Synthesis, Chromatographic Resolution, and Anti-Human Immunodeficiency Virus Activity of (±)-Calanolide A and Its Enantiomers

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The anti-HIV agent (±)-calanolide A (**1**) has been synthesized in a five-step approach starting with phloroglucinol [→ **5** → **6** → **11** → **18** → (±)-**1**], which includes Pechmann reaction, Friedel−Crafts acylation, chromenylation with 4,4-dimethoxy-2-methylbutan-2-ol, cyclization, and Luche reduction. Cyclization of chromene **11** to chromanone **18** was achieved by employing either acetaldehyde diethyl acetal or paraldehyde in the presence of trifluoroacetic acid and pyridine or PPTS. Luche reduction of chromanone **18** at lower temperature preferably yielded (±)-**1**. Reduction of chromone **12**, synthesized by Kostanecki−Robinson reaction from chromene **11**, failed to afford (±)-**1**. The synthetic (±)-**1** has been chromatographically resolved into its optically active forms, (+)- and (−)-**1**. The anti-HIV activities for synthetic (±)-**1**, as well as resultant (+)- and (−)-**1**, have been determined. Only (+)-**1** accounted for anti-HIV activity, which was similar to the data reported for the natural product, and (−)-**1** was inactive.

Introduction

Since human immunodeficiency virus (HIV) was identified as the etiological cause of acquired immune deficiency syndrome (AIDS) about 1 decade ago,[1] the provision for useful chemotherapeutic agents has been very challenging. Four antiviral nucleoside agents, AZT, ddC, ddI, and d4T, have been approved for clinical treatment of AIDS and AIDS-related complex. Although these drugs can extend the life of AIDS patients, none are capable of curing the disease, and serious side effects are induced. For example, treatment with AZT leads to a suppression of bone-marrow formation which often causes anemia and leukopenia, resulting in the need for frequent blood transfusions.[2] The use of ddI, ddC, and d4T is associated with painful sensory-motor peripheral neuropathy, as well as acute pancreatitis and hepatotoxicity in some cases.[3,4] Also, AZT, the standard antiviral therapy for initiating treatment of patients with HIV infection, has a very short half-life in the body, and high doses (250 mg) must be ingested every 4 h to maintain a constant level of drug in the body. More importantly, long-term treatment of patients with all these drugs has led to emergence of drug-resistant HIV strains;[5−7] primary infections have also been found with AZT-resistant HIV strains.[8]

The pandemic of AIDS is alarming with over 17 million people worldwide having been infected with HIV,[9] and four million people have already progressed to AIDS.[10] The need for other promising AIDS drug candidates having improved selectivity, and activity against HIV is extremely urgent.[9,11,12] Several approaches, including chemical synthesis, natural products screening, and biotechnology, have been utilized to identify compounds targeting different stages of HIV replication for therapeutic intervention such as Saquinavir, a protease inhibitor, which has been approved by the FDA very recently.[10,13−17]

As with other retroviruses, the genetic information of HIV is encoded in RNA and must be converted into DNA in the infected host cell. Only then can the viral genes be transcribed and translated into proteins in the usual sequence.[18] The virally encoded reverse transcriptase (RT) is the sole enzyme responsible for the catalytic formation of unintegrated viral DNA from the single-stranded RNA viral genome and, accordingly, plays a key role in the life cycle of HIV.[18−20] It is therefore logical to focus on this enzyme, which is not required for normal cellular processes, as a target for the development of anti-HIV drugs.[10,13,16−20]

The nucleoside analogues approved for drug therapy of AIDS target the viral RT enzyme and inhibit its enzymatic activity by competing with the normal substrates, deoxynucleoside triphosphates, after being activated in the cell into triphosphates. These nucleoside analogues lack a 2′-hydroxyl group and, when incorporated into the growing DNA chain, act as chain terminators. Recently, a series of structurally diverse nonnucleoside analogues, such as TIBO, nevirapine, pyridinone, BHAP, HEPT, TSAO, and α-APA, have been identified as HIV RT inhibitors.[10,13,21] Unlike nucleoside analogues, these nonnucleoside compounds are highly specific and selective, targeting only HIV-1 RT but not cellular DNA or other DNA polymerases such as HIV-2 RT, thereby being relatively nontoxic to human cells.

Although the mechanism of RT inhibition by HIV-1-specific compounds is not clear, it is suggested that binding of these compounds to RT is slow, reversible, and noncompetitive with respect to template-primer and deoxynucleoside triphosphates. A hydrophobic pocket, as indicated by the X-ray crystal structures of HIV-1

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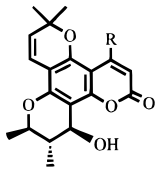
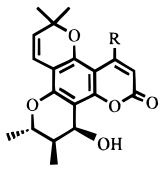
RT complexed with various nonnucleoside analogues such as nevirapine,[22−24] TIBO,[25,26] and α-APA,[27] is generally believed to be the binding site for the HIV-1-specific inhibitors to interact with HIV-1 RT. Such binding may result in conformational changes of RT to significantly slow the rate of the chemical reaction catalyzed by the enzyme[28−30] or to interfere with interaction between the enzyme and template-primer,[22,23,25,27] thus suppressing translocation of the template-primer following nucleotide incorporation. However, as in the case of the nucleoside analogues, the rapid emergence of HIV strains,[31] both *in vitro* and *in vivo*, which are resistant to these nonnucleoside inhibitors, has become a major concern that may affect the further development of these compounds as monotherapies.

Certain coumarin derivatives, isolated from several tropical plants of the genus *Calophyllum*, were recently identified as HIV-1-specific nonnucleoside inhibitors, among which (+)-calanolide A (**1**) and inophyllum B (**2**) are the most potent.[32,33] Since these compounds are active not only against the AZT-resistant strain of HIV-1 but also against the pyridinone-resistant strain A17,[34−37] they belong to a second generation of HIV-1-specific nonnucleoside inhibitors,[38] a pharmacological class different from that of the previously known nonnucleoside HIV-1 RT inhibitory drugs, and may represent a novel anti-HIV chemotype for drug development. The A17 strain contains Tyr 181 Cys and Lys 103 Asn amino acid changes and is cross-resistant to most of the other nonnucleoside inhibitors such as TIBO, pyridinone, nevirapine, HEPT, and diphenyl sulfone.[37] Combinations of (+)-calanolide A and AZT have been demonstrated *in vitro* to synergistically inhibit virus strain A17, and it has also been suggested that combination of (+)-calanolide A with other HIV-1-specific nonnucleoside inhibitors such as HEPT and diphenyl sulfone would be beneficial.[37]



1, (+)-Calanolide A (R = n-Pr)



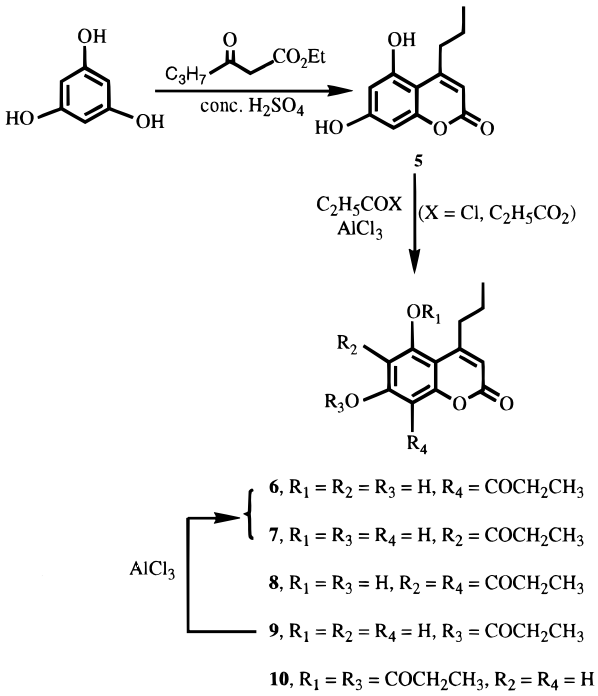
3, Costatolide or (-)-Calanolide B (R = n-Pr)

2, (+)-Inophyllum B (R = Ph)

4, Soulattrolide or (-)-Inophyllum P (R = Ph)

Supplies of the compound from plant material are extremely limited and difficult to obtain, since the original natural source of (+)-calanolide A was destroyed and other nearby members of the same species did not contain the same compound. Further pharmacological investigation of this class of compounds, as well as related analogues, is dependent upon the ready availability of significant amounts of material. Thus, a practical total synthesis of calanolide A is of great importance. Total synthesis of (±)-calanolide A has been recently reported by us[39] and others;[40,41] chiral synthesis of (+)-calanolide A has also been suggested[42] and developed.[43] Herein we wish to communicate a full account of our synthesis of (±)-calanolide A, as well as the chromatographic resolution of the synthesized racemate into its enantiomers and further characterization of the antiviral properties of the three entities, (±)-, (+)-, and (−)-calanolide A, which will be compared with the results reported for the natural product, (+)-calanolide A.

Scheme 1



Chemistry

Calanolide A has three different rings, coumarin, chromene, and chromane, built around a phloroglucinol core. Accordingly, our synthetic strategy was to utilize phloroglucinol as a starting material and then build the coumarin ring followed by the chromene ring. The chromane ring is introduced last, the stereochemistry of which plays a very important role in maintaining antiviral activity.[32,33]

Thus, Pechmann reaction[44] on phloroglucinol with ethyl butyrylacetate in the presence of concentrated sulfuric acid afforded 5,7-dihydroxy-4-propylcoumarin (**5**) quantitatively (Scheme 1). Product yield and purity were dependent on the amount of sulfuric acid used; 1 mL of H_2SO_4/g of ethyl butyrylacetate led to optimal results in a 100 g scale reaction. When polyphosphoric acid was used, only a 50% yield of coumarin **5** was obtained.

