Synthesis, Absolute Configuration, and Enantioselectivity of Antiretroviral Effect of (*R*)-(−)- and (*S*)-(+)-Cytallene. Lipase-Catalyzed Enantioselective Acylations of (±)-N^4-Acylcytallenes[1]

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Enantioselectivity of acylations of (±)-cytallene (**1b**), (±)-N^4-acetylcytallene (**11a**), (±)-N^4-benzoylcytallene (**11b**), and (±)-N^4-(9-fluorenylmethoxycarbonyl)cytallene (**11c**) using vinyl butyrate or acetate catalyzed by lipases in organic solvents was investigated. Reactions with **1b**, **11a**, and adenallene (**1a**) did not display a high enantioselectivity but all resulted in a predominant acylation of the (−)-enantiomers. Application of the Lowe–Brewster rule led to a tentative assignment of the *R*-configuration to all acylated products. Studies of the time course of acylation of (±)-N^4-benzoylcytallene (**11b**) in chloroform, tetrahydrofuran (THF), tetrahydropyran (THP), tetrahydrothiophene (THT), and dioxane with lipase PS30 and/or AK showed that the reaction in THF catalyzed by lipase AK was the most promising for resolution of **11b**. Indeed, a large-scale acylation afforded, after separation and deprotection of intermediates **3e** and **10d**, (+)- and (−)-cytallene (**3c** and **2b**) in high yield and enantioselectivity. Acylation of **11c** in THF led also to formation of **3c** and **2b** in high enantioselectivity. Single crystal X-ray diffraction established the *S*-configuration of (+)-cytallene (**3c**), thus confirming the assignment made on the basis of Lowe–Brewster rule. An improved large-scale synthesis of (±)-cytallene (**1b**) is also described. The *R*-enantiomer **2b** inhibited the replication of a primary human immunodeficiency virus (HIV-1) isolate in phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBM) with IC_{50} 0.4 and IC_{90} 1.7 μM. (±)-Cytallene (**1b**) exhibited IC_{50} 0.8 and IC_{90} 3.4 μM. Both compounds completely suppressed replication of HIV-1 at 10 μM with no detectable cytotoxicity. The *S*-enantiomer (**3c**) was inactive.

Recently, we described resolution of (±)-adenallene (**1a**), an effective anti-HIV agent,[2,3] by combination of enzymatic and chemical methods.[4] Thus, deamination of adenallene (**1a**) catalyzed by adenosine deaminase led directly to (*R*)-(−)-adenallene (**2a**) and (*S*)-(+)-hypoxallene (**3a**). The latter allene was transformed to (*S*)-(+)-adenallene (**3b**) by chemical synthesis. It was established[4] that (*R*)-(−)-adenallene (**2a**) was primarily responsible for anti-HIV effect of the racemic compound whereas the (*S*)-(+)-enantiomer **3b** exhibited only a marginal effect. The corresponding cytosine analogue, (±)-cytallene (**1b**), is also an active anti-HIV agent, the potency of which exceeds that of (±)-adenallene[2,3] (**1a**). It was then of substantial interest to resolve (±)-cytallene (**1b**) into enantiomers in order to determine their antiretroviral activity and absolute configuration.

In this paper, we report on enzyme-catalyzed acylation of cytallene (**1b**) and N^4-substituted derivatives **11a**, **11b**, and **11c** which formed the basis for resolution of (±)-cytallene (**1b**) into optically pure enantiomers **2b** and **3c** on a preparative scale. Determination of the absolute configuration of (+)-cytallene (**3c**) and anti-HIV effect of **2b** and **3c** are also described.

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Resolution of (±)-Cytallene (1b)

(±)-Cytallene (**1b**) was obtained by an improved procedure which avoids column chromatography and is especially suitable for a large-scale synthesis. Alkylation of sodium salt of cytosine (**4**) with 1-bromo-4-(benzoyloxy)-2-butyne[5] (**5**) gave intermediate **6** in 82% yield (Scheme 1). Ammonolysis of **6** furnished butynol **7** in 85% yield. The latter product was isomerized with potassium *tert*-butoxide in *N,N*-dimethylformamide[6] (DMF) to give (±)-cytallene (**1b**) in 36% yield.

(±)-Cytallene (**1b**) is not a substrate for cytidine deaminase[6] and, therefore, the approach applied for resolution of (±)-adenallene[4] (**1a**) could not be employed. Also, a method which does not lead to the transformation of heterocyclic base(s) of one or both enantiomers was considered preferable. Attempts to resolve (±)-cytallene (**1b**) by preparative chromatography on a triacetyl- or tribenzoylcellulose column were not successful. The HPLC on a triacetylcellulose-based chiral column, Chiralcel CA-1, which successfully resolved (±)-adenallene[4] (**1a**) was ineffective. By contrast, the column with tribenzoyl cellulose as a chiral phase (Chiralcel OB, 10 μm) smoothly separated[7] the (−)- and (+)-enantiomers **2b** and **3c** (Figure 1). It is also noteworthy that the order of elution of (−)- and (+)-cytallene **2b** and **3c** was reversed relative to that observed[4] with **2a** and **3b** on a Chiralcel CA-1 column. Interestingly, (±)-adenallene (**1a**) was not resolved on a Chiralcel OB column. At this point, we had in our hands a reliable method for estimating the optical purity

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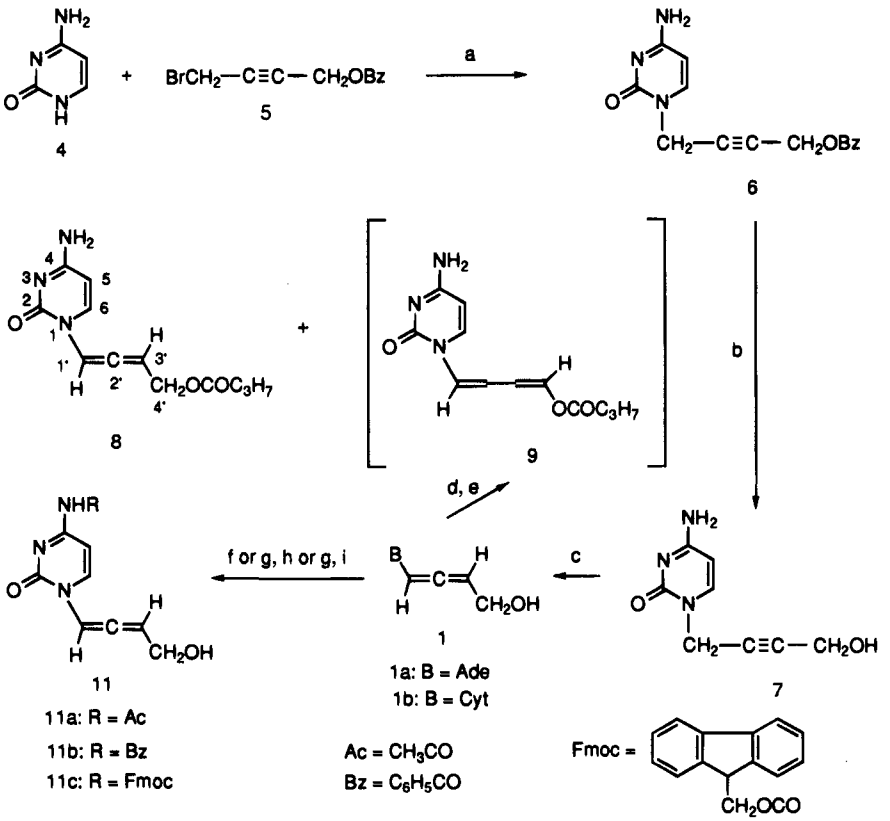
Scheme 1[a]



[a] (a) NaH, DMF; (b) NH_3, MeOH; (c) tBuOK, DMF; (d) $Me_2NCH(OMe)_2$, DMF; (e) $(C_3H_7CO)_2O$, NEt_3, $CHCl_3$; (f) Ac_2O or Bz_2O, EtOH, Δ; (g) Me_3SiCl, pyridine; (h) BzCl, pyridine; (i) FmocCl, pyridine. For abbreviations of nucleic acids components used in the schemes, see ref 35. Thus, Ade = adenin-N^9-yl; Cyt = cytosin-N^1-yl; Hyp = hypoxanthin-N^9-yl. Other abbreviations: Ac = acetyl; Bz = benzoyl; Fmoc = 9-fluorenylmethoxycarbonyl; AcCyt = N^4-acetylcytosin-N^1-yl, BzCyt = N^4-benzoylcytosin-N^1-yl, FmocCyt = N^4-(9-fluorenylmethoxycarbonyl)cytosin-N^1-yl.

