Structural Analogues of the Calanolide Anti-HIV Agents. Modification of the *trans*-10,11-Dimethyldihydropyran-12-ol Ring (Ring C)[1]

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(+)-Calanolide A is a potent inhibitor of reverse transcriptase from human immunodeficiency virus type 1 (HIV-1), which was isolated from an extract of *Calophyllum lanigerum*, along with seven related compounds. In order to examine the structure−activity relationships of the *trans*-10,11-dimethyldihydropyran-12-ol ring (designated ring C), a series of structural analogues were prepared and evaluated using a whole cell cytopathicity assay (XTT). Removal of the 10-methyl group resulted in decreased activity, with only one epimer exhibiting anti-HIV activity. Substituting the 10-methyl group with an ethyl chain maintained anti-HIV activity, with only a 4-fold reduction in potency relative to racemic calanolide A. Substitution of the 10-methyl group with an isopropyl moiety completely eliminated the anti-HIV activity. Addition of an extra methyl group at either the 10- or 11-position maintained the basic stereochemical features of the parent calanolide system while removing the chirality at the respective carbon, but resulted in decreased activity relative to calanolide A. In all the above examples, analogues containing a *cis* relationship between the 10- and 11-alkyl moieties were completely devoid of activity. Synthetic intermediates in which the 12-hydroxyl group was in the ketone oxidation state exhibited surprising anti-HIV activity, with EC_{50} values only 5-fold less potent than that of calanolide A for both the 10,11-*cis* (**6**) and -*trans* (**5**) series. These ketones represent the first derivatives in the calanolide series to exhibit anti-HIV activity while not containing a 12-hydroxyl group. Likewise, ketone derivative **6** was the first example of a compound in the calanolide series having a *cis* relationship between the 10- and 11-methyl groups found to exhibit anti-HIV activity. Analogues which showed anti-HIV activity in the CEM-SS cytoprotection assay were further confirmed to be inhibitors of HIV-1 reverse transcriptase.

Introduction

The human immunodeficiency virus (HIV), first identified in 1983,[2] is a human retrovirus commonly accepted as the causative agent of acquired immunodeficiency syndrome (AIDS), a condition which leads to destruction of the immune system and subsequent susceptibility to opportunistic infections. Since the first case of AIDS was reported in 1981,[3] the virus has spread rapidly through the human population and was recently designated as the leading cause of death of men aged 25−44 years in the United States.[4] Efforts to develop a vaccine to protect against infection have been disappointing, and to date, nine "small molecule" drugs have been approved for use in the treatment of AIDS and AIDS-related complex in the United States: the nucleoside reverse transcriptase (RT) inhibitors AZT, ddI, ddC, d4T, and 3TC; the non-nucleoside reverse transcriptase inhibitor, nevirapine; and the HIV protease inhibitors, indinavir, ritonavir, and saquinavir.

The development of additional anti-HIV drugs having novel structures is of critical importance for overcoming the problems of drug resistance, arising from both drug-promoted selection and natural random genetic mutations.[5] Exposure of HIV-infected cells, either *in vitro* or *in vivo*, to drugs targeting RT leads to rapid selection for resistant viral strains, mediated by mutations of the RT enzyme.[6] All of the RT inhibitors approved to date have been reported to select for drug resistant mutants,[7] and several mutant viral strains exhibit cross-resistance to more than one nucleoside analogue.[8] Similar problems of wide-spectrum drug resistance have been observed for the protease inhibitors as well.[9]

One area of intensive research has been the development of non-nucleoside reverse transcriptase inhibitors (NNRTIs),[10,11] which inhibit RT by occupying a binding site different than that targeted by the nucleoside analogues, and thus offer the possibility of overcoming drug resistance associated with the nucleoside analogues. Even though a variety of structurally unrelated NNRTIs have been identified which inhibit RT, all of these also lead to rapid emergence of resistant strains.[6] To date, only one NNRTI, nevirapine, has been approved for use in the treatment of HIV infection.

Combination therapy is currently believed to be one of the most promising avenues for prolonging the length and quality of life of persons afflicted with HIV infection. Combination of a variety of drugs which target different enzymes critical to the viral life cycle, or which inhibit a single enzyme by different mechanisms, would act to suppress the effects of drug resistant strains. Indeed, it was reported that the combination of AZT and 3TC provided beneficial effects *in vitro* and *in vivo* much greater than either of the drugs alone. Additionally, the drug resistant strain selected via exposure to 3TC was *hypersensitive* to AZT, providing an explanation for the enhanced effects of the combination.[12] Combinations of

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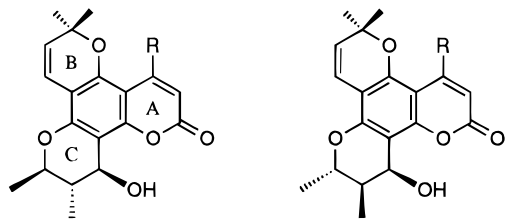
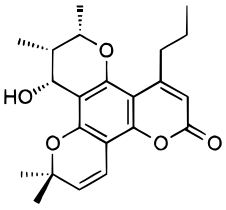
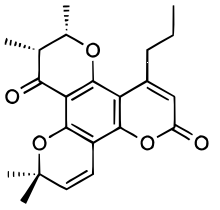


(+)-Calanolide A (R = *n*-propyl)
(+)-Inophyllum B (R = phenyl)

(-)-Calanolide B (R = *n*-propyl)
(-)-Inophyllum P (R = phenyl)



Pseudocalanolide C



Pseudocalanolide D

Figure 1.

nucleoside RT inhibitors with NNRTIs have been demonstrated to provide similar benefits *in vitro*.[13,14]

Random screens of natural products possessing unique structures have identified a series of polycyclic coumarins isolated from the *Calophyllum* genus of tropical plants, which exhibited powerful inhibition of HIV-1 RT.[15,16] The compounds having the most impressive biological activity were (+)-calanolide A,[15] (+)-inophyllum B,[16] (−)-calanolide B,[15] and (−)-inophyllum P[16] (Figure 1). The enantiomers of the calanolides, (−)-calanolide A and (+)-calanolide B, were essentially devoid of antiviral activity in an *in vitro* cytoprotection assay.[17] Calanolide A was selected for further development by the National Cancer Institute, but supplies of the drug from natural sources are extremely limited. We[18,19] and others[20−22] have reported total syntheses of racemic calanolide A and confirmed that the resolved synthetic material possessed the same antiviral activity as the natural product.[18,19] Two asymmetric approaches to construction of the 10,11-dimethyldihydropyran-12-ol ring (designated by us as ring C)[23] have been reported,[24,25] as well as a total asymmetric synthesis.[26]

In an effort to identify structural features necessary for antiviral activity, we embarked on the synthesis and evaluation of a series of compounds modified in ring C of the calanolides. Comparison of the natural products illustrated in Figure 1 suggests the importance of relative stereochemistry in this ring, with the *trans* relationship between the 10- and 11-methyl groups appearing to be crucial for activity. The absolute stereochemistry is more ambiguous, though, because (+)-calanolide A and (−)-calanolide B, which have opposite absolute stereochemistries at the 10- and 11-positions, but the same absolute stereochemistry at the 12-position, both exhibit comparable antiviral activities.[17] The same absolute stereochemical features are true for (+)-inophyllum B and (−)-inophyllum P. All of the natural compounds exhibiting significant anti-HIV activity possess a hydroxyl group at the 12-position, suggesting this feature is critical for activity. Our goals were to explore the structural requirements necessary for biological activity in ring C and, ultimately, to identify synthetic analogues having fewer chiral centers or easier preparation than the parent compound.

Chemistry

Our synthetic route[18,19] for large-scale preparation of racemic calanolide A is illustrated in Scheme 1, with several modifications.[27] Briefly, acylation of coumarin **1**[20] with propionic anhydride in the presence of $AlCl_3$ provided the corresponding 8-propionyl derivative.[19] Subsequent treatment with 1,1-dimethoxy-3-methylbutan-3-ol[28] afforded intermediate **2a**. Deprotonation with 2.1 equiv of lithium diisopropylamide (LDA) and quenching with acetaldehyde provided a mixture of *erythro*- and *threo*-aldol products **3** and **4**, respectively, in approximately 1:1 ratio.[27] Cyclization of **3** or **4** under Mitsunobu conditions provided the *trans*- and *cis*-ketones **5** and **6**, respectively, with inversion of stereochemistry at the position corresponding to C-10 of the calanolide system.[27]

Reduction of **5** with sodium borohydride/cerium chloride (Luche reduction)[29] in ethanol provided a mixture of (±)-calanolide A (**7**) and (±)-calanolide B (**8**) in a 9:1 ratio. Racemic **7** was resolved using chiral HPLC[19] to provide (+)-calanolide A [(+)-**7**] and (−)-calanolide A [(−)-**7**]. Reduction of **6** with sodium borohydride gave a mixture of **9** and **10**. Racemic calanolide C (**9**) was separated from *epi*-calanolide C (**10**) using column chromatography, and the ^{1}H NMR spectrum of **9** correlated exactly with those reported by others.[20−22,30]

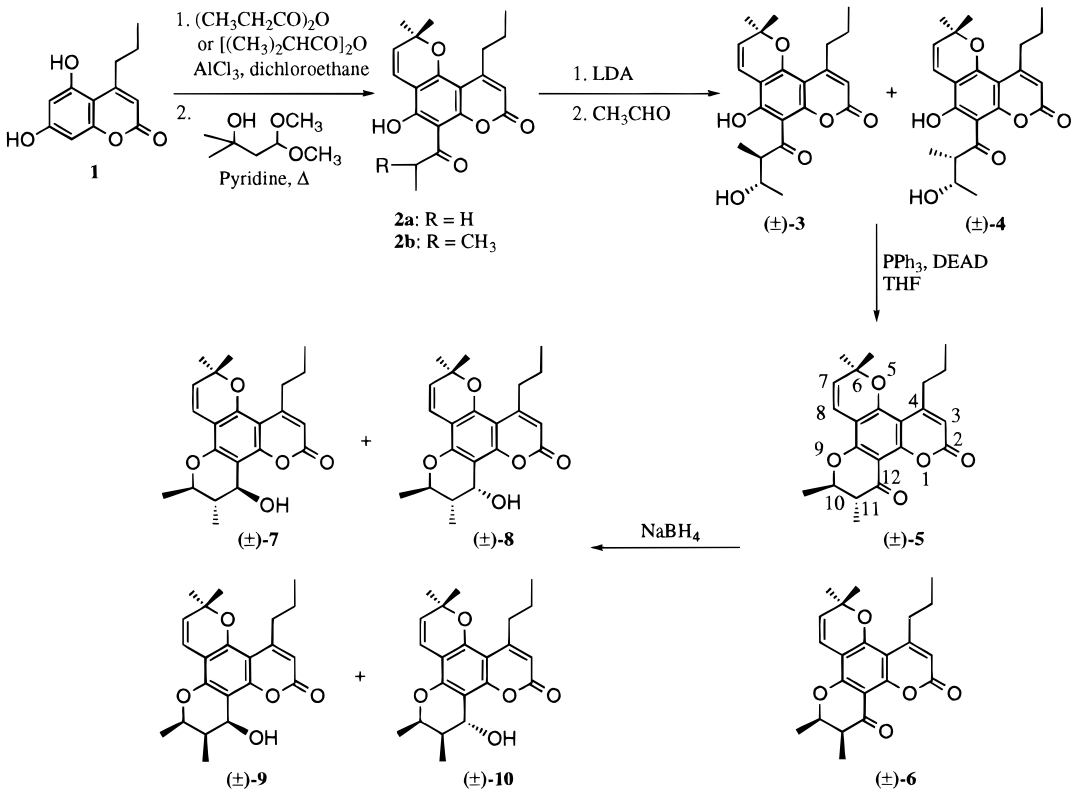
It should be noted that the ^{1}H NMR spectrum of the natural product originally designated calanolide C[15] did not correlate with synthetic samples having the structure **9**,[20−22] and it was later established that the natural product was a regioisomer of **9**.[30] Thus, structure **9** is now known as calanolide C, while the regioisomeric natural product is designated pseudocalanolide C[30] (Figure 1). Likewise, the natural product originally designated calanolide D was later found to be a regioisomer of ketone **6**. Thus, the ketone **6** is now known as calanolide D, while the regioisomeric natural product has been designated pseudocalanolide D[30] (Figure 1).