Acylation of coumarin **5** with propionyl chloride in the presence of $AlCl_3$ led to a mixture of 8-acylated coumarin **6** along with 6-acylated coumarin **7** and 6,8-bis-acylated coumarin **8** in a ratio of 1:1:1 in 50% yield (Scheme 1). The desired 8-acylated product **6** was separated from **7** and **8** by silica gel chromatography. Structural assignments of the 8- or 6-acyl derivatives **6** and **7** were made by comparison of UV spectra with analogues[45] and confirmed by Gibbs color reaction[46] and spectroscopic data. A route developed for the synthesis of *Mammea* coumarins[45] was initially attempted for preparation of compound **6**, but it proved too awkward and low yielding.

Since various factors can affect the Friedel−Crafts acylation,[47] further investigations to improve the selectivity and yield for the 8-acylated product **6** were conducted. It was found that 8-acylated coumarin **6** could be obtained as a single product in 45% yield by replacing propionyl chloride with propionic anhydride

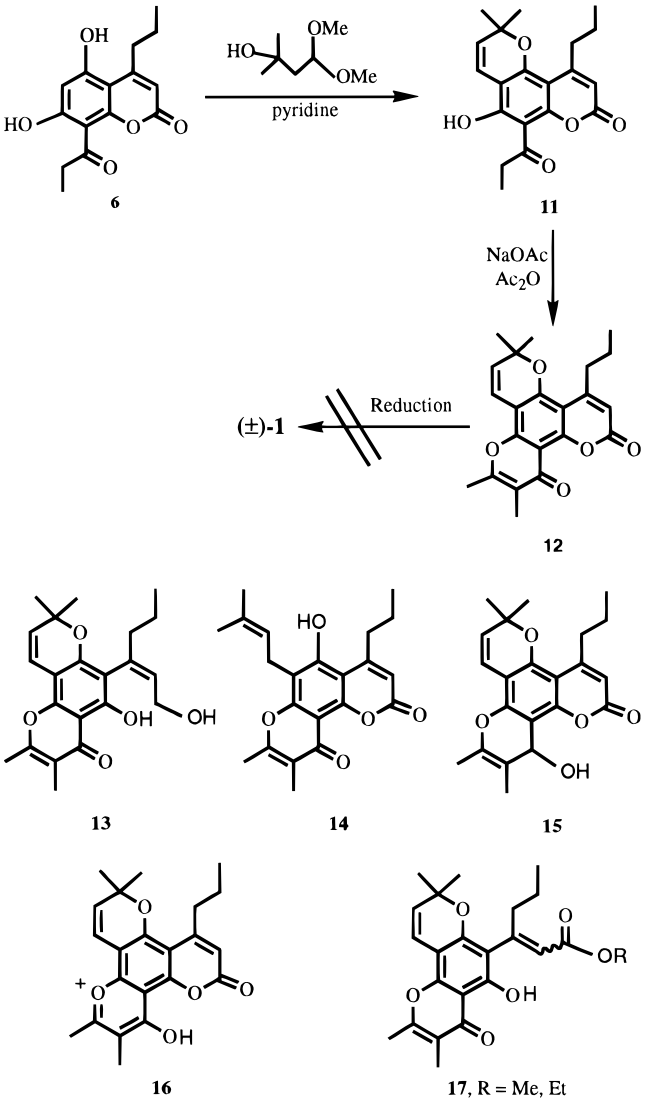
under modified conditions. No trace of **7** or **8** was observed, although a small amount of the 7-monoester **9** did form. The desired product **6** was purified without the use of column chromatography. Temperature was determined to be an important factor in this reaction. Addition of a mixture of propionic anhydride and $AlCl_3$ into a preheated (75 °C) mixture of coumarin **5** and $AlCl_3$ was essential for the selective formation of **6**. At ambient temperature, the reaction of propionic anhydride with the coumarin **5** in the presence of $AlCl_3$ led to formation of 7-monoester **9** as a single product when either dichloromethane, 1,2-dichloroethane, a mixture of dichloromethane and cyclohexane, or THF was used as solvent. Excessive propionic anhydride resulted in formation of the 5,7-diester **10** (Scheme 1). The order of addition of the reagents ($AlCl_3$, propionic anhydride, and coumarin **5**) did not affect the outcome of the reaction at ambient temperature. The structure of 7-monoester **9** was in full agreement with all of the spectroscopic data and further confirmed by a positive Gibb's test.[46]

Attempts to convert both **9** and **10** under Fries conditions into the corresponding 8-acylated coumarin **6** were unsuccessful. It was reported that 5,7-diacetoxy-4-phenylcoumarin underwent Fries rearrangement to form 6,8-diacetyl-5,7-dihydroxy-4-phenylcoumarin.[48] The same was observed for the 5,7-diester **10**. In refluxing triglyme, 5,7-diester **10** was decomposed into the starting coumarin in the presence of $AlCl_3$. The 7-monoester **9** was stable in refluxing pyridine, but in 1,2-dichloroethane with $AlCl_3$, it afforded both the 8- or 6-acylated products **6** and **7** in a ratio varying from 1:1 to 3:7, depending upon the reaction temperature (Scheme 1).

The chromene ring was then introduced upon treatment of the 8-acylated coumarin **6** with 4,4-dimethoxy-2-methylbutan-2-ol.[45,49] The reaction proceeded readily in the presence of pyridine to provide chromene **11** (Scheme 2). Azeotropic removal of water and MeOH formed during the reaction by using toluene as solvent helped to facilitate the reaction upon scaleup (see the Experimental Section). The ^1H NMR signals for chromene **11** were solvent-dependent. Thus, H-7 in the chromene ring and H-3 in the coumarin ring were shifted downfield from 5.58 ppm in $CDCl_3$ to 5.79 ppm in DMSO-d_6 and 6.00 to 6.12 ppm, respectively; however, H-8 and the CH_2 group in the propionyl side chain were shifted upfield from 6.73 ppm in $CDCl_3$ to 6.62 ppm in DMSO-d_6 and 3.35 to 3.19 ppm, respectively. It has been reported that the chemical shifts for H-8 of the chromene ring are characteristic for 8-acylchromenocoumarins, which appear at 6.62−6.67 ppm.[41,45] In contrast, in 6-acylchromenocoumarins, the corresponding olefinic protons appear at 6.75−6.85 ppm.[41,45] Therefore, the chemical shifts for H-8 of chromene **11** in both $CDCl_3$ and DMSO-d_6 (6.73 and 6.62 ppm) are consistent with the range reported for 8-acylchromenocoumarins[41,45] and further confirm the structure of chromene **11**, which, in turn, implies that the structure of compound **6** was correctly assigned.

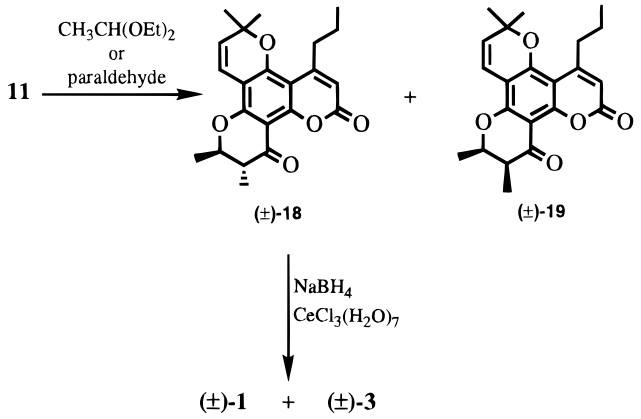
Conversion of chromene **11** to form chromone **12** under Kostanecki−Robinson[50,51] conditions was initially carried out, since chromene **11** contains an *o*-hydroxyaryl ketone moiety. Thus, in the presence of acetic anhydride and sodium acetate, the Kostanecki−Robinson reaction proceeded smoothly to generate chromone **12** in 62% yield (Scheme 2). It was hoped that chromone

Scheme 2



12 could be reduced to a saturated alcohol, that is, (±)-calanolide A (**1**) and its diastereoisomers. However, results indicated that the γ-pyrone in chromone **12** is quite resistant to reduction of the double bond and/or the keto group, and the reduction failed to afford (±)-**1**. Instead, the α-pyrone system (coumarin) in chromone **12** proved to be more reactive. A variety of reduced products were isolated depending upon the reducing agent used and the reaction conditions utilized (Scheme 2). For example, $NaBH_4/CeCl_3$,[52] $NaBH_4/CuCl_2$,[53] Baker's yeast, L-Selectride, and DIBAL preferentially reacted at the lactone position to lead to the formation of diol **13** as the major product. In contrast, *n*-Bu_3SnH[54] produced chromene ring-opened compound **14** in a 15% yield and a mixture which contained unsaturated alcohol **15**, as indicated by ^1H NMR spectroscopy. The use of SmI_2/HMPA[55] and 9-BBN resulted in a similar mixture to that produced by *n*-Bu_3SnH, while $[(Ph_3P)CuH]_6$[56] did not react with chromone **12**. It was assumed that the γ-pyrone in chromone **12**, under acidic conditions, might form a pyrylium salt (**16**) which might, in turn, be readily reduced.[57] Thus, chromone **12** was first treated with various acids such as perchloric acid, HCl, and trifluoroacetic acid followed by a variety of reducing agents such as $NaCNBH_3$, $NaBH_4$, and borane−pyridine complex. In alcoholic solvents such as MeOH and EtOH, lactone-opened ester **17** was obtained