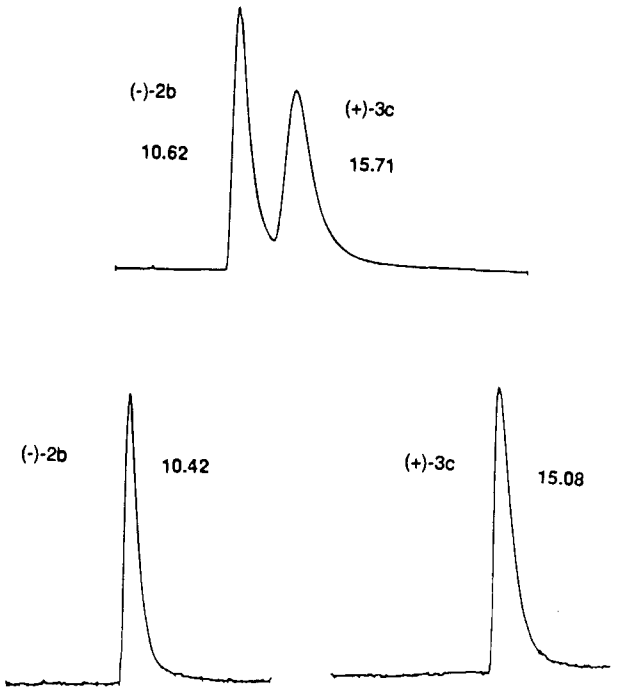


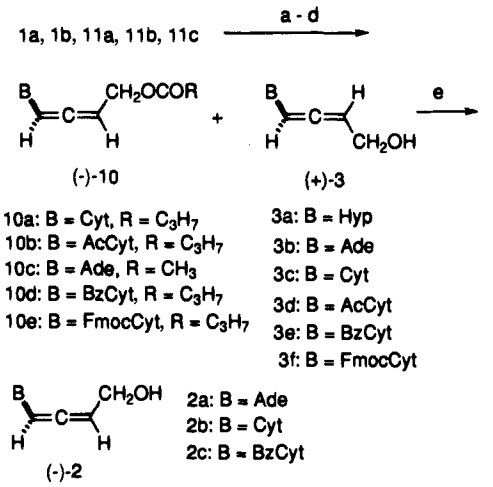
Figure 1. Resolution of (±)-cytallene (**1b**) by HPLC on a Chiralcel OB 10 μm column. Elution with solvent system S_1. Retention time (t_R, min) is indicated at each peak. For other details see the Experimental Section.

of enantiomers of cytallene although, for obvious reasons, the procedure was not practical for a preparative separation.

Our attention then turned to esterases, enzymes which were recently much exploited for synthetic purposes.[8,9] Nevertheless, the method was predominantly used for resolution of centrochiral compounds, but examples of resolving racemic mixtures of enantiomers with an axial chirality (allenes) are rare.[10] Our first attempts focused on pig liver esterase (PLE) employed in a hydrolytic mode. The enzyme was recently used for the resolution of anti-HIV agent (±)-2′,3′-dideoxy-3′-thiacytidine.[11] We were unable to prepare the starting (±)-4′-*O*-butyrylcytallene (**8**) using the method described for preparation of (±)-5′-*O*-butyryl-2′,3′-dideoxy-3′-thiacytidine[11] (acylation of cytallene hydrochloride with butyric anhydride in pyridine). More successful was the protection of **1b** with N^4-(dimethylamino)-methylene group followed by acylation with butyric anhydride in $CHCl_3$ catalyzed by NEt_3 (Scheme 1) which gave compound **8** in 52% yield. Although this product gave a correct elemental analysis, the ^1H NMR spectra indicated the presence of ca. 20% of olefinic material, possibly conjugated diene **9**, arising from an isomerization of **8** and inseparable by chromatography. This product was a substrate for PLE, but its purity was not considered sufficient for the intended purpose.

In the next stage, enzyme-catalyzed acylation of (±)-cytallene (**1b**) with vinyl butyrate in organic solvent, was investigated. Although (±)-cytallene (**1b**) is poorly soluble in most solvents, reaction with vinyl butyrate in pyridine catalyzed by subtilisin gave (−)-4′-*O*-butyrylcytallene (**10a**) in 40% yield and (+)-cytallene (**3c**, 37%, 18% ee, Scheme 2). Attempted acylations cata-

Scheme 2[a]



[a] (a) $AcOCH{=}CH_2$, Me_2CO–THF (2:1), lipase PS30; (b) $C_3H_7COOCH{=}CH_2$, Me_2CO–THF (3:2), lipase AK; (c) $C_3H_7COOCH{=}CH_2$, pyridine, subtilisin; (d) $C_3H_7COOCH{=}CH_2$, THF, lipase AK; (e) NH_3, MeOH. See the footnote to Scheme 1 for a list of abbreviations.

lyzed by lipase PS30 in DMF were unsuccessful, and reaction in DMF catalyzed by subtilisin gave only a very low conversion to acylated product. To increase the solubility in organic solvents, (±)-cytallene (**1b**) was converted to a strongly fluorescent (±)-N^4-acetylcytallene (**11a**) by reaction with acetic anhydride in ethanol[12] (87% yield, Scheme 1). Acylation of **11a** with vinyl butyrate in THF–acetone catalyzed by lipase AK afforded (−)-4′-*O*-butyrate **10b** (30%) and (+)-N^4-acetylcytallene (**3d**, 57%). In another model experiment, (±)-adenallene (**1a**) was acetylated with vinyl acetate using lipase PS30 to give (−)-4′-*O*-acetyladenallene (**10c**) in 25% yield and 31% ee based on the optical rotation of the known[4] *S*-(+)-enantiomer. (+)-Adenallene (**3b**) was obtained in 74% yield and 12% ee.

Obviously, all these acylations did not proceed with an acceptable enantioselectivity, but, interestingly, *all* esters formed were levorotatory whereas the recovered allenols were dextrorotatory. Thus, heterocyclic base (pyrimidine or purine) did not influence the sign of rotation, and, thus, it seemed possible that the Lowe–Brewster[6,13] "allene rule" but not Ulbricht's[14] "nucleoside rule" was applicable. It should be added that hindrance to rotation of heterocyclic bases and hydroxymethyl groups is absent in allenic analogues[6] in contrast to nucleosides. Such an effect is of importance for the observed difference in signs of optical rotation and Cotton effect between purine and pyrimidine nucleosides.[14] In addition, a double bond conjugated with the heterocyclic base constitutes another factor absent in nucleosides. Polarizability of CH_2OH is greater than that of hydrogen,[13] and we assume the same is true for cytosine or adenine. The absolute configuration *S* can then be tentatively assigned to dextrorotatory allenols[13] (Figure 2).

