Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 11, pp. 3315-3326, 1996

0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00433-8

Synthesis Of (+)-Calanolide A, An Anti-HIV Agent, *Via* Enzyme-Catalyzed Resolution Of The Aldol Products

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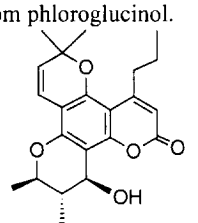
Abstract: The synthesis of (+)-calanolide A (**1**), an anti-HIV-1 agent, is described. A $TiCl_4$-mediated aldol reaction of compound **2** stereoselectively produced the desired *syn* diastereomer (±)-**5**, which was resolved by a lipase-catalyzed acylation reaction. Under Mitsunobu conditions (Ph_3P/DEAD), the *syn* aldol product (+)-**5** led to the formation of *trans*-2,3-dimethyl chroman-4-one [(+)-**3**] with 94% *ee*, while the *anti* aldol product (+)-**6** yielded both *trans* and *cis* derivatives (+)-**3** and (+)-**4** with 60% and 68% *ee*, respectively. Luche reduction on (+)-**3** led to (+)-**1** and (+)-calanolide B in a ratio of 9 : 1.

Introduction

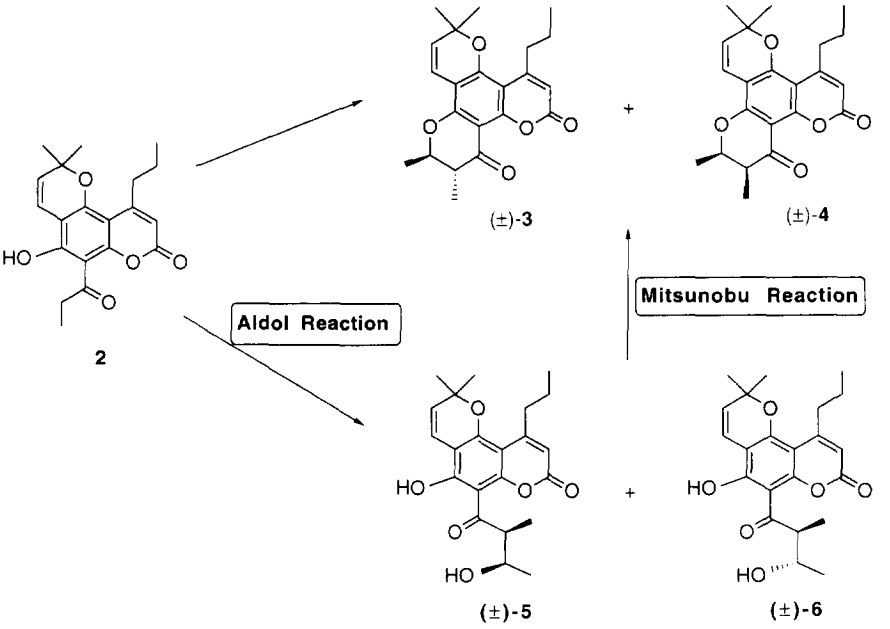
Recently, we have reported an approach for total synthesis of (±)-calanolide A (**1**),[1,2] the (+)-enantiomer of which, isolated from several tropical plants of the genus *Calophyllum*,[3] has been identified as an HIV-1-specific nonnucleoside inhibitor.[2,3] In the approach for our synthesis,[1,2] the key intermediate was 6,6-dimethyl-9-hydroxy-10-propionyl-4-propyl-2*H*,6*H*-benzo[1,2-b:3,4-b']dipyran-2-one (**2**, **Scheme 1**), which was synthesized in a 3-step process starting from phloroglucinol.



1, Calanolide A

Originally, the chromeno-coumarin **2** was cyclized in a one-step process[1,2] by treatment with acetaldehyde diethyl acetal or paraldehyde in the presence of trifluoroacetic acid and pyridine or PPTS, affording both *trans* and *cis* chromanones **3** and **4** (**Scheme 1**). It was later found that lithium-mediated aldol reaction on **2** with acetaldehyde yielded both *syn* and *anti* β-hydroxy ketones (**5** and **6**).[4] The *syn* diastereomer (±)-**5** was then cyclized by Mitsunobu reaction, selectively leading to the formation of the racemic *trans*-chromanone (±)-**3**,[4] which yielded (±)-calanolide A after reduction.

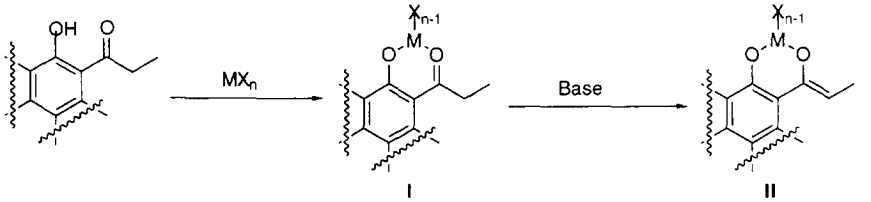
In our continuing efforts to develop (+)-calanolide A [(+)-**1**], it has been of interest to investigate methods for the highly selective synthesis of the *syn* diastereomer of the aldol products, (±)-**5**, which may be resolved into its enantiomers. The desired enantiomer of **5** can be cyclized to produce the enantiopure *trans*-chromanone **3** with the desired configurations of the two methyl groups, which, upon reduction, will lead to (+)-calanolide A.



Scheme 1

The aldol reaction is a basic method for C-C bond formation.[5,6] It has been reported very recently that complexes of β-hydroxy ketones and a Lewis acid such as titanium and a boron compound can be enolized and treated with aldehydes to afford aldol products in good to excellent yields with high regio- and diastereoselectivity.[7] For example, the boron-mediated reactions led to exclusively *anti* aldol products at the *proximal*[8] position while the titanium-mediated reactions produced primarily *syn* aldol products at the *distal*[8] position. In contrast, when enolized with two equivalents of a lithium base such as LDA at low temperature in the absence of either titanium or a boron compound, the β-hydroxy ketone led to both *proximal* and *distal*,[8] as well as *anti* and *syn* aldol products.[9] Moreover, by formation of the complexes, the β-hydroxy group required no additional protection.