Because our large-scale synthesis of calanolide A (Scheme 1) involved preparation of ring C last and provided ready access to large quantities (multikilogram) of intermediates **2a**, **3**, and **5**, we initiated our analogue study by modifying ring C. These modifications also allowed an evaluation of the structural features of ring C necessary for antiviral activity, which were ambiguous via comparison of the naturally occurring materials.

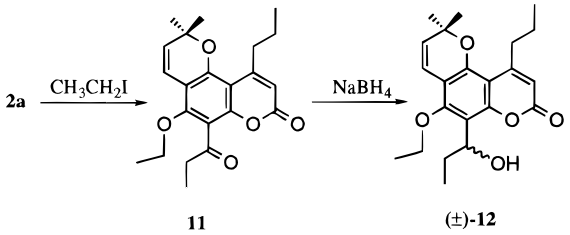
The simplest and most direct formation of a calanolide analogue was alkylation of **2a** with iodoethane (Scheme 2), which provided ketone **11**. Ketone **11** represented a nonchiral, acyclic derivative of ketones **5** and **6**. Reduction with sodium borohydride afforded analogue **12**, corresponding to an acyclic derivative of the calanolides.

Modification of ring C was accomplished by aldol alkylation of the 8-acylated coumarins **2a** and **2b** with aldehydes and ketones, followed by intramolecular Mitsunobu cyclization and reduction of the resulting ketones, illustrated in Scheme 1. The aldol addition product **13a** (Figure 2) was prepared by the action of paraformaldehyde on **2a** in the presence of pyridinium *p*-toluenesulfonate and trifluoroacetic acid in refluxing THF. Interestingly, the "protected" ketone **14** (Figure 2) was isolated as a minor byproduct, likely arising from ketal formation of small amounts of ketone **6** with bis-

Scheme 1



Scheme 2



(hydroxymethyl) ether, formed during the decomposition of paraformaldehyde. None of the desired ketone **16** was isolated directly from the reaction mixture. This is in contrast to the reaction of **2a** with paraldehyde under similar conditions,[27] in which ketones **5** and **6** were isolated directly from the reaction mixture.

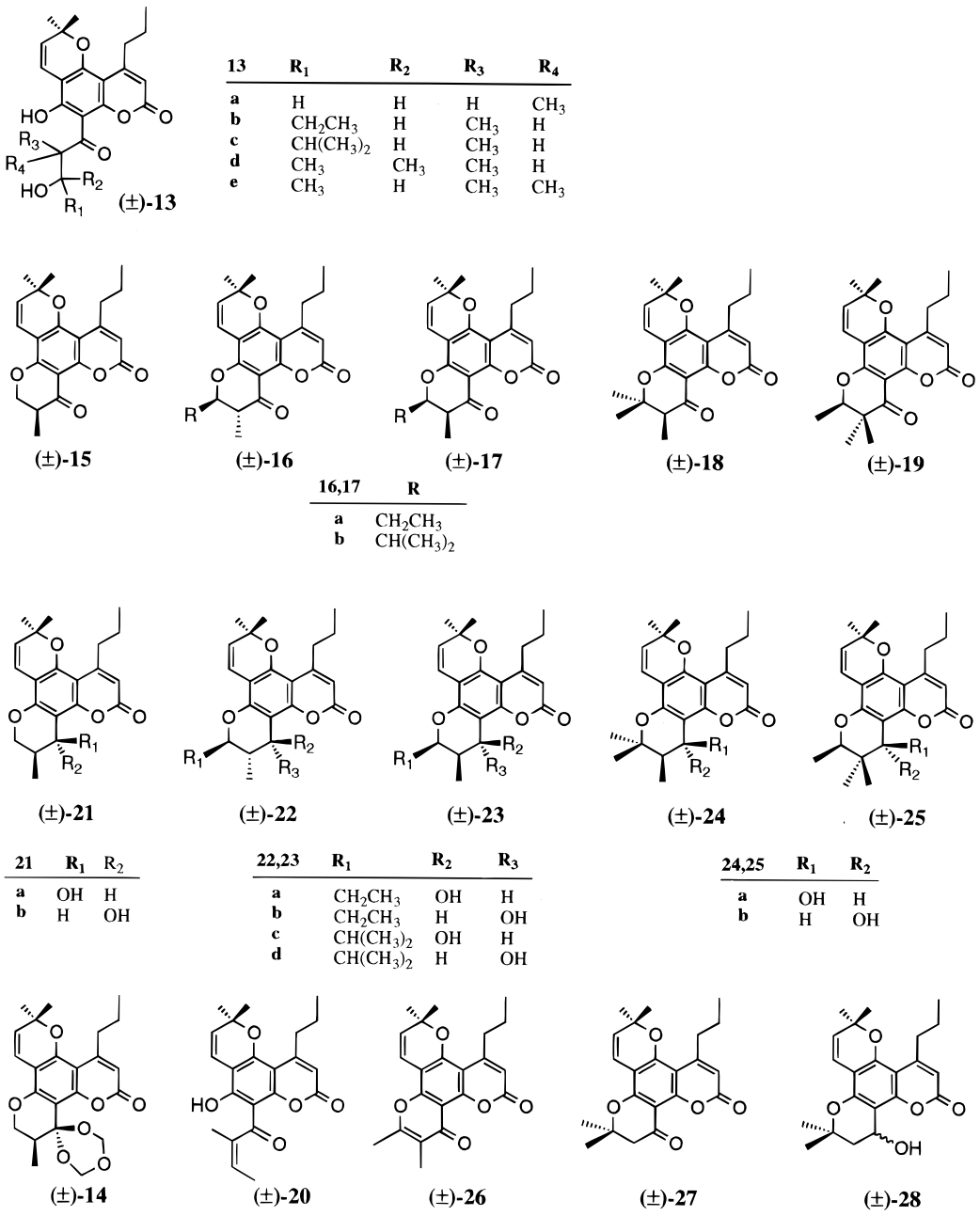
Aldol reaction of **2a** with *n*-propanal, isobutyryaldehyde, and acetone (Scheme 1) proceeded as expected to give the isolable aldol addition products **13b**−**d** (Figure 2). Intermediate **13e** was prepared via aldol alkylation of **2b** with acetaldehyde. **2b** was synthesized by Friedel−Crafts acylation of coumarin **1** with isobutyric anhydride, using conditions employed in the preparation of **2a**.[19] In some instances, the aldol products **13** were relatively unstable, being easily reverted to the starting ketone **2a**, especially during silica gel chromatography. Thus, if column chromatography was necessary for purification, it was conducted as quickly as possible. In some cases, the aldol products were not purified, but used crude for the next step in the sequence.

Mitsunobu ring closure[27] of **13** provided a series of ketone analogues **15**−**19**. The ketones which formed as diastereomeric mixtures (**16** and **17**) were separated using column chromatography. Attempts to prepare **19** via deprotonation of ketone **5** with lithium diisopropylamide or lithium hexamethyldisilazide followed by iodomethane quench produced not the desired analogue **19** but the ring-opened derivative **20** (Figure 2) as the sole product. Ring opening of methylated dihydrobenzopyran-4-ones via reverse Michael reaction has previously been described as a major reaction pathway upon deprotonation.[31] Interestingly, upon standing in solution (EtOAc or CH_2Cl_2) at room temperature, compound **20** was converted to a mixture of ketones **5** and **6**, presumably via an intramolecular Michael addition, similar to the reaction used by others[20] in the construction of ring C of the calanolides. Because of this instability, compound **20** was not evaluated for antiviral activity.

Reduction of ketones **15**−**19** with sodium borohydride provided the alcohols **21**−**25**, as epimeric mixtures. The epimeric alcohols were separated using either silica gel column chromatography, preparative TLC, or preparative normal phase HPLC. The 10-isopropyl derivatives **22c** and **22d**, obtained via reduction of ketone **16b**, could not be separated completely by any of the above methods and were thus assayed as a 1:2 mixture of **22c:22d**. Of the 10,11-*cis*-substituted epimers **23c** and **23d**, only **23c** was obtained in sufficient quantity and purity for characterization and antiviral testing.

The 10,11-unsaturated ketone **26** was prepared as previously described.[19] Attempts to reduce ketone **26** to its corresponding alcohols were unsuccessful, resulting only in intractable mixtures of products. The 10,10-dimethyl-11-desmethyl ketone **27** was prepared using a modification of a literature technique.[20] Sodium borohydride reduction of **27** provided the 10,10-dimethyl-11-desmethylcalanolide derivative **28**.

Structural assignments of ketone intermediates were made based upon comparison of the coupling constants between the 10- and 11-protons with those of ketones **5** and **6**. Ketone **5**, with a *trans* relationship between the



13	R_1	R_2	R_3	R_4
a	H	H	H	CH_3
b	CH_2CH_3	H	CH_3	H
c	$CH(CH_3)_2$	H	CH_3	H
d	CH_3	CH_3	CH_3	H
e	CH_3	H	CH_3	CH_3

16,17	R
a	CH_2CH_3
b	$CH(CH_3)_2$

21	R_1	R_2
a	OH	H
b	H	OH

22,23	R_1	R_2	R_3
a	CH_2CH_3	OH	H
b	CH_2CH_3	H	OH
c	$CH(CH_3)_2$	OH	H
d	$CH(CH_3)_2$	H	OH

24,25	R_1	R_2
a	OH	H
b	H	OH

Figure 2.

10- and 11-protons, exhibited a coupling constant of 11.0 Hz, while the 10,11-*cis* ketone had a much smaller coupling constant of 3.4 Hz. This observation was utilized in the assignment of ketones **16a** ($J_{10,11}$ = 10.4 Hz, *trans*) and **17a** ($J_{10,11}$ = 3.2 Hz, *cis*), and **16b** ($J_{10,11}$ = 10.4 Hz, *trans*) and **17b** ($J_{10,11}$ = 2.3 Hz, *cis*).

Assignments of diastereomeric derivatives bearing a 12-hydroxyl group were generally established via correlation of the ^{1}H NMR spectra with those of compounds **7**−**10**. Derivatives having a 10,11-*trans* relationship were correlated with **7** and **8**. Calanolide A (**7**), having a *trans* relationship between the 11- and 12-protons, exhibited a large coupling constant (8.0 Hz) between the *trans*-11,12 protons. Calanolide B (**8**) exhibited a much smaller coupling constant between the *cis*-11,12 protons (3.3 Hz). This correlation was used in the assignment of derivatives **22a** ($J_{11,12}$ = 6.7 Hz, *trans*) and **22b** ($J_{11,12}$ = 3.3 Hz, *cis*), and **22c** ($J_{11,12}$ = 6.9 Hz, *trans*) and **22d** ($J_{11,12}$ = 3.0 Hz, *cis*).

Compounds having a 10,11-*cis* relationship were assigned by analogy with **9** and **10**, which also exhibited differences in the coupling constants between the 11- and 12-protons, though opposite in nature to that observed for **7** and **8**. Calanolide C (**9**), having a *cis* relationship between the 11- and 12-protons, exhibited a larger coupling constant (5.4 Hz) between the *cis*-11,12 protons. *Epi*-calanolide C (**10**), having a *trans* relationship between the 11- and 12-protons, exhibited a smaller coupling constant between the *trans*-11,12 protons (2.1 Hz). This correlation was used in the assignment of derivatives **21a** ($J_{11,12}$ = 3.0 Hz, *cis*) and **21b** ($J_{11,12}$ = 1.2 Hz, *trans*), **23a** ($J_{11,12}$ = 5.2 Hz, *cis*) and **23b** ($J_{11,12}$ = 1.9 Hz, *trans*), and **23c** ($J_{11,12}$ = 6.6 Hz, *cis*).

