''}$ = 14.0 Hz, H-5′), 2.50 (1 H, m, H-5″), 4.75 (4 H, m, H-3′, H-4′, H-5′, H-6′), 5.05 (1 H, bd, H-2′), 5.75 (1 H, d, $J_{1',2'}$ = 4.5 Hz, H-1′), 6.50 (2 H, s, NH_2), 7.82 (1 H, s, H-8). Anal. ($C_{12}H_{13}N_5O_6$) C, H, N.

Compound 34. A solution of *cis*-bicyclo[3.3.0]octane-3,7-dione monoketal[6] **31** (0.5 g, 2.75 mmol), cyanoethyl acetate (0.37 g, 3.3 mmol), acetic acid (0.13 g, 2.2 mmol), and a catalytic amount of ammonium acetate in benzene (20 mL) was refluxed for 3 h. The reaction mixture was diluted with benzene (50 mL), washed with a saturated solution of $NaHCO_3$, dried ($MgSO_4$), and evaporated. The crude product **32** was dissolved in aqueous ethanol and treated with potassium cyanide (0.4 g, 6.0 mmol) at room temperature for 0.5 h. The pH of the reaction mixture was adjusted to 5 with 1 N HCl and evaporated to the crude **33**. The crude product **33** was dissolved in 6 N HCl and refluxed for 16 h. The reaction mixture was then concentrated under high vacuum, the residue was dissolved in 200 mL of EtOAc, washed with a 10% solution of $NaHCO_3$, dried ($MgSO_4$), and evaporated to a residue which was dissolved in 50 mL of THF, and the solution was cooled in an ice bath and treated with diazomethane. The solvent was then removed, and the residue was column chromatographed, eluting with 75% Et_2O–benzene to give **34** (as an oil, 0.38 g, 55%): TLC R_f = 0.70 (30% Et_2O–benzene); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (2 H, dd, $J_{3a,3}$ = $J_{1,6a}$ = 7.5, $J_{3,3'}$ = $J_{1,1'}$ = 14.00 Hz, H-1, H-3), 2.10 (2 H, dd, $J_{4,4'}$ = $J_{6,6'}$ = 16.0, $J_{3a,4}$ = $J_{6,6a}$ = 4.0 Hz, H-4, H-6), 2.55 (2 H, dd, $J_{3a,4'}$ = $J_{6',6a}$ = 9.0 Hz, H-4′, H-6′), 2.70 (2 H, dd, $J_{3',3a}$ = $J_{1',6a}$ = 8.0 Hz, H-1′, H-3′), 2.80 (2 H, s, CH_2COOCH_3), 2.90 (2 H, m, H-3a, H-6a), 3.65 and 3.75 (6 H, 2 s, 2 $COOCH_3$); HRMS calcd for $C_{13}H_{18}O_5$ (M^+ + H) 255.1232, found 255.1228.

2β-(Methoxycarbonyl)-5α-hydroxy-3aβ,6aβ-octahydropentalene-2α-acetic Acid Methyl Ester (35). A solution of **34** (0.65 g, 2.56 mmol) in 20 mL of THF was cooled to −78 °C. To this was added 3.0 mL of a 1.0 M solution of K-Selectride, and the reaction mixture was warmed to room temperature and stirred for 0.5 h. A saturated solution of NH_4Cl (10 mL) was added, the reaction mixture was concentrated, and the residue was dissolved in CH_2Cl_2, washed with water, dried ($MgSO_4$), and evaporated. The residue was column chromatographed (50% EtOAc–petroleum ether) to produce **35** as a syrup: TLC R_f = 0.50 (50% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.60–1.70 (4 H, m, H-1, H-3, H-4′, H-6′), 1.70–2.10 (2 H, m, H-4, H-6), 2.40–2.70 (4 H, m, H-1′, H-3a, H-3′, H-6a), 2.75 (2 H, s, CH_2COOCH_3), 3.65 and 3.75 (6 H, 2 s, 2 OCH_3), 4.4 (1 H, m, H-5); HRMS calcd for $C_{13}H_{20}C_5$ (M^+ + H) 257.1388, found 257.1383.

2β-(Methoxycarbonyl)-5α-[(methylsulfonyl)oxy]-3aβ,6aβ-octahydropentalene-2α-acetic Acid Methyl Ester (36). A solution of **35** (0.60 g, 2.34 mmol) in 30 mL of CH_2Cl_2 was mesylated according to the general procedure to produce crude **36** which on flash column chromaography eluting with 50% EtOAc–petroleum ether gave **36** (a syrup, 0.70 g, 90%): TLC R_f = 0.70 (50% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (1 H, dd, $J_{3,3a}$ = 6.0, $J_{3,3'}$ = 14.0 Hz, H-3), 1.85 (1 H, dd, $J_{1,1'}$ = 16.0, $J_{1,6a}$ = 10.0 Hz, H-1), 2.15 (3 H, m, H-1′, H-3′, H-4), 2.60 (2 H, m, H-4′, H-6), 2.75 (2 H, s, CH_2COOCH_3), 2.80 (1 H, m, H-3a), 3.05 (3 H, s, OSO_2CH_3), 3.65 and 3.75 (6 H, 2 s, 2 OCH_3), 5.20 (1 H, m, H-5); HRMS calcd for $C_{14}H_{22}O_7S$ (M^+ + H) 225.1165, found 335.1161.

2′β-Carboxy-5′β-(9-guanidino)-3′aβ,6′aβ-octahydropentalene-2′α-acetic Acid (10). Guaninalation of **36** (0.5 g, 1.50 mmol) was done according to the described general procedure to give crude **37** which on flash chromatography eluting with 50% EtOAc–petroleum ether gave pure **37** (0.27 g, 45%): TLC R_f = 0.25 (60% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (2 H, m, H-1′, H-3′), 1.90 (2 H, dd, $J_{3',3''}$ = $J_{1',1''}$ = 14.0, $J_{1'',6'a}$ = $J_{3'',3'a}$ = 6.0 Hz, H-3″ and H-1″), 2.20–2.45 (4 H, m, H-4′, H-4″, H-6′, H-6″), 2.65 (2 H, s, CH_2COOCH_3), 2.75 (2 H, m, H-3a′, H-6a′), 3.60 and 3.70 (6 H, 2 s, OCH_3), 4.70 (1 H, m, H-5′), 6.45 (2 H, s, NH_2), 7.90 (1 H, s, H-8). The hydrolysis of **47** was done using the described general procedure to produce **10** (a solid, mp, decomposed 240–255 °C): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (2 H, m, H-1′, H-3′), 1.90 (2 H, dd, $J_{3',3''}$ = $J_{1,1''}$ = 14.0, $J_{1'',3'a}$ = $J_{3'',3'a}$ = 6.0 Hz, H-3″ and H-1′), 2.20–2.45 (4 H, m, H-4′, H-4″, H-6′, H-6″), 2.65 (2 H, s, CH_2COOCH_3), 2.75 (2 H, m, H-3a′, H-6a′), 3.60 and 3.70 (6 H, 2 s, OCH_3), 4.70 (1 H, m, H-5′), 6.45 (2 H, s, NH_2), 7.90 (1 H, s, H-8). Anal. ($C_{16}H_{19}N_5O_5$) C, H, N.