We have reasoned that further improvement of enantioselectivity of acylation could be achieved by employing (±)-cytallene (**1b**) substituted by a more lipophilic (aromatic) function which would increase solubility and, possibly, binding affinity to the active site of esterase. We therefore prepared (±)-N^4-benzoylcytallene (**11b**) using the procedure[12] employed for the *N*-acetyl derivative **11a** (benzoic anhydride in ethanol) in 63% yield. A

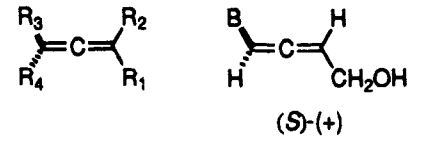


Figure 2. Assignment of absolute configuration of enantiomers of adenallene (**2a** and **3b**) and cytallene (**2b** and **3c**) based on Lowe–Brewster rule. Order of polarizabilities $R_1 > R_2$ and $R_3 > R_4$ for the *S*-(+)-enantiomer, $R_1 > R_2$ and $R_3 < R_4$ for the *R*-(−)-enantiomer. For B = Ade or Cyt, order of polarizabilities is CH_2OH > H, B > H.

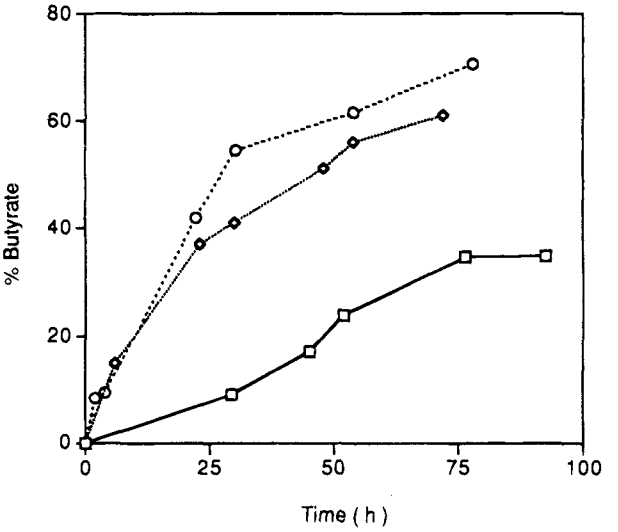


Figure 3. Time course of acylation of (±)-N^4-benzoylcytallene (**11b**) with vinyl butyrate catalyzed by lipase PS30 in selected organic solvents. For details see Experimental Section: (○) $CHCl_3$, (◇) THF, (□) dioxane.

method[15] based on benzoylation of transient 4′-*O*-(trimethylsilyl)cytallene gave 42% yield of **11b**. Interestingly, compound **11b** was also resolved on a Chiralcel OB column, although the peaks were broader and less sharp. Again, (−)-enantiomer **2c** was eluted prior to (+)-enantiomer **3e** as in case of **2b** and **3c**.

Effectivity of a kinetic resolution depends on the difference of reaction rates of both enantiomers. Therefore, it was anticipated that a time course study of a suitable derivative of (±)-cytallene (**1b**) could assist in establishing proper conditions for an efficient kinetic resolution. The time course of acylation of (±)-N^4-benzoylcytallene (**11b**) with vinyl butyrate was investigated with two lipases, PS30 and AK, in $CHCl_3$, THF, and dioxane. The reaction catalyzed by lipase PS30 in $CHCl_3$ showed little indication of significant enantioselectivity as judged from the kinetic behavior (Figure 3). In THF, a deceleration effect was noted between 50 and 60% conversion whereas in dioxane the acylation was slower. We have found with our set of substrates that lipase-catalyzed acylations in organic solvents are often accompanied by the formation of colored byproducts which increases with time. Therefore, slow reactions are not advantageous from a preparative viewpoint. With lipase AK as a catalyst, the reactions were significantly faster (Figure 4). Again, in $CHCl_3$ the acylation proceeded with a little indication that reaction rates of both enantiomers differ substantially. In THF, a significant deceleration was observed at ca. 50% conversion, suggesting an increased enantioselectivity of acylation. As in the case of lipase PS30, the acylation was much slower in dioxane.

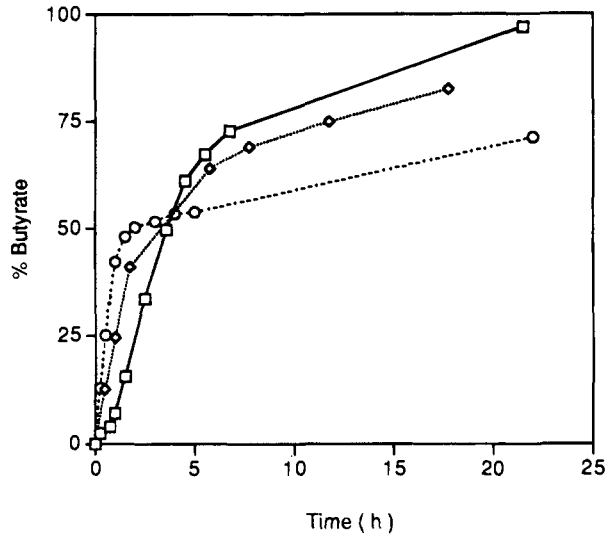


Figure 4. Time course of acylation of (±)-N^4-benzoylcytallene (**11b**) with vinyl butyrate catalyzed by lipase AK or PS30 in selected organic solvents. For details see Experimental Section: (□) $CHCl_3$, (○) THF, (◇) dioxane ($h \times 4$).

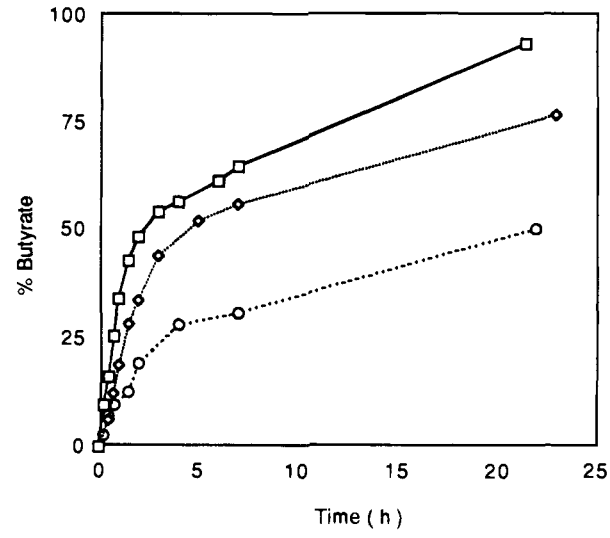


Figure 5. Time course of acylation of (±)-N^4-substituted cytallenes **11a** and **11b** with vinyl butyrate catalyzed by lipase AK in selected organic solvents. For details see Experimental Section: (□) THP (tetrahydropyran), compound **11b**; (◇) THT (tetrahydrothiophene), compound **11b**; (○) THF (tetrahydrofuran), compound **11a**, 76% conversion after 46 h.

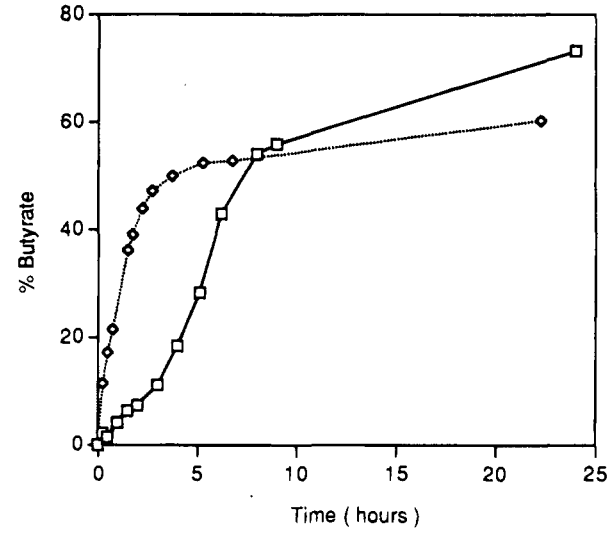


Figure 6. Time course of acylation of (±)-N^4-Fmoc cytallene (**11c**) with vinyl butyrate catalyzed by lipase AK in selected organic solvents. For details see Experimental Section: (□) $CHCl_3$, (◇) THF.

