Novel Modifications in the Alkenyldiarylmethane (ADAM) Series of Non-Nucleoside Reverse Transcriptase Inhibitors

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Received July 6, 1999

In an effort to obtain more insight into the interaction between HIV-1 reverse transcriptase and the alkenyldiarylmethanes (ADAMs), a new series of compounds has been synthesized and evaluated for inhibition of HIV-1 replication. The modifications reported in this new series include primarily changes to the alkenyl chain. The most potent compound proved to be methyl 3′,3″-dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexenoate (**28**), which displayed an EC_{50} of 1.3 nM for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cells. ADAM **28** inhibited HIV-1 reverse transcriptase with an IC_{50} of 0.3 μM. Mutations that conferred greater than 10-fold resistance to ADAM **28** clustered at residues Val 106, Val 179, Tyr 181, and Tyr 188. Results derived from this series indicate that ADAMs containing chlorines in the aromatic rings might bind to HIV-1 reverse transcriptase in a slightly different mode when compared with those analogues incorporating bromine in the aromatic rings.

Introduction

The therapeutic agents currently approved for the treatment of HIV infections include two non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs), nevirapine and delavirdine.[1,2] Although the therapeutic potential of this class of drugs has been compromised by the rapid development of resistance, its use has been recently recommended when employed in combination therapy.[1,3] Encouraging results have been reported when triple drug combinations that include one NNRTI are used.[4] In most of these studies, a reduction in HIV-1 RNA level, or an increase in CD4 lymphocyte count, or both have been observed and are associated with a delay in disease progression. However, there are certain factors that restrict the selection of the agents for combination therapy, including drug compatibilities, adverse effects, and cross-resistance.[1] Therefore, the development of new NNRTIs with pharmacokinetic characteristics and resistance mutation profiles more compatible with other anti-HIV agents is important for a more successful application of this class of drugs in combination therapy.

We previously reported the alkenyldiarylmethanes (ADAMs) as a new class of NNRTIs.[5−8] Further interest in this class of NNRTIs has been augmented by the finding that one of our compounds, methyl 3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexenoate (ADAM II, **1**), proved to be a nanomolar inhibitor (EC_{50} = 13 nM) of the cytopathic effect of HIV-1_{RF} in CEM-SS cells.[7,8] In addition, virus strains containing mutations that confer resistance to AZT showed increased sensitivity to ADAM II, which indicated the possible role of ADAM II in combination therapy with AZT.[8] In this report, we present a new series of ADAMs which has been evaluated for prevention of the cytopathic effect of HIV-1 and for inhibition of HIV-1 reverse transcriptase (RT).

Chemistry

During the design of ADAM II, it was proposed that functional groups which are capable of acting as hydrogen bond acceptors present at the end of the alkenyl chain could interact favorably with the Glu 138 or Lys 103 residue of HIV-1 RT. The alkenyl chain length and the nature of the functionality present at the end of the chain were initially investigated as described in our last report.[8] In an effort to gain more insight into the role of this part of the molecule during the binding with HIV-1 RT, we decided to explore additional modifications in the alkenyl chain, including: (1) its length; (2) the functional group present at the end of it; and (3) reduction of the double bond. Two other modifications were planned in this new series: replacement of the chlorine atoms present in **1** with other halogens and modifications of the aromatic methoxycarbonyl groups. With these considerations in mind, a new series of ADAMs was synthesized and evaluated for inhibition of HIV-1 RT and for prevention of the cytopathic effect of HIV-1 in cell culture.

A set of compounds was planned in which the methoxycarbonyl group of the alkenyl chain present in **1** would be maintained constant but the length of the chain would be varied. The first analogue of this set that incorporates a two-carbon shorter alkenyl chain was prepared from the previously reported carboxylic acid **2**.[9] Reaction of **2** with diazomethane afforded analogue **3**.

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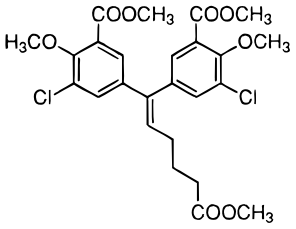
10.1021/jm990343b CCC: $18.00
Published on Web 10/21/1999



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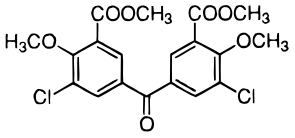

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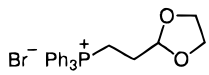

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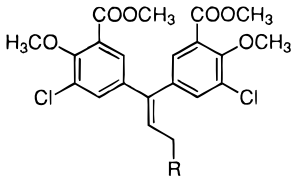
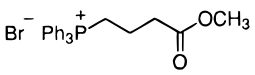


2 R = COOH
3 R = $COOCH_3$
4 R =



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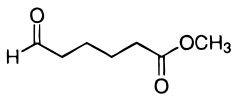

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5 R = CH_2COOCH_3
6 R = $CH_2CH_2CH_2OH$
7 R = $CH_2CH_2CH_2Br$
8 R = $CH_2CH_2CH_2Cl$
9 R = $CH_2CH_2CH_2N_3$
10 R = $CH_2CH_2CH_2OCONHCH_2CH_3$
11 R = CH_2CH_2CHO
12 R = $CH_2CH_2CH_2COOCH_3$

Wittig chemistry was used to install the alkenyl linker chain in some of the new ADAMs. Reacting ketone **13**[10] with the ylide derived from the commercially available phosphonium bromide **14** and sodium bis(trimethylsilyl)amide afforded dioxolane **4**. In a similar fashion, the ylide obtained from phosphonium bromide **15**[11] was reacted with benzophenone **13** to provide ester **5**. This analogue incorporates the same functionality at the end of the alkenyl chain as that present in ADAM II (**1**), but the chain is shortened by one carbon.