Chromeno-coumarin **2** can be considered to be a β-hydroxy ketone, but it does not present a regioselectivity problem because its *proximal* position is part of the benzene ring, thus leaving the *distal* position as the only enolizable position (**Scheme 2**). It is therefore anticipated that the transition metal enolates (**II**) with a six-membered chelation ring and ligands attached to the metal would induce a certain diastereomeric differentiation, thus achieving a preference for the *syn* aldol products with high *de* value.



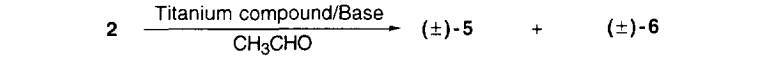
Scheme 2

Herein we wish to report our results on a titanium-mediated aldol reaction and the subsequent enzyme-catalyzed resolution which finally led to the synthesis of (+)-calanolide A (**1**). Although a chiral synthesis of (+)-**1** has been recently published,[10] the approach reported here may have certain advantages over the literature method[10] concerning the scale-up.

Results and Discussion

Titanium-Mediated Aldol Reaction: The reagent $TiCl_4$ was chosen in order to evaluate the feasibility of conducting the aldol reaction and to determine, if any, the stereoselectivity. The reaction was carried out by following a literature procedure.[7] Thus, $TiCl_4$ (1 equivalent) was added to a solution of **2** in CH_2Cl_2 at -78 °C. The resultingyellow solution was warmed to 0 °C to insure complete complex formation. Treatment of the complex with 2.1 equivalents of $(i\text{-Pr})_2NEt$ at -78 °C led to a deep red solution, which was stirred at the same temperature for 30 min. Acetaldehyde (6 equivalents) was then added rapidly and the reaction mixture was stirred at -78 °C for 4 h and then quenched by the slow addition of saturated NH_4Cl at the same temperature. Diastereomeric β-hydroxy ketones **5** and **6** were isolated by flash chromatography in a combined 32% yield and in a ratio of 95:5, as demonstrated by chiral HPLC (entry 1, **Table 1**). The unreacted compound **2** was recovered. When the reaction mixture, after addition of acetaldehyde, was allowed to warm to ambient temperature, only a retro-aldol reaction occurred, leading to recovery of the starting compound **2**. These results indicated that favorable stereoselectivity was achieved at low temperature, but that the conversion was somewhat low. It is worthwhile to note that, although the ratios of **5** and **6** can be determined by ^1H NMR,[4] a chiral HPLC approach has provided a more convenient method for conducting this assay.

Table 1. Ti-Mediated Aldol Reaction of 2 with Acetaldehyde



Entry	Ti Compound (equiv)	Base	Ratio of **5/6**[a]	Isolated Yield (%)
1	$TiCl_4$ (1.1)	$(i\text{-Pr})_2NEt$	95:5	32
2	$TiCl_4$ (1.1)	LDA	77:23	64
3	$TiCl_4$ (2.0)	LDA	83:17	68
4	$TiCl_4$ (3.0)	LDA	93:7	61
5	$TiCl_4$ (3.0)	*sec*-BuLi	-----	0
6	$TiCl_4$ (3.0)	*sec*-BuLi[b]	-----	0
7	$(i\text{-PrO})_3TiCl$ (3.0)	$(i\text{-Pr})_2NEt$	-----	0
8	$(i\text{-PrO})_3TiCl$ (3.0)	LDA	96:4	11
9	$(i\text{-PrO})_3TiCl$ (3.0)	LDA[c]	96:4	38
10	$(i\text{-PrO})_3TiCl$ (1.0)	LDA	71:29	26
11	$(i\text{-PrO})_4Ti$ (3.0)	LDA	------	0
12	$(i\text{-PrO})_4Ti$ (3.0)	LDA[c]	66:34	13

[a] Determined by chiral HPLC. [b] In THF, instead of CH_2Cl_2. [c] Titanium compound was added before LDA.

In order to increase the degree of conversion, a base stronger than $(i\text{-Pr})_2$NEt may be necessary. It was for this reason that LDA was investigated. Thus, LDA (2.2 equivalents) in THF was added to a solution of **2** in CH_2Cl_2 at -78 °C, followed by addition of $TiCl_4$ (1 equivalent). The resulting yellow solution was warmed to -40 °C and stirred for 45 min. Treatment of the titanium enolate with acetaldehyde at -78 °C afforded a diastereomeric mixture of **5** and **6** in a combined 64% yield; however, the ratio was decreased to 77:23 as determined by HPLC analysis (entry 2, **Table 1**). It was previously reported[11] that stereoselectivity of aldol reactions was affected by varying the number of equivalents of titanium compound used, and $\geq$2 equivalents of titanium was required to achieve a high level of stereoselection. We found that this was also true for compound **2**. Modifying the amount of $TiCl_4$ used from 1 equivalent to 2 equivalents led to an increase in **5**:**6** ratio (83:17); 3.0 equivalents of $TiCl_4$ yielded the highest ratio of **5**:**6** (93:7). These results are summarized in **Table 1** (Entries 3 and 4). Replacement of LDA by *sec*-BuLi was found to be detrimental to the reaction (Entries 5 and 6, **Table 1**). It should be noted that pure **5** could be obtained by carefully collecting the fractions from silica gel column chromatography. However, both retro-aldol reaction and non-selective cyclization of the aldol products (**5** and **6**) to chromanones **3** and **4** were observed during a more sophisticated chromatography, in an attempt to completely separate **5** and **6**. On gram-scale reactions, the purified **5** was utilized throughout this study.

Adding $TiCl_4$ (3 equiv) before LDA produced similar results. This procedure, however, might be more suitable for scale-up because of simplification of temperature control. Thus, $TiCl_4$ (3 equivalents) was added to a solution of **2** in CH_2Cl_2 at -40 °C. After being stirred for 30 min., the mixture was cooled to -78 °C and LDA (2.2 equivalents) was introduced, followed by treatment with acetaldehyde (see Experimental Section for details). Although the quenching process does not affect the ratio of **5**:**6**, it could lower the isolated yield due to the retro-aldol reaction. It is therefore recommended that the reaction mixture be poured into cold NH_4Cl (0 °C).