Analogues which did not contain alkyl substituents at both the 10- and 11-positions (**21**), or more than one substituent at either the 10- or 11-position (**24** and **25**, respectively), and thus no distinct *cis* or *trans* relationship for analogy with **7**−**10**, could not be reliably

assigned based upon their 11,12-proton coupling constants and were thus assigned based upon other observations. In comparing **9** and **10**, dramatic differences in resonance frequency for the methyl group attached at C-11 were noted. For compound **9**, having a *syn* relationship between the 10- and 12-protons, the doublet signal corresponding to the methyl attached at C-11 absorbed at δ 1.14, while the same methyl group in compound **10** (having an *anti* relationship between the 10- and 12-protons) absorbed dramatically upfield, at δ 0.80. This observation was used in the assignment of **25a** (δ 1.06, *syn*) and **25b** (δ 0.78, *anti*).

Comparison of the ^{1}H NMR spectra of **9** and **10** also revealed that the resonance frequency absorption for H_{12} differed by 0.23 ppm, with the absorption being further downfield (δ 5.09) for **9** relative to **10** (δ 4.86). This observation, combined with the observation regarding the absorption for the methyl group attached at C_{11}, was used for assignment of analogues **21a** (H_{12}, δ 5.01; C_{11}-methyl, δ 1.12, *cis*) and **21b** (H_{12}, δ 4.82; C_{11}-methyl, δ 1.04, *trans*), and **24a** (H_{12}, δ 4.93; C_{11}-methyl, δ 1.24, *cis*) and **24b** (H_{12}, δ 4.69; C_{11}-methyl, δ 1.13, *trans*).

Antiviral Activity

The analogues were initially evaluated for anti-HIV activity using a CEM-SS cytoprotection assay[14] with the RF strain of HIV-1, at a maximum drug concentration of 100 μM. The drug concentration required for protection of the cells from HIV-1-mediated cytopathicity by 50% relative to drug-free controls (EC_{50}) and the drug concentration which resulted in cytotoxicity of 50% of the cell population relative to drug-free controls (IC_{50}), as well as the therapeutic index (TI, IC_{50}/EC_{50}), are reported in Table 1. Additionally, analogues which showed appreciable activity in the cytoprotection assay were evaluated for inhibition of HIV-1 RT,[32] also shown in Table 1. All of the analogues assayed were racemates except for (+)-**7**, which was included for comparison purposes.

As part of our analogue program, we assessed the anti-HIV activities of several key intermediates of our calanolide synthesis. Surprisingly, both the aldol addition products (**3** and **4**, Scheme 1)[27] and the *trans*-ketone **5** showed cytoprotective activity against HIV-1 cytopathicity *in vitro* (EC_{50} values of 6.2, 2.3, and 1.2 μM, respectively). The *cis* ketone **6** exhibited antiviral activity similar to that of **5** (EC_{50} of 2.8 μM), but with a lower therapeutic index (2.3 versus 10, respectively). The simple acyclic derivative of the calanolides (**12**, Scheme 2), which contained only one chiral center and was easily prepared, was completely devoid of biological activity. Likewise, the precursor ketone **11**, representing an acyclic derivative of ketones **5** and **6**, was also inactive.

The epimeric alcohols **9** (calanolide C) and **10** (*epi*-calanolide C), obtained via reduction of the *cis* ketone **6**, were assayed for antiviral activity. Although several syntheses of calanolide C had been described,[20,22] there were no previous reports of its antiviral activity. Compound **9** was completely devoid of activity up to a concentration of 100 μM, while **10** exhibited weak activity. The naturally occurring regioisomer, pseudocalanolide C (Figure 1), also exhibited no activity.[15]

Analogues **21a** and **21b** allowed exploration of the importance of an alkyl moiety at the 10-position.

Table 1. Anti-HIV Activity of Calanolide Analogues[a]

	CEM-SS cytoprotection			reverse transcriptase
compound[b]	EC_{50}[c] (μM)	IC_{50} (μM)	TI[d] (IC_{50}/EC_{50})	EC_{50} (μM)
(±)-**3**	6.2	24	3.9	
(±)-**4**	2.3	22	9.6	
(±)-**5**	1.2	19	16	40
(±)-**6**	2.8	12	4.3	>100
(+)-**7**	0.27	23	85	0.32
(±)-**7**	0.49	23	47	0.97
(±)-**9**	NA	18	ND	
(±)-**10**	>100	20	ND	
(±)-**11**	NA	6.6	ND	
(±)-**12**	NA	16	ND	
(±)-**13d**	NA	27	ND	
(±)-**13e**	NA	37	ND	
(±)-**14**	NA	6.3	ND	
(±)-**15**	NA	6.8	ND	
(±)-**16a**	>100	18	ND	
(±)-**16b**	NA	21	ND	
(±)-**18**	NA	2.4	ND	
(±)-**19**	>100	19	ND	
(±)-**21a**	7.2	22	3.1	50
(±)-**21b**	NA	22	ND	
(±)-**22a**	2.0	18	9.0	3.2
(±)-**22b**	1.7	14	8.2	2.0
(±)-**22c/22d**	NA	6.5	ND	
(±)-**23a**	NA	12	ND	
(±)-**23b**	NA	21	ND	
(±)-**23c**	NA	6.9	ND	
(±)-**24a**	>100	21	ND	
(±)-**24b**	5.7	21	3.7	5.0
(±)-**25a**	NA	20	ND	
(±)-**25b**	2.4	15	6.2	32
(±)-**26**	NA	>100	ND	
(±)-**28**	NA	11	ND	

[a] CEM-SS cytoprotection and reverse transcriptase assays were conducted as described in the Experimental Section. [b] Data for (+)-**7** and (±)-**7** are from ref 19. [c] EC_{50} value of >100 indicates that the compound showed antiviral activity, but did not reach 50% protection at a concentration of 100 μM. NA indicates no activity at concentration of 100 μM. [d] ND indicates therapeutic index was not determined because an EC_{50} was not available.

Compound **21a**, having a *cis* relationship between the 11-methyl and 12-hydroxyl groups (similar to calanolide B), showed anti-HIV activity with an EC_{50} of 7.2 μM. The epimer **21b**, with a *trans* relationship between the 11- and 12-positions (similar to calanolide A), was devoid of biological activity. The corresponding 11-desmethyl derivatives were previously described by other investigators[33] and found to have weak activity against reverse transcriptase relative to (+)-inophyllum B and (−)-inophyllum P; thus, these analogues were not pursued by us.

Substituting the 10-methyl group with an ethyl substituent provided the 10,11-*trans* derivatives **22a** and **22b**, as well as the 10,11-*cis* derivatives **23a** and **23b**. Both **22a** and **22b** exhibited anti-HIV activity, with nearly identical EC_{50} values (2.0 and 1.7 μM, respectively), while **23a** and **23b** were both inactive. The antiviral activities of **22a** and **22b** were approximately 4-fold less potent than that of racemic calanolide A. The relative stereochemistry of **22a** was that of calanolide A, and **22b** that of calanolide B, both of which possessed similar anti-HIV activity. The 10,11-*cis* derivatives **23a** and **23b** had the same relative stereochemistry as calanolide C and *epi*-calanolide C, respectively, neither of which exhibited significant antiviral activity.

Replacement of the 10-methyl group with an isopropyl group provided the 10,11-*trans* derivatives **22c** and **22d**, and the 10,11-*cis* derivatives **23c** and **23d**. The *trans*

derivatives **22c** and **22d** could not be completely separated by any of the attempted techniques (column chromatography, preparative HPLC) and thus were assayed as a 1:2 epimeric mixture. This mixture showed no antiviral activity at a maximum concentration of 100 μM. Of the *cis* derivatives **23c** and **23d**, only **23c** was obtained in sufficient quantity and purity for antiviral testing, and this analogue was also devoid of activity.

Addition of a second methyl group at ring positions 10 or 11 effectively removed the chirality at those carbons, yet preserved the relative stereochemical features of the calanolides, in that a *trans* dimethyl relationship still existed between the 10- and 11-positions. Thus, the 10,10-dimethyl calanolide analogues **24a** and **24b** were prepared, as well as the 11,11-dimethyl analogues **25a** and **25b**. Compound **24a**, which had a *cis* relationship between the 11-methyl group and the 12-hydroxyl (similar to calanolide B), exhibited anti-HIV activity with an EC_{50} of 5.7 μM. Epimer **24b**, which had a *trans* relationship between the 11-methyl and the 12-hydroxyl groups (similar to calanolide A), was inactive. Similar results were obtained for the 11,11-dimethyl derivatives **25a** and **25b**, in that only one epimer exhibited anti-HIV activity. The 10,12-*syn*-analogue **25a** (similar to calanolide A) demonstrated antiviral activity with an EC_{50} of 2.4 μM, while the 10,12-*anti* derivative **25b** (similar to calanolide B) was inactive. The 10,10-dimethyl-11-desmethylcalanolide derivative **28**, which contained only one chiral center, was also inactive. Neither of the ketones **16a**, **16b**, **18**, **19**, or **26** possessed significant antiviral activity. Because neither of the 10,11-*cis*-alcohols **23** exhibited anti-HIV activity, their corresponding ketones **17** were not evaluated. The aldol addition products **13a**, **13b**, and **13c** were unstable, tending to revert to starting material **2a** upon attempted chromatographic purification; thus, these analogues were not examined for biological activity.

Analogues which showed appreciable anti-HIV activity in the CEM-SS cytoprotection assay were evaluated for inhibition of HIV-1 RT, as previously described.[32] The aldol addition products **3** and **4** were not evaluated because of their tendency to rapidly cyclize to a mixture of ketones **5** and **6** upon standing in solvent. All of the derivatives tested for RT activity exhibited inhibition of the enzyme (Table 1). The relative magnitude of enzyme inhibition correlated with that of cytoprotectivity for alcohols **7**, **9**, **22a**, **22b**, and **24b**. However, correlations were poorer for ketones **5**, **6**, **21a**, and **25b**, which showed appreciable activity in the cytoprotection assay, but less activity in the RT assay. However, these results are most likely due to the poor solubility of these ketones in the buffer system used for the RT assay. Ketone **5** was evaluated as an inhibitor of both HIV protease and HIV integrase (data not shown) and found to have no activity against either, supporting the postulate that RT is indeed the target of these ketones.

Discussion

Many of the observations derived from this structure−activity relathionship study correlate with results obtained via comparisons of the naturally occurring compounds. Surprisingly, we found that several of the synthetic intermediates in our pathway to the calanolides exhibited anti-HIV activity with potency similar to racemic calanolide A. Both ketones **5** and **6** showed impressive activity against HIV-mediated cytopathicity, with EC_{50} values of 1.2 and 2.8 μM, respectively. These values are only 5-fold less active than racemic calanolide A [(±)-**7**], but the ketones have significantly lower therapeutic indices (16 and 4.3) than (±)-**7** (46). Although both ketones were previously prepared by others, ours is the first observation of anti-HIV activity for these compounds.[1,34] The corresponding *trans*- and *cis*-ketones in the inophyllum series (inophyllum C and inophyllum E, respectively), which contain a phenyl moiety at the 4-position in place of the *n*-propyl group, were reported to exhibit weak activity against HIV-1 reverse transcriptase, with inophyllum C being slightly more active than inophyllum E, but no data was reported regarding inhibition of HIV-mediated cytopathicity.[16] Interestingly, the 10,11-unsaturated ketone **26** was completely devoid of antiviral activity, suggesting a requirement for a certain degree of flexibility in ring C.

Aldol products **3** and **4** possessed antiviral activity, with EC_{50} values and therapeutic indices similar to those of the ketones **5** and **6**. It is unclear whether this activity is actually mediated by the aldol products themselves or the result of intramolecular cyclization to the ketones during the course of the *in vitro* assay. It was observed that both **3** and **4** rapidly cyclize to an approximate 1:1 mixture of ketones **5** and **6** upon standing in solvent (ethanol or ethyl acetate). Thus, because of their instability, these derivatives were not pursued further.