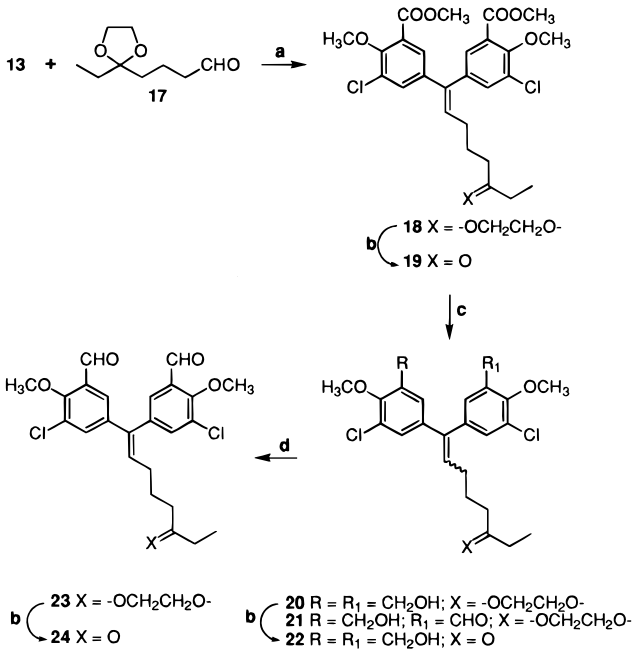
The incorporation of the azido group at the end of the alkenyl chain is a modification that provided interesting results in our previous work.[8] Therefore, we planned to prepare a series of derivatives that would incorporate this functionality, halogens, or a carbamate group. The latter functionality was considered because it contains three moieties that can be involved in hydrogen bonding with the enzyme. The new groups would be attached at the end of a five-carbon alkenyl chain. This chain length was chosen because it would situate the new functionality in the same position as the carbonyl oxygen of the alkenyl chain ester group of ADAM II. It is believed that this oxygen is relevant in the binding of ADAM II with RT. This new series of analogues was prepared from alcohol **6**.[8] Bromide **7** was obtained when **6** was reacted with carbon tetrabromide and triphenylphosphine in acetonitrile.[12,13] Reacting alcohol **6** with methanesulfonyl chloride in dichloromethane gave chloride **8**.[14] In a separate experiment, the crude product was reacted with sodium azide in DMF to afford azide **9**. Conversion of alcohol **6** to the *N*-ethylcarbamate **10** was achieved by reacting the alcohol with excess ethyl isocyanate and a catalytic amount of DMAP.[15,16]

Aldehyde **11** was planned based not only on the consideration that this functionality might interact with the enzyme through hydrogen bonding in a similar fashion as the methoxycarbonyl present in **1** but also on the fact that this group might react with Lys 103 to give a covalently bonded inhibitor. Analogue **11** would incorporate the same chain length present in ADAM II. This compound was obtained by Swern oxidation of alcohol **6**.[17]

The McMurry reaction was used for the preparation of additional ADAM II analogues.[18,19] Among the different titanium reagents reported for this reaction, we have employed the commercially available titanium tetrachloride−tetrahydrofuran complex (1:2) and zinc dust for all our reactions. In this way, we have been able to carry out several couplings between benzophenone **13** and different aldehydes, providing the corresponding cross-coupled products in good yields (50−75%). Under these conditions, benzophenone **13** was reacted with aldehyde **16**[20] in THF to give ester **12**. This compound incorporates the methoxycarbonyl group attached at the end of a six-atom chain, which makes it the one-carbon-extended chain ADAM II analogue.

For the preparation of congener **18**, benzophenone **13** was coupled with aldehyde **17** (obtained in three steps from methyl 5-chloro-5-oxovalerate)[21,22] under conditions previously described (Scheme 1). Interestingly, the ketal group of the alkenyl chain of **18** was not affected under the acidic conditions of the McMurry coupling. This compound was then used for the preparation of several analogues. Reduction of **18** with DIBAL-H (4.3 equiv) in dichloromethane−toluene afforded diol **20** as the main product, along with some aldehyde **21**. Conversion of diol **20** into the corresponding dialdehyde **23** was achieved by Swern oxidation.[17] The direct conversion of diester **18** to dialdehyde **23** was attempted using DIBAL-H (2.2 equiv), but only mixtures of **20** and **21** were obtained. Removal of the ketal group from **18**, **20**, and **23** with pyridinium *p*-toluenesulfonate (PPTS) in

Scheme 1[a]

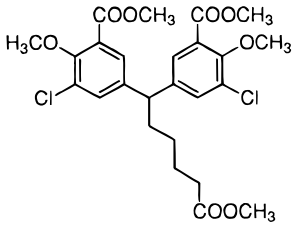


[a] Reagents and conditions: (a) $TiCl_4$·2THF, Zn(0), THF, reflux, 2 h; then **13** and **17**, THF, reflux, 1.5 h, 75.5%; (b) PPTS, $CH_3COCH_3-H_2O$, reflux, 2.25 h; **19**: 62.5%; **22**: 64.2%; **24**: 64.8%; (c) DIBAL-H, $CH_2Cl_2-PhCH_3$, −78 °C, 4 h, 93.5%; (d) (i) $(COCl)_2$, DMSO, −78 °C; (ii) Et_3N, −78 °C to rt, 60%.

refluxing acetone−water provided the corresponding ketones **19**, **22**, and **24**, respectively.[23]

Hydrogenation of the double bond has not been previously studied. This is a significant change to the conformational rigidity of this class of compounds. This modification would provide valuable information about the rigidity required for optimal interaction of this class of NNRTIs with the enzyme. Hydrogenation of **1** under atmospheric pressure in ethyl acetate, using platinum oxide as catalyst, afforded **25**.

Replacement of the chlorines of ADAM II by bromines was then considered. In our previous series,[5,8] the interchange of these two halogens provided congeners with similar antiviral potency. Therefore, we decided to prepare analogue **28**. The McMurry coupling between ketone **26**[6] and aldehyde **27**[24] under the conditions discussed above gave ester **28**. Compound **28** was also prepared using Wittig chemistry. However, preparation of **28** by the Wittig reaction was not consistently reproducible.