Other titanium compounds such as $(i\text{-PrO})_3$TiCl and $(i\text{-PrO})_4$Ti were also investigated for the aldol reaction of **2** with acetaldehyde. Once again, the number of equivalents of titanium compound used was found to be critical for stereoselection (Entry 8 vs. 10, **Table 1**). In contrast to the $TiCl_4$ reaction, the order of addition of $(i\text{-PrO})_3$TiCl or $(i\text{-PrO})_4$Ti and LDA influenced the reaction yield (Entry 8 vs. 9 and Entry 11 vs. 12, **Table 1**). Although the use of $(i\text{-PrO})_3$TiCl maintained the same level of stereoselectivity as $TiCl_4$, the yield of the reaction was significantly lower. Both stereoselection and yield were reduced when $(i\text{-PrO})_4$Ti was used in the reaction (**Table 1**).

Titanium, being able to form hexa- and even octacoordinate complexes, is generally expected to provide both chelation-controlled and stereocontrolled aldol products.[11,12] Zimmerman-Traxler's pericyclic model has been widely accepted for correlating metal enolate geometry and the relative stereochemistry of aldol adducts.[13,14] Thus, in the transition structure, the enolate and the reacting aldehyde are coordinated in a chair conformation, with *Z* enolates, in general, favoring the formation of *syn* aldols and *E* enolates favoring the formation of *anti* aldols. Accordingly, one could speculate that the titanium enolates with *Z* configuration, derived from **2** and $TiCl_4$ or $(i\text{-PrO})_3$TiCl, may be predominantely formed and maintained up to addition of the aldehyde.

Enzyme-Catalyzed Resolution of the Aldol Products: Hydrolytic enzymes such as esterases and lipases, due to their commercial availability, relatively low cost, broad substrate specificity, and the fact that no cofactors are required for their action, have been widely used for the resolution of racemic alcohols and

carboxylic acids through asymmetric hydrolysis or acylation.[15,16] However, attempts to directly resolve (±)-**1** by lipase-catalyzed acylation or hydrolysis met with only limited success.[2] It is possible that the hydroxyl group in (±)-**1** might be so sterically hindered that it does not fit into the catalytic pocket of hydrolytic enzymes. It was expected that the hydroxyl group of the β-hydroxy ketone, (±)-**5**, could freely rotate and fit into the catalytic pocket of hydrolytic enzymes so that it could be enantioselectively acylated under enzymatic conditions.

A number of lipases, such as lipases PP, PS-13, PS-30, AY-30, AK, AP-12, FAP-15, CC-7, and N, as well as porcine liver esterase (PLE), were tested in the acylation of (±)-**5** (**Scheme 3**). Among the enzymes investigated (**Table 2**), PS-13, PS-30, and AK were found to catalyze, in the presence of vinyl acetate and molecular sieves, the acetylation of (±)-**5** in good yields (entries 4, 5, and 10), while the other enzymes did not exhibit activity for acetylation of (±)-**5** to a significant extent. It is interesting to note that the recovered **5** in entries 4 and 5 (**Table 2**) was almost enantiomerically pure. These results indicated that three enzymes (PS-13, PS-30 and AK) exhibited significant enantioselectivity. Since baseline separation was not achieved between the two enantiomers of **5** in the chiral HPLC, it was difficult to determine the *ee* values for unreacted **5**. However, the two esters which were formed, (+)- and (-)-**7** from (+)- and (-)-**5** respectively, demonstrated baseline separation in the HPLC, and the *ee* values for **7** could be easily determined, as presented in **Table 2**.

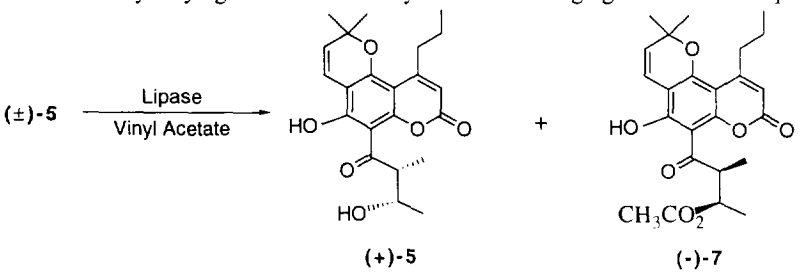
Table 2. Acetylation of (±)-5 with Vinyl Acetate Catalyzed by Lipases[a]

Entry	Lipase	Unreacted **5** (%)	Ester (-)-**7** (%)
1	no enzyme	99	1
2	CC-7	97	3
3	PP	100	0
4	PS-30	11.8	88.2 (14)[b]
5	AK	40.4	59.6 (72)[b]
6	AP-12	90	10
7	N	100	0
8	AY-30	100	0
9	FAP-15	96	4
10	PS-13	0	100 (0)[b]
11	PLE	100	0

[a] The experiments were carried out as follows: To a stirred solution of *tert*-butyl methyl ether (0.52 mL) and vinyl acetate (0.17 mL) were added (±)-**5** (20 mg), lipase (52 mg) and molecular sieves (20 mg). The mixture was stirred at ambient temperature for 3.5 days, whereupon it was filtered through celite. The crude product was then analyzed by chiral HPLC. [b] Number in the parenthesis was *ee* value as determined by chiral HPLC.

Then acetylation of (±)-**5** catalyzed by PS-13, PS-30 and AK was further monitored by chiral HPLC over the course of the reaction (**Table 3**). Vinyl acetate selectively acylated (-)-**5**[17] into the corresponding ester **7**, and the desired enantiomer (+)-**5**[17,18] was unreacted in the presence of these enzymes (**Scheme 3**). However, the enantioselectivity varied with enzymes, with lipase AK yielding the highest level of enantioselectivity,

followed by PS-30 and PS-13; the order of catalytic activity was completely reversed (**Table 3**). The general theory of enzyme-catalyzed kinetic resolutions suggests that the optical purity of the ester formed decreases and that of the remaining alcohol increases upon an increase in the degree of conversion. In order to obtain the desired enantiomer (+)-**5** with a higher level of enantiopurity, therefore, it was helpful to allow the reaction to exceed 50% conversion at the expense of (+)-**5**. Also, it was found that the reaction rate and the optical purity of (+)-**5** could be attenuated by varying the amount of enzyme used or changing the reaction temperature.