Compound 40. To a solution of (+)-3(*R*)-acetoxy-5(*S*)-hydroxycyclopent-1-ene[7,8] **39** (8.10 g, 56.7 mmol) and *N*-iodosuccinimide (13.4 g, 59.53 mmol) in CH_2Cl_2 (200 mL) at −20 °C was added a solution of ethyl vinyl ether (5.3 g, 7.0 mL) in CH_2Cl_2 (100 mL) dropwise, and the reaction was maintained at −20 °C for 4 h. The reaction was warmed to room temperature, washed with water, dried ($MgSO_4$), and evaporated to give an isomeric mixture of **40** (17.40 g, 90%, an oil): TLC R_f = 0.90 (40% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (3 H, t, CH_2CH_3), 1.78 (1 H, ddd, $J_{4,4'}$ = 15.00, $J_{3,4}$ = 4.00, $J_{4,5}$ = 8.5 Hz, H-4), 2.05 (3 H, s, $OCOCH_3$), 2.70 (1 H, ddd, $J_{3,4'}$ = 5.0, $J_{4',5}$ = 7.5 Hz, H-4′), 3.22 (2 H, d, CH_2I), 3.60 (1 H, m, CH_2CH_3), 4.65 (1 H, m, H-5), 5.50 (1 H, bt, H-3), 6.00 and 6.10 (2 H, 2 m, H-1, H-2); HRMS calcd for $C_{11}H_{17}IO_4$ (M^+ + H) 341.0251, found 341.0249.

Compound 41. To a refluxing solution of **40** (10.0 g, 29.45 mmol) and AIBN (0.7 g) in benzene (175 mL) was added a 0.03 M solution of tri-*n*-butyltin hydride (35.35 mmol) over a period of 4 h.[9] Reaction mixture was further refluxed for 0.5 h and was stirred overnight at room temperature. Solvent was then removed, and the residue was dissolved in Et_2O (750 mL), washed with 10% KF solution, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel, eluting with 30% EtOAc–petroleum ether to give **41** as a mixture of two anomers (an oil, 4.85 g, 77%): TLC R_f = 0.42 and 0.47 (30%

EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, MHz) δ 2.05 (3 H, s, $OCOCH_3$), 2.88 (1 H, m, H-3a), 4.65 (1 H, m, H-6a), 5.15 (1 H, m, H-5a), 5.20 (1 H, d, $J_{2,3}$ = 5.5 Hz, H-2); HRMS calcd for $C_{11}H_{18}O_4$ (M^+ + H) 215.1283, found 255.1281.

Compound 42. A solution of 41 (9.53 g, 44.53 mmol) in CH_2Cl_2 (1000 mL) was cooled to −78 °C. To this were added TMSCN (11.0 g, 110.0 mmol) and TMSOTf (3.85 g, 17.8 mmol), and the reaction mixture was warmed to −40 °C and kept at that temperature for 2 h, washed with a saturated solution of $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel, eluting with 50% Et_2O–hexane to give **42a** and **42b** in 8:1 ratio. For compound **42a** (a solid, mp 69–70 °C): TLC R_f = 0.20 (50% Et_2O–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.75 (1 H, ddd, $J_{6,6'}$ = 12.8, $J_{6,6a}$ = 1.2, $J_{5,6}$ = 8.0 Hz, H-6), 2.05 (3 H, s, $OCOCH_3$), 2.08 (1 H, ddd, $J_{5,6'}$ = 2.8, $J_{6',6a}$ = 7.2 Hz, H-6'), 2.22 (1 H, ddd, $J_{3,3'}$ = 12.8, $J_{2,3}$ = 7.2 Hz, H-3), 2.50 (1 H, ddd, $J_{2,3'}$ = 4.0, $J_{3',3a}$ = 9.2 Hz, H-3'), 3.0 (1 H, m, H-3a), 4.80 (1 H, dt, $J_{6,6a}$ = 1.2, $J_{6',6a}$ = 7.2 Hz, H-6a), 4.85 (1 H, dd, H-2), 5.18 (1 H, ddt, $J_{4,5}$ = $J_{5,6'}$ = 2.8, $J_{4',5}$ = 5.60 Hz, H-5). Anal. ($C_{10}H_{13}NO_3$) C, H, N. For minor isomer **42b** (an oil): TLC R_f = 0.15 (50% Et_2O–hexane); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.10 (3 H, s, $OCOCH_3$), 2.90 (1 H, m, H-3a), 4.60 (2 H, m, H-6a, H-2), 5.20 (1 H, m, H-5); HRMS calcd for $C_{10}H_{13}NO_3$ (M^+ + H) 196.0973, found 196.0969.

Ethyl 3a,β,6aβ-Hexahydro-5-α-hydroxy-2*H*-cyclopenta-[*b*]furan-2α-carboxylate (43a). A solution of **42a** (2.0 g, 10.25 mmol) in ethanol (50 mL) saturated with hydrogen chloride gas was refluxed for 1 h. Solvent was then removed, and the residue was treated with a cold saturated solution of $NaHCO_3$, extracted with CH_2Cl_2 (2 × 100 mL), dried, and evaporated to produce **43a** as a syrup (1.95 g, 90%): TLC R_f = 0.5 (EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.28 (3 H, t, J = 7.2 Hz, OCH_2CH_3), 1.68 (1 H, m, H-6), 1.92 (1 H, dt, $J_{2,3}$ = $J_{2,3a}$ = 5.7, $J_{3,3'}$ = 14.7 Hz, H-3), 2.05 (1 H, m, H-4), 2.10 (1 H, dd, H-3'), 2.30 (1 H, dd, $J_{4,5'}$ = 5.7 Hz, H-4'), 2.35 (1 H, ddd, $J_{6,6'}$ = 12.6 Hz, $J_{5,6}$ = 6.5 Hz, $J_{6,6a}$ = 6.5 Hz, H-6), 4.20 (2 H, q, OCH_2CH_3), 4.30 (1 H, m, H-5), 4.72 (1 H, d, $J_{3a,6a}$ = 7.4 Hz, $J_{6,6a}$ = 6.5 Hz, H-6a), 4.80 (1 H, bt, $J_{2,3}$ = $J_{2,3'}$ = 5.7 Hz, H-2); HRMS calcd for $C_{10}H_{16}O_4$ (M^+ + H) 201.1127, found 201.1126.