In general, all of the compounds possessing a 10,11-*cis*-dialkyl substitution pattern except ketone **6** exhibited no anti-HIV activity, consistent with the results of calanolide C (**9**) and *epi*-calanolide C (**10**). The natural product pseudocalanolide C, which is regioisomeric with **9** regarding orientation of the B and C rings (Figure 1), was also reported to be devoid of activity.[15] The corresponding compounds in the inophyllum series (inophyllum A, similar to **9**, and inophyllum D, similar to **10**), which contain a phenyl moiety at the 4-position in place of the *n*-propyl group, were reported to be weakly active against HIV-1 reverse transcriptase.[16] However, no data regarding inhibition of HIV-mediated cytopathicity was provided. Ketone **6** represents the first example of a compound in the calanolide series having a *cis* relationship between the 10- and 11-methyl groups which exhibited significant anti-HIV activity.

Removal of the 10-methyl group provided the epimers **21a** and **21b**. The 11,12-*cis* derivative **21a**, which had the same relative 11,12-stereochemistry as calanolide B, showed significant antiviral activity (EC_{50} of 7.2 μM), while the 11,12-*trans* derivative **21b**, which had the relative 11,12-stereochemistry of calanolide A, was inactive up to a concentration of 100 μM. The precursor, 10-desmethyl ketone **15**, was completely inactive. The corresponding 11-desmethyl derivatives were previously reported to be only weakly active by other investigators[33] and thus were not prepared by us. These results suggest a requirement for alkyl substituents in the 10- and 11-positions for effective anti-HIV activity.

Substitution of the 10-methyl group for other alkyl substituents demonstrated that antiviral activity was retained when the 10-methyl moiety was replaced with

a slightly larger group, such as ethyl (**22a** and **22b**). **22a**, which had the same relative stereochemistry as calanolide A, and **22b**, which has the relative stereochemistry of calanolide B, were essentially equally active (EC_{50} values of 2.0 and 1.7 μM, respectively), and only approximately 4-fold less active than racemic calanolide A. As expected, both of the 10,11-*cis* derivatives (**23a** and **23b**) were inactive, anticipated from the inactivity of **9** and **10**. Increasing the size of the 10-substituent to isopropyl (**22c**/**22d** and **23c**) completely eliminated the antiviral activity, irrespective of the *trans*- or *cis*-relationship of the 10,11-dialkyl substituents. These results suggest that substitution of the 10-methyl group with other alkyl moieties is tolerated to a certain point, but the antiviral activity is rapidly diminished with increasing size of the substituent. However, neither the 10-ethyl nor the 10-isopropyl ketone derivatives **16a** and **16b** possessed anti-HIV activity, suggesting that the tolerance for larger alkyl substituents at the 10-position is not extended to the ketone series of analogues. Because the 10,11-*cis*-alcohols **23a** and **23b** possessed no anti-HIV activity, their corresponding ketones **17a** and **17b** were not examined.

Introduction of a second methyl group at either the 10- or 11-position effectively removed the chirality at the respective carbon and thus provided a simpler target molecule. Also, it might be expected that addition of a methyl group to either of these carbons would not have a serious deleterious effect on biological activity, because the same spatial relationships exist between the 10- and 11-positions (two methyl groups in a *trans*-relationship). Compounds **24a** and **24b**, possessing two methyl groups at the 10-position and the same relative stereochemical features of calanolide B and calanolide A, respectively, each exhibited antiviral activity. The calanolide B-like derivative (**24a**) was only weakly active, with an EC_{50} greater than 100 μM, while the calanolide A-like derivative (**24b**) was much more active, with an EC_{50} value of 5.7 μM. However, this activity is 12-fold less potent than racemic calanolide A, suggesting that inclusion of an additional methyl group at position 10 suppresses the binding of the calanolide nucleus to its RT binding site. It is possible that the additional methyl group at the 10-position affects the conformation of the ring, forcing the 11-methyl and 12-hydroxyl groups into orientations unfavorable for enzymatic binding. This was supported by the observation that derivative **28**, which also has two methyl groups at position 10 but no methyl group at position 11, was devoid of anti-HIV activity. Ketone **18** was completely inactive, also demonstrating the dramatic changes in antiviral activity imparted by addition of a second methyl group to the 10-position.

Introduction of a second methyl group at the 11-position resulted in analogues **25a** and **25b**, possessing the same relative stereochemical features of calanolide A and calanolide B, respectively. In this series, the calanolide A-like analogue (**25a**) was completely devoid of antiviral activity, while the calanolide B-like derivative (**25b**) was active, with an EC_{50} value of 2.4 μM, only 5-fold less potent than racemic calanolide A. The therapeutic index of **25b** (6.2) was 7.6-fold lower than that of racemic calanolide A. As for the 10,10-dimethyl derivatives **24**, introduction of a second methyl group at position 11 seems to suppress binding of the calanolide nucleus to its RT binding site, likely for the same reasons postulated for **24**. Ketone **19** showed slight antiviral activity, but the EC_{50} value was above 100 μM.

From these data, several key structural features of ring C necessary for antiviral activity can be concluded. First, the requirement of an oxygen moiety at position 12 appears to be crucial, whether it be in the form of an alcohol (**7**, **8**, **21a**, **22a**, **22b**, **24b**, and **25b**) or a ketone (**5** and **6**). This was previously suggested[19] because the naturally occurring calanolide A 12-methyl ether was reported to be inactive.[15] The naturally occurring 12-acetyl calanolide A demonstrated antiviral activity in a cell culture assay,[15] but it is conceivable that the acetyl group was hydrolyzed during the course of the assay. Our observations regarding the anti-HIV activity of ketones **5** and **6** represent the first examples of polycyclic coumarins of this class lacking a hydroxyl group in the 12-position having anti-HIV activity. The RT inhibition data did not correlate well with the cytoprotectivity data for ketones **5** and **6** (Table 1), but this is most likely due to the poor solubility of the ketones in the buffer utilized in the RT assay.

The presence of alkyl substituents at both the 10- and 11-positions having a *trans* relationship also appear to be important for significant antiviral activity, with a tolerance for small alkyl groups at the 10-position. Except for the *cis*-ketone **6**, none of the analogues containing a *cis* relationship between the 10- and 11-substituents showed appreciable activity. Ketone **6** represents the first analogue of this class having a *cis*-10,11 relationship to exhibit anti-HIV activity. Conversion of ring C to an acyclic derivative (**12**) having the same number of carbon atoms, but deleting the carbon−carbon bond between the 10- and 11-positions, eliminated the biological activity, suggesting the rigid ring system is necessary for correct orientation of the 10- and 11-alkyl groups into the enzymatic binding site.

On the basis of the above results, it is concluded that the *trans*-10,11-dimethyldihydropyran-12-ol ring of the calanolides is very sensitive to modification and that even the most minute of changes result in dramatic attenuation of biological activity. The inophyllum coumarins, (+)-inophyllum B and (−)-inophyllum P (Figure 1), which contain a phenyl substituent in the 4-position, are nearly equally as active as (+)-calanolide A and (−)-calanolide B, which contain a *n*-propyl moiety in the 4-position. These observations suggest that the 4-position, which is also likely important for tight binding to the target RT enzyme, could be more easily modified to provide potent, novel derivatives without eliminating the desired biological activity. Similarly, no studies of the structure−activity relationships of the pyran ring (ring B) have been reported, and this ring could also serve as the basis for analogue development. The modifications reported in our present study confirm that the dihydropyran ring (ring C) is critical for antiviral activity and that subtle manipulations of this ring result in drastic reductions in activity. However, ketone derivatives **5** and **6**, which are the first examples of calanolide analogues not having a 12-hydroxyl group but still exhibiting anti-HIV activity, represent novel analogues which could serve as lead compounds for development of further series of structural analogues.

Experimental Section

General Experimental. ^{1}H NMR spectra (300 MHz) were recorded on a Varian Gemini 2000 spectrometer. Chemical shifts are reported on the δ scale downfield from tetramethylsilane. Mass spectra were obtained with a Finnegan MAT 90 mass spectrometer. Infrared spectra were recorded with a Midac M series FTIR spectrophotometer. Elemental analyses were obtained from Midwest Microlab, Indianapolis, IN, and agree to within ±0.4% of the theoretical values. Melting points were determined with a Mel-Temp melting point apparatus and are corrected. Column chromatography was conducted with EM Science silica gel 60 (70−230 mesh) with indicated eluents. Analytical thin layer chromatography was performed with silica gel 60 F_{254} precoated glass-backed plates (250 μm) with indicated eluents. Preparative thin layer chromatography was performed with silica gel 60 F_{254} glass-backed plates (1 mm) with indicated eluents. Analytical HPLC was conducted with a normal phase silica gel column (250 mm × 4.6 mm Zorbasil with 5 μm particle size, MAC-MOD Analytical, Inc.). Semipreparative HPLC was conducted with a 250 mm × 22 mm i.d. Econosil silica gel column with 10 μm particle size (Alltech Associates, Inc.). Reagents and solvents were purchased from commercial sources.

6,6-Dimethyl-9-hydroxy-10-isobutyryl-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (2b). Into a flame-dried 500 mL three-necked round-bottom flask were placed 10.0 g (48.1 mmol) of 5,7-dihydroxy-4-propylcoumarin (**1**)[19,20] and 12.0 g (90 mmol) of $AlCl_3$, under a positive nitrogen atmosphere. 1,2-Dichloroethane (120 mL) was then added, and the solution was warmed to 75 °C in a water bath with mechanical stirring. After 15 min of stirring at 75 °C, a homogeneous solution was obtained. To this solution was added a mixture of isobutyric anhydride (7.61 g, 48.1 mmol) and 12.0 g $AlCl_3$, dissolved in 60 mL 1,2-dichloroethane, dropwise over 1 h. After addition was complete, the solution was stirred 1 h further at 75 °C and then cooled to room temperature. The solution was poured over a mixture of 100 g of crushed ice and 100 mL of 2 M HCl, at which point a white precipitate formed. The mixture was diluted with 1.8 L of EtOAc, and the organic layer was separated. The organic solution was washed sequentially with 500 mL each of 1 M HCl and saturated brine, dried over magnesium sulfate, filtered, and evaporated to provide an orange powder. The powder was triturated with 80 mL of acetone, collected on a Büchner funnel, rinsed with 80 mL of Et_2O, and air-dried to provide a cream-colored solid (4.22 g). The product was purified via recrystallization from 200 mL of EtOH to give 5,7-dihydroxy-8-isobutyryl-4-propylcoumarin as colorless plates (3.63 g, 26.0%): mp 263−265 °C (lit.[28] mp 272−273 °C), with softening at 250 °C; ^{1}H NMR (DMSO-d_6) δ 0.95 (t, 3 H, J = 7.4 Hz), 1.08 (d, 6 H, J = 6.9 Hz), 1.59 (sextet, 2 H, J = 7.4 Hz), 2.87 (t, 2 H, J = 7.4 Hz), 3.24 (heptet, 1 H, J = 6.9 Hz), 5.93 (s, 1 H), 6.37 (s, 1 H), 11.16 (s, 1 H), 11.44 (s, 1 H): FTIR (KBr) 3216, 1684 cm^{-1}; EIMS *m/e* 290 (M^+, 23), 247 (100), 219 (11). Anal. ($C_{16}H_{18}O_5$) C, H.

The above product (2.90 g, 10.0 mmol) and 1,1-dimethoxy-3-methylbutan-3-ol[28] (1.49 g, 10.1 mmol) were dissolved in 5 mL of pyridine and refluxed for 40 h. The solvent was evaporated *in vacuo*, and the residue was taken up into 50 mL of EtOAc. The solution was washed with 2 M HCl (2 × 50 mL) and saturated brine (50 mL), dried over magnesium sulfate, filtered, and evaporated to give an orange-brown solid. The product was dissolved in 100 mL of $CHCl_3$, 3.56 g of silica gel was added, and the solvent was evaporated to give an orange powder. The powder was placed atop a column of 125 g of silica gel (EtOAc/hexane, 1:4) and eluted with the same. Pooling of appropriate fractions and evaporation provided a bright orange crystalline solid (2.51 g, 70.5%): mp 70−72 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3 H, J = 7.3 Hz), 1.26 (d, 6 H, J = 6.7 Hz), 1.54 (s, 6 H), 1.66 (tq, 2 H, J = 7.7, 7.3 Hz), 2.91 (br t, 2 H, J = 7.7 Hz) 4.06 (heptet, 1 H, J = 6.7 Hz), 5.58 (d, 1 H, J = 9.9 Hz), 6.01 (s, 1 H), 6.73 (d, 1 H, J = 9.9 Hz), 14.45 (s, 1 H); FTIR (KBr) 1732 cm^{-1}; EIMS *m/e* 356 (M^+, 48), 341 (100), 323 (15), 313 (65). Anal. ($C_{21}H_{24}O_5$) C, H.