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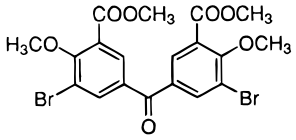

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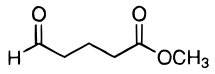

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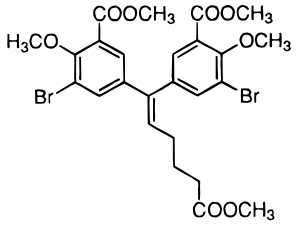

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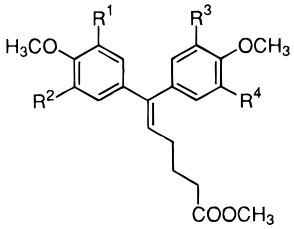
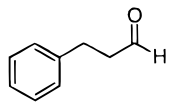


28a $R^1 = R^3 = COOCH_3$; $R^2 = R^4 = Br$
28b $R^1 = R^4 = Br$; $R^2 = R^3 = COOCH_3$
28c $R^1 = R^3 = Br$; $R^2 = R^4 = COOCH_3$
28d $R^1 = R^4 = COOCH_3$; $R^2 = R^3 = Br$



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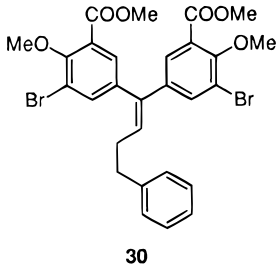

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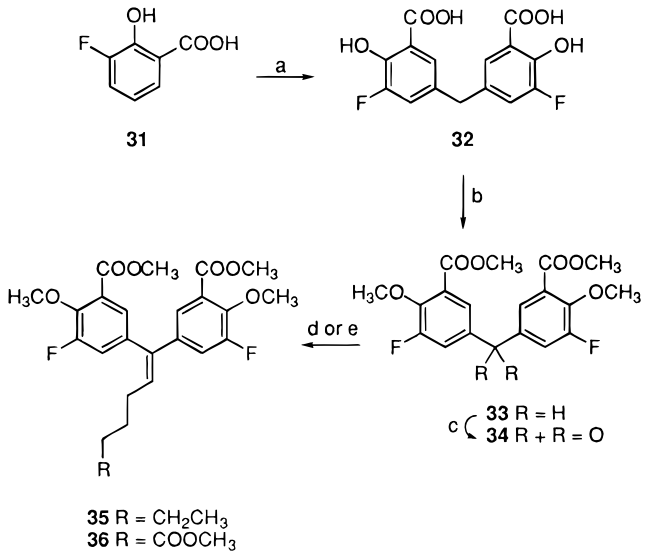
The potent activity of the ADAM analogues with functionality in the side chain suitably situated to form hydrogen-bonding or electrostatic interactions with Lys 103 led us to consider other compounds with potential for a cation−π interaction.[25] This interaction has been shown recently to be approximately energetically equivalent to a hydrogen bond.[26] ADAM **30** was shown by molecular modeling to have the alkenyl chain phenyl ring situated for optimal cation−π interaction with Lys 103. Compound **30** was prepared by a McMurry coupling of benzophenone **26** with hydrocinnamaldehyde (**29**) under the conditions described above.

In our previous series, we reported the replacement of the aromatic chlorines or bromines with iodines. To complete the aromatic halogen series, difluoro ADAM analogues **35** and **36** were planned (Scheme 2). The synthesis of these compounds started with 3-fluorosalicylic acid (**31**).[27] This compound was condensed with formaldehyde under acidic conditions in methanol to yield the difluorodisalicylmethane intermediate **32**. Permethylation of **32** with dimethyl sulfate in refluxing acetone using potassium carbonate as the base provided **33**. Oxidation of **33** to the benzophenone **34** was accomplished with chromic anhydride. McMurry coupling of benzophenone **34** with hexanal or methyl 5-oxopentanoate (**27**) afforded difluoro analogues **35** and **36**, respectively.

Scheme 2[a]



[a] Reagents and conditions: (a) H_2SO_4, MeOH, H_2O, −78 to 0 °C, 1 h; then HCHO, −78 °C to ambient temp, overnight, 95%; (b) Me_2SO_4, K_2CO_3, acetone, reflux, 20 h, 79%; (c) Ac_2O, CrO_3, 20 h, 81%; (d) $TiCl_4$·2THF, Zn(0), THF, reflux, 1 h; then **34** and hexanal, THF, reflux, 30 min, 66%; (e) $TiCl_4$·2THF, Zn(0), THF, reflux, 1 h; then **34** and **27**, THF, reflux, 45 min, 46%.

Biological Results and Discussion

The 20 new ADAMs synthesized in this study were evaluated for prevention of the cytopathic effect of HIV-1_{RF} in CEM-SS cells and for cytotoxicity in uninfected CEM-SS cells, and the results are listed in Table 1. These compounds were also tested for inhibition of HIV-1 RT, and the resulting IC_{50} values are also included in Table 1. Nine ADAMs prevented the cytopathic effect of HIV-1_{RF} with EC_{50} values ranging from 50.5 to 0.0013 μM. These analogues were also found to inhibit HIV-1 RT with poly(rC)·oligo(dG) as the template primer with IC_{50} values ranging from 100 to 0.3 μM.