On the basis of these data, reaction conditions were selected for a preparative kinetic resolution of (±)-N^4-benzoylcytallene (**11b**). The acylation was performed in THF with vinyl butyrate using lipase AK. Molecular sieves 4A were added to scavenge acetaldehyde released during the reaction.[16] The progress of acylation was monitored by TLC. After 2 h at room temperature, the mixture was filtered and the products were separated by flash-chromatography on silica gel. (−)-Ester **10d** and (+)-allenol **3e** were deacylated with NH_3 in methanol to afford, after chromatography, (−)- and (+)-cytallene **2b** and **3c** in 100 and 82% yield, respectively, with enantioselectivity exceeding 95% ee. After a single recrystallization, optically pure (99% ee) enantiomers were obtained (Figure 1). As expected, the optical rotation of enantiomers **2b** and **3c** is high, in fact greater than that of their adenallene counterparts.[4] By contrast, Cotton effect of **2b** and **3c** exhibits a lower molecular ellipticity than **2a** and **3b**.

The favorable effect of THF on enantioselectivity of acylation of **11b** is difficult to explain. In many cases, the nonpolar solvents immiscible with water are considered optimal,[8] whereas those capable of stripping water from the enzyme are usually less favorable. However, THF and other *cyclic* solvents gave the best enantioselectivity in acetylation of racemic β-nitro alcohols with vinyl acetate catalyzed by lipase AK irrespective of their distribution coefficients,[17] *P*. We have examined time courses of acylation of **11b** in two additional cyclic solvents structurally close to THF, tetrahydrothiophene (THT), and tetrahydropyran (THP, Figure 5) which are immiscible with water and less polar than THF. In both solvents, a significant decelertion was observed at ca. 50% conversion, but they do not seem to offer a particular advantage over THF. With (±)-N^4-acetylcytallene (**11a**), the time course of acylation in THF resembled that obtained with (±)-N^4-benzoylcytallene (**11b**) and lipase PS30 (Figure 3), but the reaction was noticeably slower. This again underlines the importance of the N^4-benzoyl group for reactivity and enantioselectivity of acylation.

Although the latter function proved satisfactory for the purpose of resolution of (±)-cytallene (**1b**), it was of interest to examine the influence of more bulky groups on the enantioselectivity of acylation. It should be mentioned that bulky protecting groups can impart enantioselectivity on suitable molecules.[18] The (±)-N^4-(9-fluorenylmethoxycarbonyl) (Fmoc)-cytallene (**11c**) was chosen as an example. Compound **11c** was readily obtained by a "transient protection" method[15,19] (Scheme 1). The time course of the reaction of **11c** with vinyl butyrate catalyzed by lipase AK in THF and, somewhat surprisingly, $CHCl_3$ indicated a significant enantioselectivity (Figure 6). Likewise, acylation in THF furnished intermediates (−)-**10e** and (+)-**3f** which were deacylated by NH_3 in MeOH. Chiral chromatography of the obtained products showed a high enantioselectivity of the formation of (+)-cytallene (**3c**, ca. 90% ee after 4 and 6 h) and somewhat lower in case of

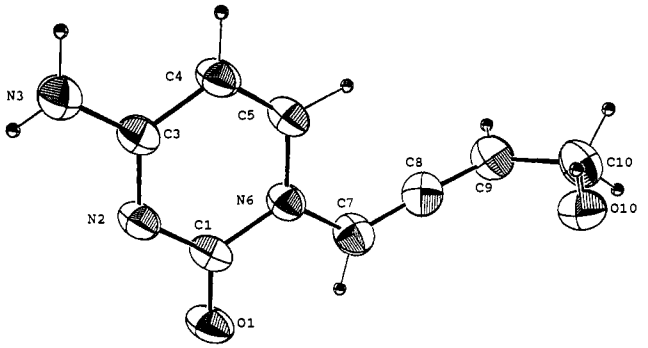


Figure 7. ORTEP drawing of the crystal structure of (*S*)-(+)-cytallene (**3c**).

(−)-enantiomer **2b** (65% ee). The latter result may indicate certain limitations of using substrates with a bulky lipophilic groups for enantioselective acylation.

It is tempting to put these results into a context of recently proposed[20] active site model of lipase AK. This model was derived from acylation of a few relatively simple *centrochiral* alcohols. It comprises a large and small binding pocket along with a site accommodating the hydroxy group and closely resembles that of PLE.[21] Nevertheless, we must caution that all models of the active sites of lipases are preliminary, and ambiguous interpretations do exist.[22] It is not likely that *both* aromatic and heterocyclic moieties of **11b** (ca. 8 Å long) and, especially, **11c** can be accommodated in the large pocket of lipase AK in their entirety. A possibility then exists that only aromatic portions of **11b** and **11c** are bound in this site and the heterocyclic moiety is excluded. There is also an ambiguity as to what portion of the molecule is bound in the small pocket, although the CH_2 group of hydroxymethyl and adjacent part of allene system appear to be suitable candidates.

The influence of the nature of solvent on biological activity of enzymes in organic solvents has not been satisfactorily explained by any of the proposed models. A host of factors such as dielectric constant, partition coefficient, and dipole moment[23] were considered, but one was apparently overlooked: A possible participation of solvent (THF) in the binding of substrate in the small or large pocket through a hydrogen bonding with the hydroxy group of a substrate.

Crystallographic Results

The (+)-cytallene (**3c**) has the *S* absolute configuration in the notation of Cahn, Ingold, and Prelog[24] (Figure 7). The absolute configuration was determined with greater statistical precision than in our previous work on adenallene.[4] Numerical details are given in the Experimental Section, and the improvement in reliability can be explained by the use of a different statistical method on a sample of better crystallographic quality. The physical appearance of the crystals was the criterion for choice of enantiomer and, in contrast to the previous investigation,[4] the determination of absolute configuration employed the crystals of the (+)-enantiomer **3c**. It is important to emphasize that these results show that the (+)-enantiomers of both analogues possess the same absolute configuration *S*. Thus, tentative conclusions based on the Lowe−Brewster rule (Figure 2) were confirmed.

Unlike in (*R*)-(−)-adenallene[4] (**2a**), the molecular packing is relatively simple, with only one molecule in the asymmetric unit of the common space group $P2_12_12_1$. There is no indication of parallel stacking, and hydrogen bonds involving all probable hydrogen atoms link the molecules into a thick sheet extending through the crystal. Full details are available as supplementary material, but the bonds are N3···O10 (screw axis *a*), N3···O (screw axis *b*), and O10···N2 (screw axis *a*) with lengths of 2.971(2), 2.950(2) and 2.871(2) Å, respectively. Crystals of (+)-cytallene (**3c**) have a density of 1.38 g/cm³, nearly as high as reported[4] for (*R*)-(−)-adenallene (**2a**, 1.42 g/cm³). In the adenallene structure, the two independent molecules had different conformations of the hydroxymethyl group; one essentially *cis* with a torsion angle of −9° and the other roughly *trans* torsion angle 146°. In (*S*)-(+)-cytallene (**3c**), the O10−C10−C9−C8 torsion angle is −6°. In both compounds, the values of this torsion angle are probably a result of packing. The N6−C7−C9−C10 torsion angle is 91.2(2)°, as might be expected from the structure. Apart from some hydrogen atoms, C10, and O10, the molecule is relatively if not rigorously flat. The C10 is −1.26 Å from the least squares plane of C1, N2, C3, C4, C5, N6, C7, C8, and C9 (deviations −0.04 to +0.03 Å). The cytosine ring has a deviation of ±0.02 Å from planarity, and C8, O1, and N3 are +0.08, +0.09, and +0.03 Å, respectively, from this plane. The torsion angle for C5−N6−C7−C8, corresponding to the χ angle of nucleosides, is 4.8°. Similar torsion angles of 5° and 11° were reported for (*R*)-(−)-adenallene[4] (**2a**). Thus, the base (cytosine) conformation can be described as *anti*. It should be noted that a similar conformation was found in the crystal structure of the anti-AIDS drug 2′,3′-dideoxycytidine (ddCyd).[25]