Scheme 3

Thus, on a gram-scale reaction, the (±)-**5** was treated with vinyl acetate in the presence of lipase PS-30 at ambient temperature. The reaction was terminated after 4 days to yield (+)-**5** (37% yield) and (-)-**7** (57% yield with 52% *ee*), respectively, after purification by silica gel column chromatography. On a kilogram-scale reaction, lipase PS-30 was changed to lipase AK and the amount of enzyme used was doubled. The reaction temperature was also increased to 40 °C. After such modifications, the reaction was accelerated significantly without sacrificing the yields and optical purities of the products (see the Experimental Section for details).

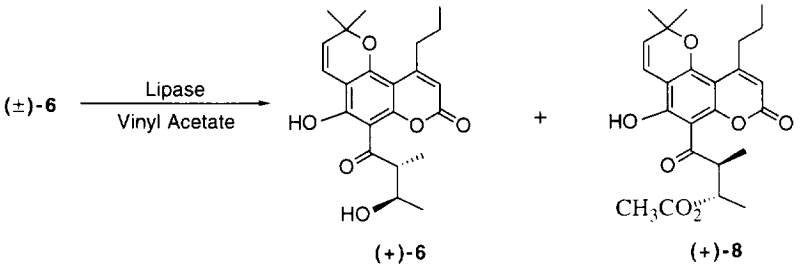
Table 3. Enantioselective Acetylation of (±)-5 with Vinyl Acetate Catalyzed by Lipases[a,b]

Entry	Lipase	17 h		40 h		65 h	
		5 (%)	(-)-**7** (%)	**5** (%)	(-)-**7** (%)	**5** (%)	(-)-**7** (%)
1	AK	83.7	16.3 (92.6)	68.6	31.2 (90.6)	58.4	41.6 (91.3)
2	PS-30	46.4	53.6 (75.6)	37.9	62.1 (61.4)	35.3	64.7 (53.2)
3	PS-13	57.0	43.0 (27.0)	14.2	85.8 (0.4)	0	100 (0)

[a] The experiments were carried out as follows: To a stirred solution of *tert*-butyl methyl ether (1.0 mL) and vinyl acetate (0.25 mL) were added (±)-**5** (40 mg), lipase (100 mg) and molecular sieves. The mixture was stirred at ambient temperature. An aliquot was taken at the time indicated in the table, filtered through celite, and analyzed by chiral HPLC. [b] Number in the parenthesis was *ee* value as determined by chiral HPLC.

The racemic *anti* aldol product (±)-**6**,[4] prepared by lithium-mediated aldol reaction and purified by silica gel column chromatography,[4] was also resolved by lipase-catalyzed acylation into (+)-**6** and the ester **8** from (-)-**6** (**Scheme 4**), which were separated in 46% and 50% yields, respectively, with >99% *ee*. In contrast to

(±)-**5**, the acetylation reaction of (±)-**6** proceeded with excellent selectivity, and appeared to cease upon consumption of (-)-**6**, with no apparent acylation of (+)-**6**.

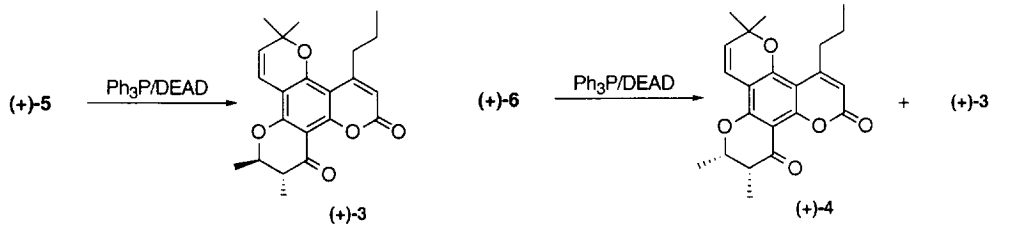


Scheme 4

Attempts to hydrolyze the esters, (-)-**7** and (+)-**8**, by either chemical or enzymatic reactions failed to afford (-)-**5** and (-)-**6**. For example, when (-)-**7** was treated with phosphate buffer (pH=7.5) in the presence of lipase AK and PS-30 at ambient temperature, only racemic chromanones, **3** and **4** (**Scheme 1**), were formed. Also, transesterification of (-)-**7** with EtOH catalyzed by lipase PS-30 yielded **3** and **4** as the only detectable products.

Mitsunobu Cyclization of the Aldol Products into Chromanones: The Mitsunobu reaction, employing a mixture of triphenylphosphine and a dialkyl azodicarboxylate, is a very useful reaction in the refunctionalization of alcohols and, in particular, inversion of this moiety.[19-21] We have recently applied the Mitsunobu reaction to the synthesis of racemic chromanones **3** and **4** from (±)-**5** and (±)-**6** (**Scheme 1**).[4] It was found that (±)-**5** selectively led to the formation of the *trans*-chromanone (±)-**3** after the Mitsunobu reaction, using Ph_3P and DEAD.[4] However, (±)-**6**, under the same conditions, resulted in a mixture of both *trans* and *cis* chromanones **3** and **4** (**Scheme 1**).

The enantiopure (+)-**5** obtained by enzymatic resolution was subjected to Mitsunobu reaction (Ph_3P and DEAD). The *trans* chromanone (+)-**3** was isolated in 63% yield and the optical purity was determined to be 94% *ee* by chiral HPLC (**Scheme 5**). By contrast, Mitsunobu reaction on (+)-**6** led to formation of both *trans* and *cis* chromanones (+)-**3** and (+)-**4** with the latter being the major product, the optical purities of which were 60% and 68% *ee*, respectively. These results are consistent with that obtained from the racemates[4] and demonstrate, once again, that Mitsunobu reaction of *syn* aldol product (+)-**5** proceeds by an S_N2 mechanism with inversion of the alcoholic center, while, for the *anti* aldol product (+)-**6**, S_N2, S_N1 and dehydration followed by Michael addition may be involved.