Ethyl 3a,β,6aβ-Hexahydro-5α-[(methylsulfonyl)oxy]-2*H*-cyclopenta[*b*]furan-2α-carboxylate (44a). A solution of compound **43a** (1.0 g, 7.0 mmol) was mesylated using the described general procedure, and the crude product was purified using flash column chromatography eluting with 70% EtOAc–hexane to give **44a** (an oil, 1.3 g, 95%): TLC R_f = 0.35 (EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 (3 H, t, J = 7.2, CH_2CH_3), 2.2–2.50 (6 H, m), 2.85 (1 H, m, H-3a), 3.05 (3 H, s, OSO_2CH_3), 4.85 (1 H, dt, $J_{6,6a}$ = $J_{3a,6a}$ = 7.5, $J_{6',6a}$ = 12.0 Hz, H-6a), 5.20 (1 H, tt, $J_{4,5}$ = $J_{5,6}$ = 3.0, $J_{4',5'}$ = $J_{5',6'}$ = 6.0 Hz, H-5); HRMS calcd for $C_{11}H_{18}O_6S$ (M^+ + H) 279.0902, found 279.0899.

Ethyl 3a,β,6aβ-Hexahydro-5α-[(methylsulfonyl)oxy]-2*H*-cyclopenta[*b*]furan-2β-carboxylate (44b). Compound **42b** (1.0 g, 5.12 mmol) was converted to **43b** (oil, 0.97 g, 89%) using the procedure described for **43a**. A solution of compound **43b** (0.5 g, 3.5 mmol) was mesylated using the described general procedure to produce the crude product. A flash chromatography on the crude product eluting with 30% hexane–EtOAc gave **44b** (0.65 g, 95%): TLC R_f = 0.32 (30% hexane–EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 (3 H, t, J = 7.2 Hz, OCH_2CH_3), 1.90–2.60 (6 H, m, H-3, H-3', H-4, H-4', H-6, H-6'), 2.85 (1 H, m, H-3a), 3.10 (3 H, s, OSO_2CH_3), 4.35 (1 H, ddd, $J_{3a,6a}$ = 7.60, $J_{6,6a}$ = 8.80, $J_{6',6a}$ = 1.10 Hz, H-6a), 4.55 (1 H, bt, $J_{2,3}$ = $J_{2,3'}$ = 7.0 Hz, H-2), 5.15 (1 H, m, H-5); HRMS calcd for $C_{11}H_{18}O_6S$ (M^+ + H) 279.0902, found 279.0897.

1,6-Dihydro-2-amino-9-(2'β-carboxy-3'aβ,6'aβ-hexahydro-2*H*-cyclopenta[*b*]furanyl)-6-chloropurine (15). A solution of **44b** (0.5 g, 1.80 mmol) in 2 mL of DMF was guaninalated with 2-amino-6-chloropurine using the described general procedure to give crude **45b** which on flash column chromatography, eluting with EtOAc, gave **45b** (white solid, 0.28 g, 45%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ, 1.35 (3 H, t, J = 7.20 Hz, CH_2CH_3), 1.85 (1 H, ddd, $J_{2',3'}$ = 8.9, $J_{3',3''}$ = 13.0, $J_{3',3a'}$ = 6.10 Hz, H-3'), 2.20–2.50 (2 H, m), 2.66 (1 H, bdd, $J_{2',3''}$ = 7.10, $J_{3',3''}$ = 13.0 Hz, H-3''), 3.20 (1 H, m, H-3a'), 4.33 (1 H, dd, $J_{2',3'}$ = 8.90, $J_{2,3''}$ = 7.10 Hz, H-2'), 5.05 (1 H, tt, $J_{4',5'}$ = $J_{5',6'}$ = 6.70, $J_{4'',5'}$ = $J_{5',6''}$ = 10.90 Hz, H-5'), 5.30 (2 H, s, NH_2), 7.80 (1 H, s, H-8). A solution of **45b** (0.25 g, 0.071 mmol) in 2 mL of aqueous ethanol was treated with 1 N NaOH for 4 h. The reaction mixture was than neutralized with 1 N HCl, and solvent was removed. The residue was dissolved in 2 mL of water and put on CHP^{20}P gel column and eluted, first with water then with 50% acetone–water, to produce **15** (0.19 g, 84%): ^{1}H NMR (CD_3OD, 300 MHz) δ (1 H, ddd, $J_{2',3'}$ = 6.80, $J_{3',3''}$ = 12.80, $J_{3',3a'}$ = 9.30 Hz, H-3'), 2.65 (1 H, ddd, $J_{2',3''}$ = 9.30, $J_{3'',3a'}$ = 6.80 Hz, H-3''), 3.05 (1 H, m, H-3a'), 4.30 (1 H, dd, $J_{2',3'}$ = 6.80, $J_{2',3''}$ = 9.30 Hz, H-2'), 4.63 (1 H, t, $J_{6',6a'}$ = $J_{3a',6a'}$ = 6.10 Hz, H-6a'), 5.05 (1 H, m, H-5'), 8.15 (1 H, s, H-8). Anal. ($C_{13}H_{14}N_5O_3Cl$) C, H, N.

1,6-Dihydro-2-amino-9-(2'α-carboxy-3'aβ,6'aβ-hexahydro-2*H*-cyclopenta[*b*]furanyl)-6-chloropurine (16). A solution of compound **44a** (0.37 g, 1.33 mmol) was converted to **45a** (0.19 g, 40%) using the procedure described for **45b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (3 H, t, OCH_2CH_3), 2.00–2.20 (2 H, m), 2.30–2.60 (4 H, m), 3.05 (1 H, m, H-3a'), 4.67 (1 H, dd, $J_{2',3'}$ = 4.0 Hz, $J_{2',3''}$ = 8.10 Hz, H-2'), 4.85 (1 H, m, H-6a'), 4.95 (1 H, m, H-5'), 5.15 (2 H, bs, NH_2), 7.80 (1 H, s, H-8). The hydrolysis of **45a** (0.15 g, 0.43 mmol) was done as described for **45b** to produce compound **16** (0.12 g, 90%): ^{1}H NMR (CD_3OD, 300 MHz) δ 2.45 (1 H, ddd, $J_{3',3''}$ = 13.00, $J_{2',3'}$ = 4.0, $J_{3'3a'}$ = 9.0 Hz, H-3'), 3.10 (1 H, m, H-3a'), 4.50 (1 H, dd, $J_{2',3'}$ = 4.0, $J_{2',3''}$ = 8.0 Hz, H-2'), 4.90 (1 H, m, H-5), 5.10 (2 H, s, NH_2), 8.00 (1 H, s, H-8). Anal. ($C_{13}H_{14}N_5O_3Cl$) C, H, N.