Scheme 3



as the major product of these reactions. Attempts to reduce the γ-pyrone in ester **17** by utilizing $NaBH_4$ in MeOH failed to produce any products, with only recovery of ester **17** observed. The results are understandable if one considers that chromone **12** contains both α- and γ-pyrone moieties and that the carbonyl group of γ-pyrones is generally less active than a normal ketone, due to aromatic character, while α-pyrones behave as $\alpha,\beta,\gamma,\delta$-unsaturated lactones.[58] Consequently, the double bond in the γ-pyrone of chromone **12** might not be electron-deficient (or electrophilic) enough to coordinate with copper[56] or samarium,[55,59] which is crucial for initiating the conjugate reduction of α,β-unsaturated carbonyl compounds. It has been reported that reduction of benzo-α-pyrones (coumarins) with $LiAlH_4$ led to diol derivatives.[58,60] As to benzo-γ-pyrones (chromones), the reduction of the ketone and double bond to form γ-pyranols and 2,3-dihydro-γ-pyranols (chromanones) has also been reported.[58]

Attention was then shifted to cyclization of chromene **11** into chromanone **18**, which could be readily reduced to (±)-**1**. In the presence of a base such as K_2CO_3 or LDA, reaction of chromene **11** with acetaldehyde was unsuccessful in forming **18**.[61] However, treatment of chromene **11** with acetaldehyde diethyl acetal in the presence of trifluoroacetic acid and pyridine formed racemic chromanone **18** with the desired stereochemical arrangement, characterized by spectroscopic data and consistent with previously reported structures[40] (Scheme 3). The yield for this reaction, however, was somewhat inconsistent, with the best being ca. 30%.[39] The process was improved by replacing acetaldehyde diethyl acetal with paraldehyde as the acetaldehyde equivalent and under modified conditions (for details, see the Experimental Section); the formation of racemic chromanone **18** (30% yield), along with the *cis*-isomer (±)-**19** (calanolide D) as a minor product, was reproducible and scalable.

Reduction of (±)-ketone **18** with $NaBH_4$ at room temperature afforded the hydroxyl epimers in ca. a 1:1 ratio. Luche reduction[52] of ketone (±)-**18** using $NaBH_4/CeCl_3$ at −10 to −40 °C was highly selective in affording 90% (±)-calanolide A (**1**) over (±)-calanolide B (**3**)[39] (Scheme 3), while at room temperature the selectivity for calanolide A over calanolide B was only 60:40. On a relative larger scale, selection of calanolide A was slightly decreased to 80% and unreacted starting material remained, due in part to the incomplete solubility of (±)-**18** in EtOH at the reaction temperature (see the Experimental Section for details). (±)-Calanolide A (**1**) and (±)-calanolide B (**3**) were separated by semipreparative HPLC, and (±)-calanolide A (**1**) thus obtained exhibited initial mp 52−54 °C (lit.[40] mp 56−58 °C) which increased to 102 °C after being thoroughly dried.

Attempts to resolve the synthetic (±)-**1** into its enantiomers by enzyme-catalyzed acylation or hydrolysis met with only limited success. Enzymes that have been investigated include lipase CC (*Candida cylindracea*), lipase AK (*C. cylindracea*), lipase AY (*C. cylindracea*), lipase PS (*Pseudomonas* species), lipase AP (*Aspergillus niger*), lipase N (*Rhizopus nieveus*), lipase FAP (*Rhizopus nieveus*), lipase PP (porcine pancrease), pig (porcine) liver esterase (PLE), pig liver acetone powder (PLAP), subtilisin, and their immobilized forms on Celite, molecular sieves, or ion exchange resin. Among the enzymes mentioned above, only lipase PS-13 (Sigma) enantioselectively acylated (−)-**1**, with the (+)-enantiomer left unreacted. However, reaction was very slow and impractical. For example, after 20 days, 21% of (−)-**1**, as detected by chiral HPLC, was converted into its butyrate ester when vinyl butyrate was used as acylating agent. There were no significant changes observed for different vinyl esters such as acetate, butyrate, and propionate. These results imply that the hydroxyl group in (±)-**1** might be so sterically hindered that it does not fit into the catalytic pocket of the enzymes examined above.[62,63]

Practically, the synthetic (±)-**1** was resolved into its enantiomers, (+)- and (−)-**1**, by using a semipreparative chiral HPLC column.[64] Thus, on a normal phase silica gel HPLC column, only one peak was observed with a retention time of 10.15 min when hexane/ethyl acetate (70:30) was used as the mobile phase at a flow rate of 1.5 mL/min and a wavelength of 290 nm was used as the UV detector setting. However, on a chiral HPLC column packed with amylose carbamate, two peaks with retention times of 6.39 and 7.15 min in a ratio of 1:1 were observed. The mobile phase was hexane/ethanol (95:5) and the UV detector was set at a wavelength of 254 nm. These two components were then separated using a semipreparative chiral HPLC column packed with the same material as the analytical column to provide the enantiomers of calanolide A. The chemical structures of the separated enantiomers, which were assigned based on their optical rotations and compared with the reported natural[32] and synthetic product,[43] were further characterized by spectroscopic data.

In conclusion, (±)-calanolide A (**1**) has been synthesized in a five-step approach (overall yield ca. 6.6%) starting with phloroglucinol [→ **5** → **6** → **11** → **18** → (±)-**1**] and has been chromatographically resolved into its optically active forms, (+)- and (−)-**1**.

Biological Activity

The synthetic (±)-**1** and resolved enantiomers [(+)- and (−)-**1**] were tested *in vitro* against both laboratory strains and clinical isolates of HIV. The results are shown in Table 1. Essentially, (+)- and (−)-**1** exhibit the same level of cytotoxicity in the cell lines examined and do not exhibit synergistic toxicity, since the cytotoxicity of (±)-**1** is approximately the same. However, only (+)-**1** accounted for the anti-HIV activity, which was similar to the data reported for the natural product,[32] and (−)-**1** was inactive. Both (±)- and (+)-**1** showed the same relative activity against RF_{II} and III_B strains of HIV-1 as well as clinical isolates H112-2 in CEM or MT2 cell lines; however, (+)-**1** was generally

Table 1. Anti-HIV Activity of (±)-, (+)-, and (−)-Calanolide A (**1**)

strain/cell		(±)-**1**	(+)-**1**	(−)-**1**	AZT	ddC
RF_{II}/CEM	EC_{50} (μM)	0.486	0.267		0.023	0.189
	IC_{50} (μM)	22.81	22.96	18.70	301.60	47.34
	IC_{50}/EC_{50} (TI)	47	86		13 113	250
III_B/MT2	EC_{50} (μM)	0.108	0.053		0.029	0.900
	IC_{50} (μM)	6.86	14.80	7.31	51.64	83.80
	IC_{50}/EC_{50} (TI)	64	279		1780	93
H112-2[d]/MT2	EC_{50} (μM)	0.135	0.107	(2.67[a])	0.037	1.562
	IC_{50} (μM)	6.53	7.15	6.21 (4.05[b])	119.84	258.97
	IC_{50}/EC_{50} (TI)	48	67	(2[c])	3236	166
G910-6[e]/MT2	EC_{50} (μM)	0.108	0.027	(0.747[a])		0.994
	IC_{50} (μM)	7.42	7.17	6.16 (3.97[b])	131.71	212.10
	IC_{50}/EC_{50} (TI)	69	266	(5[c])		213
A17[f]/MT2	EC_{50} (μM)	0.297	0.427		0.014	0.331
	IC_{50} (μM)	6.94	6.99	5.89	83.44	134.93
	IC_{50}/EC_{50} (TI)	23	16		5960	408

[a] EC_{25} (μM). [b] IC_{25} (μM). [c] IC_{25}/EC_{25}. [d] Pre-AZT treatment isolate. [e] Post-AZT treatment isolate. [f] Pyridinone-resistant isolate.

Table 2. Inhibitory Effects of (±)-, (+)-, and (−)-Calanolide A (**1**) on Nucleic Acid Polymerase[a]

compd	HIV-1 RT[b]		HIV-2 RT	TIBO-resistant HIV-1 RT	AMV[c] RT	DNA polymerases		RNA polymerases
	DDDP	RDDP				α	β	
(+)-**1**	0.38	0.32	inactive	10.1	inactive	inactive	205	inactive
	(83.9%)	(97.4%)	(0%)	(91.5%)	(20.6%)	(22.9%)	(62%)	(16%)
(±)-**1**	0.7	0.97	inactive	17.6	inactive	inactive	279	inactive
	(80.1%)	(97.6%)	(10%)	(81.2%)	(13.3%)	(24.9%)	(61%)	(27.8%)
(−)-**1**	155.7	27.8	inactive	inactive	inactive	inactive	214	inactive
	(52.7%)	(68.1%)	(5%)	(0%)	(16.8%)	(22.6%)	(77.1%)	(12.1%)

[a] Values are IC_{50} (μM); percent inhibition values at 200 μg/mL are shown in the parentheses. [b] HIV RT was assessed for DNA-dependent DNA polymerase (DDDP) and RNA-dependent DNA polymerase (RDDP) activities. IC_{50} value for AZT-TP against RDDP activity of HIV-1 RT was 0.051 μM. [c] AMV RT: avian myeloblastosis virus reverse transcriptase.

2-fold more active, consistent with expectations if only one enantiomer is active. Both the AZT-resistant strain G910-6 and the pyridinone-resistant strain A17 were fully inhibited by (±)- and (+)-**1**.[32,37] The (+)-**1** was more active than (±)-**1** against the AZT-resistant strain G910-6 with EC_{50} values of 0.03 and 0.1 μM, respectively, while, surprisingly, the activity of (±)-**1** against the pyridinone-resistant strain A17 was more comparable with that of (+)-**1** (EC_{50} = 0.3 and 0.4 μM, respectively) (Table 1).

Direct cytotoxicity of (+)-**1** upon the target cells was apparent only at concentrations of ca. 100- or 200-fold efficacy; the concentrations of (±)-**1** leading to cytotoxicity were lower. Although the separation of antiviral and cytotoxicity concentrations (therapeutic index) for (±)- and (+)-**1** is relatively narrow compared with other nonnucleoside inhibitors[13,21] such as TIBO, pyridinone, and nevirapine, it is reasonable when compared with ddC.