Scheme 5

Reduction of (+)-3 into (+)-Calanolide A: A low-temperature (-30 °C) Luche reduction on (+)-**3** led to the formation of (+)-calanolide A, (+)-**1**, which contained 10% of (+)-calanolide B. It should be noted that addition of triphenylphosphine oxide enhances the solubility of (+)-**3** in EtOH at -30 °C without affecting the stereoselection. The desired compound, (+)-calanolide A [(+)-**1**], was further separated from (+)-calanolide B by normal phase HPLC and was identical with an authentic sample. In addition, (+)-**1** thus synthesized was tested for anti-HIV avtivity. In the *in vitro* XTT assay (CEM-SS cells infected with HIV_{RT} strain),[2] the EC_{50} and IC_{50} were determined to be 0.16 and 18.6 μM. The values for the natural product were reported to be 0.1 and 22 μM.[3]

Stereochemistry: We did not conduct any further studies in determining the absolute stereochemistries of the aldol products (**5**, **6**) and the chromanones (**3**, **4**) at present. However, the absolute configuration of (+)-calanolide A (**1**) has been determined to be [10*R*, 11*R*, 12*S*].[3] Based on the chemistries described above, it is therefore reasonable to believe that chromanone (+)-**3** and the aldol product (+)-**5** should be [10*R*, 11*R*], and [2'*R*, 3'*S*], respectively, while the ester (-)-**7** has the configuration [2'*S*, 3'*R*]. In addition, since both (+)-**3** and (+)-**4** were generated from the Mitsunobu reaction of (+)-**6**, it is assumed that the absolute configuration of C11 in (+)-**4** is 11*R* , the same as that in (+)-**3**, while C10 of (+)-**4** is 10*S*, opposite to that in (+)-**3**. Accordingly, (+)-**6** and (+)-**8** should possess the configurations [2'*R*, 3'*R*] and [2'*S*, 3'*S*].

Experimental

General. Melting points were taken on a Laboratory Devices Mel-Temp apparatus and are corrected. TLC analyses were performed on analytical thin layer plates coated with silica gel 60 F_{254} (Merck) and components were visualized under UV light and/or stained with iodine. Column chromatography was performed using silica gel 60 (70-230 mesh from EM Science). NMR spectra were recorded on a 300 MHz Varian Gemini 2000 NMR spectrometer. Chemical shifts are reported in parts per million (δ ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. IR spectra were recorded on a Midac M series FT-IR. Mass spectra were carried out on a Finnegan MAT 90 mass spectrometer. UV spectra were measured in MeOH or aqueous solutions on a Hitachi U-2000 UV-VIS spectrophotometer. Elemental analyses were carried out at Midwest Microlab, Indianapolis, Indiana.

An isocratic liquid chromatograph (Hitachi Instruments, Inc., Naperville, IL, USA) was used for HPLC which consisted of a L-6200A pump, a L-4000H/L-4200H UV/UV-Vis detector and a D-2500 chromato-integrator. The analytical normal phase silica gel HPLC column was 250 mm x 4.6 mm I.D. Zorbasil with 5 μm particle size (MAC-MOD Analytical, Inc., PA, USA), and the analytical chiral HPLC column was packed with amylose carbamate (250 mm x 4.6 mm I.D. Chiralpak AD, 10 μm particle size, Chiral Technologies, Inc., PA, USA). The UV detector was set at a wavelength of 254 nm, and the mobile phase was 5% isopropanol in hexane for the chiral HPLC, or stated otherwise, and 30% ethyl acetate in hexane for normal HPLC at flow rate of 1.0 mL/min. The semi-preparative normal phase HPLC column was 250 mm x 22 mm I.D. Econosil silica with 10 μm particle size (Alltech Associates Inc., IL, USA), which was eluted with 30% ethyl acetate in hexane at flow rate of 9.0 mL/min.

All chemical reagents and anhydrous solvents were purchased from Aldrich Chemical Co. Lipases PP, PS-13, CC-7, and porcine liver esterase (PLE) were purchased from Sigma (St. Louis, MO), while lipases AK, AY-30, AP-12, PS-30, FAP-15, and N were from Amano (Troy, VA).

Aldol Reaction of Chromeno-coumarin 2 in the Presence of LDA/$TiCl_4$: Procedure A. To a stirred solution of **2** (200 mg, 0.58 mmol) in dry CH_2Cl_2 (10 mL) at -78 °C under N_2 was added a 2 M solution of LDA in heptane/THF/ethylbenzene (0.64 mL, 1.28 mmol). The reaction mixture was stirred at -78 °C for 30 min and then $TiCl_4$ (0.13 mL, 1.17 mmol) was added. The resulting yellow solution was warmed to -40 °C and stirred for 45 min. The mixture was re-cooled to -78 °C and acetaldehyde (150 mg, 3.5 mmol) was added *via* syringe. After 4 h, the reaction was quenched by the slow addition of pre-cooled saturated NH_4Cl (10 mL). Water (3 mL) was added to dissolve the oily solid. The mixture was extracted with ethyl acetate (50 mL x 3). The combined extracts were washed with brine (100 mL) and dried over $MgSO_4$. The crude product obtained by evaporation was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (5 : 1) to afford unreacted **2** (30 mg, 15% yield) and a mixture of (±)-**5** and (±)-**6** (140 mg, 61% yield), the ratio of which was 93 : 7 as determined by chiral HPLC and confirmed by ^1H NMR.[4]

Procedure B. To a stirred solution of **2** (20 g, 58.4 mmol) in dry CH_2Cl_2 (300 mL) at -40 °C under N_2 was added $TiCl_4$ (19 mL, 175 mmol). The mixture was then cooled to -78 °C, followed by the slow addition of a 2 M solution of LDA in heptane/THF/ethylbenzene (64 mL, 128 mmol). After 30 min at the same temperature, acetaldehyde (9 mL, 175 mmol) was added *via* syringe. The reaction mixture was stirred at -78 °C for 2 h. TLC analysis (hexane/ethyl acetate, 5 : 1) indicated that approximately 90% of **2** had been converted. The mixture was then poured into pre-cooled saturated NH_4Cl (240 mL). Water (120 mL) was added to dissolve the oily solid and the mixture was stirred for 20 min. Layers were separated and the aqueous solution was extracted with ethyl acetate (600 mL x 3). The combined extracts were washed with brine (600 mL) and dried over $MgSO_4$. Removal of solvents *in vacuo* afforded a reddish oil (23 g), which was taken up into ether (250 mL). The undissolved residue was filtered and the etheral solution was concentrated to half volume and then slowly added into rapidly stirring hexane cooled at -78 °C. Precipitates thus formed were collected by filtration to afford a mixture of (±)-**5** and (±)-**6** (11.1 g, 49% yield), the ratio of which was 96 : 4 as determined by chiral HPLC.