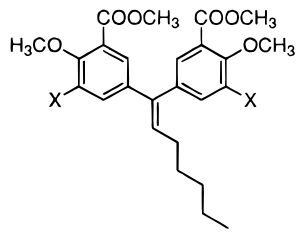
Table 1. Anti-HIV-1 Activities of ADAMs

	RT IC_{50} (μM)[a]		XTT assay		
compd	rCdG	rAdT	EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
1	0.3	1.9	0.013	31.6	2430
3	2.2	>100	NA	13.3	
4	3.2	20.5	6.5	26.0	20.5
5	7.2	>100	4.8	29.4	6.1
7	11.0	1.9	2.8	98.7	35.2
8	70	NI	50.5	>316	6.2
9	1.3	0.8	7.1	175.5	24.7
10	3.2	1.2	NA	8.4	
11	4.9	4.2	NA	9.6	
12	0.47	3.35	1.5	152.5	101
18	NI	NI	NA	19.9	
19	1.0		2.8	24.6	8.8
20	3.2	31.6	NA	16.9	
21	31.6	NI	NA	5.8	
22			NA	14.2	
24			NA	1.13	
25	100		NA	21.3	
28	0.3	1.0	0.0013	13.0	10000
30			NA	31.50	
35	10		NA	13.4	
36			1.9	68.3	35.9

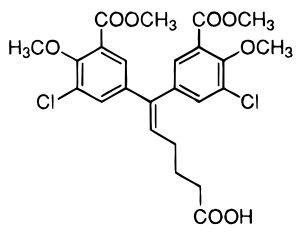
[a] Inhibitory activity vs HIV-1 RT with either rCdG or rAdT as the template primer. [b] The EC_{50} is the 50% inhibitory concentration for cytopathicity of HIV-1_{RF} in CEM-SS cells. [c] The CC_{50} is the 50% cytotoxic concentration for mock-infected CEM cells. [d] The TI is the therapeutic index, which is the CC_{50} divided by the EC_{50}. NA means there was no observed inhibition of HIV-1 cytopathicity up to the cytotoxic concentration in uninfected cells.

Ester **28** was the most potent compound of the new series. This compound only differs from **1** in the halogens present on the aromatic ring, incorporating bromines instead of chlorines. ADAM **28** displayed an EC_{50} of 0.0013 μM for inhibition of the cytopathic effect of HIV-1_{RF} in CEM cells, which represents a 10-fold increase in potency over ADAM II (**1**) (EC_{50} = 0.013 μM). The therapeutic index was also increased by a factor of 4. The other new compounds that displayed anti-HIV-1 activity are methyl esters **12** and **36**, ketone **19**, bromide **7**, methyl ester **5**, dioxolane **4**, azide **9**, and chloride **8**. The remaining new ADAMs did not prevent the cytopathic effect of HIV-1 at concentrations up to those resulting in cytotoxicity in uninfected cells.

As predicted from our previous series,[8] the increase in antiviral potency observed for **28** vs **37** (EC_{50} = 9.2 μM) and **1** (EC_{50} = 0.013 μM) is not associated with an increase in potency for inhibition of HIV-1 RT with poly(rC)·oligo(dG) as the template primer, even though the anti-HIV activity of these compounds clearly results from inhibition of RT. ADAM II (**1**), ester **28**, and our initial lead compound ADAM I (**37**)[6] have very similar IC_{50} values for inhibition of HIV-1 RT (**1**: IC_{50} = 0.3 μM; **28**: IC_{50} = 0.3 μM; **37**: IC_{50} = 0.38 μM) with poly(rC)·oligo(dG) as the template primer, but still there is a >7000-fold difference in antiviral potency between **28** (EC_{50} = 0.0013 μM) and **37** (EC_{50} = 9.2 μM). ADAM **28** was also tested for inhibition of HIV-1 RT with poly(rA)·oligo(dT) as the template primer. Only a small difference in IC_{50} values is observed between **1** and **28** (**1**: IC_{50} = 1.9 μM; **28**: IC_{50} = 1.0 μM). The remaining new ADAMs were less potent than **28** as inhibitors of HIV-1 RT with poly(rC)·oligo(dG) as the template primer. Interestingly, the second most potent analogue of this series, methyl ester **12**, was also the only new ADAM that inhibited the enzyme at submicromolar concentrations with poly(rC)·oligo(dG) as the template primer (IC_{50} = 0.47 μM). Several congeners were found to inhibit HIV-1 RT at low micromolar concentrations, but they did not display antiviral activity. Most of the results obtained in the present and previous studies indicate there is not a strong correlation in the ADAM series between potency for inhibition of RT in cell-free systems with poly(rC)·oligo(dG) as the template primer and potency for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cells. The in vitro IC_{50} for compound **28** vs HIV-1 RT with poly(rC)·oligo(dG) as the template/primer (0.3 μM) is significantly higher than its EC_{50} for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cell culture (1.3 nM). This difference is not at all unusual for the NNRTIs.[28−31] As discussed elsewhere, the discrepancy may simply reflect the differences between the in vitro assay, in which synthetic template/primer has been added, and the cellular system.[30] This might imply that another template primer may be more advantageous for evaluating the inhibitory activity of these and other NNRTIs on HIV-1 RT and correlating the results with antiviral potency. In certain cases, primed ribosomal RNA (16S/23S) appears to more accurately resemble native viral RNA than synthetic templates in the inhibition of HIV-1 RT assay.[32]



37 X = Br
39 X = Cl



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To gain a better understanding of the interaction of ADAM **28** with HIV-1 RT, we constructed a hypothetical model for the docking of this compound in the NNRTI binding pocket. The model was derived in the following manner. Using Sybyl (Tripos, Inc., version 6.5, 1998), the X-ray crystal structure of HIV-1vRT complexed with nevirapine (1VRT)[33] was clipped to include enzyme information within a 9 Å sphere of nevirapine. Compound **28** was then overlayed onto the structure of nevirapine, and nevirapine was deleted from the complex. The protein was frozen except for Lys 103 using the aggregate option of Sybyl. The Lys 103 side chain amino group was rotated toward the side chain methyl ester of the ligand and then frozen in order to facilitate minimization in the direction of the local minimum which includes the hydrogen-bonding interaction between these two moieties. Gasteiger−Hückel charges were calculated for compound **28**, and the charges from the Kollman united-atom force field were loaded for the enzyme portion of the complex. The complex was minimized using the conjugate gradient method employing the current charges option in the energy setup. The model obtained in this manner is displayed in Figure 1. Further support for this hypothetical binding model was obtained by minimizing a series of enzyme-bound conformers designated by structures **28a**−**d**. The "right" and "left" aromatic rings of these structures correspond to the "right" and "left" aromatic rings of the