To further investigate the activity of the calanolides, inhibition profiles have been evaluated with HIV-1 RT and a variety of other nucleic acid polymerases. As expected,[32] both (±)- and (+)-**1** specifically inhibited HIV-1 RT and were not active (<30% inhibition at 200 μg/mL) against HIV-2 RT and avian myeloblastosis virus (AMV) RT (Table 2). Therefore, calanolide A does function as a nonnucleoside HIV-1-specific RT inhibitor. Compounds (±)- and (+)-**1** were effective inhibitors of the DNA-dependent DNA polymerase (IC_{50} = 0.7 and 0.38 μM, respectively) and RNA-dependent DNA polymerase (IC_{50} = 0.97 and 0.32 μM, respectively) activities of HIV-1 RT. Accordingly, calanolide A may be considered a potent inhibitor of the polymerase activity of HIV-1 RT, since the activity of (+)-**1** is only 6-fold less than that observed with AZT-TP (IC_{50} = 0.051 μM).[65] Furthermore, calanolide A [both (±)- and (+)-**1**] inhibited TIBO-resistant HIV-1 RT. Although (−)-**1** was not active against HIV-1 in cell culture assays, it was moderately active against the HIV-1 RT enzyme with IC_{50} = 155.7 μM for DNA-dependent DNA polymerase and IC_{50} = 27.8 μM for RNA-dependent DNA polymerase (Table 2).

It may be anticipated that cytotoxicity associated with calanolide A is due to its relatively nonspecific inhibition of polymerases. A number of studies have reported that cellular DNA polymerase α, the enzyme primarily involved in cell replication, is resistant to nucleoside analogues, but both polymerases β and γ are sensitive to nucleoside analogues[66] (e.g., IC_{50}'s for AZT with polymerases β and γ were 11 and 16 μM, respectively). This has been suggested to account for the toxicity of AZT and the resulting serious limitation of clinical usefulness.[67] The potential of calanolide A to inhibit nucleic acid polymerases was therefore investigated (Table 2). Among the cellular polymerases tested, DNA polymerase β seems to be the most sensitive to all three entities of calanolide A, (±)-, (+)-, and (−)-**1**, but activity was weak and only observed at higher concentrations (i.e., 60% inhibition for (±)- and (+)-**1** at 279 and 205 μM and 77% inhibition for (−)-**1** at 214 μM). Neither DNA polymerase α nor RNA polymerase was inhibited significantly in the presence of high concentrations of calanolide A (Table 2). Comparison of the IC_{50} values obtained with HIV-1 RT (0.3−1.0 μM) with the IC_{50} values obtained with polymerase β (around 200 μM) may indicate that the cytotoxicity of calanolide A could be, at least in part, due to the inhibition of this cellular enzyme. Similarly, other coumarin compounds such as costatolide (**3**) and soulattrolide (**4**) have been determined to very weakly inhibit DNA polymerase β with IC_{50} values being >500 μM.[68]

Structure−Activity Relationships

On the basis of the published preliminary SAR studies related to calanolides and inophyllums, one can specu-

late that the saturated lower pyran ring plays a very important role in maintaining antiviral activity for this series of compounds because the stereochemistry of the methyl groups at C_{10} and C_{11} and the existence of the hydroxyl group at C_{12} are essential. For example, the methyl groups at C_{10} and C_{11} must be *trans* to each other in order to observe the maximum anti-HIV activity as evidenced by (+)-calanolide A (**1**),[32] (+)-inophyllum B (**2**),[33] costatolide [or (−)-calanolide B] (**3**),[64] and soulattrolide[69] [or (−)-inophyllum P] (**4**).[70] The anti-HIV activity for compounds with *cis*-methyl groups at C_{10} and C_{11} such as (+)-inophyllum A[33] and (+)-inophyllum D[33] is dramatically decreased. The compound (−)-calanolide C was first reported to be very weakly active against HIV,[32] but its structure was later revised to pseudocalanolide C.[71] The corresponding 12-keto compounds, e.g., (+)-inophyllum C[33,72] and (+)-inophyllum E,[33] were inactive, as was the 12-methoxy (+)-calanolide A.[32] However, the 12-acetoxy derivatives[32,33] of (+)-calanolide A (**1**) and (+)-inophyllum B (**2**) exhibited anti-HIV-1 activity, although their antiviral activity was significantly lower as compared to that of the corresponding free hydroxyl compounds. These results suggest that much of the antiviral activity of the calanolides and inophyllums is associated with a free 12-hydroxyl group or a functional group which can be hydrolyzed to a hydroxyl group.

The stereochemistry of 12-OH in the calanolides and inophyllums was originally believed not to be critical for anti-HIV activity because (+)-calanolide A (**1**), (+)-inophyllum B (**2**), and their 12-OH epimers, (+)-calanolide B and (+)-inophyllum P, were all reported to be active against HIV.[32,33] Recently, it was found that the naturally occurring (+)-calanolide B was a partially racemized mixture containing 45% (−)-calanolide B (**3**).[64] It was further discovered that only (−)-calanolide B (**3**) was active against HIV and the (+)-enantiomer was inactive.[64] This result, combined with the fact that (−)-calanolide A (Tables 1 and 2), the 12-OH epimer of (−)-calanolide B (**3**), is inactive against HIV, implies that the 12-OH must be in the β-position in order to confer the maximum anti-HIV activity. This may be also true for the inophyllums. Although both (+)- and (−)-inophyllum P[70] were reported to be active against HIV,[33,69] the fact that the optical rotation for (+)-inophyllum P (+19.8°)[33] was lower than that of (−)-inophyllum P (**4**)[70] (−29.6°)[73] led us to believe that the naturally occurring (+)-inophyllum P[33] was also partially racemized (ca. 67% ee), as with (+)-calanolide B.[64] Consequently, only (−)-inophyllum P (**4**)[70] possesses the β-hydroxyl group at the C_{12} position for activity against HIV,[69] while (+)-inophyllum P with the α-hydroxyl group is predicted to be inactive. Ultimate proof of this hypothesis must await synthesis of both (+)- and (−)-inophyllum P or resolution of the racemate followed by the appropriate biological assays.

Experimental Section

Chemistry. All melting points are corrected. NMR spectra were run on either a Hitachi 60 MHz R-1200 NMR or a Varian VX-300 NMR spectrometer. The chemical shifts are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. IR spectra were obtained using a Midac M series FT-IR spectrometer. Mass spectral data were recorded using a Finnegan MAT 90 mass spectrometer. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F_{254} from EM Science), and components were visualized with UV light and/or stained with iodine. Column chromatography was performed with silica gel 60 (70−230 mesh from EM Science). An isocratic liquid chromatograph (Hitachi Instruments, Inc., Naperville, IL) was used for HPLC which consisted of a L-6200A pump, a L-4000H/L-4200H UV/UV−vis detector, and a D-2500 chromatointegrator. The analytical normal phase silica gel HPLC column was 250 mm × 4.6 mm i.d. Zorbasil with 5 μm particle size (MAC-MOD Analytical, Inc., PA), and the analytical chiral HPLC column was packed with amylose carbamate (250 mm × 4.6 mm i.d. Chiralpak AD, 10 μm particle size; Chiral Technologies, Inc., PA). The semipreparative normal phase HPLC column was 250 mm × 22 mm i.d. Econosil silica gel with 10 μm particle size (Alltech Associates Inc., IL), and the semipreparative chiral HPLC column was packed with the same material as the chiral analytical column but with a column size of 250 mm × 20 mm i.d. All chemical reagents and anhydrous solvents were purchased from Aldrich Chemical Co. The reagent 4,4-dimethoxy-2-methylbutan-2-ol was prepared according to the literature procedure.[45]

5,7-Dihydroxy-4-propylcoumarin (5). Concentrated sulfuric acid (200 mL) was added into a mixture of phloroglucinol dihydrate (150 g, 0.926 mol) and ethyl butyrylacetate (161 g, 1.02 mol). The resulting mixture was mechanically stirred at 90 °C for 2 h, after which it was poured onto ice. The solid product was collected by filtration and then dissolved in ethyl acetate. The solution was washed with brine and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was triturated with hexane to provide essentially pure **5** (203 g) in near quantitative yield, mp 233−235 °C (lit.[40] mp 236−238 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95 (3H, t, J = 7.3 Hz, CH_3), 1.59 (2H, apparent sextet, J = 7.4 Hz, CH_2), 2.85 (2H, t, J = 7.5 Hz, CH_2), 5.84 (1H, s, H-3), 6.19 (1H, d, J = 2.3 Hz, H-6), 6.28 (1H, d, J = 2.3 Hz, H-8), 10.32 (1H, s, OH), 10.60 (1H, s, OH); MS (EI) 220 (100, M^+), 205 (37.9, M − CH_3), 192 (65.8, M − C_2H_4), 177 (24.8, M − C_3H_7), 164 (60.9, M − $CHCO_2$ + 1), 163 (59.6, M − $CHCO_2$); IR (KBr) 3210 (vs, br, OH), 1649 (vs, sh), 1617 (vs, sh), 1554 (s) cm^{-1}. Anal. Calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.04; H, 5.43.