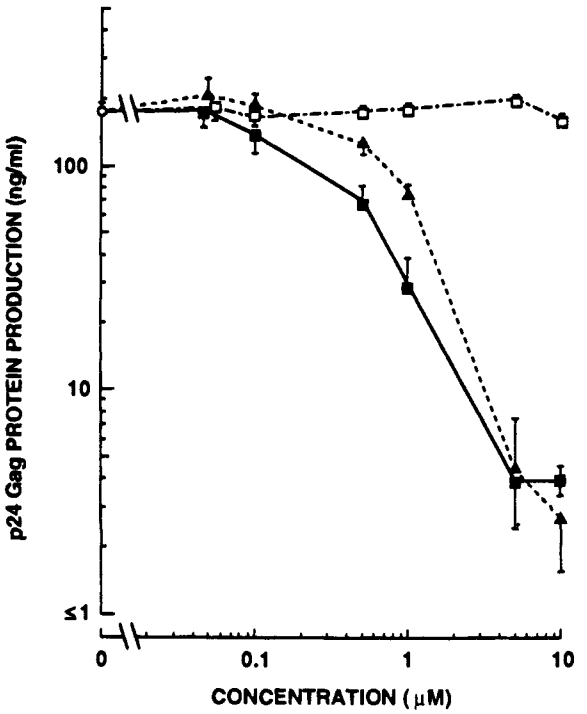


Figure 8. Suppression of HIV-1 replication in PHA-PBM cells by (±)-cytallene (**1b**), (*R*)-(−)-cytallene (**2b**), and (*S*)-(+)-cytallene (**3c**). The data represent the geometric means (±standard deviation) of quadruplicate determinations. For details, see the Experimental Section: (▲) **1b**, (■) **2b**, (□) **3c**.

Antiretroviral Activity

Enantioselectivity of the anti-HIV effect of (±)-cytallene (**1b**) was investigated with both enantiomers **2b**

and **3c**. Phytohemagglutinin-stimulated peripheral blood mononuclear (PHA-PBM) cells infected with a primary HIV-1 isolate were employed as a testing system (Figure 8). It is clear that (*R*)-(−)-enantiomer **2b** (IC_{50} 0.4 and IC_{90} 1.7 μM) is the most active agent. The IC_{50} and IC_{90} values of (±)-cytallene (**1b**) were 0.8 and 3.4 μM, respectively. It is important to note that both analogues virtually completely suppressed viral replication at 10 μM with no detectable toxicity. The (*S*)-(+)-enantiomer (**3c**) was inactive in the testing range employed (IC_{50} > 10 μM). Thus, as predicted earlier,[3] in both cases of antiretroviral allenes, adenallene[4] (**1a**) and cytallene (**1b**), the (*R*)-(−)-enantiomers **2a** and **2b** are responsible for activity of the racemic compounds.[2]

It can be concluded that in both purine and pyrimidine derivatives **1a** and **1b**, the (*R*)-(−)-allenols **2a** and **2b** are the most active components of the racemic mixtures. Enantiomers **2a** and **2b** are now readily accessible by simple chemoenzymatic procedures. This should further facilitate their evaluation as potential drugs for AIDS.

Experimental Section

General Methods (see refs 4 and 6). High-performance liquid chromatography (HPLC) was performed using Synchropak RP-P (C18, 6.5 μm, 2.1 × 250 mm, SynChrom, Inc., Lafayette, IN) or Altex Ultrasphere-Octyl (5 μm, 4.6 × 250 mm, Beckman Instruments, Inc., Fullerton, CA) reverse phase columns. The chiral HPLC was performed on a Chiralcel OB column (Chiral Technologies, Inc., Exton, PA) tribenzoyl cellulose column (10 μm, 4.6 × 250 mm, flow rate 1 mL/min) in the following solvents: S_1 = 80% hexane (0.3% Et_2NH)–20% 2-propanol at 40 °C and S_2 = 85% hexane (0.2% Et_2NH)–15% 2-propanol at room temperature. The peaks were detected at 290 (**2b** and **3c**) or 340 nm (**2c**, **3e**, and **10d**) and retention times (t_R) are in minutes. The NMR spectra were determined in $(CD_3)_2SO$. Tribenzoylcellulose for column chromatography was the product of Riedel-de Haën, Seelze, Germany. For column flash chromatography, 40 g of silica gel/g of the mixture to be separated was used. Tetrahydrofuran (THF) was freshly distilled from benzophenone ketyl before use. Dioxane was passed through activated basic alumina[26] to remove peroxides. For FAB-MS thioglycerol (TG, M 108) was used as a matrix.

Enzymes. Lipases AK (lot no. 10131SS20, activity 22 units/mg) and PS30 (lot no. LPSAR01520, activity 32.6 units/mg) from *Pseudomonas sp.* were the products of Amano Enzyme U.S.A., Ltd., Troy, VA. Subtilisin Carlsberg, Type VIII (lot no. 73H0666, activity 11 units/mg) from Bacillus licheniformis and pig liver esterase (PLE, activity 260 units/mg) were obtained from Sigma Chemical Co., St. Louis, MO.

***N*¹-(4-(Benzoyloxy)-2-butyn-1-yl)cytosine (6). A. Isolation by Chromatography.** A mixture of cytosine (**4**, 4 g, 36 mmol) and NaH (60%, 1.5 g, 37 mmol) in DMF (100 mL) was stirred for 30 min at room temperature. 1-(Benzoyloxy)-4-bromo-2-butyne[5] (**5**, 9.5 g, 37 mmol) was then added slowly with stirring, which was then continued for 15 h. The solvent was evaporated (oil pump), and the crude product was chromatographed on a silica gel column using CH_2Cl_2–MeOH (85:15) as the eluent. The major UV-absorbing fraction was evaporated to give compound **6** (6.26 g, 63%), mp 212–213 °C (lit.[27] mp 189–191 °C), after crystallization from 5% aqueous methanol: UV max (ethanol) 273 nm (ϵ 9500), 229 (ϵ 21 300), 205 (ϵ 21 300); ^1H NMR δ 7.93 (1, d, H_6), 7.92 (1, overlapped with H_6), 7.63 (2, m) and 7.50 (2, m) /C_6H_5/, 7.17 and 7.15 (2, 2 poorly resolved s, NH_2), 5.69 (1, d, H_5), 4.97 and 4.55 (4, 2 s, CH_2); ^{13}C NMR 166.47 (CO), 165.74, 155.99, 145.56, and 94.88 (cytosine), 134.34, 129.83, 129.48, and 129.40 (Ph), 82.60 and 79.18 ($C_{2'}$ and $C_{3'}$), 53.10 ($C_{4'}$), and 38.30 ($C_{1'}$); FAB-MS 783 (1.1, 2M + 2 × 108 + H), 675 (1.5, 2M + TG + H), 567 (4.9, 2M + H), 500 (4.6, M + 2 × TG + H), 392 (6.4, M + TG + H), 284 (86.7, M + H), 105 (100.0, benzoyl). Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

B. Large-Scale Synthesis. Sodium hydride (60%, 15 g, 0.28 mol) was added in portions to a suspension of cytosine (**4**, 40 g, 0.36 mol) in DMF (1.2 L) at room temperature with stirring under N_2. After all of the NaH was added, the suspension was stirred for additional 30 min. 1-(Benzoyloxy)-4-bromo-2-butyne[5] (**5**, 94.5 g, 0.38 mol) was added dropwise over 45 min. The resulting orange/brown solution was stirred at room temperature for 16 h whereupon it was evaporated in vacuo (oil pump). The brown syrupy residue was triturated with CH_2Cl_2 (1 L), and the beige solid that formed was filtered off. This material was stirred with water (5 × 0.8 L) until the washings remained clear. Product **6** was recrystallized from methanol to give white crystals which were dried in vacuo (83.39 g, 82%), mp 205–207 °C.