Ethyl 2β-(Ethoxycarbonyl)-3aβ,6aβ-hexahydro-5α-hydroxy-2*H*-cyclopenta[*b*]furan-2α-acetate (46a) and Ethyl 2α-(Ethoxycarbonyl)-5-α-hydroxy-3aβ,6aβ-hexahydro-2*H*-cyclopenta[*b*]furan-2β-acetate (46b). A solution of **43** (1.0 g, 5.0 mmol), 3,4-dihydro-2*H*-pyran (0.5 mL, 5.5 mmol), and pyridinium *p*-toluenesulfonate (0.05 g) in CH_2Cl_2 (20 mL) was stirred overnight at room temperature. The reaction mixture was than diluted with CH_2Cl_2 (50 mL), washed with saturated solution of $NaHCO_3$, dried ($MgSO_4$), and evaporated. The crude compound was passed through short column to give the THP derivative: TLC R_f = 0.85 (60% Et_2O–hexane). The solution of this THP derivative (0.55 g, 1.94 mmol) in THF (5 mL) was added to the solution of LDA (generated with 0.255 g of diisopropylamine and 1.0 mL of 2.5 M *n*-BuLi) at −78 °C, and the reaction was kept at this temperture for 0.5 h. Ethyl iodoacetate (0.53 g, 0.29 mL, 2.5 mmol) was then added, and the reaction was warmed to room temperature slowly. A saturated solution of ammonium chloride (5 mL) was added, extracted with ethyl acetate, dried ($MgSO_4$), and evaporated. The crude product was dissolved in EtOH (10 mL) and treated with TsOH (0.02 g) for 6 h. Solvent was then removed, and the residue was dissolved in CH_2Cl_2 (50 mL), washed with a saturated solution of $NaHCO_3$ and water, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography eluting with 10% hexane–EtOAc to give **46a** (an oil) and **46b** (an oil) in a 4:1 ratio, respectively (0.92 g, 65%). For compound **46a**: TLC R_f = 0.60 (EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 and 1.30 (6 H, 2 t, 2 OCH_2CH_3), 1.76 (1 H, bd, $J_{6,6'}$ = 13.0 Hz, H-6), 1.92 (1 H, dt, $J_{4,4'}$ = $J_{3a,4}$ = 13.0, $J_{4,5}$ = 5.0 Hz, H-4), 2.18 (1 H, d, H-3'), 2.45–2.55 (2 H, m, H-6', H-4'), 2.68 (1 H, t, $J_{3,3'}$ = $J_{3,3a}$ = 10.5 Hz, H-3), 2.78 (1 H, m, H-3a), 2.83 and 3.02 (2 H, AB, $J_{A,B}$ = 15.80 Hz, CH_2COOEt), 4.22 and 4.13 (4 H, 2 q, 2 OCH_2CH_3), 4.32 (1 H, bt, $J_{4,5}$ = $J_{5,6}$ = 5.0 Hz, H-5), 4.85 (1 H, $J_{6',6a}$ = $J_{3a,6a}$ = 6.0 Hz, H-6a); HRMS calcd for $C_{14}H_{22}O_6$ (M^+ + H) 287.1494, found 287.1491. For compound **46b**: TLC R_f = 0.55 (EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.68 (1 H, bd, $J_{4,4'}$ = 14.5 Hz, H-4), 1.78 (1 H, dt, $J_{4,5}$ = 5.5 Hz, H-4'), 2.12 (1 H, ddd, $J_{5,6}$ = 10.0 Hz, $J_{6,6'}$ = 15.0, $J_{6,6a}$ = 5.5 Hz, H-6), 2.22 (1 H, dd, $J_{5,6'}$ = 5.0 Hz, H-6'), 2.32 (1 H, bd, $J_{3,3a}$ = 1.2 Hz, H-3), 2.75 (1 H, m, H-3a), 2.47 (1 H, dd, $J_{3,3'}$ = 14.00, $J_{3',3a}$ = 10 Hz, H-3'), 4.30 (1 H, m, H-5), 4.66 (1 H, bt, H-6a); HRMS calcd for $C_{14}H_{22}O_6$ (M^+ + H) 287.1494, found 287.1489.

Ethyl 2β-(Ethoxycarbonyl)-3aβ,6aβ-hexahydro-5α-[(methylsulfonyl)oxy]-2*H*-cyclopenta[*b*]furan-2α-acetate (47). A solution of **46a** (0.30 g, 1.05 mmol) in 20 mL of CH_2Cl_2 was meslyated according to the general procedure to produce a crude product which on flash chromatography eluting with 50% EtOAc–hexanes gave **47** (0.34 g, 90%): TLC R_f = 0.69 (EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.90 (1 H, bd, $J_{4,4'}$ = 14.00 Hz, H-4), 2.00 (1 H, dd, $J_{3,3a}$ = 4.5, $J_{3,3'}$ = 16.0 Hz, H-3), 2.10 (1 H, dd, $J_{5,6}$ = 3.0, $J_{6,6'}$ = 13.50 Hz, H-6), 2.40 (1 H, bd, $J_{3,3'}$ = 16.0 Hz, H-3'), 2.65

(1 H, dd, $J_{4,4'}$ = 14.0, $J_{4,5}$ = 10.0 Hz, H-4), 2.80 and 3.05 (2 H, AB, $J_{A,B}$ = 16.0 Hz, CH_2COOEt), 2.90 (1 H, m, H-3a), 3.01 (3 H, s, OSO_2CH_3), 4.95 (1 H, bt, $J_{3a,6a}$ = $J_{6,6a}$ = 6.0 Hz, H-6a), 5.19 (1 H, bdd, $J_{4,5}$ = 10.0, $J_{5,6}$ = 3.0 Hz, H-5); HRMS calcd for $C_{15}H_{24}O_8S$ (M^+ + H) 365.1270, found 365.1261.

2'β-Carboxy-5'β-(9-guanidino)-3'aβ,6'aβ-hexahydro-2H-cyclopenta[b]furan-2'α-acetic Acid (9). A solution of **47** (0.33 g, 0.9 mmol) in 1 mL of DMF was guaninalated with 2-amino-6-chloropurine using the described general procedure to produce crude **49**. A flash column chromatography on the crude product eluting with 10% ether–EtOAc gave **49** (a solid, decomp at 240–260 °C, 0.18 g, 45%): TLC R_f = 0.75 (7% MeOH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.82 (1 H, dd, $J_{6',6''}$ = 14.0, $J_{5',6'}$ = 8.3 Hz, H-6'), 2.10 (1 H, ddd, $J_{3a',4'}$ = 1.0, $J_{4',5'}$ = 6.6, $J_{4',4''}$ = 13.50 Hz, H-4'), 2.20 (1 H, ddd, $J_{3a',4''}$ = 1.2, $J_{4'',5'}$ = 8.2 Hz, H-4''), 2.28 (1 H, dd, $J_{3',3a'}$ = 3.3, $J_{3',3''}$ = 14.0 Hz, H-3'), 2.48 (1 H, ddd, $J_{5',6''}$ = 1.5, $J_{5',6''}$ = 14.0 Hz, H-6''), 2.76 (1 H, dd, $J_{3',3''}$ = 14.0, $J_{3'',3a'}$ = 10.0 Hz, H-3''), 2.96 (2 H, $J_{A,B}$ = 16.5 Hz, CH_2COOEt), 3.00 (1 H, m, H-3a'), 4.84 (1 H, m, H-5'), 5.10 (2 H, bs, NH_2), 7.80 (1 H, s, H-8). The hydrolysis of compound **49** (0.15 g, 0.35 mmol) was done according to the described general procedure to produce **9** (white solid, mp decomposed at 255–265 °C, 0.11 g, 85%): TLC R_f = 0.12 (iPrOH–MeOH–H_2O–NH_4OH, 70:10:5:15); ^{1}H NMR (DMSO, 200 MHz) δ 1.65 (1 H, dd, $J_{6',6''}$ = 14.0, $J_{5',6'}$ = 8.3 Hz, H-6'), 1.95 (1 H, dd, $J_{3',3''}$ = 13.3, $J_{3'',3a'}$ = 10.0 Hz, H-3''), 2.60 (1 H, m, H-3'), 2.64 (2 H, AB, J_{AB} = 16.5 Hz, CH_2COOEt), 3.05 (1 H, m, H-3a'), 4.40 (2 H, m, H-5' and H-6a'), 6.20 (2 H, s, NH_2), 7.90 (1 H, s, H-8). Anal. ($C_{15}H_{17}N_5O_6$) C, H, N.