Acylation of 5,7-Dihydroxy-4-propylcoumarin (5): A. With Propionyl Chloride. A three-necked flask (500 mL) equipped with an efficient mechanical stirrer, thermometer, and addition funnel was charged with 4-propyl-5,7-dihydroxycoumarin (**5**) (25.0 g, 0.113 mol) and aluminum chloride (62.1 g, 0.466 mol). Nitrobenzene (150 mL) was then added, and the mixture was stirred until a solution was obtained, which was then cooled to 0 °C in an ice bath. A solution of propionyl chloride (15.2 g, 0.165 mol) in carbon disulfide (50 mL) was added dropwise at such a rate that the reaction temperature was held at 8−10 °C. Addition was completed over a period of 1 h with vigorous stirring. The reaction was monitored by TLC using a mobile phase of 50% ethyl acetate/hexane. After 3 h, an additional portion of propionyl chloride (2.10 g, 0.0227 mol) in carbon disulfide (10 mL) was added, and the mixture was stirred for an additional 30 min, whereupon the reaction mixture was poured onto ice and allowed to stand overnight. The nitrobenzene was removed by steam distillation, and the remaining solution was extracted several times with ethyl acetate. The extracts were combined and dried over Na_2SO_4. The crude product obtained by evaporation *in vacuo* was purified by chromatography on a silica gel column eluting with 50% ether/hexane to provide compounds **8**, **6**, and **7**, successively.

5,7-Dihydroxy-8-propionyl-4-propylcoumarin (6): 5.3 g (17% yield) was obtained, mp 244−246 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.96 (3H, t, J = 7.3 Hz, CH_3), 1.09 (3H, t, J = 7.1 Hz, CH_3), 1.57 (2H, apparent sextet, J = 7.3 Hz, CH_2), 2.86 (2H, t, J = 7.6 Hz, CH_2), 3.03 (2H, q, J = 7.1 Hz, CH_2), 5.95 (1H, s, H-3), 6.28 (1H, s, H-6), 11.50 and 12.75 (2H, 2s, 2 OH); MS (EI) 277 (6.6, M + 1), 276 (39.0, M^+), 247 (100, M − C_2H_5); IR (KBr) 3239 (s, br, OH), 1693 (s, C═O), 1625 and 1593 (s) cm^{-1}; UV λ_{max} (MeOH): 219, 288, 319 nm. Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 64.92; H, 5.83.

5,7-Dihydroxy-6-propionyl-4-propylcoumarin (7): 5.0 g (16% yield) was obtained, mp 252−253 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95 (3H, t, J = 7.3 Hz, CH_3), 1.09 (3H, t, J = 7.1 Hz, CH_3), 1.57 (2H, apparent sextet, J = 7.4 Hz, CH_2), 2.86 (2H, t, J = 7.4 Hz, CH_2), 3.14 (2H, q, J = 7.1 Hz, CH_2),

5.93 (1H, s, H-3), 6.29 (1H, s, H-6), 11.94 and 15.55 (2H, 2s, 2 OH); MS (CI) 277 (21.7, M + 1), 276 (92.6, M^+), 261 (15.7, M − CH_3), 247 (100, M − C_2H_5), 233 (32.6, M − C_3H_7), 219 (57.6, M − C_2H_5CO); IR (KBr) 3241 (m, br, OH), 1696 (s, C=O), 1626 (s) cm^{-1}; UV λ_{max} (MeOH) 201, 278, 324 nm. Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 64.79; H, 5.89.

5,7-Dihydroxy-6,8-dipropionyl-4-propylcoumarin (8): 6.1 g (16% yield) was obtained, mp 128−129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.3 Hz, CH_3), 1.21 (3H, t, J = 7.1 Hz, CH_3), 1.24 (3H, t, J = 7.1 Hz, CH_3), 1.64 (2H, apparent sextet, J = 7.5 Hz, CH_2), 2.96 (2H, t, J = 7.6 Hz, CH_2), 3.23 (2H, q, J = 7.1 Hz, CH_2), 3.34 (2H, q, J = 7.1 Hz, CH_2), 6.00 (1H, s, H-3), 16.59 and 16.67 (2H, 2s, 2 OH); MS (CI) 333 (100, M + 1), 332 (11.5, M^+), 305 (9.6, M − C_2H_4 + 1), 277 (12.4, M − C_2H_4CO + 1); IR (KBr) 3453 and 2461 (br-w, OH), 1746 (s, C=O), 1630 (s), 1588 (vs), 1551 (s) cm^{-1}. Anal. Calcd for $C_{18}H_{20}O_6 \cdot {}^1/_3H_2O$: C, 63.90; H, 6.16. Found: C, 64.25; H, 5.89.

B. With Propionic Anhydride. 5,7-Dihydroxy-4-propylcoumarin (**5**) (5 g, 22.7 mmol) and $AlCl_3$ (6 g, 45 mmol) were mixed in 1,2-dichloroethane (60 mL). The resulting orange suspension was stirred and heated to 75 °C for 30 min, after which a solution was obtained. Then, a mixture of propionic anhydride (3.3 g, 22.7 mmol) and $AlCl_3$ (6 g, 45 mmol) in 1,2-dichloroethane (30 mL) was added dropwise over 1 h. The reaction mixture was allowed to stir at 75 °C for an additional 1 h. After the mixture was cooled to room temperature, the reaction was quenched with ice water and 1 N HCl. The precipitated product was taken into ethyl acetate, and the aqueous solution was extracted with the same solvent (400 mL × 3). The combined ethyl acetate solutions were dried over Na_2SO_4 and concentrated *in vacuo* to provide a solid product which was taken up into acetone and cooled at −40 °C. Pure compound **6** (total 2.8 g, 45%) was collected by filtration. The product was identical with that obtained from the propionyl chloride reaction described above.

5-Hydroxy-7-(propionyloxy)-4-propylcoumarin (9). Into a suspension of coumarin **5** (5 g, 22.7 mmol) in dichloromethane (25 mL) was added dropwise a solution of propionic anhydride (3.3 g, 22.7 mmol) and $AlCl_3$ (6 g, 45 mmol) in dichloromethane (25 mL) at ambient temperature with stirring. After 16 h, the reaction was quenched with ice water. The organic layer was separated and washed with 1 N HCl, water, and brine. After being dried over Na_2SO_4, the crude product was obtained from rotary evaporation and contained only the starting material and a product as shown by TLC. Recrystallization from ethyl acetate and hexane afforded 0.6 g (10% yield) of 7-monoester **9**, which led to a greenish blue color on Gibbs test, mp 170−171 °C: ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.96 (3H, t, J = 7.2 Hz, CH_3), 1.13 (3H, t, J = 7.2 Hz, CH_3), 1.61 (2H, apparent sextet, J = 7.4 Hz, CH_2), 2.62 (2H, q, J = 7.5 Hz, CH_2), 2.92 (2H, t, J = 7.5 Hz, CH_2), 6.10 (1H, s, H-3), 6.56 (1H, d, J = 2.3 Hz, H-6 or H-8), 6.67 (1H, d, J = 2.3 Hz, H-8 or H-6), 11.11 (1H, s, OH); MS (CI) 277 (100, M^+), 249 (16.4, M − C_2H_5 + 1), 221 (21.9, M − C_2H_5CO + 1); IR (KBr) 3218 (w, OH), 1769 (m, C=O), 1680 (vs, C=O), 1612 (s) cm^{-1}. Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 64.57; H, 5.77.

5,7-Bis(propionyloxy)-4-propylcoumarin (10). Into a solution of coumarin **5** (0.5 g, 2.3 mmol) in THF (5 mL) at −78 °C was added a precooled (−78 °C) suspension of $AlCl_3$ (0.3 g, 2.2 mmol) in THF (5 mL) followed by addition of propionic anhydride (1.0 g, 7.8 mmol). The resulting mixture was warmed to ambient temperature and refluxed overnight (∼16 h), whereupon the reaction was quenched with ice water and the mixture extracted with ethyl acetate. The organic layer was separated and washed with 1 N HCl, H_2O, and brine. After being dried over Na_2SO_4, the crude solid product was obtained from evaporation and reprecipitated from cold ether to afford 0.44 g (57% yield) of 5,7-diester **10**, mp 118−120 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (3H, t, J = 7.3 Hz, CH_3), 1.27 (3H, t, J = 7.6 Hz, CH_3), 1.30 (3H, t, J = 7.5 Hz, CH_3), 1.67 (2H, apparent sextet, J = 7.5 Hz, CH_2), 2.62 (2H, q, J = 7.5 Hz, CH_2), 2.66 (2H, q, J = 7.5 Hz, CH_2), 2.78 (2H, t, J = 7.6 Hz, CH_2), 6.21 (1H, s, H-3), 6.87 (1H, d, J = 2.4 Hz, H-6 or H-8), 7.06 (1H, d, J = 2.3 Hz, H-8 or H-6); MS (CI) 333 (100, M + 1), 277 (85.0, M − C_2H_4CO + 1), 221 (19.9, M − C_2H_4CO − C_2H_5CO + 1); IR (KBr) 1767 (s, C=O), 1730 (s, C=O), 1612 (s) cm^{-1}. Anal. Calcd for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.79; H, 5.95.

6,6-Dimethyl-9-hydroxy-10-propionyl-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (11): A. In Pyridine. A mixture of compound **6** (2.60 g, 9.42 mmol) and 4,4-dimethoxy-2-methylbutan-2-ol[45] (5.54 g, 37.7 mmol) was dissolved in dry pyridine (6.5 mL). This mixture was refluxed under nitrogen for 3 days. After removal of the solvent *in vacuo*, the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed several times with 1 N HCl and brine. It was then dried over Na_2SO_4. The crude product obtained by evaporation *in vacuo* was purified by chromatography on a silica gel column eluting with 25% ethyl acetate/hexane to afford 2.55 g of **11** in 78% yield, mp 96−98 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (3H, t, J = 7.3 Hz, CH_3), 1.23 (3H, t, J = 7.1 Hz, CH_3), 1.53 (6H, s, 2 CH_3), 1.66 (2H, m, J = 7.6 Hz, CH_2), 2.90 (2H, t, J = 7.7 Hz, CH_2), 3.35 (2H, q, J = 7.1 Hz, CH_2), 5.58 (1H, d, J = 10.2 Hz, H-7), 6.00 (1H, s, H-3), 6.73 (1H, d, J = 10.2 Hz, H-8), 14.50 (1H, s, OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00 (3H, t, J = 7.3 Hz, CH_3), 1.11 (3H, t, J = 7.1 Hz, CH_3), 1.49 (6H, s, 2 CH_3), 1.59 (2H, m, J = 7.8 Hz, CH_2), 2.88 (2H, t, J = 7.7 Hz, CH_2), 3.19 (2H, q, J = 7.0 Hz, CH_2), 5.79 (1H, d, J = 10.1 Hz, H-7), 6.12 (1H, s, H-3), 6.62 (1H, d, J = 10.1 Hz, H-8); MS (EI) 343 (5.7, M + 1), 342 (22.5, M^+), 327 (100, M − CH_3); IR (KBr) 1728 (vs, C=O) cm^{-1}. Anal. Calcd for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48. Found: C, 70.45; H, 6.92.