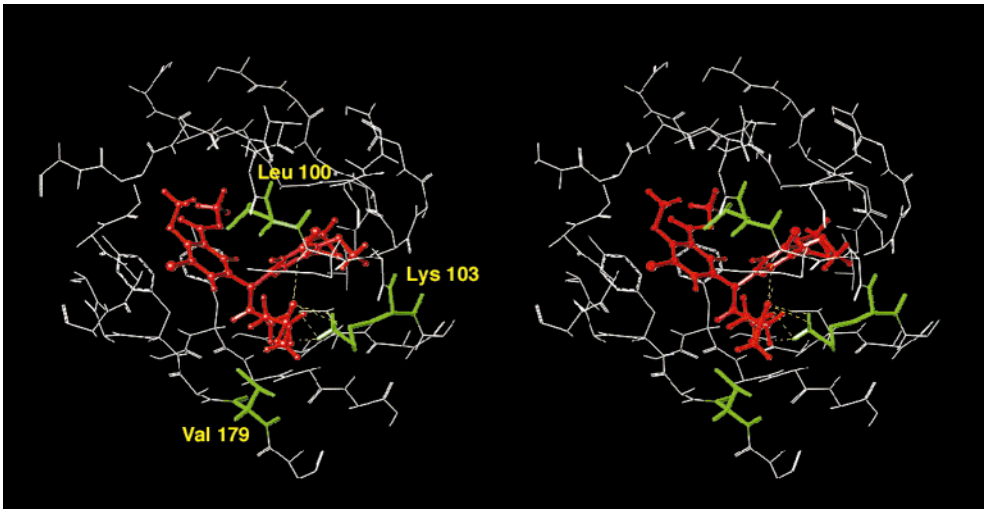


Figure 1. Hypothetical model of ADAM **28** docked in the NNRTI binding pocket of HIV-1 RT. The ligand is shown in red, while the Leu 100, Lys 103, and Val 179 residues are shown in green. Potential hydrogen-bonding interactions between the alkenyl chain methoxycarbonyl group of **28** and the backbone amide of Lys 101 and the side chain amino group of Lys 103 are shown as yellow dotted lines.

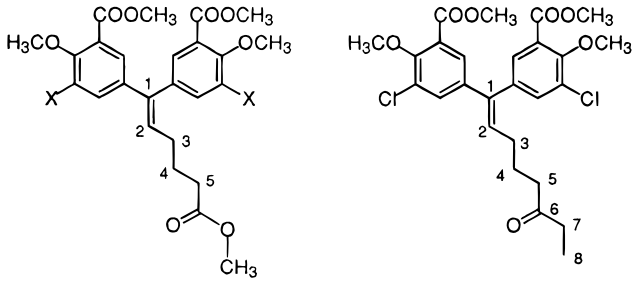
Table 2. Energy Calculations for Enzyme-Bound Conformers of **28**

conformer	total E^a	steric E	electrostatic E
28a	−47.456	−49.691	−2.235
28b	−33.341	−31.665	−1.649
28c	−25.024	−25.570	−0.546
28d	−29.550	−30.043	0.493

[a] Energy values are in kcal/mol.

ligand in the complex as designated by the structure of the ligand−enzyme complex displayed in Figure 1. The conformers chosen for these minimizations are near logical local minima on the potential energy surface. Each conformer was minimized by the procedure described above for the generation of the hypothetical model. After minimization, the energy of the ligand−enzyme complex was calculated using the Dock utility within the Sybyl platform. The data obtained in this manner is given in Table 2. Note that rotation of either aromatic ring relative to its orientation in the hypothetical model results in an increase in the energy of the ligand−enzyme complex. In addition, as would be expected, rotation of both aromatic rings relative to their orientations in the hypothetical model results in the highest energy complex. This hypothetical model provides interesting features, including: (a) the overall conformation of enzyme-bound ADAM **28** closely approximates the general "butterfly" structure observed by X-ray crystallography for a variety of other NNRTIs;[34,35] (b) the aromatic rings of **28** are in close proximity to several residues suitable for hydrophobic interactions; (c) the methyl group of the alkenyl chain ester moiety is likely to be engaged in hydrophobic contacts with Val 179, as the distance between the methyl moiety and the Val Cγ is <3.7 Å; (d) the alkenyl chain carbonyl oxygen is involved in bifurcated hydrogen bonding with the backbone amide of Lys 101 and with the side chain amino group of Lys 103; (e) hydrogen bonding is also possible between the ϵ-amino group of Lys 103 and the carboxyl oxygen of the alkenyl chain ester group. As discussed in the following sections, support for some of the interactions observed in the model can be derived from the structure−activity relationships. Moreover, the resistance mutation results of ADAMs **1** and **28** provide additional information regarding the mode of binding of this class of NNRTIs with HIV-1 RT that can be correlated with the hypothetical model.

Chart 1. Alkenyl Chain Numbering



Structure−Activity Relationships. 1. Length of the Alkenyl Chain. Comparison of ADAMs **1**, **3**, **5**, **12**, and **28** containing a methoxycarbonyl group at the end of the chain but differing in chain length strongly indicates that an eight-atom alkenyl chain (not counting hydrogens; for numbering see Chart 1) provides the most potent compounds. Shortening or extending this chain by only one methylene unit causes a significant drop in the antiviral potency, as observed for esters **5** and **12**. Shortening the chain by one more methylene group as in analogue **3** completely abolishes the anti-HIV-1 activity.