Enzymatic Resolution of (±)-5 with Vinyl Acetate: Procedure A, Catalyzed by Lipase PS-30 at Ambient Temperature. Into a stirred solution of (±)-**5** (7.6 g, 19.7 mmol) in *tert*-butyl methyl ether (130 mL) at ambient temperature under N_2 were added successively vinyl acetate (33 mL), 4 Å molecular sieves (17 g), and lipase PS-30 (3.8 g). The resulting mixture was vigorously stirred at ambient temperature for 4 days, whereupon it was filtered through celite and the celite was washed with ethyl acetate (20 mL). The crude product obtained from evaporation was subjected to silica gel column chromatography eluting with an incontinuous gradient of 5%, 10%, 15%, 25%, 30% and 40% of ethyl acetate in hexane to afford 4.8 g (57% yield) of the acetate (-)-**7** with 52% *ee* as determined by HPLC and 2.8 g (37% yield) of (+)-**5**.

6,6-Dimethyl-9-hydroxy-10-[2(*S*)-methyl-3(*R*)-acetoxybutyro]-4-propyl-2*H*,6*H*-benzo[1,2-b:3,4-b'] dipyran-2-one [(-)-**7**]. m.p.137-142 °C; $[\alpha]^{25}_D$= -9.6 ($CHCl_3$, *c* 0.72); ^1H NMR ($CDCl_3$): 1.05 (3H, t, J=7.4 Hz, CH_3), 1.29 (3H, d, J=6.9 Hz, CH_3), 1.38 (3H, d, J=6.6 Hz, CH_3), 1.54 (6H, s, 2 CH_3), 1.67 (2H, apparent sextet, J=7.6 Hz, CH_2), 2.00 (3H, s, CH_3CO), 2.91 (2H, dd, J=6.2 Hz, J=9.2 Hz, CH_2), 4.15 (1H, dq, J=4.5 Hz, J=6.8 Hz, $H_{2'}$), 5.41 (1H, dq, J=4.5 Hz, J=6.4 Hz, $H_{3'}$), 5.58 (1H, d, J=10.2 Hz, H_7), 6.01 (1H, s, H_3), 6.73 (1H, d, J=10.2 Hz, H_8), 14.16 (1H, s, OH); ^{13}C NMR ($CDCl_3$): 11.07 (CH_3), 13.82 (CH_3), 18.44 ($\boldsymbol{C}H_3CO_2$), 20.95 (CH_3), 23.17 (CH_2), 28.15 and 28.21 (2 CH_3), 38.97 (CH_2), 50.05 ($\boldsymbol{C}HCO$), 71.07 (CH-O), 79.75 (C-O), 102.83 (C_{8a}), 104.00 (C_{4a}), 106.15 (C_{10}), 110.52 (C_3), 115.94 (C_8), 126.43 (C_7), 156.90 and 157.04 (C_9 and C_{10a}), 158.72 (C_{4b}), 159.09 (C_4), 163.29 (CO_2), 170.70 ($CH_3\boldsymbol{C}O_2$), 207.29 (C=O); MS (CI):

429 (13.9, M+1), 369 (100, M-AcO); IR (KBr): 3462 (w, OH), 1736 (vs, C=O) cm^{-1}; Anal. calcd. for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59; Found: C, 67.23; H, 6.69.

6,6-Dimethyl-9-hydroxy-10-[2(*R*)-methyl-3(*S*)-hydroxybutyro]-4-propyl-2*H*, 6*H*-benzo[1,2-b:3,4-b']dipyran-2-one [(+)-**5**]. m.p. 82-85 °C; $[\alpha]^{25}_D$= 0 ($CHCl_3$, *c* 0.7); $[\alpha]^{25}_D$= 0 ($CHCl_3$, *c* 0.35); ^1H NMR ($CDCl_3$): 1.05 (3H, t, J=7.4 Hz, CH_3), 1.31 (3H, d, J=5.6 Hz, CH_3), 1.33 (3H, d, J=6.9 Hz, CH_3), 1.54 (6H, s, 2 CH_3), 1.67 (2H, apparent sextet, J=7.6 Hz, CH_2), 2.75 (1H, broad-s, OH), 2.91 (2H, t, J=7.8 Hz, CH_2), 3.98 (1H, dq, J=2.7 Hz, J=7.0 Hz, $H_{2'}$), 4.30 (1H, dq, J=2.7 Hz, J=6.5 Hz, $H_{3'}$), 5.59 (1H, d, J=10.2 Hz, H_7), 6.01 (1H, s, H_3), 6.72 (1H, d, J=10.3 Hz, H_8), 14.10 (1H, s, OH); ^{13}C NMR ($CDCl_3$): 10.42 (CH_3), 14.00 (CH_3), 20.61 (CH_3), 23.32 (CH_2), 28.31 (2 CH_3), 39.05 (CH_2), 50.93 (***C***HCO), 68.03 (CH-O), 79.92 (C-O), 102.95 (C_{8a}), 103.69 (C_{4a}), 106.12 (C_{10}), 110.60 (C_3), 115.80 (C_8), 126.51 (C_7), 157.03 and 157.11 (C_9 and C_{10a}), 158.58 (C_{4b}), 159.01 (C_4), 163.13 (CO_2), 210.61 (C=O); MS (CI): 388 (33.4, M+2), 387 (100, M+1), 386 (8.5, M^+), 369 (36.3, M-OH), 343 (97.2, M-C_3H_7); IR (KBr): 3507 and 3443 (m, OH), 1713 (vs, C=O) cm^{-1}; Anal. calcd. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78; Found: C, 68.02; H, 6.62.