B. In Toluene/Pyridine. A mixture of compound **6** (510.6 g, 1.85 mol) and 4,4-dimethoxy-2-methylbutan-2-ol (305.6 g, 2.06 mol) was dissolved in a mixture of toluene (1.5 L) and dry pyridine (51 mL). This mixture was stirred and refluxed; water and MeOH formed during the reaction were removed azeotropically via a Dean−Stark trap. The reaction was monitored by TLC. After 6 days, the reaction had proceeded to completion. The mixture was then cooled to ambient temperature and diluted with ethyl acetate (1 L) and 1 N HCl (1 L). The ethyl acetate solution was separated and washed with 1 N HCl (500 mL) and brine (1 L). After being dried over Na_2SO_4 and evaporated *in vacuo*, a quantity of 590 g (93% yield) of compound **11** was obtained which was >95% pure without further purification and compared with an authentic sample by TLC and spectroscopic data.

6,6,10,11-Tetramethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (12). A mixture of chromene **11** (1.76 g, 5.11 mmol) and sodium acetate (0.419 g, 5.11 mmol) in acetic anhydride (12 mL) was refluxed for 4 h, and the solvent was then removed *in vacuo*. The residue was purified by chromatography on a silica gel column eluting first with 25% ethyl acetate/hexane followed by 50% ethyl acetate/hexane to provide 1.16 g of chromone **12** (62% yield) as a yellow solid, mp 209−209.5 °C after recrystallization from ethyl acetate: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00 (3H, t, J = 7.2 Hz, CH_3), 1.51 (6H, s, 2 CH_3), 1.60 (2H, apparent sextet, J = 7.6 Hz, CH_2), 1.88 (3H, s, CH_3), 2.39 (3H, s, CH_3), 2.90 (2H, t, J = 7.7 Hz, CH_2), 5.89 (1H, d, J = 10.0 Hz, H-7), 6.26 (1H, s, H-3), 6.75 (1H, d, J = 10.2 Hz, H-8); MS (EI) 366 (29.6, M^+), 351 (100, M − CH_3), 323 (16.5, M − C_3H_7); IR (KBr) 1734 (vs, C=O), 1657, 1640, 1610, 1562 (s) cm^{-1}. Anal. Calcd for $C_{22}H_{22}O_5$: C, 72.12; H, 6.05. Found: C, 72.14; H, 6.15.

Reduction of Chromone 12 under Luche Conditions. To a solution of chromone **12** (50 mg, 0.14 mmol) in EtOH (1.8 mL) were added sodium borohydride (20 mg, 0.52 mmol) and $CeCl_3(H_2O)_7$ (51.7 mg, 0.14 mmol) at ambient temperature. After stirring for 30 min, the mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by preparative TLC using ethyl acetate/hexane (1:1) as the mobile phase to afford the diol **13** (40 mg, 77%), mp 109−110 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, t, J = 7.9 Hz, CH_3), 1.39 (2H, m, CH_2), 1.40 (3H, s, CH_3), 1.47 (3H, s, CH_3), 2.01 (4H, s, CH_3 + OH), 2.35 (2H, t, J = 7.6 Hz, CH_2), 2.42 (3H, s, CH_3), 3.84 (2H, m, CH_2), 5.56 (1H, d, J = 9.6 Hz, H-3), 5.97 (1H, t, J = 7.6 Hz, C=C***H***CH_2OH), 6.70 (1H, d, J = 10.0 Hz, H-4), 13.51 (1H, s, OH); MS (EI) 370 (23.7, M^+), 339 (100, M − CH_2OH); IR (KBr) 1649 (vs), 1584, 1437 (s) cm^{-1}.

Reduction of Chromone 12 with *n*-Bu_3SnH. A mixture of chromone **12** (300 mg, 0.82 mmol) and tri-*n*-butyltin hydride

(600 mg, 2.05 mmol) in dry dioxane (2.4 mL) was refluxed under nitrogen for 24 h, whereupon the reaction was quenched with water (20 mL). The mixture was extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over Na_2SO_4. The crude product (500 mg) obtained by evaporation *in vacuo* was chromatographed on a silica gel column. Elution with 30% ethyl acetate in hexane afforded 45 mg (15% yield) of compound **14**, mp 137−138 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (3H, t, J = 7.3 Hz, CH_3), 1.67 (3H, s, CH_3), 1.68 (2H, apparent sextet, J = 8.1 Hz, CH_2), 1.86 (3H, s, CH_3), 2.05 (3H, s, CH_3), 2.47 (3H, s, CH_3), 3.01 (2H, t, J = 7.6 Hz, CH_2), 3.59 (2H, d, J = 7.3 Hz, C=CCH_2), 5.21 (1H, t, J = 7.4 Hz, C=CH), 6.06 (1H, s, H-3), 14.78 (1H, s, OH); MS (EI) 368 (89.3, M^+), 339 (17.8, M − CH_2CH_3), 325 (100, M − $CH_2CH_2CH_3$); IR (KBr) 1740 (vs, C=O), 1649, 1591, 1451 (s) cm^{-1}. Anal. Calcd for $C_{22}H_{24}O_5 \cdot {}^1/_6H_2O$: C, 71.14; H, 6.60. Found: C, 71.14; H, 6.72.

Continuing elution with 80% ethyl acetate in hexane afforded 80 mg of an oil, which was subjected to further purification using preparative TLC. The fraction with an R_f of ca. 0.4 was collected and concentrated to afford 13.3 mg of an oil, which was still a mixture, with unsaturated alcohol **15** being a predominant component: ^{1}H NMR (60 MHz, $CDCl_3$) δ 0.92 (3H, t, J = 6.0 Hz, CH_3), 1.26 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.63 (2H, m, CH_2), 1.96 (3H, s, CH_3), 2.36 (3H, s, CH_3), 2.45 (2H, t, J = 6.0 Hz, CH_2), 3.65 (1H, s, H-12), 5.51 (1H, d, J = 10.0 Hz, H-7), 6.06 (1H, s, H-3), 6.67 (1H, d, J = 10.0 Hz, H-8), 13.25 (1H, br s, OH); MS (EI) 369 (3.8, M + 1), 368 (4.4, M^+), 367 (8.3, M − 1), 366 (28.4, M − 2), 351 (100, M − OH); IR (KBr) 1651 (s), 1589 (m) cm^{-1}.

Reduction of Chromone 12 with $NaBH_4$ in the Presence of $HClO_4$. To a stirred solution of chromone **12** (50 mg, 0.14 mmol) in THF (2 mL) at ambient temperature and under N_2 was added 1 equiv of $HClO_4$ dropwise. After 24 h, $NaBH_4$ (10.3 mg, 0.27 mmol) was added, and the suspension was stirred at ambient temperature for 3 days. Analysis of the reaction mixture by TLC indicated that no reaction took place. The precipitates were dissolved by adding EtOH (2 mL). Then 4 equiv of $HClO_4$ and an additional 1 equiv of $NaBH_4$ were added. The resulting solution was stirred at ambient temperature for an additional 24 h. Analysis by TLC indicated that a product had formed. However, the TLC profile did not change after prolonged reaction time (total 7 days) and elevated temperature (at 55 °C for 10 h). The reaction was quenched with water (10 mL) and the mixture extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over Na_2SO_4. The crude product (35 mg) obtained by evaporation *in vacuo* was chromatographed on a silica gel column. Elution with 5% MeOH in CH_2Cl_2 afforded 11 mg (30% yield) of compound **17** (R = Et) and 22 mg (60%) of the starting chromone **12**. Compound **17** (R = Et): mp 140−141 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 0.97 (3H, t, J = 7.3 Hz, CH_3), 1.08 (3H, t, J = 7.2 Hz, CH_3), 1.39 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.50 (2H, m, CH_2), 2.01 (3H, s, CH_3), 2.46 (3H, s, CH_3), 2.48 (2H, m, CH_2), 3.98 (2H, q, J = 7.1 Hz, OCH_2), 5.60 (1H, d, J = 10.2 Hz, H-3), 6.03 (1H, s, C=CH), 6.72 (1H, d, J = 10.1 Hz, H-4); MS (CI) 413 (100, M + 1), 412 (19.6, M^+), 367 (34.1, M − OCH_2CH_3); IR (KBr) 3387 (w, br, OH), 1726 (s, C=O), 1645, 1586 and 1443 (s) cm^{-1}.