2. Functionality Present at the End of the Alkenyl Chain. When attached at the end of a five-atom alkenyl chain, two functional groups have provided compounds with anti-HIV-1 activity. A methoxycarbonyl group is present in esters **1** and **28**, which are the most potent ADAMs prepared up to this date. The propionyl group present in ketone **19** was tolerated, but a significant decrease in antiviral potency was observed. The aldehyde group present in **11**, or the carboxylic acid moiety contained in the previously reported analogue **38**, did not provide compounds capable of inhibiting the

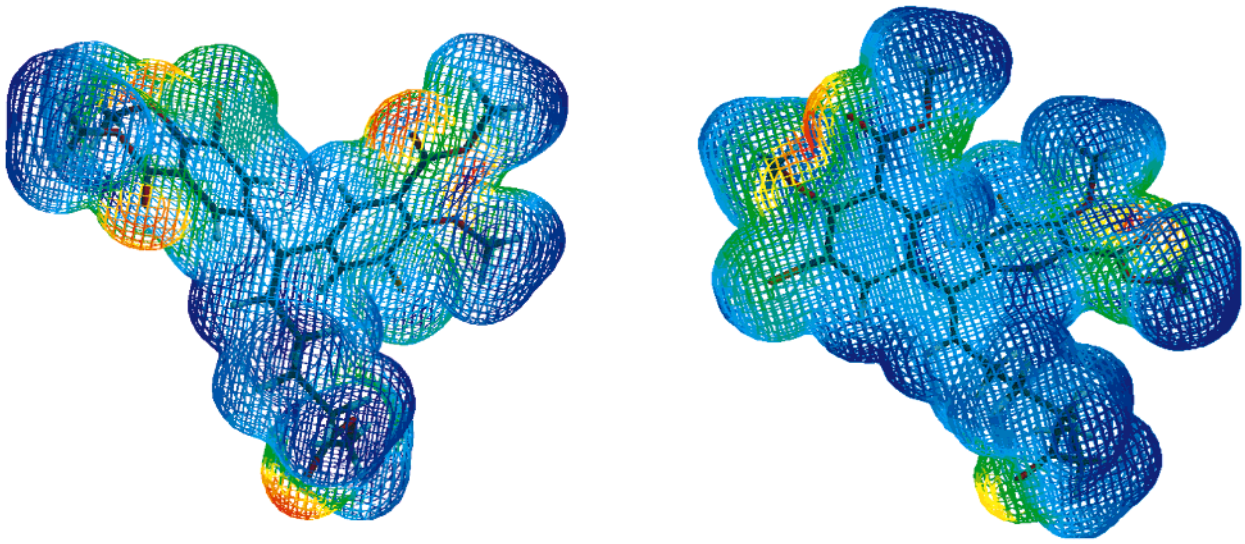


Figure 2. Value of the electrostatic potential of ADAM II (**1**) (a) and ketone **19** (b) is shown color encoded onto the total electron density surface. Colors toward red indicate electron-rich regions of the molecule.

cytopathic effect of HIV-1. Functional groups tolerated when attached at the end of a six-atom chain include bromine, chlorine, azide, and methoxycarbonyl. These groups are incorporated in derivatives **7**−**9** and **12**, respectively. However, the antiviral potency of these compounds is only in the low micromolar range. Functionality that is not tolerated at this position includes alcohol and carbamate groups. Congener **30**, which includes a phenyl ring incorporated at the end of a four-atom chain, did not show any anti-HIV-1 activity. As discussed above, the alkenyl chain phenyl ring in **30** was considered to be situated for optimal cation-π interaction with Lys 103. The lack of antiviral activity of this analogue suggests that the Lys 103 side chain is not protonated and, therefore, is uncharged. Consequently, this residue is probably capable of hydrogen bonding, but it may not be involved in electrostatic interactions.

It is clear that there is a very strong correlation of anti-HIV-1 potency with the length of the alkenyl chain and the functionality attached at its end. It seems to be important that the length of the chain should be appropriate to position a moiety for interaction with the side chain amino group of Lys 103 and another group for hydrophobic contact with Val 179 (see hypothetical model displayed in Figure 1). Hydrogen bonding is probably the mode of interaction between the ϵ-amino group of Lys 103 and the moiety present in the inhibitor. In ADAM II (**1**) and **28**, this moiety is believed to be the oxygen of the carbonyl group in the ester functionality. Following the numbering shown in Chart 1, this oxygen is attached to the sixth carbon atom in the chain. Therefore, it might be expected that analogues containing moieties capable of hydrogen bonding at this position would provide active compounds. The results obtained with azide **9** and ketone **19** provide support for this idea. Moreover, high electron density is probably required in this moiety for successful interaction with the ϵ-amino group of Lys 103. This might explain the significant difference in antiviral potency between ADAM II and ketone **19**. The carbonyl oxygen of the ester has higher electron density than the carbonyl oxygen of the ketone due to a resonance effect of the methoxy group of the ester. This property was calculated for ADAMs **1** and **19** using MacSpartan (version 1.1, Wavefunction, Inc., Irvine, CA). The atomic charge for the carbonyl oxygen of **1** is −0.56 electrostatic units, while that of ketone **19** is −0.48 electrostatic units. This difference between ADAMs **1** and **19** is evident in the graphical representation showing the electrostatic potential combined with the electron density surfaces of these ADAMs. The graphics were generated using MacSpartan and are displayed in Figure 2. In these graphics, the value of the electrostatic potential is color encoded onto the total electron density surface, with colors toward red indicating electron-rich regions of the molecule.

As mentioned above, the length of the chain should also position a moiety capable of hydrophobic interaction with Val 179. In ADAM **28**, this moiety might be the methoxy group of the ester (see model Figure 1). Hydrophobic interactions between Val 179 and different NNRTIs have been reported, including the contacts with the 5-methyl group on the diazepin ring of Cl-TIBO,[36] the carboxamide moiety of α-APA,[33] and the methylthiomethylene group of the quinoxaline HBY 097.[37]

3. Alkenyl Chain Double Bond. Hydrogenation of ADAM II provided analogue **25**, which was completely devoid of anti-HIV-1 activity. This is a very drastic modification in this series because it provides a significant increase in conformational freedom. According to this result, the double bond is essential to secure the molecular geometry required for interaction with HIV-1 RT.