Reduction of Ketone 6: Synthesis of Calanolide C (9) and *Epi*-calanolide C (10). To a solution of **6** (736 mg, 2.00 mmol) in 40 mL of THF/EtOH (1:1) was added sodium borohydride (75.6 mg, 2.00 mmol), and the solution was stirred at room temperature for 2 h. The reaction was quenched via addition of 1 mL of saturated NH_4Cl, and then the solvent was evaporated *in vacuo*. The residue was partitioned between 50 mL each of H_2O and EtOAc, and the organic layer was separated and washed sequentially with 50 mL each of 1 M HCl, 5% $NaHCO_3$, and saturated brine. After being dried over magnesium sulfate, the solution was filtered and evaporated to provide a colorless gum. TLC (silica gel, EtOAc/hexane, 1:1) showed two components, a faster-moving major component and a slower minor component. These materials were separated via column chromatography through 75 g of silica gel (EtOAc/hexane, 1:3) to provide complete separation. The faster-moving major component corresponded to calanolide C (**9**, 416 mg, 56.2%), and the slower component was *epi*-calanolide C (**10**, 53.4 mg, 7.2%). Both materials were obtained as white foams.

(±)-(10,11-*cis*,11,12-*cis*)-10,11-Dihydro-12-hydroxy-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-one (9, calanolide C): ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7.2 Hz), 1.14 (d, 3 H, J = 7.2 Hz), 1.41 (d, 3 H, J = 6.6 Hz), 1.49 (s, 6 H), 1.66 (m, 2 H), 2.28 (m, 1 H), 2.90 (m, 2 H), 3.16 (br s, 1 H), 4.39 (dq, 1 H, J = 3.3, 6.6 Hz), 5.09 (d, 1 H, J = 5.4 Hz), 5.53 (d, 1 H, J = 9.9 Hz), 5.94 (s, 1 H), 6.63 (d, 1 H, J = 9.9 Hz); FTIR (thin film) 3443, 1724, 1586, 1134 cm^{-1}; EIMS *m/e* 370 (M^+, 56), 355 (100), 337 (25), 299 (61), 271 (22). Anal. ($C_{22}H_{26}O_5$) C, H.

(±)-(10,11-*cis*,11,12-*trans*)-10,11-Dihydro-12-hydroxy-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-one (10, *epi*-calanolide C): ^{1}H NMR ($CDCl_3$) δ 0.80 (d, 3 H, J = 7.2 Hz), 1.04 (t, 3 H, J = 7.2 Hz), 1.43 (d, 3 H, J = 6.6 Hz), 1.49 (s, 6 H), 1.66 (m, 2 H), 2.03 (m, 1 H), 2.90 (dt, 2 H, J = 3.0, 7.2 Hz), 4.52 (dq, 1 H, J = 1.8, 6.6 Hz), 4.86 (d, 1 H, J = 1.8 Hz), 5.54 (d, 1 H, J = 9.9 Hz), 5.96 (s, 1 H), 6.66 (d, 1 H, J = 9.9 Hz); FTIR (thin film) 3430, 1713, 1586, 1134 cm^{-1}; EIMS *m/e* 370 (M^+, 45), 355 (100), 337 (22), 299 (34), 271 (12). Anal. ($C_{22}H_{26}O_5 \cdot {}^3/_4H_2O$) C, H.

6,6-Dimethyl-9-ethoxy-10-propionyl-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (11). A solution of 5,7-dihydroxy-8-propionyl-4-propylcoumarin[19] (**2a**, 684 mg, 2.00 mmol), iodoethane (390 mg, 2.50 mmol), and K_2CO_3 (553 mg, 4.00 mmol) in 25 mL of acetone was stirred under reflux for 8 h. The solvent was evaporated *in vacuo* and the residue partitioned between 30 mL each of EtOAc and H_2O. The organic phase was collected and washed with 30 mL each of H_2O and saturated brine. After being dried over magnesium sulfate, the solution was filtered and evaporated to provide a brown oil. Column chromatography through 60 g of silica gel (EtOAc/hexane, 1:1) gave a light yellow oil. After being dried under vacuum overnight, the product was obtained as a light-yellow crystalline solid (526 mg, 71%): mp 71 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3 H, J = 7.3 Hz), 1.22 (t, 3 H, J = 7.3 Hz), 1.35 (t, 3 H, J = 7.0 Hz), 1.52 (s, 6 H), 1.66 (m, 2 H), 2.91 (m, 4 H), 3.95 (q, 2 H, J = 7.0 Hz), 5.66 (d, 1 H, J = 9.8 Hz), 6.03 (s, 1 H), 6.54 (d, 1 H, J = 9.8 Hz); FTIR (KBr) 1742, 1570, 1200, 1138 cm^{-1}; EIMS *m/e* 370 (M^+, 32), 355 (100), 341 (51), 327 (14). Anal. ($C_{22}H_{26}O_5$), C, H.

(±)-6,6-Dimethyl-9-ethoxy-10-(1-hydroxypropyl)-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (12). A solution of **11** (306 mg, 0.827 mmol) in 10 mL of ethanol/THF (9:1) was reduced with sodium borohydride (31.4 mg, 0.827 mmol), as described above for the reduction of ketone **6**. Column chromatography of the crude product through 30 g of silica gel (EtOAc/hexane, 1:4) provided the desired product as a light-yellow oil (217 mg, 71%): ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3 H, J = 7.4 Hz), 1.04 (t, 3 H, J = 7.3 Hz), 1.48 (s, 3 H), 1.52 (s, 3 H), 1.66 (sextet, 2 H, J = 7.5 Hz), 1.87 (tq, 1 H, J = 6.3, 7.4 Hz), 2.48 (tq, 1 H, J = 6.3, 7.4 Hz), 2.91 (dd, 2 H, J = 9.3, 6.3 Hz), 3.13 (d, 1 H, J = 11.3 Hz), 3.97 (q, 2 H, J = 7.4 Hz), 5.01 (ddd, 1 H, J = 6.3, 6.4, 11.3 Hz), 5.64 (d, 1 H, J = 9.9 Hz), 6.03 (s, 1 H), 6.52 (d, 1 H, J = 9.9 Hz); FTIR (thin film) 3445, 2971, 1734, 1578, 1132 cm^{-1}; EIMS *m/e* 372 (M^+, 12), 357 (9), 343 (100), 339 (15), 325 (8), 299 (7). Anal. ($C_{22}H_{28}O_5$) C, H.

(±)-6,6-Dimethyl-9-hydroxy-10-(3-hydroxy-2-methylpropionyl)-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (13a). To a mixture of **2a** (3.48 g, 10.0 mmol), paraformaldehyde (9.0 g, 300 mmol), and pyridinium *p*-toluenesulfonate (5.02 g, 20.0 mmol) was added 50 mL of THF. The resulting suspension was heated at 65−70 °C with magnetic stirring for 2 h. The suspension was allowed to slowly cool to room temperature and then cooled to −78 °C. Trifluoroacetic acid (10 mL) was slowly added to the suspension. After the addition was finished, the mixture was first warmed slowly to room temperature and then heated at reflux for 32 h. The mixture was cooled to 25 °C and then quenched with $NaHCO_3$ until pH 6−7. The solution was extracted with ethyl acetate (150 mL × 3), and the combined extracts were washed with saturated $NaHCO_3$ and brine. After being dried over magnesium sulfate, the solution was filtered and concentrated to give an oily residue (3.86 g). The crude product was purified by silica gel column chromatography, eluting with EtOAc/hexane (1:2). The desired fractions were combined, concentrated under reduced pressure, and dried under high vacuum in the presence of P_2O_5 overnight to afford an off-white solid of **13a** (779 mg, 20.6%): mp 88−90 °C; ^{1}H NMR ($CDCl_3$) δ 1.06 (t, 3 H, J = 7.4 Hz), 1.34 (d, 3 H, J = 6.6 Hz), 1.55 (s, 6 H), 1.66 (m, 2 H), 2.66 (dd, 1 H, J = 6.0, 7.5 Hz), 2.93 (m, 2 H), 3.71 (m, 1 H), 4.06 (m, 2 H), 5.61 (d, 1 H, J = 9.9 Hz), 6.04 (s, 1 H), 6.74 (d, 1 H, J = 10.2 Hz), 14.28 (s, 1 H); FTIR (KBr) 3507, 1719, 1721 cm^{-1}; EIMS *m/e* 372 (M^+, 40), 357 (38), 339 (100). Anal. ($C_{21}H_{24}O_6$) C, H.

(±)-10,11-Dihydro-4-propyl-6,6,11-trimethyl-12-(2,4,6-trioxano)-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (14). Obtained as a byproduct in the preparation of **13a**, above (290 mg, 6.9%): mp 216−218 °C; ^{1}H NMR ($CDCl_3$) δ 0.62 (d, 3 H, J = 6.9 Hz), 1.03 (t, 3 H, J = 7.4 Hz), 1.50 (s, 3 H), 1.51 (s, 3 H), 1.65 (sextet, 2 H, J = 7.4 Hz), 2.84 (m, 1 H), 2.95 (m, 1 H), 3.51 (m, 1 H), 3.81 (t, 1 H, J = 11.0 Hz), 3.92 (dd, 1 H, J = 5.3, 11.0 Hz), 4.92 (d, 1 H, J = 6.0 Hz), 5.23 (d, 1 H, J = 6.0 Hz), 5.29 (d, 1 H, J_{AB} = 5.2 Hz), 5.33 (d, 1 H, J_{AB} = 5.2 Hz), 5.59 (d, 1 H, J = 10.0 Hz), 6.03 (s, 1 H), 6.58 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 1725, 1580, 1360 cm^{-1}; EIMS *m/e* 414 (M^+, 38), 384 (52), 369 (25), 354 (56), 339 (100), 312 (21), 297 (17). Anal. ($C_{22}H_{26}O_7$) C, H.

General Procedure for Aldol Alkylation of Coumarins 2a and 2b. To a solution of the coumarin **2** (1.0 equiv) in anhydrous THF at −78 °C under N_2 was added a solution of lithium diisopropylamide (LDA) in heptane/THF/ethylbenzene (Aldrich, 2 M, 2.2 equiv), and the reaction mixture was stirred for 30 min. A solution of the aldehyde or ketone (3 equiv), dissolved in a small volume of THF, was then added, and the mixture was stirred for 2 h at −78 °C. The reaction was then quenched at −78 °C with 2 M methanolic HCl or saturated aqueous NH_4Cl and then allowed to warm to room temperature. The solvent was evaporated *in vacuo* and the residue partitioned between EtOAc and H_2O. The organic phase was separated, washed with saturated brine, dried over magnesium sulfate, filtered, and evaporated to provide the crude aldol products **13**. In most cases, the crude material was used without purification for the Mitsunobu cyclization step. Compound **13a** was prepared using a different method, described in the previous section.

(±)-6,6-Dimethyl-9-hydroxy-10-(3-hydroxy-2-methylpentanoyl)-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (13b). Prepared from **2a** (1.75 g, 5.11 mmol) and propionaldehyde as described above, to provide a diastereomeric mixture of **13b** as a red oil (2.44 g, 100%). This material was used without purification for the next step, because of its tendency to revert to starting material **2a**.

(±)-6,6-Dimethyl-9-hydroxy-10-(2,4-dimethyl-3-hydroxypentanoyl)-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (13c). Prepared from **2a** (4.00 g, 11.7 mmol) and isobutyraldehyde as described above, to give a diastereomeric mixture of **13c** as a red oil (5.40 g, 100%). This material was used without further purification for the next step, because of its tendency to revert to starting material **2a**.