Procedure B, Catalyzed by Lipase AK at 40 °C. Compound (±)-**5** (1.0 kg, 2.59 mol) was dissolved in *tert*-butyl methyl ether (9 L) at ambient temperature under N_2, into which were added, successively, lipase AK (1.0 kg), 4 Å molecular sieves (327 g), and vinyl acetate (850 mL). The resulting mixture was heated at 40 °C while being vigorously stirred. The reaction was monitored by chiral HPLC. After 24 h, the reaction mixture was filtered through celite and the celite was washed with ethyl acetate (9 L). The crude product obtained from evaporation under vacuum was purified by silica gel column chromatography eluting with 20% ethyl acetate in hexane to afford 600 g (54% yield) of the acetate (-)-**7** with 77% *ee* as determined by chiral HPLC and 413 g (41% yield) of (+)-**5**, the two compounds being identical with those prepared by procedure A. (*Caution: The column should be finished as quickly as possible. Otherwise, (+)-**5** may be converted to **2**, (±)-**3** and (±)-**4** during the chromatography.*)

Enzymatic Resolution of (±)-6: Into a stirred solution of (±)-**6** (3.0 g, 7.8 mmol) in *tert*-butyl methyl ether (78 mL) at ambient temperature under N_2 were added successively vinyl acetate (26 mL), 4 Å molecular sieves (3.0 g), and Lipase PS-30 (1.5 g). The resulting mixture was vigorously stirred at ambient temperature for 41 h, whereupon it was filtered through through celite and the celite was washed with ethyl acetate (20 mL). The crude yellowish solid product (3.2 g) obtained from evaporation was purified by silica gel column chromatography eluting with a step gradient of 5%, 10%, 15%, 25%, 30% and 40% of ethyl acetate in hexane to afford 1.68 g (50% yield) of the acetate (**8**) with >99% *ee* as determined by chiral HPLC and 1.37 g (46% yield) of (+)-**6** with >99% *ee* as determined by chiral HPLC.

6,6-Dimethyl-9-hydroxy-10-[2(*S*)-methyl-3(*S*)-acetoxybutyro]-4-propyl-2*H*, 6*H*-benzo[1,2-b:3,4-b']dipyran-2-one [(+)-**8**]. m.p. 61-64 °C; $[\alpha]^{25}_D$= +30.0 ($CHCl_3$, *c* 0.73); ^1H NMR ($CDCl_3$): 1.06 (3H, t, J=7.2 Hz, CH_3), 1.29 (3H, d, J=6.2 Hz, CH_3), 1.32 (3H, d, J=6.7 Hz, CH_3), 1.54 (6H, s, 2 CH_3), 1.67 (2H, apparent sextet, J=7.6 Hz, CH_2), 1.93 (3H, s, CH_3CO), 2.91 (2H, m, CH_2),4.18 (1H, dq, J=8.3 Hz, J=6.9 Hz, $H_{2'}$), 5.34 (1H, dq, J=8.2 Hz, J=6.4 Hz, $H_{3'}$), 5.59 (1H, d, J=10.1 Hz, H_7), 6.02 (1H, s, H_3), 6.73 (1H, d, J=10.1 Hz, H_8), 14.02 (1H, s, OH); ^{13}C NMR ($CDCl_3$): 13.52 (CH_3), 13.82 (CH_3), 17.01 (***C***H_3CO_2), 20.99 (CH_3), 23.16 (CH_2), 28.11 and 28.20 (2 CH_3), 38.91 (CH_2), 50.34 (***C***HCO), 71.98 (CH-O), 79.76 (C-O), 102.89 (C_{8a}), 104.32 (C_{4a}), 106.00 (C_{10}), 110.71 (C_3), 115.90 (C_8), 126.51 (C_7), 156.94 and 157.14 (C_9 and C_{10a}), 158.47 (C_{4b}), 159.03 (C_4), 162.87 (CO_2), 170.35 (CH_3***C***O_2), 207.41 (C=O); MS (CI): 430 (37.1, M+2), 429 (95.2, M+1), 428 (7.2, M^+), 369 (100, M-AcO); IR (KBr): 3449 (vw, OH), 1734 (vs, C=O) cm^{-1}; Anal.

calcd. for $C_{24}H_{28}O_7$: C, 67.31; H, 6.59; Found: C, 67.75; H, 6.90.

6,6-Dimethyl-9-hydroxy-10-[2(*R*)-methyl-3(*R*)-hydroxybutyro]-4-propyl-2*H*, 6*H*-benzo[1,2-b:3,4-b']dipyran-2-one [(+)-**6**]. m.p. 131-134 °C; $[\alpha]^{25}_D$= +45.3 ($CHCl_3$, *c* 0.72); ^{1}H NMR ($CDCl_3$): 1.06 (3H, t, J=7.3 Hz, CH_3), 1.25 (3H, d, J=6.6 Hz, CH_3), 1.29 (3H, d, J=6.7 Hz, CH_3), 1.55 (6H, s, 2 CH_3), 1.67 (2H, apparent sextet, J=7.6 Hz, CH_2), 2.92 (2H, t, J=7.8 Hz, CH_2), 2.96 (1H, d, J=7.1 Hz, OH), 3.98 (1H, apparent quintet, J=6.1 Hz, $H_{2'}$), 4.22 (1H, apparent sextet, J=6.0 Hz, $H_{3'}$), 5.60 (1H, d, J=10.1 Hz, H_7), 6.03 (1H, s, H_3), 6.73 (1H, d, J=10.1 Hz, H_8), 14.25 (1H, s, OH); ^{13}C NMR ($CDCl_3$): 13.09 (CH_3), 13.80 (CH_3), 19.32 (CH_3), 23.18 (CH_2), 28.15 and 28.16 (2 CH_3), 38.95 (CH_2), 53.17 (***C***HCO), 69.45 (CH-O), 79.85 (C-O), 102.91 (C_{8a}), 104.53 (C_{4a}), 106.13 (C_{10}), 110.36 (C_3), 115.85 (C_8), 126.53 (C_7), 157.07 and 157.13 (C_9 and C_{10a}), 159.02 (C_{4b}), 159.41 (C_4), 163.24 (CO_2), 209.49 (C=O); MS (CI): 388 (41.4, M+2), 387 (100, M+1), 386 (13.0, M^+), 369 (42.8, M-OH), 343 (63.8, M-C_3H_7); IR (KBr): 3470 (s, OH), 1715 (vs, C=O) cm^{-1}; Anal. calcd. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78; Found: C, 68.50; H, 6.91.