4. Halogen on the Aromatic Rings. Taking into consideration the results obtained with this new series of ADAMs, there is more evidence that suggests that analogues containing bromine are more potent than those containing chlorine. This is observed when one compares ADAM II (**1**) (EC_{50} = 0.013 μM) vs ester **28** (EC_{50} = 0.0013 μM). This hypothesis is also supported with results from our initial work in this series, from which ADAM I (**37**) (EC_{50} = 9.2 μM) was observed to be more potent than **39** (EC_{50} = 16.0 μM). When chlorine is replaced by fluorine as in derivative **36**, a drastic decrease in antiviral potency is observed. The significant drop in potency is probably related to a reduction in the van der Waals radius when going from chlorine (1.80

Table 3. Activity of ADAMs **1**, **28**, and **37** Against a Site-Directed Panel of Resistant Isolates in CEM-SS Cells

mutation in RT	ADAM **1**[a]		ADAM **28**		ADAM **37**[b]	
	EC_{50} (μM)	fold resistance	EC_{50} (μM)	fold resistance	EC_{50} (μM)	fold resistance
NL4-3[c]	1.78		0.06		12.3	
L74V	2.23	NC[d]	0.019	S[e]	5.47	S[e]
A98G	0.84	NC[d]	0.22	3.7	24.1	2
L100I	>0.16	S[e]	0.26	4.3		
K101E	3.05	NC[d]	0.07	NC[d]	>100	>8
K103N	>50	>28	0.42	7	23.6	NC[d]
V106A	12.7	7.13	9.51	158.5	>100	>8
V108I	12.0	6.74	0.67	10		
V179D	50	28	40.2	670	>100	>8
Y181C	>50	>28	6.13	102	>100	>8
Y188C	10.80	6.07	1.73	28.8	>100	>8
4X AZT[f]	0.90	NC[d]	0.07	NC[d]	0.93	S[e]
4X AZT/L100I[f]	0.27	S[e]	0.33	5.5		
4X AZT/Y181C[f]	>50	>28	3.5	58.3		

[a] Taken from ref 8. [b] Taken from ref 6. [c] Wild-type enzyme. [d] No significant change in sensitivity. [e] Enhanced sensitivity. [f] AZT-resistant.

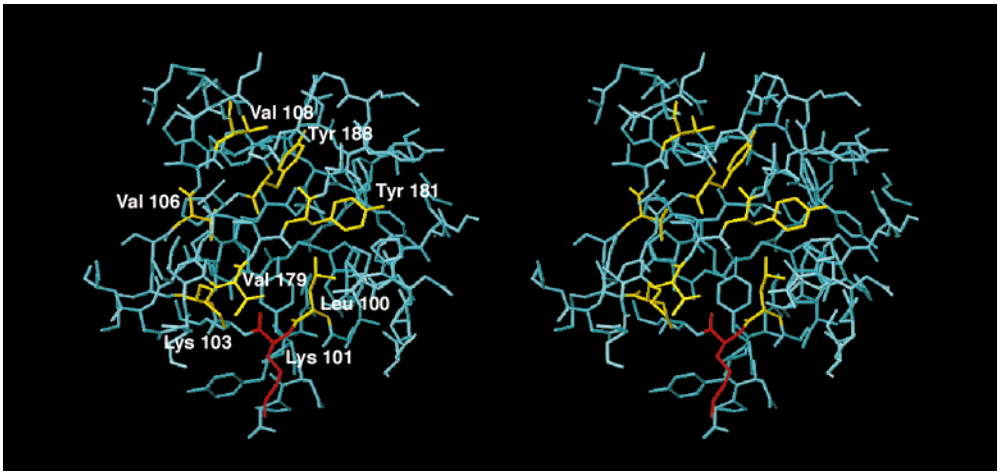


Figure 3. HIV-1 NNRTI binding site. Mutation of the yellow- and red-colored residues results in resistance to known NNRTIs. Mutation of the yellow residues also results in resistance to ADAM **28**, while mutation of the red residue results in no change in sensitivity to ADAM **28**.

Å) or bromine (1.95 Å) to fluorine (1.35 Å). We previously correlated the lack of anti-HIV-1 activity in analogues containing iodine to a possible size limitation for activity.[8] Combined, these results indicate that only chlorine and bromine are tolerated. Other halogens (iodine or fluoride), or hydrogen, compromise the biological activity significantly.

5. Aromatic Methoxycarbonyl Group. In our initial report of this class of NNRTIs,[6] the aromatic methoxycarbonyl groups were replaced by primary, secondary, or tertiary amides and by carboxylic acids. These modifications led only to inactive compounds. In this report, the aromatic methyl esters were replaced by benzylic alcohols or aldehydes, which provided analogues **22** and **24**, respectively. These congeners can be compared directly with ketone **19** since this compound incorporates the same functionality in the alkenyl chain as that present in **22** and **24**, but includes methoxycarbonyl groups on the aromatic rings. The moderate antiviral potency of ketone **19** ($EC_{50} = 2.8\ \mu M$) contrasts with the lack of antiviral activity in **22** and **24** and emphasizes the importance of methoxycarbonyl groups at these positions. Without an X-ray crystal structure of ADAMs **1** or **28** bound with HIV-1 RT, it is difficult to assess the role of this functionality in the interaction of this class of NNRTIs with the enzyme. The internal surface of the NNRTI binding pocket is largely hydrophobic, and therefore, hydrophobic contacts account for the majority of interactions in the enzyme−inhibitor complex.[38,39] Consequently, it can be expected that the aromatic methoxycarbonyl groups in the ADAMs are involved in such interactions. Further support for this hypothesis comes from the lack of antiviral activity observed in the analogues incorporating a carboxylic acid or a benzylic alcohol, which are not as likely to be involved in hydrophobic interactions.