2'β-Carboxy-5'β-(9-inosidino)-3'aβ,6'aβ-hexahydro-2H-cyclopenta[b]furan-2'α-acetic Acid (11). A solution of **47** (0.3 g, 0.82 mmol) in 2 mL of DMF was guaninalated with 6-chloropurine (0.19 g, 1.24 mmol) using the general procedure to give crude product which on flash chromatography eluting with EtOAc to give **48** (0.21 g, 60%): TLC 0.50 (EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 and 1.35 (6 H, 2 t, 2 $COOCH_2CH_3$), 1.88 (1 H, dd, $J_{6'6''}$ = 13.2 Hz, $J_{5',6'}$ = 7.70 Hz, H-6'), 2.20 (1 H, dd, $J_{3',3''}$ = 12.75 $J_{6',6a'}$ = 6.35, H-6''), 2.30–2.60 (2 H, m, H-4', H-3''), 2.78 (1 H, dd, $J_{3',3a'}$ = 9.70, H-3''), 3.05 (1 H, m, H-3a), 3.05 (2 H, AB, $J_{A,B}$ = 15.80, CH_2COOEt), 5.00 (1 H, t, $J_{6',6a'}$ = $J_{3a',6a'}$ = 6.35 Hz, H-6a'), 5.05 (1 H, m, H-5'), 8.15 (1 H, s, H-2), 8.75 (1 H, s, H-8). The hydrolysis of compound **48** (0.18 g, 0.44 mmol) was done according to the general procedure to produce **11** (0.15 g, white solid, decomposed 220–225 °C): ^{1}H NMR (DMSO, 200 MHz) δ 1.58 (1 H, dd, $J_{6',6''}$ = 14.0 Hz, $J_{5',6'}$ = 8.2 Hz, H-6'), 2.0 (1 H, dd, $J_{3',3''}$ = 13.1 Hz, $J_{3'',3a'}$ = 10.2 Hz, H-3''), 2.65 (1 H, m, H-3'), 2.70 (2 H, AB, J_{AB} = 16.3 Hz, CH_2OOEt), 3.05 (1 H, m, H-3'a). Anal. ($C_{15}H_{16}N_4O_6$) C, H, N.

1,6-Dihydro-2-amino-9-(2'β-carboxy-2'α-(carboxymethyl)-3'aβ,6'aβ-hexahydro-2H-cyclopenta[b]-5'β-furanyl)-6-chloropurine (12). A solution of **49** (0.30 g, 0.70 mmol) in 2 mL of aqueous ethanol was treated with 1 N NaOH (4 equiv) for 4 h. The reaction mixture was then neutralized with 1 N HCl, and the solvent was removed. The residue was dissolved in 2 mL of water and put on CHP$^{2)}$P gel column and eluted, first with water and then with 50% acetone–water, to give **12** (white solid): ^{1}H NMR (DMSO, 200 MHz) δ 1.62 (1 H, dd, $J_{5',6'}$ = 8.0, $J_{6',6''}$ = 13.80 Hz, H-6'), 1.88 (1 H, dd, $J_{3',3''}$ = 14.00 Hz, $J_{3'',3a'}$ = 10.0 Hz, H-3''), 2.62 (2 H, AB, $J_{A,B}$ = 15.8 Hz, CH_2COOH), 3.00 (1 H, m, H-3a'), 4.45 (2 H, m, H-5' and H-6a'), 6.30 (2 H, s, NH_2), 7.98 (1 H, s, H-8). Anal. ($C_{14}H_{16}N_5O_5Cl$) C, H, N.

Compound 63. Sodium borohydride was added to a cold 0.2 M solution of **62**[10] (2.10 g, 5.0 mmol) and $CeCl_3 \cdot 7H_2O$ (1.69 g, 5.0 mmol) in methanol, and the reaction mixture was stirred at room temperature for 3 h. The pH of the reaction was then adjusted to 7 with acetic acid, and the solvent was removed. The residue was taken up in diethyl ether, washed with a solution of brine, dried, and evaporated. The ice-cold solution of this crude product in pyridine (10 mL) was treated with benzoyl chloride (1.12 mL, 10.0 mmol) dropwise, and the reaction mixture was stirred at room temperature for 4 h. It was then poured into ice/water and extracted with methylene chloride (2 × 100 mL), and the combined organic layers were washed with 1 N HCl, a saturated solution of sodium bicarbonate, and water, dried, and evaporated to produce **63** (as a syrup): TLC R_f = 0.60 (20% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40 and 1.45 (6 H, s, $C(CH_3)_2$), 3.80 and 4.00 (2 H, AB, $J_{A,B}$ = 14.20 Hz, CH_2OTr), 4.95 (1 H, d, $J_{3,4}$ = 5.7 Hz, H-3), 5.07 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 5.7 Hz, H-4), 5.66 (1 H, m, H-5), 6.18 (1 H, bs, H-1), 7.1–7.45 (15 H, m, CPh_3), 7.65 and 8.12 (5 H, 2 m, C_6H_5). Anal. ($C_{35}H_{32}O_5$) C, H.

Compound 64. A solution of compound **63** (6.1 g, 11.46 mmol) in methylene (500 mL) was cooled to −78 °C, and a 25% wt solution of diethylaluminum iodide in toluene (32 mL, 7.78 g, 36.0 mmol) was carefully added. The reaction was complete in 5 min by TLC. The reaction mixture was then poured into an ice-cold solution of $NaHCO_3$. The organic layer was washed with water, dried and evaporated. The residue was column chromatographed eluting with 20% EtOAc–petroleum ether to produce **64** (an oil, 3.0 g, 90%): TLC R_f = 0.15 (20% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.35 and 1.40 (6 H, s, $C(CH_3)_2$), 4.5 (2 H, m, CH_2OH), 5.15 (2 H, m, H-3 and H-4), 5.65 (1 H, m, H-5), 5.90 (1 H, m, H-1), 7.4–7.7 and 8.15 (5 H, m, C_6H_5); HRMS calcd for $C_{16}H_{18}O_5$ (M^+ + H) 291.1232, found 291.1226.