10,11-*trans*-Dihydro-6,6,10,11-tetramethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione or 12-Oxocalanolide A (18): A. From Acetaldehyde Diethyl Acetal. A solution containing chromene **11** (344 mg, 1.0 mmol), acetaldehyde diethyl acetal (473 mg, 4.0 mmol), pyridinium tosylate (35 mg, 0.14 mmol), trifluoroactic acid (1.5 mL), and dry pyridine (0.7 mL) was heated at 140 °C under N_2. The reaction was monitored by TLC analysis. After 4 h, the reaction mixture was cooled to room temperature, diluted with ethyl acetate, and washed several times with 10% aqueous $NaHCO_3$ and brine. The organic layer was separated and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the crude product was purified by chromatography on a silica gel column eluting with ethyl acetate/hexane (2:3). Chromanone **18** (110 mg, 30% yield) was obtained, mp 176−177 °C (lit.[40] mp 130−132 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (3H, t, J = 7.3 Hz, CH_3), 1.22 (3H, d, J = 6.9 Hz, CH_3), 1.56 (3H, d, J = 6.3 Hz, CH_3), 1.54 and 1.57 (6H, 2 s, 2 CH_3), 1.63 (2H, apparent sextet, J = 7.6 Hz, CH_2), 2.57 (1H, dq, J = 6.9, 11.0 Hz, H-11), 2.91 (2H, t, J = 7.6 Hz, CH_2), 4.33 (1H, dq, J = 6.3, 11.1 Hz, H-10), 5.64 (1H, d, J = 10.1 Hz, H-7), 6.05 (1H, s, H-3), 6.66 (1H, d, J = 10.1 Hz, H-8); MS (CI) 369 (100, M + 1); IR 1736 (vs, C=O), 1686 (s, C=C−C=O), 1645 (m), 1606 (m), 1578 (s), 1557 (vs) cm^{-1}. Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.71; H, 6.70.

B. From Paraldehyde. To a stirring solution of chromene **11** (350 mg, 1.0 mmol) and PPTS (250 mg, 1.0 mmol) in 1,2-dichloroethane (2 mL) at ambient temperature under N_2 was added 3 mL of paraldehyde (22.5 mmol). The resulting mixture was refluxed for 7 h. Then, CF_3CO_2H (1 mL), an additional 1 equiv of PPTS, and 1 mL of paraldehyde were added; the mixture was refluxed overnight. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate (50 mL × 3). The crude product obtained by evaporation under reduced pressure was washed with hexane. The residue was purified by column chromatography eluting with ethyl acetate/hexane (1:2) to afford 100 mg (27% yield) of chromanone **18**, which was identical with an authentic sample by comparison of TLC, HPLC, and spectroscopic data, and 30 mg (8% yield) of **19**, the analytical data of which are shown below.

10,11-*cis*-Dihydro-6,6,10,11-tetramethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione or (±)-calanolide D (19): mp 122−124 °C after recrystallization from ethyl acetate (lit.[40] mp 130−132 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.3 Hz, CH_3), 1.16 (3H, d, J = 7.2 Hz, CH_3), 1.42 (3H, d, J = 6.6 Hz, CH_3), 1.54 (6H, 2s, 2 CH_3), 1.64 (2H, apparent sextet, J = 7.7 Hz, CH_2), 2.68 (1H, dq, J = 3.4, 7.2 Hz, H-11), 2.88 (2H, apparent dd, J = 6.2, 9.1 Hz, CH_2), 4.70 (1H, dq, J = 3.4, 6.6 Hz, H-10), 5.60 (1H, d, J = 10.2 Hz, H-7), 6.04 (1H, s, H-3), 6.66 (1H, d, J = 10.2 Hz, H-8); MS (EI) 368 (41.9, M^+), 353 (100, M − CH_3), 325 (7.1, M − C_3H_7), 312 (7.7, M − C_4H_8), 297 (45.8, M − CH_3-C_4H_8), 269 (15.3, M − CH_3 − C_5H_8O); IR 1738 (vs, C=O), 1718 (s, C=C−C=O), 1677 (m), 1624 (m), 1557 (vs) cm^{-1}. Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.75; H, 6.53.

Reduction of Chromanone 18 under Luche Conditions. To a stirring solution of chromanone **18** (51.5 g, 0.14 mol) in EtOH (1.5 L) was added $CeCl_3(H_2O)_7$ (102 g, 274 mmol). The mixture was stirred for 1.5 h at room temperature under N_2 and then cooled to −30 °C with an ethylene glycol/H_2O (1:2, w/w) dry ice bath. After the temperature was equilibrated to −30 °C, $NaBH_4$ (21.3 g, 563 mmol) was added and the mixture stirred at the same temperature for 8.5 h, at which time the reaction was quenched with water (2 L) and extracted with ethyl acetate (2 L × 3). The extracts were combined, washed with brine (2 L), and dried over Na_2SO_4. The crude product obtained by removal of solvent under reduced pressure was passed through a short silica gel column to provide 53 g of mixture which contained 68% (±)-calanolide A, 14% (±)-calanolide B, and 13% starting chromanone **18** as shown by HPLC. This material was subjected to further purification by semipreparative HPLC. The mobile phase for the semipreparative HPLC was hexane/ethyl acetate (70:30) at a flow rate of 9.0 mL/min, and the UV detector was set at a wavelength of 290 nm. The fractions for (±)-calanolide A (**1**) and (±)-calanolide B (**3**) were pooled, combined, and concentrated.

(±)-Calanolide A (1):[32,40] mp 52−54 °C, which increased to 102 °C after it was dried thoroughly (lit.[40] mp 56−58 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.3 Hz, CH_3), 1.15 (3H, d, J = 6.8 Hz, CH_3), 1.46 (3H, d, J = 6.8 Hz, CH_3), 1.47 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.65 (2H, apparent sextet, J = 7.4 Hz, CH_2), 1.93 (1H, apparent sextet, J = 7.8 Hz, H-11), 2.86, 2.92 (2H, t-AB type, J = 8.0 Hz, J_{AB} = 21.3 Hz, CH_2), 3.60 (1H, br-s, OH), 3.93 (1H, dq, J = 6.4, 9.0 Hz, H-10), 4.72 (1H, d, J = 8.0 Hz, H-12), 5.54 (1H, d, J = 9.9 Hz, H-7), 5.94 (1H, s, H-3), 6.62 (1H, d, J = 9.9 Hz, H-8); MS (CI) 371 (75.4, M + 1), 370 (16.1, M^+), 353 (100, M − OH); IR 3590 (w), 3449 (m, br, OH), 1726 (sh), 1701 (vs, C=O), 1640 (m), 1609 (m), 1581 (vs) cm^{-1}. Anal. Calcd for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07. Found: C, 71.63; H, 7.21.

(±)-Calanolide B (3):[32] liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.3 Hz, CH_3), 1.14 (3H, d, J = 7.0 Hz, CH_3), 1.43 (3H, d, J = 6.3 Hz, CH_3), 1.48 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.65 (2H, apparent sextet, J = 7.5 Hz, CH_2), 1.73 (1H,

m, H-11), 2.53 (1H, br-s, OH), 2.86, 2.92 (2H, t-AB type, J = 7.5 Hz, J_{AB} = 21.1 Hz, CH_2), 4.26 (1H, dq, J = 6.3, 10.7 Hz, H-10), 4.98 (1H, br, H-12), 5.53 (1H, d, J = 10.0 Hz, H-7), 5.95 (1H, s, H-3), 6.63 (1H, d, J = 9.9 Hz, H-8).

Resolution of the Synthetic (±)-1 by Chiral HPLC. The synthetic (±)-**1** was resolved using a semipreparative chiral HPLC column packed with amylose carbamate. The mobile phase was hexane/ethanol (95:5) at a flow rate of 6.0 mL/min, and the UV detector was set at a wavelength of 254 nm. The enantiomers of calanolide A, (+)- and (−)-**1**, were collected, and their chemical structures were assigned based on their optical rotations and spectroscopic data, which were in full agreement with the data reported for the natural[32] and synthetic products.[43]

(+)-Calanolide A (1):[32,43] mp 47−50 °C (lit.[32] oil, lit.[43] mp 45−48 °C); $[\alpha]^{25}_D$ = +68.8° ($CHCl_3$, *c* 0.7) {(lit.[32] $[\alpha]^{25}_D$ = +60° ($CHCl_3$, *c* 0.5), lit.[43] $[\alpha]^{25}_D$ = +66° ($CHCl_3$, *c* 0.5)}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.3 Hz, CH_3), 1.15 (3H, d, J = 6.8 Hz, CH_3), 1.46 (3H, d, J = 6.4 Hz, CH_3), 1.47 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.66 (2H, apparent sextet, J = 7.5 Hz, CH_2), 1.93 (1H, apparent sextet, J = 7.8 Hz, H-11), 2.87, 2.92 (2H, t-AB type, J = 7.9 Hz, J_{AB} = 21.7 Hz, CH_2), 3.52 (1H, d, J = 2.9 Hz, OH), 3.93 (1H, dq, J = 6.4, 9.1 Hz, H-10), 4.72 (1H, dd, J = 2.7, 7.8 Hz, H-12), 5.54 (1H, d, J = 9.9 Hz, H-7), 5.95 (1H, s, H-3), 6.62 (1H, d, J = 9.9 Hz, H-8); ^{13}C NMR ($CDCl_3$) δ 13.99 (CH_3), 15.10 (CH_3), 18.93 (CH_3), 23.26 (CH_2), 27.38 (CH_3), 28.02 (CH_3), 38.66 (CH_2), 40.42 (CH), 67.19 (CH-OH), 77.15 (CH-O), 77.67 (C-O), 104.04 (C-4a), 106.36 (C-8a, C-12a), 110.14 (C-3), 116.51 (C-8), 126.97 (C-7), 151.14 (C-4b), 153.10 (C-8b), 154.50 (C-12b), 158.88 (C-4), 160.42 (C=O); MS (CI) 371 (100, M + 1), 370 (23.6, M^+), 353 (66.2, M − OH); IR 3611 (w), 3426 (m, br, OH), 1734 (vs, C=O), 1643 (m), 1606 (m), 1587 (vs) cm^{-1}; UV λ_{max} (MeOH) 204 (32 100), 228 (23 200), 283 (22 200), 325 (12 700) nm. Anal. Calcd for $C_{22}H_{26}O_5 \cdot {}^1/_4H_2O$: C, 70.47; H, 7.12. Found: C, 70.64; H, 7.12.