***N*¹-(4-Hydroxy-2-butynyl)cytosine (7).** A suspension of compound **6** (80.6 g, 0.285 mol) in methanolic ammonia (20%, 3 L) was stirred at 0 °C for 1 h and then at room temperature for 24 h. The resulting yellow solution was concentrated in vacuo to ca. 0.5 L to give a pale yellow solid which was filtered off and washed with methanol. This product was recrystallized from methanol to obtain a white solid (43.18 g, 85%), mp 191–193 °C, identical with compound **7** described previously.[6] It gave a single peak on HPLC (Synchropak RP-P, H_2O, 0.5 mL/min) t_R 4.83.

(±)-Cytallene (1b). Potassium *tert*-butoxide (12.53 g, 0.112 mol, 0.5 equiv) was added to a stirred solution of compound **7** under N_2 (40.00 g, 0.223 mol) in DMF (3 L) cooled in an ice bath. The reaction mixture was stirred for 1 h and then at room temperature for 17 h. Water (100 mL) was then added, and the solution was evaporated in vacuo (oil pump). The residue was triturated with ether (100 mL), and the resultant solid was suspended in water (1 L). (±)-Cytallene (**1b**) was filtered off, and it was recrystallized from methanol after addition of Norit A (5.68 g, 14.2%). The aqueous filtrate was lyophilized, and the residue was suspended in water (200 mL) to give after recrystallization another portion of **1b** (7.02 g, 17.6%). Repeating the whole procedure gave additional **1b** (1.69 g, 4%). The total yield was 14.39 g (36%) of **1b**, identical (UV, IR, NMR) with the compound described previously.[6] The product (all fractions combined) was homogeneous on TLC (CH_2Cl_2–MeOH, 4:1) and HPLC (Synchropak RP-P, H_2O, 0.5 mL/min., t_R 10.41; Altex Ultrasphere, MeOH–H_2O (7:3), 0.8 mL/min), t_R 6.68: EI-MS 179 (M, 7.4), 178 (M − H, 6.5), 162 (M − OH, 100.0), 111 (cytosine, 38.7).

(±)-*N*¹-(4-(Butyryloxy)-1,2-butadien-1-yl)cytosine (8). A mixture of cytallene (**1b**, 0.20 g, 1.12 mmol) and *N,N*-dimethylformamide dimethyl acetal (0.16 mL, 1.23 mmol) in DMF (10 mL) was stirred at room temperature for 21 h. The solution was evaporated in vacuo (oil pump) to a syrup which was dissolved in $CHCl_3$ (10 mL). Butyric anhydride (0.27 mL, 1.67 mmol) and NEt_3 (0.47 mL, 3.35 mmol) were added, and the mixture was stirred at room temperature for 48 h. The reaction was quenched with water, and the solution was evaporated to a syrup which was dissolved in 1-butanol–AcOH–H_2O (5:3:2, 20 mL). The solution was allowed to stand at room temperature for 16 h to remove the N^4-(dimethylamino)methylene group,[28] and then it was evaporated. The crude product was chromatographed on a silica gel column using CH_2Cl_2–MeOH (95:5 and 9:1) as eluents. The appropriate fractions were combined and evaporated to give a syrup which was triturated with ether to give a solid **8** uniform on TLC in CH_2Cl_2–MeOH (9:1). This product was recrystallized from benzene (0.12 g, 52%): mp 102–104 °C; UV max (EtOH) 296 nm (ϵ 12 000), 247 (ϵ 8400), 224 (ϵ 11 500), 204 (ϵ 16 500); ^1H NMR[29] δ 0.84 (3, t, CH_3), 1.49 (2, m) and 2.33 (2, t) /CH_2 of C_3H_7/, 4.66 (2, qt, $H_{4'}$), 5.84 (1, d, H_5), 6.19 (1, q, $H_{3'}$), ca. 7.40 (4, m, H_6, $H_{1'}$ and NH_2); ^{13}C NMR[29] 13.34 (CH_3), 17.86 and 35.19 (CH_2 of C_3H_7), 60.76 ($C_{4'}$), 95.82, 140.54, 153.46, and 165.43 (cytosine), 100.12 and 101.66 ($C_{1'}$ and $C_{3'}$), 172.44 (C=O, ester), 195.05 ($C_{2'}$); FAB-MS 71 (C_3H_7CO, 58.6), 112 (cytosine + H, 95.0), 180 (cytallene + H, 48.2), 162 (M − C_3H_7COO, 100.0), 250 (M + H, 87.6), 358 (M + TG + H, 9.3), 499 (2M + H, 11.0). Anal. ($C_{12}H_{15}N_3O_3$) C, H, N.

(±)-*N*⁴-Acetylcytallene (11a). A mixture of cytallene (**1b**, 0.40 g, 2.23 mmol) and Ac_2O (0.5 mL, 5.3 mmol) in ethanol (50 mL) was heated at 60 °C for 1 h. Another portion of Ac_2O

(0.5 mL, 5.3 mmol) was added, and heating was continued for another hour. After cooling, the solution was concentrated in vacuo to approximately 10 mL and the product began to precipitate. Ether (3 mL) was added and the mixture was allowed to stand at 0 °C overnight. Product **11a** was filtered off, and it was washed thoroughly with ether (0.43 g, 87%). A strongly fluorescent compound 11a was homogeneous on TLC (CH_2Cl_2–MeOH, 9:1): UV max (pH 7) 204 nm (ε 17 300), 220 (ε 14 100), 257 (ε 14 800), 319 (ε 12 700); UV max (ethanol) 205 nm (ε 18 200), 221 (ε 12 900), 261 (ε 14 200), 323 (ε 11 700); ^{1}H NMR δ 2.06 (3, s, CH_3), 4.06 (2, poorly resolved m, $H_{4'}$), 5.10 (1, t, OH), 6.21 (1, q, $H_{3'}$), 7.22 (1, d, H_5), 7.30 (1, qt, $H_{1'}$), 7.95 (1, d, H_6), 10.93 (1, s, NH); ^{13}C NMR 24.44 (CH_3), 58.80 ($C_{4'}$), 96.66, 145.42, 153.31, and 162.32 (cytosine ring), 99.30 ($C_{1'}$), 107.71 ($C_{3'}$), 171.12 (C=O, acetyl), 195.08 ($C_{2'}$); FAB-MS 73 (100.0), 222 (M + H, 21.7) and 330 (M + TG + H, 16.2). Anal. ($C_{10}H_{11}N_3O_3$) C, H, N.