Mitsunobu Cyclization of (+)-5: Into a stirred solution of (+)-**5** (2.0 g, 5.2 mmol) in THF (50 mL) were added triphenylphosphine (1.9 g, 7.2 mmol) and diethyl azodicarboxylate (DEAD, 1.2 mL, 7.6 mmol). The resulting reddish solution was stirred at ambient temperature under N_2 for 5 h, after which the reaction mixture was quenched with saturated aqueous NH_4Cl (20 mL) and extracted with ethyl acetate (50 mL x 3). The combined extracts were washed with brine (50 mL) and dried over Na_2SO_4. The crude product (5.8 g) obtained by evaporation was purified by column chromatography on silica gel eluting with an incontinous gradient of 10%, 20%, 30% and 40% of ethyl acetate in hexane to afford 1.2 g (63% yield) of (+)-**3** with 94% *ee* as determined by chiral HPLC.

10(*R*),11(*R*)-*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 [(+)-**3**]. mp 171-175 °C; $[\alpha]^{25}_D$= +37.9 ($CHCl_3$, *c* 0.73); ^{1}H NMR ($CDCl_3$/CD_3OD, 3 : 1): 1.06 (3H, t, J=7.3 Hz, CH_3), 1.22 (3H, d, J=7.0 Hz, CH_3), 1.54 (3H, s, CH_3), 1.57 (3H, d, J=6.0 Hz, CH_3), 1.58 (3H, s, CH_3), 1.67 (2H, apparent sextet, J=7.6 Hz, CH_2), 2.59 (1H, dq, J=6.9 Hz, J=11.1 Hz, H_{11}), 2.92 (2H, t, J=7.8 Hz, CH_2), 4.37 (1H, dq, J=6.3 Hz, J=11.1 Hz, H_{10}), 5.66 (1H, d, J=10.1 Hz, H_7), 6.05 (1H, s, H_3), 6.67 (1H, d, J=10.1 Hz, H_8); ^{13}C NMR ($CDCl_3$/CD_3OD, 3 : 1): 9.87 (CH_3), 13.34 (CH_3), 18.97 (CH_3), 22.85 (CH_2), 27.40 and 27.73 (2 CH_3), 38.38 (CH_2), 46.82 (***C***HCO), 79.17 (CH-O and C-O), 102.91 (C_{8a}), 104.11 (C_{4a}), 105.46 (C_{12a}), 111.09 (C_3), 115.21 (C_8), 126.90 (C_7), 154.83 and 155.86 (C_{8b} and C_{12b}), 157.89 (C_{4b}), 158.99 (C_4), 160.27 (CO_2), 190.50 (C=O); MS (CI): 370 (49.0, M+2), 369 (100, M+1), 368 (17.2, M^+); IR (KBr): 1738 (vs, C=O) cm^{-1}; Anal. calcd. for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57; Found: C, 71.46; H, 6.60.

Mitsunobu Cyclization of (+)-6: The reaction was carried out by the same procedure presented above. From (+)-**6** (1.0 g, 2.6 mmol), triphenylphosphine (918 mg, 3.5 mmol) and diethyl azodicarboxylate (DEAD, 610 mg, 3.5 mmol) in THF (25 mL), 250 mg (26% yield) of (+)-**3** and 700 mg (73% yield) of (+)-**4** were obtained after purification by silica gel column chromatography, eluting with 20% ethyl acetate in hexane. The optical purity of (+)-**3** was determined to be 60% ee by both chiral HPLC and optical rotation {$[\alpha]^{25}_D$= +22.5 ($CHCl_3$, *c* 0.72)}, while (+)-**4** was 68% *ee*, as assayed by chiral HPLC, with $[\alpha]^{25}_D$= −49.8 ($CHCl_3$, *c* 0.69), and was spectroscopically identical with (±)-**4**.[2]

(+)-Calanolide A: To a stirred solution of (+)-**3** (660 mg, 1.79 mmol) in EtOH (18 mL) were added $CeCl_3 \cdot 7H_2O$ (2.7 g, 7.17 mmol) and triphenylphosphine oxide (2.0 g, 7.17 mmol). The mixture was stirred for 1 h at ambient 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, $NaBH_4$ (271 mg, 7.17 mmol) was added and stirred at

the same temperature for 5.5 h, at which time the reaction was quenched with saturated NH_4Cl (20 mL) and extracted with ethyl acetate (3 x 30 mL). The combined extracts were washed with brine (50 mL) and dried over Na_2SO_4. The crude product obtained by removal of solvent under reduced pressure was purified by column chromatography on silica gel eluting with 20% ethyl acetate in hexane to afford 520 mg (78% yield) of a mixture containing 90% of (+)-calanolide A [(+)-**1**] and 10% of (+)-calanolide B, which were further separated by normal phase HPLC.[2] (+)-Calanolide A [(+)-**1**] thus obtained had $[\alpha]^{25}_D$= +56.7 ($CHCl_3$, *c* 0.73) {Lit. $[\alpha]^{25}_D$= +60 ($CHCl_3$, *c* 0.5);[3] $[\alpha]^{25}_D$= +66 ($CHCl_3$, *c* 0.5),[10] $[\alpha]^{25}_D$= +68.8 ($CHCl_3$, *c* 0.7)[2]} and 94% *ee* as determined by chiral HPLC eluting with 5% ethanol in hexane, and was spectroscopically identical with an authentic sample.

Acknowledgement: This work was partially supported by a Phase II Small Business Innovation Research (SBIR) grant (2R44 AI34805) from the National Institutes of Health, Bethesda, MD.

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17. The optical rotation for compound (+)-**5** was determined to be 0 in $CHCl_3$ and was independent of the concentration. For the convenience of comparison and discussion, the desired enantiomer was assigned as (+)-**5**.
18. Since baseline separation was not achieved between the two enantiomers of (±)-**5** in the chiral HPLC, it was difficult to determine the *ee* values for (+)-**5**.[17] However, after the Mitsunobu cyclization, the enantiomers of *trans* chromanone **3** were completely separated with chiral HPLC and the *ee* value for (+)-**3** was determined to be 94%. Based on the nature of the Mitsunobu reaction,[19-21] the optical purity of (+)-**5** should be at the same level as that of (+)-**6**.
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(*Received in USA* 10 *September* 1996)