(−)-Calanolide A (1):[32,43] mp 47−50 °C; $[\alpha]^{25}_D$ = −75.6° ($CHCl_3$, *c* 0.7) {(lit.[43] $[\alpha]^{25}_D$ = −66° ($CHCl_3$, *c* 0.5)}; ^{1}H NMR ($CDCl_3$) δ 1.03 (3H, t, J = 7.4 Hz, CH_3), 1.15 (3H, d, J = 6.8 Hz, CH_3), 1.46 (3H, d, J = 6.3 Hz, CH_3), 1.47 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.66 (2H, apparent sextet, J = 7.4 Hz, CH_2), 1.93 (1H, apparent sextet, J = 7.3 Hz, H-11), 2.87, 2.92 (2H, t-AB type, J = 7.8 Hz, J_{AB} = 22.3 Hz, CH_2), 3.50 (1H, d, J = 2.9 Hz, OH), 3.93 (1H, dq, J = 6.4, 9.0 Hz, H-10), 4.72 (1H, dd, J = 2.7, 7.8 Hz, H-12), 5.54 (1H, d, J = 10.0 Hz, H-7), 5.94 (1H, s, H-3), 6.62 (1H, d, J = 10.0 Hz, H-8); ^{13}C NMR ($CDCl_3$) δ 13.99 (CH_3), 15.10 (CH_3), 18.93 (CH_3), 23.36 (CH_2), 27.38 (CH_3), 28.02 (CH_3), 38.66 (CH_2), 40.42 (CH), 67.19 (CH-OH), 77.15 (CH-O), 77.67 (C-O), 104.04 (C-4a), 106.36 (C-8a, C-12a), 110.14 (C-3), 116.51 (C-8), 126.97 (C-7), 151.14 (C-4b), 153.11 (C-8b), 154.50 (C-12b), 158.90 (C-4), 160.44 (C=O); MS (CI) 371 (95.2, M + 1), 370 (41.8, M^+), 353 (100, M − OH); IR 3443 (m, br, OH), 1732 (vs, C=O), 1643 (m), 1606 (m), 1584 (vs) cm^{-1}; UV λ_{max} (MeOH) 200 (20 500), 230 (19 400), 283 (22 500), 326 (12 500) nm. Anal. Calcd for $C_{22}H_{26}O_5 \cdot {}^1/_4H_2O$: C, 70.47; H, 7.12. Found: C, 70.27; H, 7.21.

Antiviral Assays. Antiviral assays were performed at Southern Research Institute, Birmingham, AL. Materials and methods employed were the same as described in the literature.[37] CEM-SS and MT-2 cells used for the screening were maintained in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 25 mM HEPES, and 20 μg/mL gentamicin. The medium used for dilution of compounds and maintenance of cultures during the assay was the same as above. Cultures were maintained in disposable tissue culture labware at 37 °C in a humidified atmosphere of 5% CO_2 in air. The appropriate amounts of the compounds for anti-HIV evaluations were dissolved in 100% DMSO and then diluted with the medium to the desired initial concentration. Each dilution was added to plates in the amount of 100 μL/well. Compounds were tested in triplicate wells per dilution with infected cells and in duplicate wells per dilution with uninfected cells for evaluation of cytotoxicity. After addition of cells to the plates, the high drug concentration was 100 μg/mL and the high DMSO concentration was 0.25%.

Infected cells were charged with HIV-1 virus to yield an approximate multiplicity of infection (MOI) of 0.03 $TCID_{50}$/cell on MT-2 cells in a volume of 1 mL/10^6 cells and ca. 0.12 $TCID_{50}$/cell on CEM-SS cells in a volume of 1 mL/10^6 cells. The cell-virus suspension was incubated at 37 °C for 4 h, whereupon the cells were centrifuged and the virus supernatant was discarded. Medium was added to the cells to attain a cell density of 10^5 cells/mL. The infected cells as well as uninfected cells, which were prepared in the same manner but without the addition of virus, were added to a 96-well microtiter plate in the amount of 100 μL/well to give a starting cell number of 10^4 cells/mL. After being incubated for 6 days (CEM-SS cells) and 7 days (MT-2 cells), respectively, the cellular viability was then measured with a tetrazolium dye, MTT (5 mg/mL), added to the test plates, as described in the literature.[74] Antiviral and toxicity data are reported as the quantity of drug required to inhibit 50% of virus-induced cell killing or virus production (EC_{50}) and the quantity of drug required to inhibit virus-induced cell viability by 50% (IC_{50}).

HIV Reverse Transcriptase (RT) Enzyme Inhibition Assays. [*Methyl*-^{3}H]TTP (15 Ci/mmol) and [*methyl*-^{3}H]UTP (6.5 Ci/mmol) were purchased from ICN Radiochemicals (Irvine, CA), [^{3}H]poly(rA) was from Amersham (IL), DNA polymerases α and β were from Molecular Biology Resources, Inc. (Milwaukee, WI), RNA polymerase (*Escherichia coli*) and poly(dT) were from Pharmacia (Piscataway, NJ), and DEAE-cellulose filter discs (DE-81, Whatman) were from VWR Scientific (Batavia, IL). Activated calf thymus DNA, native DNA, herring sperm DNA, TTP, ATP, CTP, GTP, dATP, dCTP, dGTP, poly(rA), oligo(dT), dithiothreitol, glutathione, gelatin, and BSA were purchased from Sigma Chemical Co. (St. Louis, MO).

Dimeric HIV-1 (p66/p51) RT was purified by modification of the literature procedures.[75] HIV-2 (p66/p51) RT is a recombinant enzyme consisting of two polypeptides subunits, which was synthesized in an *E. coli* expression system using a genetically engineered plasmid.[76] The enzyme possesses DNA-dependent DNA polymerase (DDDP), RNA-dependent DNA polymerases (RDDP), and ribonuclease H (RNase H) activities typical of retroviral RT's.

HIV-2, mutant (TIBO-resistant) HIV-1, and AMV RT's were assessed for RDDP activities, and HIV-1 RT was assessed for DDDP and RDDP activities. All RT assays were performed following a standard protocol.[77,78] For RDDP assays, the reaction mixture contained the following: 50 mM Tris·HCl buffer (pH 8.0), 150 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 0.3 mM glutathione, 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 250 μg/mL bovine serum albumin, 41 μM poly(rA) [ϵ_{260} (mM) = 7.8], 9.5 μM oligo(dT) [ϵ_{260} (mM) = 5.6], 20 μM TTP, and 0.5 μCi [^{3}H]-TTP. The required amount of each test compound in DMSO (10 μL) was added to the reaction mixture (80 μL) in 96-well microtiter plates (2 wells/concentration). The reaction was started by the addition of 10 μL (0.08 μg) of RT followed by incubation at 37 °C for 1 h. The reaction was terminated by heating to 80 °C (5 min) and then chilling on ice (15 min). Aliquots of each reaction mixture (90 μL) were spotted uniformly onto circular 2.5 cm DE-81 (Whatman) filters and kept at ambient temperature for 15 min before washing four times with 5% aqueous $Na_2HPO_4 \cdot 7H_2O$. This was followed by two more washings with distilled H_2O. Finally, the filters were thoroughly dried and subjected to scintillation counting in a nonaqueous scintillation fluid.

Each test compound was prescreened at 200 μg/mL. If inhibition at 200 μg/mL was >50%, 50% inhibition concentration (IC_{50}) values were determined by assaying at 10 different concentrations (in duplicate) prepared by half-log serial dilutions. Fagaronine chloride (IC_{50} = 13 μM) and DMSO (10 μL) were used as positive and negative controls, respectively. Percentage inhibition was calculated as (1 − test/negative control) × 100%, and IC_{50} values were estimated from dose−response curves.

DDDP assays with HIV-1 RT were performed using similar procedures, except activated calf thymus DNA (final concentration, 20 μg/mL), dGTP, dCTP, and dATP (each at a final concentration of 50 μM), and 5 μCi [^{3}H]TTP were used.

DNA Polymerase Assays. These assays were performed using the same procedure as the RT assays. The reaction mixture contained the following: 80 mM Tris·HCl buffer (pH

7.5), 5 mM KCl, 10 mM $MgCl_2$, 1.5 mM dithiothreitol, 25 μg/mL bovine serum albumin, 12% (v/v) glycerol, 41 μM activated calf thymus DNA, 80 μM each of dATP, dCTP, dGTP, and ATP, and 2.0 μCi [*methyl*-^{3}H]TTP. The required amount of each test compound in DMSO (10 μL) was added to 80 μL of the reaction mixture, and the reaction was initiated by adding 0.5 unit of either DNA polymerase α or β. One unit is the amount of enzyme required to incorporate 1 nmol of total nucleotide into acid-insoluble form in 60 min at 37 °C.

RNA Polymerase Assays. These assays were performed using the same procedure as the RT assays. The assay mixture contained the following: 40 mM Tris·HCl buffer (pH 7.9), 150 mM KCl, 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 25 μg/mL bovine serum albumin, 41 μM native calf thymus DNA, 80 μM each of ATP, CTP, and GTP, and 1.5 μCi [*methyl*-^{3}H]-UTP. The required amount of each test compound in DMSO (10 μL) was added to 80 μL of the assay mixture, and the reaction was initiated by adding 0.5 unit of RNA polymerase. One unit is the amount of enzyme required to incorporate 1 nmol of AMP into acid-insoluble product using poly(dA)·poly-(dT) as template-primer in 10 min at 37 °C.

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Supporting Information Available: Copies of ^{1}H NMR spectra of (±)-, (+)-, and (−)-**1**, (±)-**3**, **5**−**15**, and **17**−**19** and copies of ^{13}C NMR spectra of (+)- and (−)-**1** (20 pages). Ordering information is given on any current masthead page.

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