(±)-6,6-Dimethyl-10-(2,3-dimethyl-3-hydroxybutanoyl)-9-hydroxy-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (13d). Prepared in quantitative yield from **2a** and acetone, as described above. The crude material was used without further purification for the next step. An analytical sample was obtained via silica gel column chromatography (EtOAc/hexane, 1:4) as an off-white solid: mp 99−102 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3 H, J = 7.3 Hz), 1.29 (s, 3 H), 1.32 (s, 3 H), 1.39 (d, 3 H, J = 6.8 Hz), 1.55 (s, 6 H), 1.67 (sextet, 2 H, J = 7.7 Hz), 2.91 (t, 2 H, J = 7.7 Hz), 3.52 (s, 1 H), 4.03 (q, 1 H, J = 6.8 Hz), 5.60 (d, 1 H, J = 9.9 Hz), 6.03 (s, 1 H), 6.73 (d, 1 H, J = 10.1 Hz), 13.81 (s, 1 H); FTIR (KBr) 3547, 3449, 1734 cm^{-1}; EIMS *m/e* 400 (M^+, 21), 385 (6), 327 (100). Anal. ($C_{23}H_{28}O_6$) C, H.

(±)-6,6-Dimethyl-10-(2,2-dimethyl-3-hydroxybutanoyl)-9-hydroxy-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (13e). Prepared from **2b** (1.25 g, 3.51 mmol) and acetaldehyde as described above. After workup, the crude product was obtained as a brown solid. The product was triturated with EtOAc/hexane, collected on a Büchner funnel, rinsed with fresh solvent, and air-dried to give the desired product as a white powder (654 mg, 46.6%). This material was used without further purification for the next step. An analytical sample was obtained via recrystallization from EtOAc/hexane (1:1): mp 190−191 °C; ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7.4 Hz), 1.25 (s, 3 H), 1.29 (d, 3 H, J = 6.4 Hz), 1.33 (s, 3 H), 1.48 (s, 3 H), 1.52 (s, 3 H), 1.66 (sextet, 2 H, J = 7.5 Hz), 2.39 (br s, 1 H), 2.88 (m, 2 H), 4.47 (q, 1 H, J = 6.4 Hz), 5.56 (d, 1 H, J = 10.0 Hz), 5.92 (s, 1 H), 6.64 (d, 1 H, J = 10.0 Hz), 8.99 (br s, 1 H); FTIR (KBr) 3246, 2967, 1686, 1572, 1364, 1132 cm^{-1}; EIMS *m/e* 400 (M^+, 1), 356 (37), 341 (100), 313 (68). Anal. ($C_{23}H_{28}O_6$) C, H.

General Procedure for Mitsunobu Cyclization of Aldol Addition Products (13). To a solution of aldol product **16** (1.0 equiv) and triphenylphosphine (1.5 equiv) in THF at 0 °C was slowly added diethyl azodicarboxylate (DEAD, 1.5 equiv) under N_2. A slightly exothermic reaction ensued. The solution was warmed to room temperature and stirred for 2.5 h. The solvent was evaporated *in vacuo* and the residue extracted with EtOAc, washed with H_2O and brine, and dried over magnesium sulfate. After filtration, the solution was concentrated *in vacuo* to provide the crude product.

(±)-10,11-Dihydro-4-propyl-6,6,11-trimethyl-2*H*,6*H*,-12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (15). Prepared from **13a** (513 mg, 1.38 mmol) as described above. The crude product was purified via silica gel column chromatography eluting with ether/hexane (3:1). The desired fractions were combined, concentrated under reduced pressure, and dried under high vacuum in the presence of P_2O_5 to afford **17** as an off-white solid (254 mg, 52.1%): mp 134−136 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7.2 Hz), 1.22 (d, 3 H, J = 6.9 Hz), 1.53 (s, 3 H), 1.55 (s, 3 H), 1.64 (m, 2 H), 2.86 (m, 3 H), 4.20 (t, 1 H, J = 10.8 Hz), 4.57 (dd, 1 H, J = 5.1, 11.4 Hz), 5.61 (d, 1 H, J = 10.2 Hz), 6.05 (s, 1 H), 6.63 (d, 1 H, J = 9.6 Hz); FTIR (KBr) 1741, 1557 cm^{-1}; EIMS *m/e* 354 (M^+, 30), 339 (100), 297 (15). Anal. ($C_{21}H_{22}O_5$) C, H.

Mitsunobu Cyclization of 13b: Synthesis of 16a and 17a. Prepared from **13b** (2.44 g, 5.11 mmol) as described above. The crude product was purified via column chromatography through silica gel, eluting with Et_2O/hexane (1:1). Pooling of appropriate fractions and evaporation provided **16a** (765 mg, 39.2%) and **17a** (350 mg, 17.9%), both as white solids.

(±)-10,11-*trans*-Dihydro-10-ethyl-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (16a): mp 155−158 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7.4 Hz), 1.13 (t, 3 H, J = 7.4 Hz), 1.22 (d, 3 H, J = 6.9 Hz), 1.53 (s, 3 H), 1.56 (s, 3 H), 1.64 (m, 2 H), 1.85 (m, 2 H), 2.62 (dq, 1 H, J = 7.0, 10.4 Hz), 2.88 (t, 2 H, J = 7.7 Hz), 4.14 (ddd, 1 H, J = 3.5, 7.8, 10.7 Hz), 5.61 (d, 1 H, J = 10.0 Hz), 6.04 (s, 1 H), 6.66 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 1738 cm^{-1}; EIMS *m/e* 382 (M^+, 37), 367 (100). Anal. ($C_{23}H_{26}O_5$) C, H.

(±)-10,11-*cis*-Dihydro-10-ethyl-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (17a): mp 100−102 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7.3 Hz), 1.07 (t, 3 H, J = 7.4 Hz), 1.14 (d, 3 H, J = 7.3 Hz), 1.54 (s, 3 H), 1.55 (s, 3 H), 1.65 (m, 2 H), 1.89 (m, 2 H), 2.70 (dq, 1 H, J = 3.2, 7.3 Hz), 2.88 (t, 2 H, J = 7.6 Hz), 4.39 (ddd, 1 H, J = 3.4, 5.3, 8.8 Hz), 5.60 (d, 1 H, J = 10.0 Hz), 6.05 (s,

1 H), 6.66 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 1732 cm^{-1}; EIMS *m/e* 382 (M^+, 55), 367 (100). Anal. ($C_{23}H_{26}O_5$) C, H.

Mitsunobu Cyclization of 13c: Synthesis of 16b and 17b. Prepared from **13c** (5.40 g, 11.7 mmol) as described above. The crude product was purified via silica gel column chromatography using EtOAc/hexane (1:4.5) as eluent. The desired fractions were combined, concentrated under reduced pressure, and dried under high vacuum in the presence of P_2O_5 to afford an off-white solid, a mixture of **16b** and **17b**. Repeated recrystallizations from EtOAc/hexane provided pure **16b** (782 mg, 13.1%). Column chromatography of the mother liquors provided pure samples of **17b** (964 mg, 16.2%).

(±)-10,11-*trans*-Dihydro-10-isopropyl-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (16b): mp 181−183 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 8.1 Hz), 1.04 (d, 3 H, J = 6.9 Hz), 1.16 (d, 3 H, J = 6.9 Hz), 1.21 (d, 3 H, J = 7.0 Hz), 1.53 (s, 3 H), 1.56 (s, 3 H), 1.64 (m, 2 H), 2.06 (m, 1 H), 2.72 (dq, 1 H, J = 7.0, 10.4 Hz), 2.88 (m, 2 H), 4.01 (dd, 1 H, J = 3.9, 10.4 Hz), 5.60 (d, 1 H, J = 10.0 Hz), 6.03 (s, 1 H), 6.64 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 1730, 1557, 1201, 1115 cm^{-1}; EIMS *m/e* 396 (M^+, 42), 381 (100), 297 (39). Anal. ($C_{24}H_{28}O_5$) C, H.

(±)-10,11-*cis*-Dihydro-10-isopropyl-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (17b): mp 156−158 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 3 H, J = 6.8 Hz), 1.04 (t, 3 H, J = 7.3 Hz), 1.15 (d, 3 H, J = 7.3 Hz), 1.21 (d, 3 H, J = 6.4 Hz), 1.55 (s, 3 H), 1.58 (s, 3 H), 1.66 (m, 2 H), 2.15 (m, 1 H), 2.71 (dq, 1 H, J = 2.3, 7.4 Hz), 2.90 (m, 2 H), 3.96 (dd, 1 H, J = 2.3, 9.9 Hz), 5.62 (d, 1 H, J = 10.0 Hz), 6.06 (s, 1 H), 6.68 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 1738 cm^{-1}; EIMS *m/e* 396 (M^+, 37), 381 (100). Anal. ($C_{24}H_{28}O_5$) C, H.

(±)-10,11-Dihydro-6,6,10,10,11-pentamethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (18). Prepared from **13d** (980 mg, 2.45 mmol) as described above. Purification by silica gel column chromatography (EtOAc/hexane, 1:10) provided, after overnight drying under high vacuum in the presence of P_2O_5, the desired product as an off-white solid (417 mg, 44.6%): mp 140−141 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3H, J = 7.3 Hz), 1.19 (d, 3 H, J = 7.0 Hz), 1.34 (s, 3 H), 1.53 (s, 6 H), 1.55 (s, 3 H), 1.65 (sextet, 2 H, J = 7.8 Hz), 2.79 (q, 1 H, J = 7.0 Hz), 2.88 (m, 2 H), 5.60 (d, 1 H, J = 10.1 Hz), 6.03 (s, 1 H), 6.65 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 1728 cm^{-1}; EIMS *m/e* 382 (M^+, 61), 367 (82), 297 (100). Anal. ($C_{23}H_{26}O_5$) C, H.

(±)-10,11-Dihydro-6,6,10,11,11-pentamethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (19). Prepared from **13e** (500 mg, 1.25 mmol) as described above. Column chromatography through silica gel (EtOAc/hexane, 1:2) provided the desired product as a white crystalline solid (449 mg, 94.0%). An analytical sample was obtained via recrystallization from EtOAc/hexane (2:1): mp 157 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7.3 Hz), 1.09 (s, 3 H), 1.19 (s, 3 H), 1.43 (d, 3 H, J = 6.5 Hz), 1.53 (s, 3 H), 1.55 (s, 3 H), 1.64 (m, 2 H), 2.88 (br t, 2 H, J = 7.7 Hz), 4.34 (q, 1 H, J = 6.4 Hz), 5.60 (d, 1 H, J = 10.0 Hz), 6.04 (s, 1 H), 6.66 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 2969, 1730, 1686, 1557, 1140 cm^{-1}; EIMS *m/e* 382 (M^+, 61), 367 (100), 312 (50), 297 (75), 269 (23). Anal. ($C_{23}H_{26}O_5$) C, H.

Attempted 11-Methylation of Ketone 5 with LDA/Iodomethane: 6,6-Dimethyl-9-hydroxy-10-*Z*-(2-methyl-2-butenoyl)-4-propyl-2*H*,6*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (20). To a solution of (±)-**5** (1.00 g, 2.72 mmol) in 25 mL of anhydrous THF at −78 °C under N_2 was added lithium diisopropylamide (Aldrich, 2.0 M, 1.50 mL, 2.99 mmol) dropwise via syringe, and the resulting bright orange solution was stirred for 30 min. Iodomethane (463 mg, 3.26 mmol), dissolved in 2 mL of THF, was then added dropwise via syringe, and the reaction mixture was then stirred for 3 h at −78 °C. The reaction was allowed to warm to room temperature and then quenched with 1 mL of H_2O, and the solvent was evaporated *in vacuo*. The residue was dissolved in 100 mL of EtOAc, washed sequentially with 100 mL each of 1 M HCl, 5% $NaHCO_3$, and saturated brine, and then dried over magnesium sulfate. The solution was filtered and evaporated to provide an orange oil. TLC analysis (silica gel, EtOAc/hexane, 1:1) showed a new product at R_f 0.60. Column chromatography through 80 g of silica gel (EtOAc/hexane, 1:2) provided the ring-opened product **20** as a pale-yellow waxy solid: ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3 H, J = 7.4 Hz), 1.54 (s, 6 H), 1.66 (m, 2 H), 1.84 (d, 3 H, J = 7.0 Hz), 1.98 (s, 3 H), 2.89 (t, 2 H, J = 7.7 Hz), 5.59 (d, 1 H, J = 10.0 Hz), 5.96 (s, 1 H), 6.17 (dq, 1 H, J = 1.4, 7.0 Hz), 6.72 (d, 1 H, J = 10.0 Hz), 12.03 (s, 1 H); FTIR (KBr) 1725, 1580 cm^{-1}; EIMS *m/e* 368 (M^+, 24), 353 (100), 325 (14). Anal. ($C_{22}H_{26}O_7$) C, H.