Compound 65. To a cold solution of **64** (3.0 g, 10.34 mmol) in methylene chloride (100 mL) was added triphenylphosphine (2.98 g, 11.38 mmol) and carbon tetrabromide (3.78 g, 11.38 mmol), and the reaction mixture was then stirred at room temperature for 2 h. The solvent was then removed, and the residue was column chromatographed eluting with 20% EtOAc–petroleum ether to produce **65**: TLC R_f = 0.50 (20% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.45 (6 H, 2 s, $C(CH_3)_2$), 4.10 (2 H, s, CH_2Br), 5.10 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 5.5 Hz, H-4), 5.20 (1 H, d, $J_{3,4}$ = 5.5 Hz, H-3), 5.65 (1 H, bd, H-5), 6.00 (1 H, bs, H-1), 7.4–7.7 and 8.15 (5 H, m, C_6H_5); HRMS calcd for $C_{16}H_{17}O_4Br$ (M^+ + H) 353.0388, found 353.0298.

Compound 66. The 1.0 M solution of LDA (5 mL) in THF was cooled to −78 °C, and a solution of diethyl (phenylsulfonyl)-succinate (1.6 g, 5.1 mmol) was added. A solution of compound **65** (1.5 g, 4.26 mmol) in THF was introduced after 0.5 h, and the reaction mixture was removed from the cold bath and was allowed to warm to room temperature. A saturated solution of ammonium chloride was added, and the solvent was removed. The residue was extracted with methylene chloride, washed with water, dried, and evaporated. A solution of this crude product in benzene was refluxed for 2 h in the presence of pyridine. The solvent was removed, and residue was column chromatographed eluting with 20% EtOAc–petroleum ether to produce **66** (a thick syrup, 0.85 g, 45%): TLC R_f = 0.35 (20% EtOAc–petroleum ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40 (6 H, 2 s, $C(CH_3)_2$), 3.60 (2 H, bs, CH_2-CCOOEtCHCOOEt), 5.00 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 5.3 Hz, H-4), 5.30 (1 H, d, $J_{3,4}$ = 5.3 Hz, H-3), 5.70 (1 H, bd, H-5), 6.00 (1 H, bs, H-1), 6.50 (1 H, bs, CHCOOEt), 7.4–7.7 and 8.15 (5 H, m, C_6H_5); HRMS calcd for $C_{24}H_{28}O_8$ (M^+ + H) 445.1862, found 445.1856.

Ethyl 2β-(Ethoxycarbonyl)-5α-(benzoyloxy)-6α-hydroxy-3,5,6,6aβ-tetrahydro-2H-cyclopenta[b]furan-2α-acetate (67a). A solution of compound **66** (0.5 g, 1.13 mmol) in ethanol was treated with 2 mL of a 1.0 mM solution of H_2SO_4 for 1 day. The reaction mixture was then neutralized with sodium bicarbonate solution, and solvent was removed. The residue was extracted with ethyl acetate, dried, and evaporated. The solution of the crude product in toluene was refluxed for 2 h with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Solvent was then evaporated, and the residue was extracted with CH_2Cl_2, washed with water, dried ($MgSO_4$), and evaporated. The crude product was column chromatographed eluting with 60% EtOAc–hexane to give two isomeric products (0.18 g, 40%) in a 3:1 ratio. For major isomer **67a**: TLC R_f = 0.30 (50% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ, 1.20 and 1.30 (6 H, 2 t, 2 OCH_2CH_3), 2.65 (1 H, d, $J_{3,3'}$ = 16.00 Hz, H-3), 2.90 and 3.10 (2 H, $J_{A,B}$ = 15.00 Hz, CH_2COOEt), 3.20 (1 H, d, $J_{A,B}$ = 16.0 Hz, H-3'), 5.00 (1 H, m, H-6), 5.65 (1 H, m, H-6a), 5.95 (1 H, d, $J_{5,6}$ = 5.2 Hz, H-5), 6.15 (1 H, bs, H-4); HRMS calcd for $C_{21}H_{24}O_8$ (M^+ + H) 405.1549, found 405.1541.

Ethyl 2β-(Ethoxycarbonyl)-5α-[(methylsulfonyl)oxy]-6α-[(tert-butyldimethylsilyl)oxy]-3,5,6,6aβ-tetrahydro-2H-cyclopenta[b]furan-2α-acetate (68). To a solution of **67a** (1.0 g, 2.47 mmol) in dry DMF (10 mL) was added imidazole (0.20 g, 3.0 mmol), *tert*-butyldimethylsilyl chloride (0.45 g, 3.0 mmol), and 4-(dimethylamino)pyridine (0.25 g), and reaction mixture was kept at 60 °C for 4 h and then cooled and poured over ice/water. It was then extracted with diethyl ether (2 × 100 mL), the organic layer was washed with water, dried, and evaporated.

This crude product was dissolved in absolute ethanol (150 mL) and treated with a freshly prepared solution of sodium methoxide in ethanol. After 4 h it was carefully neutralized with acetic acid, and the solvent was removed. The residue was extracted with methylene chloride, washed with water, and dried ($MgSO_4$). The crude product was dissolved in methylene chloride and cooled in an ice bath. Methanesulfonyl chloride (0.3 g, 0.2 mL, 2.60 mmol) was added followed by a dropwise addition of triethylamine (0.36 g, 0.36 mL), and reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with methylene chloride and washed with water, dried, and evaporated. The residue was column chromatographed eluting with 50% EtOAc–hexane to produce **68** as a syrup (0.67 g, 55%): TLC R_f = 0.60 (50% ETOAc–hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.85 (9 H, s, $C(CH_3)_3$), 1.25 and 1.30 (6 H, 2 t, 2 OCH_2CH_3), 2.65 (1 H, d, $J_{3,3'}$ = 16.00 Hz, H-3), 2.90 and 3.10 (2 H, $J_{A,B}$ = 15.00 Hz, CH_2COOEt), 3.05 (3 H, s, OMs), 3.20 (1 H, d, $J_{A,B}$ = 16.0 Hz, H-3′), 5.00 (1 H, m, H-6), 5.65 (1 H, m, H-6a), 5.95 (1 H, d, $J_{5,6}$ = 5.2 Hz, H-5), 6.15 (1 H, bs, H-4); HRMS calcd for $C_{21}H_{36}O_9SiS$ (M^+ + H) 493.1927, found 493.1920.