(±)-N^4-Benzoylcytallene (11b). A. From Benzoic Anhydride in Ethanol. Benzoic anhydride (0.13 g, 0.56 mmol) was added to a stirred, refluxing solution of cytallene (**1b**, 0.10 g, 0.56 mmol) in ethanol (13 mL). The reflux was continued for 1 h. Five further portions of benzoic anhydride (0.13 g each) were added every hour. After an additional 1 h of reflux, the mixture was cooled and evaporated. The residue was partitioned between CH_2Cl_2 (75 mL) and saturated aqueous $NaHCO_3$ (2 × 50 mL). The organic phase was washed with water (50 mL), dried ($MgSO_4$), and evaporated, and the residue was chromatographed on a silica gel column. The column was first eluted with CH_2Cl_2 and then with CH_2Cl_2–MeOH (95:5). The latter solvent eluted product **11b** (114 mg, 72%) which was recrystallized from ethanol (99 mg, 63%): mp 92–94 °C (melting then resolidification and decomposition at 145–150 °C); chiral HPLC (S_2) t_R 25.79 [(−)-**2c**] and 33.26 [(+)-**3e**]; UV max (pH 7) 325 nm (ε 15 300), 267 (ε 19 500), and 219 (ε 14 600); (ethanol) 330 (ε 14 500), 269 (ε 20 100), 206 (ε 21 100); ^{1}H NMR (500 MHz) δ 11.31 (bs, 1, NH), 8.04 (d, 1, H_6), 7.99 (m, 2), 7.61 (m, 1) and 7.50 (m, 2) /C_6H_5/, 7.38 (poorly resolved d, 1, H_5), (1, m, $H_{1'}$), 6.26 (q, 1, $H_{3'}$), 5.13 (t, 1, $J_{OH,4'}$ = 6.5 Hz, OH), 4.33 (t, 1, J = 5.0 Hz, OH, EtOH), 4.10 (m, 2, $H_{4'}$), 3.42 (q, 1, CH_2, EtOH), 1.04 (t, 1, J = 7.0 Hz, CH_3, EtOH); ^{13}C NMR (125.70 Hz) 195.60 (d, $C_{2'}$), 167.00 (CO, benzoyl), 163.90, 154.00, 145.70, and 97.87 (cytosine ring), 133.48, 133.25, 128.93, and 128.90 (phenyl), 108.18 ($C_{3'}$), 99.70 ($C_{1'}$), 59.24 and 56.48 ($C_{4'}$ and CH_2, EtOH), 19.00 (CH_3, EtOH); FAB-MS 392 (M + TG + H, 7.1), 284 (M + H, 12.6), 216 (N^4-benzoylcytosine, 18.9), 105 (benzoyl, 100.0), 77 (Ph, 30.9). Anal. ($C_{15}H_{13}N_3O_3 \cdot C_2H_5OH$) C, H, N.

B. Using Trimethylsilyl Chloride and Benzoyl Chloride. Trimethylsilyl chloride (1.77 mL, 14 mmol) was added to a suspension of cytallene (**1b**, 1.00 g, 5.6 mmol) in pyridine (40 mL), and the mixture was stirred for 15 min at room temperature. Benzoyl chloride (0.78 mL, 6.7 mmol) was added, and the stirring was continued for 2 h. The reaction mixture was stored at 0–4 °C for 12 h, allowed to warm to ambient temperature, and stirred for 1 h. Water (20 mL) was added, and the solution was stirred for 20 min. After addition of aqueous ammonia (30%, 20 mL) and stirring for another 20 min, the mixture was evaporated to dryness. The residue was triturated with ether (25 mL) to give a precipitate which was partitioned between CH_2Cl_2 (1 L) and saturated aqueous $NaHCO_3$ (2 × 200 mL). The organic phase was washed with water (200 mL), dried ($MgSO_4$), and evaporated to give a solid which was recrystallized from ethanol with the aid of Norit A (0.41 g, 22%). The combined mother liquor and ether portions were evaporated, and the residue was recrystallized as described above to give **11b** (0.37 g, 20%). This product was identical with a sample prepared by method A (UV, NMR and TLC).

(±)-N^4-(9-Fluorenylmethoxycarbonyl)cytallene (11c). Pyridine (3 × 10 mL) was evaporated from cytallene (**1b**, 0.10 g, 0.56 mmol) which was then suspended in the same solvent (3.3 mL), Me_3SiCl (0.18 mL, 1.40 mmol) was added, and the mixture was stirred at room temperature for 15 min. After addition of 9-fluorenylmethyl chloroformate (0.17 g, 0.67 mmol), the stirring was continued for another 2 h. The mixture was then cooled in an ice bath, and water (0.57 mL) was added followed, after 5 min, by NH_4OH (30%, 0.57 mL). The solution was stirred for 15 min at room temperature, whereupon it was evaporated and the residue was chromatographed on a silica gel column using CH_2Cl_2 and CH_2Cl_2–MeOH (98:2) as eluents. The Fmoc derivative **11c** was eluted with CH_2Cl_2–MeOH (95:5), 196 mg (87%), after recrystallization from methanol 159 mg (71%): mp >115 °C dec; UV max (EtOH) 318 nm (ε 11 200), 300 (ε 12 700), 290 (ε 9000), 261 (ε 28 100), 213 (ε 31 700); ^{1}H NMR δ 11.10 (bs, 1, NH), 7.93 (d, 1, H_6, $J_{6,5}$ = 7.5 Hz), 7.85, 7.76, 7.39 and 7.30 (m, 9, aromatic H's + $H_{1'}$), 7.04 (d, 1, H_5, $J_{5,6}$ = 7.5 Hz), 6.22 (qt, 1, $H_{3'}$), 5.15 (poorly resolved t, 1, OH), 4.35 (d, 2, CH_2 of Fmoc), 4.28 (t, 1, CH of Fmoc), 4.07 (apparent bs, 2, $H_{4'}$). Compound **11c** is not completely stable in CD_3SOCD_3 at ambient temperature. It suffered elimination of the Fmoc group to give cytallene **1b** which was confirmed by TLC in CH_2Cl_2–MeOH (9:1): FAB-MS 803 (2M + H, 0.7), 510 (M + TG + H, 2.7), 402 (M + H, 21.6), 179 (cytallene, 100.0), 165 (fluorene − H, 32.5), 112 (cytosine + H, 26.3). Anal. ($C_{23}H_{19}N_3O_4$) C, H, N.

Enzymatic Acetylation of (±)-Adenallene (1a). (±)-Adenallene[6] (**1a**, 50 mg, 0.25 mmol) was dissolved in warm acetone–THF (2:1, 9 mL). After cooling, vinyl acetate (68 μL, 0.74 mmol) and lipase PS30 (50 mg, 1630 units) were added, and the mixture was magnetically stirred at room temperature for 24 h. The solvents were evaporated, and the crude product was chromatographed on a column of silica gel (10 g). The (−)-4′-*O*-acetyladenallene (**10c**) was eluted with CH_2Cl_2–MeOH (95:5), and the appropriate fractions were combined and concentrated under reduced pressure to give solid **10c** (15 mg, 25%), $[\alpha]^{25}_D$ −58° (*c* 0.15, MeOH), 30.5% ee based on $[\alpha]^{25}_D$ of (*S*)-(−)-4′-*O*-acetyladenallene,[4] 190°. (+)-Adenallene (**3b**) was eluted with CH_2Cl_2–MeOH (9:1): yield 37 mg (74%), $[\alpha]^{25}_D$ 23°, 12% ee based on $[\alpha]^{25}_D$ (ref 4) 181°.

Enzymatic Acylation of (±)-N^4-Acetylcytallene (11a). N^4-Acetylcytallene (**11a**, 50 mg, 0.23 mmol) was dissolved in acetone–THF (3:2, 5 mL) by warming and sonication. After cooling, vinyl butyrate (86 μL, 0.68 mmol) and lipase AK (45 mg, 990 units) were added, and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated under reduced pressure, and the two compounds were separated by column chromatography as described in the previous experiment. (−)-Ester **10b** was eluted with CH_2Cl_2–MeOH (95:5): yield 20 mg (30%); $[\alpha]^{25}_D$ −58° (*c* 0.1, MeOH). Continuing elution with CH_2Cl_2–MeOH (9:1) gave N^4-acetylcytallene (**3d**): 28 mg (57%), $[\alpha]^{25}_D$ 54° (*c* 0.1, MeOH).

Enzymatic Acylation of (±)-Cytallene (1b). (±)-Cytallene (**1b**, 60 mg, 0.33 mmol) was dissolved in pyridine (8 mL) with warming (~40 °C). The solution was allowed to cool to room temperature, and vinyl butyrate (0.13 mL, 1.1 mmol) was added followed by subtilisin (50 mg, 550 units). The reaction was stirred at room temperature for 21 h, but TLC indicated only ca. 30% reaction. Additional vinyl butyrate (42 μL, 0.33 mmol) and subtilisin (20 mg, 220 units) were added, and the reaction mixture was stirred at room temperature for a further 24 h. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed as described in the previous experiments. (−)-4′-*O*-Butyrylcytallene (**10a**) was eluted with CH_2Cl_2–MeOH (9:1), and the appropriate fractions were evaporated. A syrupy residue was triturated with ether, and the resulting precipitate was collected by filtration (33 mg, 39.5%), $[\alpha]^{25}_D$ −14° (*c* 0.1, MeOH). (+)-Cytallene (**3c**) was eluted with CH_2Cl_2–MeOH (4:1), 22 mg (37%), $[\alpha]^{25}_D$ 42° (*c* 0.1, MeOH), 18% ee.