General Procedure for Reduction of Ketones 15−19. To a solution of the ketone (1.0 equiv) in absolute EtOH or a mixture of EtOH/THF (depending upon solubility) was added $NaBH_4$ (1.0 equiv) in several portions. The solution was stirred for 30 min and then quenched by the addition of saturated aqueous NH_4Cl. The solvent was evaporated *in vacuo* and the residue partitioned between EtOAc and 1 M HCl. The organic phase was separated and washed with 5% $NaHCO_3$ and saturated brine, dried over magnesium sulfate, and evaporated to provide the crude alcohols as epimeric mixtures. The epimers were separated using either column chromatography, preparative TLC, or preparative HPLC.

Reduction of 15: Synthesis of 21a and 21b. Prepared via reduction of **15** (189 mg, 0.53 mmol) as described above. The crude product was purified via silica gel column chromatography, eluting with EtOAc/hexane (1:3). The desired fractions were combined, concentrated, and dried under high vacuum in the presence of P_2O_5 to afford **21a** (86.3 mg, 45.4%) and **21b** (90.3 mg, 47.4%) as off-white foams.

(±)-11,12-*cis*-10,11-Dihydro-12-hydroxy-4-propyl-6,6,-11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (21a): ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7.4 Hz), 1.12 (d, 3 H, J = 6.9 Hz), 1.48 (s, 3 H), 1.49 (s, 3 H), 1.65 (m, 2 H), 2.09 (m, 1 H), 2.70 (br s, 1 H), 2.89 (dt, 2 H, J = 4.2, 7.7 Hz), 4.09 (m, 2 H), 5.01 (d, 1 H, J = 3.0 Hz), 5.54 (d, 1 H, J = 9.9 Hz), 5.95 (s, 1 H), 6.62 (d, 1 H, J = 9.9 Hz); FTIR (KBr) 3435, 1705 cm^{-1}; EIMS *m/e* 356 (M^+, 19), 341 (100). Anal. ($C_{21}H_{24}O_5$), C, H.

(±)-11,12-*trans*-10,11-Dihydro-12-hydroxy-4-propyl-6,6,-11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (21b): ^{1}H NMR ($CDCl_3$) δ 0.99 (d, 3 H, J = 7.5 Hz), 1.04 (t, 3 H, J = 7.4 Hz), 1.49 (s, 3 H), 1.50 (s, 3 H), 1.66 (m, 2 H), 2.18 (m, 1 H), 2.90 (dt, 2 H, J = 2.8, 7.4 Hz), 4.07 (dd, 1 H, J = 1.4, 3.2 Hz), 4.11 (dd, 1 H, J = 1.2, 3.3 Hz), 4.82 (dd, 1 H, J = 1.2, 3.0 Hz), 5.55 (d, 1 H, J = 9.9 Hz), 5.96 (s, 1 H), 6.63 (d, 1 H, J = 9.9 Hz); FTIR (KBr) 3420, 1726 cm^{-1}; EIMS *m/e* 356 (M^+, 23), 341 (100). Anal. ($C_{21}H_{24}O_5$) C, H.

Reduction of 16a: Synthesis of 22a and 22b. Prepared via reduction of **16a** (455 mg, 1.19 mmol) as described above. Column chromatography through silica gel (EtOAc/hexane, 1:4) provided the pure epimeric mixture of **22a** and **22b**, which was subsequently separated using preparative HPLC (EtOAc/hexane, 3:7), to provide **22a** (138 mg, 30.1%, white solid) and **22b** (46.5 mg, 10.1%, pale yellow oil).

(±)-10,11-*trans*,11,12-*trans*-10,11-Dihydro-10-ethyl-12-hydroxy-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (22a): mp 103−105 °C; ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7.3 Hz), 1.07 (t, 3 H, J = 7.4 Hz), 1.13 (d, 3 H, J = 6.9 Hz), 1.47 (s, 3 H), 1.51 (s, 3 H), 1.66 (sextet, 2 H, J = 7.6 Hz), 1.84 (m, 2 H), 2.05 (m, 1 H), 2.90 (m, 2 H), 3.53 (s, 1 H), 3.78 (dq, 1 H, J = 4.1, 8.1 Hz), 4.73 (d, 1 H, J = 6.7 Hz), 5.54 (d, 1 H, J = 10.0 Hz), 5.95 (s, 1 H), 6.63 (d, 1 H, J = 9.9 Hz); FTIR (KBr) 3493, 3432, 1699, 1586, 1128 cm^{-1}; EIMS *m/e* 384 (M^+, 31), 369 (100). Anal. ($C_{23}H_{28}O_5$) C, H.

(±)-10,11-*trans*,11,12-*cis*-10,11-Dihydro-10-ethyl-12-hydroxy-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (22b): ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7.3 Hz), 1.10 (t, 3 H, J = 7.6 Hz), 1.13 (d, 3 H, J = 6.8 Hz), 1.48 (s, 3 H), 1.49 (s, 3 H), 1.65 (sextet, 2 H, J = 7.4 Hz), 1.82 (m, 2 H), 1.90 (m, 1 H), 2.80−2.92 (m, 3 H), 4.10 (ddd, 1 H, J = 2.9, 7.9, 10.7 Hz), 4.98 (d, 1 H, J = 3.3 Hz), 5.54 (d, 1 H, J = 9.9 Hz), 5.94 (s, 1 H), 6.63 (d, 1 H, J = 9.9 Hz); FTIR (thin film) 3432, 1709 cm^{-1}; EIMS *m/e* 384 (M^+, 36), 369 (78), 337 (100). Anal. ($C_{23}H_{28}O_5$) C, H.

Reduction of 16b: Synthesis of 22c and 22d (1:2 Mixture). Prepared via reduction of **16b** (721 mg, 1.82 mmol)

as described above. The crude product was purified by silica gel column chromatography using EtOAc/hexane (1:1) as eluent. The desired fractions were concentrated *in vacuo* and dried under high vacuum to afford a yellow foam (620 mg, 85.6%), which contained a mixture of **22c** and **22d** (1:2) as determined by HPLC. Attempts to separate the epimers by preparative HPLC were unsuccessful; thus these analogues were characterized and assayed for antiviral activity as the 1:2 mixture.

(±)-10,11-*trans*,11,12-*trans*-10,11-Dihydro-12-hydroxy-10-isopropyl-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (22c) and (±)-10,11-*trans*,11,12-*cis*-10,11-Dihydro-12-hydroxy-10-isopropyl-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (22d): 2:1 epimeric mixture of **22c**:**22d**; EIMS *m*/*e* 398 (M^+, 44), 383 (100), 337 (53), 299 (30); FTIR (KBr) 3447, 1736, 1586 cm^{-1}. Anal. ($C_{24}H_{30}O_5$) C, H.

22c: ^1H NMR ($CDCl_3$) δ 1.00 (d, 3 H, J = 6.9 Hz), 1.03 (t, 3 H, J = 7.5 Hz), 1.11 (d, 3 H, J = 6.9 Hz), 1.12 (d, 3 H, J = 6.9 Hz), 1.47 (s, 3 H), 1.51 (s, 3 H), 1.64 (sextet, 2 H, J = 7.5 Hz), 1.88 (m, 1 H), 2.17 (m, 1 H), 2.90 (m, 2 H), 3.61 (dd, 1 H, J = 8.2, 5.1 Hz), 4.73 (d, 1 H, J = 6.9 Hz), 5.54 (d, 1 H, J = 10.0 Hz), 6.62 (d, 1 H, J = 10.0 Hz).

22d: ^1H NMR ($CDCl_3$) δ 0.95 (d, 3 H, J = 6.6 Hz), 1.03 (t, 3 H, J = 7.5 Hz), 1.13 (d, 3 H, J = 6.9 Hz), 1.20 (d, 3 H, J = 6.9 Hz), 1.49 (s, 6 H), 1.64 (sextet, 2 H, J = 7.5 Hz), 2.03 (m, 1 H), 2.17 (m, 1 H), 2.90 (m, 2 H), 4.06 (dd, 1 H, J = 11.0, 2.2 Hz), 5.01 (d, 1 H, J = 3.0 Hz), 5.53 (d, 1 H, J = 10.0 Hz), 5.94 (s, 1 H), 6.62 (d, 1 H, J = 10.0 Hz).

Reduction of 17a: Synthesis of 23a and 23b. Prepared via reduction of **17a** (290 mg, 0.76 mmol) as described above. The crude product was purified by silica gel preparative TLC (EtOAc/hexane, 2:1). The desired bands were scraped, combined, extracted, concentrated *in vacuo*, and dried under high vacuum in the presence of P_2O_5 to afford the desired products **23a** (229 mg, 95% pure by HPLC) and **23b** (55.9 mg, 92% pure by HPLC). The two compounds were further purified via preparative HPLC (normal phase, EtOAc/hexane, 3:7) to provide analytically pure **23a** (128 mg, 44%, yellow foam) and **23b** (7.1 mg, 2.4%, yellow oil).

(±)-10,11-*cis*,11,12-*cis*-10,11-Dihydro-10-ethyl-12-hydroxy-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (23a): ^1H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7.4 Hz), 1.04 (t, 3 H, J = 7.3 Hz), 1.12 (d, 3 H, J = 7.1 Hz), 1.49 (s, 3 H), 1.66 (sextet, 2 H, J = 7.3 Hz), 1.90 (m, 2 H), 2.34 (m, 1 H), 2.90 (m, 2 H), 3.30 (br s, 1 H), 4.06 (td, 1 H, J = 3.3, 10.1 Hz), 5.10 (d, 1 H, J = 5.2 Hz), 5.55 (d, 1 H, J = 10.0 Hz), 5.94 (s, 1 H), 6.63 (d, 1 H, J = 10.0 Hz); FTIR (KBr) 3449, 1734 cm^{-1}; EIMS *m*/*e* 384 (M^+, 27), 369 (100). Anal. ($C_{23}H_{28}O_5$) C, H.

(±)-10,11-*cis*,11,12-*trans*-10,11-Dihydro-10-ethyl-12-hydroxy-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (23b): ^1H NMR ($CDCl_3$) δ 0.79 (d, 3 H, J = 7.3 Hz), 1.04 (t, 3 H, J = 7.3 Hz), 1.11 (t, 3 H, J = 7.3 Hz), 1.49 (s, 3 H), 1.51 (s, 3 H), 1.60 (m, 1 H), 1.67 (m, 2 H), 1.92 (m, 2 H), 2.10 (tq, 1 H, J = 2.0, 7.3 Hz), 2.79 (br s, 1 H), 2.90 (m, 2 H), 4.23 (ddd, 1 H, J = 1.9, 5.4, 8.7 Hz), 4.87 (s, 1 H), 5.54 (d, 1 H, J = 10.0 Hz), 5.96 (s, 1 H), 6.66 (d, 1 H, J = 9.9 Hz); FTIR (thin film) 3410, 1732 cm^{-1}; EIMS *m*/*e* 384 (M^+, 26), 369 (100). Insufficient material for elemental analysis.

Reduction of 17b: Synthesis of 23c and 23d. Prepared via reduction of **17b** (874 mg, 2.20 mmol) as described above. The crude product was purified via silica gel column chromatography using ether/hexane (2:1) as eluent. The desired fractions were concentrated *in vacuo* and dried under high vacuum in the presence of P_2O_5 to afford a yellow oil (538 mg). The epimeric mixture was separated using preparative HPLC using EtOAc/hexane (1:4) as mobile phase. The desired fractions were combined, concentrated *in vacuo*, and dried under high vacuum in the presence of P_2O_5 to afford **23c** as a yellow foam (160 mg, 18.2%) and **23d** as a yellow foam (17.7 mg). The **23d** was only 60% pure by analytical HPLC, with the balance being **23c**. Because of the scarcity of this material, no further purification was attempted, and thus, this analogue was not evaluated for antiviral activity.