2′β-Carboxy-5′β-(9-guanidino)-6′α-hydroxy-3′,5′,6′,6′aβ-tetrahydro-2*H*-cyclopenta[*b*]furan-2′α-acetic Acid (8). A solution of **68** (0.3 g, 0.6 mmol) in 1 mL of DMF was guaninalated with 2-amino-6-chloropurine using the described general procedure to produce crude **69**. A flash column chromatography on the crude product eluting with 10% ether–EtOAc gave **69** (a solid, decomposed at 250–265 °C, 0.09 g, 25%): TLC R_f = 0.80 (5% MeOH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (9 H, s, $C(CH_3)_3$), 2.68 (1 H, d, $J_{3',3''}$ = 15.0 Hz, H-3′), 3.50 (1 H, d, $J_{3',3''}$ = 15.0, H-3″), 2.90 (2 H, $J_{A,B}$ = 15.0 Hz, CH_2COOEt), 5.60 (1 H, d, $J_{5',6'}$ = 4.5 Hz, H-5′), 5.30 (2 H, bs, NH_2), 6.00 (1 H, bs, H-4′), 7.80 (1 H, s, H-8). The hydrolysis of compound **69** (0.08 g, 0.14 mmol) was done according to the described general procedure to produce **8** (white solid, mp decomposed at 270–280 °C, 0.04 g, 80%); TLC R_f = 0.25 (i-PrOH–MeOH–H_2O–NH_4OH, 70:10:5:15); ^{1}H NMR (DMSO, 200 MHz) δ 2.75 (1 H, d, $J_{3',3''}$ = 13.3 Hz, H-3′), 2.70 (2 H, AB, J_{AB} = 15.5 Hz, CH_2COOEt), 3.65 (1 H, d, $J_{3',3''}$ = 13.3 Hz, H-3″), 5.80 (1 H, d, $J_{5',6'}$ = 4.2 Hz, H-5′), 6.15 (1 H, bs, H-4′), 6.20 (2 H, s, NH_2), 8.0 (1 H, s, H-8). Anal. ($C_{15}H_{15}N_5O_7$) C, H, N.

Molecular Modeling. Modeling of cGMP and the selected PDE inhibitors was done using SYBYL Version 5.4 operating on a Silicon Graphics Power Series 4D/420 using the IRIS graphics interface or with a 4D/240s using the NITRO graphics interface installed on a Macintosh IIfx. Other parameters for minimization, fitting, and conformational searching are as described in the text. Graphics representations (Figure 1) of the wireframe structures and vector maps were produced using the Macintosh computer. The graphics were copied out of NITRO and pasted into a PowerPoint (Microsoft Corporation) document, recolored for clarity and processed into 8-in. × 11-in. color prints by the Genigraphics Corporation service bureau. Coordinates of the three modeled structures (SYBYL MOL2 files) are included in the supplementary material.

Enzyme Assays. cGMP, cAMP, calmodulin, and most other chemicals were obtained from Sigma Chemical Co. [^{3}H]cGMP (specific activity 42.5 Ci/mmol) and [^{3}H]cAMP (specific activity 31.2 Ci/mmol) purchased from Du Pont NEN were purified as described previously.[13] The cGMP hydrolyzing enzyme, bovine brain calmodulin sensitive PDE (CaM PDE), was purified by successive chromatography on DEAE-sephacel column and Calmodulin afflgel column as previously reported.[14] Two cAMP enzymes bovine heart cGMP-inhibited PDE (cGiPDE or type III PDE) was kindly provided by Dr. Beavo.[15] cAMP PDE (fraction III) which was a mixture of cGi-PDE and cGMP-insensitive PDE (type IV PDE) was isolated from rabbit aortic extracts by DEAE-Sephacel chromatography as previously described.[13] The cGi PDE represents a more highly purified form of the more commonly used cAMP PDE fraction.

Preparation of Drug Solution and Statistical Analysis. Stock solutions (10 mM) of tested compounds were prepared in water, 0.1 N HCl, 0.1 N NaOH, or 100% DMSO. Subsequent dilution was made with reagent-grade water. Final concentrations of organic vehicle were 1% or lower, and the minimal vehicle effect, if any, was substracted from the effect of drug. The IC_{50} (concentration required for 50% inhibition of PDE activity) was obtained by linear regression. The student *t*-test was used to analyze the statistical significance of the effect of any agent.

CaM PDE Assay. The activity of CaM PDE (type I PDE) was measured by the method previously reported.[16] Briefly, the final reaction mixture (0.2 mL) contained 50 mM Tris-HCl (pH 7.5), mM $MgCl_2$, 0.1 μM CaM, 1.0 mM $CaCl_2$, 1 μM cGMP, [^{3}H]-cGMP (about 80 000 cpm), and CaM-PDE (20–40 ng). The mixture was incubated at room temperature for 25 min. At the end of the incubation, IBMX (1-methyl-3-isobutylxanthine) was added to the mixture to a final concentration of 10 mM. The reaction mixture was boiled for 1 min and incubated for 10 min with 0.5 mg of Crotalus atrox (snake venom) to liberate guanosine. After addition 0.8 mL of a 1:2 (by volume) suspension of AG 1-X2 resin (chloride form)–water, the reaction mixture was vortex-mixed and centrifuged at 2000*g* for 10 min. The resulting supernatant fraction (0.3 mL) was transferred to counting vials with 3.5 mL of Ready Safe cocktail for the counting of radioactivity.

cGi-PDE or cA-PDE Assay. The final reaction mixture contained 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 0.1 mM EGTA, 1 μM cAMP, [^{3}H]cAMP (about 80 000 cpm), and cGi-PDE (20–40 ng) or cAMPPDE (50 μL eluate) in a total volume of 0.2 mL. The mixture was incubated at room temperature for 25 min and processed as above for the CaM PDE assay.

Supplementary Material Available: Molecular structure files in Sybyl MOL2 format for cGMP, compound 4, and compound 7 (14 pages). Ordering information is given on any current masthead page.

References

(1) (a) Nakagawa, F.; Okazaki, T.; Naito, A. Griseolic Acid, An Inhibitor of Cyclic Adenosine 3′,5′-Monophosphate Phosphodiesterase I. Taxonomy, Isolation and Characterization. *J. Antibiot.* **1985**, *XXXVIII*, 823–829. (b) Takahashi, S.; Nakagawa, F.; Kawazoe, K.; Furukawa, Y.; Sato, S.; Tamura, C.; Naito, A. Griseolic Acid, An Inhibitor of Cyclic Adenosine 3′,5′-Monophosphate Phosphodiesterase II. The Structure of Griseolic Acid. *J. Antibiot.* **1985**, *XXXVIII*, 830–834. (c) Iijima, Y.; Nakagawa, F.; Handa, S.; Oda, T.; Naito, A.; Yamazaki, M. Biological Properties of Griseolic Acid, a Cyclic AMP Phosphodiesterase Inhibitor With and Adenine Group, *FEBS Lett.* **1985**, *192*, 179–183.