Time Course of the Reaction of (±)-N^4-Acetyl-, N^4-Benzoyl-, and N^4-Fmoc-cytallene (11a, 11b, and 11c) with Vinyl Butyrate Catalyzed by Lipase AK or PS30 in Organic Solvents. A mixture of compound **11a**, **11b**, or **11c** (23 μmol), lipase AK (4.5 mg, 99 units) or PS30 (4.5 mg, 147 units), and vinyl butyrate (0.17 mL, 1.4 mmol) in organic solvent (1 mL) was magnetically stirred at room temperature. At appropriate time intervals aliquots were removed, and they were subjected to TLC in CH_2Cl_2–MeOH (95:5). The spots were eluted with ethanol, and the amounts of products were determined by UV spectrophotometry at 330 nm (**11c** at 300 nm). For further details see Figures 3–6.

(*R*)-(−)- and (*S*)-(+)-Cytallene (2b and 3c). A mixture of N^4-benzoylcytallene ethanolate (**11b**, 1.00 g, 3.0 mmol), vinyl butyrate (1.5 mL, 12 mmol), molecular sieves 4A (6.03 g), and lipase AK (1.01 g, 22 220 units) in THF (160 mL) was stirred at room temperature for 2 h. The esterification was monitored by TLC in CH_2Cl_2–MeOH (9:1). The reaction mixture was then filtered, and the filtrate was evaporated to give a solid which was flash chromatographed on a silica gel column using $CHCl_3$ as an eluent to give (−)-ester **10d** as a hygroscopic foam, chiral HPLC (S_2) t_R 43.34. Further elution with $CHCl_3$–MeOH (4:1) furnished (+)-N^4-benzoylcytallene (**3e**), chiral HPLC (S_2) t_R 32.67. (−)-Ester **10d** was dissolved in NH_3/methanol (20%, 30 mL), and the mixture was stirred at room temperature for 4 h. The resultant solution was evaporated, and the product was chromatographed on a silica gel column using first chloroform (100 mL), then $CHCl_3$–MeOH (9:1, 100 mL), and, finally, $CHCl_3$–MeOH (7.5:2.5) to give (−)-cytallene (**2b**, 0.27 g, 100%) which was >95% optically pure by chiral HPLC. Recrystallization from methanol gave two crops of **2b** (0.2 g, 74%) and raised the optical purity to 99% (Figure 1): mp >170 °C dec; $[\alpha]^{25}_D$ −232.4° (*c* 0.2, MeOH); HPLC (Synchropak RP-P, H_2O, 0.5 mL/min) t_R 10.56, t_R of (±)-**1b** 10.65; UV max (pH 7) 291 nm (ϵ 12 600), 224 (ϵ 12 300), 201 (ϵ 15 400); CD_{max} (pH 7) 246 nm (Θ −13 400). The ^1H and ^{13}C NMR spectra were identical with those of (±)-cytallene[6] (**1b**). Anal. ($C_8H_9N_3O_2$) C, H, N.

(+)-N^4-Benzoylcytallene (**3e**) was deprotected and chromatographed as described for (−)-ester **10d** to give (+)-cytallene (**3c**, 0.22 g, 82% overall yield) which was >95% optically pure. Recrystallization from methanol afforded two crops of **3c** (0.19 g, 70%) of 99% optical purity (Figure 1): mp > 170 °C dec; $[\alpha]^{25}_D$ 229.5° (*c* 0.2, MeOH); HPLC (see **2b**) t_R 10.59. UV max (pH 7) 291 nm (ϵ 12 300), 224 (ϵ 12 000), 201 (ϵ 14 600); CD_{max} (pH 7) 246 nm (Θ 13 100). The ^1H and ^{13}CNMR spectra were identical with those of (±)-cytallene[6] (**1b**). Anal. ($C_8H_9N_3O_2$) C, H. N.

Crystal Data. The crystals for X-ray diffraction obtained by a slow crystallization of (+)-cytallene (**3c**) from methanol were colorless, orthorhombic prismatic, 0.20 × 0.10 × 0.04 mm, Cu Kα radiation, λ = 1.541 82 Å; *F*(000) 376; temperature 23 ± 1 °C; Enraf-Nonius diffractometer.[30] Space group: $P2_12_12_1$ (No. 19); a = 6.843(1) Å; b = 9.563(1) Å; c = 13.198(1) Å; V = 863.6 Å^3; D (calculated) = 1.378 g/cm^3. Corrections: Lorentz-polarization; linear decay (from 1.000 to 1.007 on I); empirical absorption (max. 88.5% on I); extinction[31] (coefficient = 1.05 × 10^{-5}); maximum 2Θ 148.0°; *hkl* ranges (Friedel pairs collected) h = 0:8, k = 0:11, l = 0:16; number of reflections measured 2030 total (including Friedel pairs); phase solution carried out by direct methods using MolEN[32] (also used for all further standard calculations) with a unique set of data (Friedel pairs averaged, $R(I)$ = 1.9%).

Least Squares Refinement. A total of 1812 reflections with $F_o^2 > 3.0\sigma(F_o^2)$; hydrogen atoms, fixed; parameters refined, 146; R = 0.028 (both Friedel pairs); ESD of observation of unit weight, 1.32; convergence, largest shift 0.01σ; minimization function, $\sum w(|F_o| - |F_c|)^2$; least-squares weights, $4F_o^2/\sigma(F_o^2)$; instrument instability factor, 0.040; high peak in final difference map, 0.22(4) e/Å^3; low peak in final difference map, −0.27(4) e/Å^3.

Absolute Configuration. Both possible enantiomers of cytallene produced essentially identical least squares residual when anomalous dispersion was included, and thus the method of LePage et al.[33] was employed. Calculations were carried out with *ad hoc* programs written by one of us (J.V.S.) for an IBM 3090 computer. The 52 reflections having $F_c > 3.5$ and $(F_{c+}^2/F_{c-}^2 - 1.0) > 0.015$ were employed and the intensities of seven measurements, at ψ values of ±30°, ±20°, ±10°, and 0°, were added together. The maximum time for each measured was 120 s, and Friedel pairs were measured sequentially. Of the 52 data, 6 measurements proved correct and 46 incorrect, thus the opposite enantiomer from that randomly chosen for refinement was indicated with a probability error of 1 in 10^6 according to LePage et al.[33] The indicated absolute configuration is used in all tables and the ORTEP picture of the molecule (Figure 7).

Inhibition of HIV-1 Replication in Phytohemagglutinin-Activated Peripheral Blood Mononuclear (PHA-PBM) Cells. The PHA-PBM cells (5 × 10^5 cells/mL) from an HIV-1 seronegative donor were incubated for 2 h in the presence of various concentrations of the tested analogue in culture medium/1 mL of RPMI1640 containing 15% fetal serum, 4 mM L-glutamine, penicillin (50 units/mL), and exposed to a clinical HIV-1 isolate,[34] HIV-$1_{ERS104pre}$, at a 500 $TCID_{50}$ (50% tissue culture infectious dose). The cells were continuously exposed to the analogue, and they were cultured in 5% CO_2-containing humidified air. The amounts of p24 *gag* protein in culture medium were determined by radioimmunoassay on day 7 of the culture. The results are given in Figure 8.

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Supplementary Material Available: Crystallographic data in Tables 1–9 (27 pages). Ordering information is given on any current masthead page.

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