(±)-10,11-*cis*,11,12-*cis*-10,11-Dihydro-12-hydroxy-10-isopropyl-4-propyl-6,6,11-trimethyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (23c): ^1H NMR ($CDCl_3$) δ 0.96 (d, 3 H, J = 7.2 Hz), 0.97 (d, 3 H, J = 6.9 Hz), 1.03 (t, 3 H, J = 7.4 Hz), 1.15 (d, 3 H, J = 6.3 Hz), 1.45 (s, 3 H), 1.52 (s, 3 H), 1.65 (apparent sextet, 2 H, J = 7.6 Hz), 2.11 (m, 1 H), 2.52 (m, 1 H), 2.89 (m, 2 H), 3.53 (d, 1 H, J = 9.9 Hz), 3.91 (d, 1 H, J = 2.4 Hz), 5.25 (dd, 1 H, J = 2.4, 6.6 Hz), 5.53 (d, 1 H, J = 10.2 Hz), 5.94 (s, 1 H), 6.62 (d, 1 H, J = 10.2 Hz); FTIR (KBr) 3451, 1736 cm^{-1}; EIMS *m*/*e* 398 (M^+, 30), 383 (100). Anal. ($C_{24}H_{30}O_5$) C, H.

Reduction of 18: Synthesis of 24a and 24b. Prepared via reduction of **18** (290 mg, 0.75 mmol) as described above. Silica gel column chromatography (EtOAc/hexane, 1:5) of the crude product provided **24a** (100 mg, 34.3%) as an off-white foam. The epimer **24b** was purified via preparative TLC (silica gel, Et_2O/hexane, 2:1) to give 56.0 mg (19.2%) as an off-white foam.

(±)-11,12-*cis*-10,11-Dihydro-12-hydroxy-6,6,10,10,11-pentamethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]-tripyran-2-one (24a): mp 44−45 °C; ^1H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7.3 Hz), 1.24 (d, 3 H, J = 7.1 Hz), 1.38 (s, 3 H), 1.45 (s, 3 H), 1.47 (s, 3 H), 1.51 (s, 3 H), 1.66 (m, 2 H), 2.04 (m, 1 H), 2.89 (m, 2 H), 3.02 (d, 1 H, J = 4.0 Hz), 4.94 (dd, 1 H, J = 4.0, 4.2 Hz), 5.53 (d, 1 H, J = 10.0 Hz), 5.94 (s, 1 H), 6.65 (d, 1 H, J = 9.9 Hz); FTIR (KBr) 3451, 1709 cm^{-1}; EIMS *m*/*e* 384 (M^+, 61.8), 369 (71) 299 (100). Anal. ($C_{23}H_{28}O_5$) C, H.

(±)-11,12-*trans*-10,11-Dihydro-12-hydroxy-6,6,10,10,11-pentamethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (24b): mp 40−42 °C; ^1H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7.3 Hz), 1.13 (d, 3 H, J = 7.0 Hz), 1.21 (s, 3 H), 1.46 (s, 3 H), 1.48 (s, 3 H), 1.52 (s, 3 H), 1.66 (m, 2 H), 2.03 (dq, 1 H, J = 7.1, 7.2 Hz), 2.90 (m, 2 H), 3.66 (s, 1 H), 4.69 (d, 1 H, J = 7.4 Hz), 5.54 (d, 1 H, J = 10.0 Hz), 5.94 (s, 1 H), 6.63 (d, 1 H, J = 9.9 Hz); FTIR (KBr) 3437, 1734 cm^{-1}; EIMS *m*/*e* 384 (M^+, 36), 369 (66) 299 (100). Anal. ($C_{23}H_{28}O_5$) C, H.

Reduction of 19: Synthesis of 25a and 25b. Prepared via reduction of **19** (252 mg, 0.66 mmol) as described above. Analysis of the crude reduction product via TLC (silica gel, EtOAc/hexane, 1:2) showed the two epimeric alcohols **25a** and **25b** at R_f 0.30 and 0.25, as well as a minor impurity at R_f 0.55. Separation via column chromatography (silica gel, EtOAc/hexane, 1:2) provided the R_f 0.30 component (**25a**, 128 mg, 50.3%, white foam) and the R_f 0.25 component (**25b**, 19 mg, 7.4% off-white waxy solid).

(±)-10,12-*cis*-10,11-Dihydro-12-hydroxy-6,6,10,11,11-pentamethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]-tripyran-2-one (25a): mp 52−55 °C; ^1H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7.3 Hz), 1.06 (s, 6 H), 1.40 (d, 3 H, J = 6.7 Hz), 1.47 (s, 3 H), 1.50 (s, 3 H), 1.66 (sextet, 2 H, J = 7.3 Hz), 2.89 (m, 2 H), 3.39 (d, 1 H, J = 3.2 Hz), 3.99 (q, 1 H, J = 6.7 Hz), 4.70 (d, 1 H, J = 3.2 Hz), 5.54 (d, 1 H, J = 9.9 Hz), 5.94 (s, 1 H), 6.63 (d, 1 H, J = 9.9 Hz); FTIR (KBr) 3432, 2969, 1734, 1584, 1377, 1130 cm^{-1}; EIMS *m*/*e* 384 (M^+, 59), 369 (100), 351 (13), 314 (45), 299 (89), 271 (24). Anal. ($C_{23}H_{28}O_5$) C, H.

(±)-10,12-*cis*-10,11-Dihydro-12-hydroxy-6,6,10,11,11-pentamethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]-tripyran-2-one (25b): mp 142 °C; ^1H NMR ($CDCl_3$) δ 0.78 (s, 3 H), 1.04 (t, 3 H, J = 7.3 Hz), 1.11 (s, 3 H), 1.36 (d, 3 H, J = 6.5 Hz), 1.49 (s, 6 H), 1.64 (m, 2 H), 2.89 (m, 2 H), 4.35 (q, 1 H, J = 6.5 Hz), 4.63 (br s, 1 H), 5.54 (d, 1 H, J = 9.8 Hz), 5.96 (s, 1 H), 6.65 (d, 1 H, J = 9.8 Hz); EIMS *m*/*e* 384 (M^+, 41), 369 (100), 351 (11), 314 (14), 299 (48), 271 (10). Anal. ($C_{23}H_{28}O_5$) C, H.

10,11-Dihydro-6,6,10,10-tetramethyl-4-propyl-2*H*,6*H*,-12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2,12-dione (27). A solution of 3,3-dimethylacryloyl chloride (9.0 g, 75 mmol) in CS_2 (6 mL) was added to a suspension of **2a** (15.0 g, 68.2 mmol) and $AlCl_3$ (38.7 g, 286 mmol) ikn CS_2 (150 mL), and the resulting mixture was stirred at room temperature for 30 min. Nitrobenzene (60 mL) was then added, and the mixture was heated to 75 °C. After 14 h, reaction was complete, and the mixture was poured into a mixture of ice (150 g) and 1 M HCl (600 mL). Nitrobenzene was removed by steam distillation, and the residue was extracted with EtOAc (3 × 300 mL). The extracts were combined and dried over Na_2SO_4. The crude

product obtained by evaporation under vacuum was purified using silica gel chromatography with a mobile phase of EtOAc/hexane (1:3 to 1:1) to provide, after recrystallization from EtOAc, white crystals of 2,2-dimethyl-9-hydroxy-8-propyl-4*H*,6*H*-benzo[1,2-*b*:1,2-*b*′]dipyran-4,6-dione (8.0 g, 39%): mp 280−282 °C; ^{1}H NMR (CD_3OD) δ 1.01 (t, 3 H, J = 7.4 Hz), 1.46 (s, 6 H), 1.65 (sextet, 2 H, J = 7.5 Hz), 2.73 (s, 2 H), 2.95 (t, 2 H, J = 7.5 Hz), 6.02 (s 1 H), 6.26 (s, 1 H); EIMS *m*/*e* 302 (M^+, 79), 287 (100), 259 (38), 247 (64), 246 (93), 218 (38), 203 (26), 190 (28); FTIR (KBr) 1734, 1650, 1611, 1553 cm^{-1}. Anal. ($C_{17}H_{18}O_5$) C, H.

The above product (3.0 g, 9.9 mmol) was reacted with 1,1-dimethoxy-3-methylbutan-3-ol (1.46 g, 9.9 mmol) in pyridine (15 mL) for 72 h, as described above for the preparation of **2b**. Purification of the crude product using silica gel column chromatography (EtOAc/hexane, 1:3), followed by recrystallization from EtOAc, furnished 2.0 g (55%) of **27** as white crystals: mp 159−160 °C; ^{1}H NMR (acetone-d_6) δ 1.06 (t, 3 H, J = 7.3 Hz), 1.50 (s, 6 H), 1.55 (s, 6 H), 1.68 (m, 2 H), 2.77 (s, 2 H), 2.94 (t, 2 H, J = 7.7 Hz), 5.73 (d, 1 H, J = 10.2 Hz), 6.06 (s, 1 H), 6.67 (d, 1 H, J = 10.1 Hz); EIMS *m*/*e* 368 (M^+, 56), 353 (100), 297 (80); FTIR (KBr) 1730, 1688 cm^{-1}. Anal. ($C_{22}H_{24}O_5$) C, H.

(±)-10,11-Dihydro-12-hydroxy-6,6,10,10-tetramethyl-4-propyl-2*H*,6*H*,12*H*-benzo[1,2-*b*:3,4-*b*′:5,6-*b*″]tripyran-2-one (28). Reduction of ketone **27** (300 mg, 8.1 mmol) was performed as described above in the general procedure for reduction of ketones. The crude product was purified using silica gel column chromatography (EtOAc/hexane, 1:3) to provide **28** as white crystals: mp 140−141 °C; ^{1}H NMR (acetone-d_6) δ 1.04 (t, 3 H, J = 7.4 Hz), 1.42 (s, 3 H), 1.48 (s, 3 H), 1.49 (s, 3 H), 1.50 (s, 3 H), 1.67 (sextet, 2 H, J = 7.4 Hz), 2.10 (m, 2 H), 2.81−2.98 (m, 2 H), 3.21 (d, 1 H, J = 2.6 Hz), 5.16 (m, 1 H), 5.54 (d, 1 H, J = 9.9 Hz), 5.95 (s, 1 H), 6.65 (d, 1 H, J = 9.9 Hz); EIMS *m*/*e* 370 (M^+, 46), 355 (100), 337 (39), 299 (88); FTIR (KBr) 3453, 1705, 1647, 1586 cm^{-1}. Anal. ($C_{22}H_{26}O_5$) C, H.

Cytoprotection Assay. Anti-HIV cytoprotection assays were conducted as previously described.[14] CEM-SS cells used for the assay 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. Cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere in air. Appropriate concentrations of test compounds were prepared in DMSO, diluted with culture medium to the desired concentrations, and then added in the amount of 100 μL to each test well. Compounds were tested in triplicate wells per dilution with infected cells and in duplicate wells per concentration for uninfected cells.

Infected cells were prepared by charging with HIV-1 to yield an approximate multiplicity of infection (MOI) of 0.12 $TCID_{50}$/cell 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 discarded. Medium was added to the cells to attain a density of 10^5 cells/mL. The infected cells, as well as uninfected cells, were added to a 96-well microtiter plate in the amount of 100 μL/well to give a cell number of 10^4 cells/well. After being incubated for 6 days, the cell viability was measured via addition of the tetrazolium dye, XTT (5 mg/mL), to the test plates, as previously described. Cytoprotection and toxicity are reported as the concentration of drug required to inhibit viral-mediated cytopathicity by 50% of infected controls (EC_{50}) and to cause reduction of cell growth by 50% of uninfected controls (IC_{50}), respectively.

Reverse Transcriptase Assay. Inhibition of HIV-1 RT was evaluated as previously described,[32] using a poly(rC):oligo-$(dG)_{12-18}$ template:primer system.

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