(2) Takahashi, S.; Nakagawa, F.; Sato, S. Griseolic Acids B and C, An Inhibitor of Cyclic Adenosine 3′,5′-Monophosphate Phosphodiesterase *J. Antibiot.* **1988**, *XLI*, 705–706.

(3) Kaneko, M.; Murofushi, Y.; Kimura, M.; Yamazaki, M.; Iijima, Y. Studies on Griseolic Acid Derivatives I. Synthesis of Substituted Derivatives of Griseolic Acid at the N1, C6 C2′ or C7′ Position and Their Biological Activities. *Nucleic Acids Res.* **1985**, (*Symp. Ser. No. 16*), 89–92. (b) Murofushi, Y.; Kimura, M.; Kuwano, H.; Iijima, Y.; Yamazaki, M.; Kaneko, M. Studies on Griseolic Acid Derivatives III. Synthesis and Biological Activities of the Adducts of Griseolic Acid and their Base Exchanged Derivatives. *Nucleic Acids Res.* **1986**, (*Symp. Ser. No. 17*), 45–48. (c) Murofushi, Y.; Kimura, M.; Iijima, Y.; Yamazaki, M.; Kaneko, M. Studies on Griseolic Acid Derivatives IV. Synthesis and Phosphodiesterase Inhibitory Activity of Acylated Derivatives of Griseolic Acid. *Chem. Pharm. Bull.* **1987**, *35*, 1036–1043. (d) Murofushi, Y.; Kimura, M.; Iijima, Y.; Yamazaki, M.; Kaneko, M. Studies on Griseolic Acid Derivatives V. Synthesis and Phosphodiesterase Inhibitory Activity of Substituted Derivatives of the Hydroxy Group at the 2′- or 7′ Position in Griseolic Acid *Chem. Pharm. Bull.* **1987**, *35*, 4442–4453. (e) Murofushi, Y.; Kimura, M.; Iijima, Y.; Yamazaki, M.; Kaneko, M. Studies on Griseolic Acid Derivatives VI. Synthesis and Phosphodiesterase Inhibitory Activity of 6- and N^1-Derivatives of Griseolic Acid *Chem. Pharm. Bull.* **1988**, *36*, 1309–1320. (f) Murofushi, Y.; Kimura, M.; Kuwano, H.; Iijima, Y.; Yamazaki, M.; Kaneko, M. Studies on Griseolic Acid Derivatives VII. Synthesis and Phosphodesterase Inhibitory Activity of the C4′-C5′ Hydrogenated Products of Griseolic Acid and their Base Exchanged Derivatives. *Chem. Pharm. Bull.* **1988**, *36*, 3760–3769. (g) Kaneko, M.; Kimura, M.; Murofushi, Y.; Iijima, Y.; Yamazaki, M. Studies on Griseolic Acid Derivatives VIII. Synthesis and Phosphodiesterase Inhibitory Activity of a 2-Substituted Derivative of Griseolic Acid. *Nucleic Acids Res.* **1988**, (*Symp. Ser. No. 20*), 43–44. (h) Kaneko, M.; Murofushi, Y.; Kimura, M.; Sato, S.; Hata, T.; Iijima, Y.; Yamazaki, M. Studies on Griseolic Acid Derivatives IX. Synthesis and Phosphodiesterase Inhibitory Activity of a 8-Substituted Derivative of Griseolic Acid. *Sankyo Kenkyusho Nempo* **1989**, *41*, 87–103.

(4) Ignarro, L. J.; Kadowitz, P. J. The Pharmacological and Physiological Role of Cyclic GMP in Vascular Smooth Muscle Relaxation. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 171–191.
(5) Tulshian, D.; Doll, R. J.; Stansberry, M. F.; McPhail, A. T. Total Synthesis of Griseolic Acid Derivatives from D-Glucose. *J. Org. Chem.* **1991**, *56*, 6819–6822.
(6) Shibasaki, M.; Ueda, J.; Ikegami, S. New synthetic routes to 9(O)-methanoprostacyclin. A highly stable and biologically potent analog of prostacyclin. *Tetrahedron Lett.* **1979**, 433–436.
(7) Deardorff, D. R.; Myles, D. C.; MacFerrin, K. D. A palladium-catalyzed route to mono- and diprotected cis-2-cyclopentene-1,4-diols. *Tetrahedron Lett.* **1985**, *26*, 5615–5618.
(8) Deardorff, D. R.; Matthews, A. J.; McMeekin, D. S.; Craney, C. L. A highly enantioselective hydrolysis of cis-3,5-diacetoxycyclopent-1-ene. An enzymic preparation of 3(R)-acetoxy-5(S)-hydroxycyclopent-1-ene. *Tetrahedron Lett.* **1986**, *27*, 1255–1256.
(9) Ueno, Y.; Chino, K.; Watanabe, M.; Moriya, O.; Okawara, M. Homolytic carbocyclization by use of a heterogeneous supported organotin catalyst. A new synthetic route to 2-alkoxytetrahydrofurans and gamma-butyrolactones. *J. Am. Chem. Soc.* **1982**, *104*, 5564–5566.
(10) Altenbach, H.; Holzapfel, W.; Smerat, G.; Finkler, S. H. *Tetrahedron Lett.* **1985**, *26*, 6329–6332.
(11) For a recent application and description of the vector mapping technique, see: Whitten, J. P.; Harrison, B. L.; Weintraub, H. J. R.; McDonald, I. A. Modeling of Competitive Phosphono Amino Acid NMDA Receptor Antagonists. *J. Med. Chem.* **1992**, *35*, 1509–1514.
(12) Vorbruggen, H.; Kreolikiewicz, K.; Bennua, B. Nucleoside Synthesis with Trimethylsilyl Triflate and Perchlorate as Catalysts. *Chem. Ber.* **1981**, *114*, 1234–1255.
(13) Ahn, H. S.; Crim, W.; Romano, M.; Sybertz, E. J.; Pitts, B. J. R. Effects of selective inhibitors on cyclic nucleotide phosphodiesterases of rabbit aorta. *Biochem. Pharmacol.* **1989**, *38*, 3331–3339.
(14) Ahn, H. S.; Foster, M.; Cable, M.; Pitts, B. J. R.; Sybertz, E. J. Calcium/CaM-stimulated and cGMP-specific phosphodiesterases in vascular and non-vascular tissues. *Adv. Exp. Med. Biol.* **1991**, *308*, 191–197.
(15) Harrison, S. A.; Reifsnyder, D. H.; Gallis, B.; Cadd, G. G.; Beavo, J. A. Isolation and characterization of bovine cardiac muscle cGMP-inhibited phosphodiesterase: a receptor for new cardiotonic drugs. *Mol. Pharmacol.* **1986**, *29*, 506–514.
(16) Ahn, H. S.; Foster, M.; Foster, C.; Sybertz, E. J.; Wells, J. N. Evidence for essential histidine and cysteine residues in calcium/calmodulin-sensitive cyclic nucleotide phosphodiesterase. *Biochemistry* **1991**, *30*, 6754–6760.