Resistance Mutations. ADAM **28** was evaluated for inhibition of the cytopathic effect of a variety of HIV-1 strains containing defined mutations in RT, and the results are presented in Table 3. Mutations that conferred resistance to the antiviral activity of **28** were clustered within the amino acid residues comprising the NNRTI binding pocket.[34,38,40,41] The mutations conferring greater than 10-fold resistance to ADAM **28** were located at residues Val 106, Val 179, Tyr 181, and Tyr 188.

The mutations conferring resistance to ADAM **28** are displayed graphically in Figure 3, which was constructed by erasing nevirapine from the structure of the nevirapine−RT complex.[40] This shows the amino acid residues in the p66 subunit surrounding the NNRTI binding pocket. Mutation of the yellow-colored residues in Figure 3 results in resistance to the antiviral activity of **28**, while mutation of the red-colored residue did not

change the sensitivity significantly. The clustering of the resistance mutations around the NNRTI binding pocket of HIV-1 RT provides convincing evidence that inhibition of RT is responsible for the antiviral activity of **28** and the other NNRTIs in this series. Similar, but not identical, resistance profiles were also observed previously for NNRTIs **37** and **1**.[6,8] Furthermore, we have previously shown with compound **37** that the ADAMs are RT inhibitors by exclusion of other possible mechanisms of action.[6]

A comparison can be made between the results obtained in this study with those obtained previously for ADAM II (**1**),[6,8] which are included in Table 3. There is an apparent, close correlation between antiviral potency and degree of resistance. Ester **28** is the most potent compound of this class of NNRTIs, but it is also the one causing stronger resistance for the V106A, V179D, Y181C, and Y188C mutants. Analysis of these mutations is providing a better understanding of the residues involved in the binding of these compounds with HIV-1 RT. The V179D and Y181C mutants have been observed to provide the most significant resistance to ADAM II (**1**) and **28**. Both mutations have been reported before for other NNRTIs, and in fact, the Y181C is one of the mutations conferring the most generalized cross-resistance.[42] More interesting is the V179D mutant because it emphasizes the important role of this residue in the binding of **28** with the enzyme. The V179D mutant showed a 670-fold resistance against ADAM **28** and was by far the mutation providing the highest level of resistance against this ADAM. This high level of resistance was not observed for ADAM II (**1**) and may provide an indication that these two inhibitors interact slightly differently with the NNRTI binding pocket. As discussed above, we believe that hydrophobic contacts between Val 179 and the methoxy group of the ester attached at the end of the alkenyl chain may be in part responsible for the high affinity of **28** for HIV-1 RT. Possible contacts of ADAM **28** with Val 179 were also detected in the hypothetical model displayed in Figure 1. This interaction would be lost in the V179D mutant.

High resistance against the K103N mutant was observed for ADAM II (**1**).[8] Surprisingly, this mutation was not as significant for ADAM **28**, resulting in only a 7-fold increase in the EC_{50} value. The role of this residue in the binding of these two ADAMs with the enzyme was discussed above. Again, the different degrees of resistance might indicate that ADAMs **1** and **28** may interact differently with RT. The change of Lys to Asn does not eliminate the possibility of hydrogen bonding with the amino acid residue. The fact that the K103N mutation does not affect the activity of **37**, which does not have a hydrogen bond acceptor at the end of the alkenyl chain, is consistent with the hypothetical hydrogen bond donor and acceptor roles proposed for Lys 103 and the ligand side chain ester carbonyl groups, respectively.

The activity against the L100I mutant also deserves discussion. Increased sensitivity versus this mutant was detected with ADAM II (**1**),[8] but a 4.3-fold resistance was observed with ADAM **28**. It is interesting that the replacement of the aromatic bromines present in **28** by the aromatic chlorines of ADAM **1** restores the activity toward this mutant. This situation has been noted previously for other NNRTIs, including compounds in the TIBO and pyridinone series, where minor alterations restored activity against a mutant strain of HIV-1.[43,44] The distance of 4.14 Å calculated from one of the γ-carbons of Leu 100 to the closer bromine atom of **28** makes possible interactions of the halogens of the ligand and the side chain of the amino acid residue at position 100 plausible (see Figure 1).

Taking into consideration all of the results discussed above, ADAM II (**1**) and its congener **28** appear to interact slightly differently with HIV-1 RT. The aromatic portion of both ADAMs probably makes contacts with the hydrophobic residues of the NNRTI binding pocket including Val 106, Val 108, Tyr 181, and Tyr 188. This mode of interaction correlates with the observations derived from the hypothetical model of ADAM **28** bound to RT (see Figure 1). The replacement of the aromatic chlorines present in ADAM **1** by the bulkier bromines present in ADAM **28** may force this analogue to change the mode of interaction with the binding pocket. In the case of ADAM **1**, the alkenyl chain methoxycarbonyl group might be suitably positioned for hydrogen bonding with the side chain amino moiety of Lys 103. Probably, this is one of the most important interactions of the alkenyl chain ester moiety of **1**. This hypothesis is supported by the results of the resistance mutation studies. For ADAM **28**, the most significant interaction of the alkenyl chain methyl ester group may be through hydrophobic contacts with Val 179. The hypothetical model of ADAM **28** bound to HIV-1 RT offers some plausibility for interaction with this residue. However, this model also shows **28** hydrogen bonding with Lys 103. It is important to mention that reports of other classes of NNRTIs have indicated that analogues containing minor structural changes interact with RT in similar ways, but the specific interactions with the enzyme and the shape of the NNRTI binding pocket may be different.[45,46] This might be the case in the ADAM series.

ADAM **28** was also evaluated against the virus containing the 4X AZT enzyme, which has four mutations in its RT and is resistant to AZT. ADAM **28** displayed similar antiviral potency against this AZT-resistant strain as that observed for the wild-type virus containing the NL4-3 enzyme. Moreover, the combination of the 4X AZT mutation with two typical NNRTI mutations (L100I and Y181C) does not significantly change the antiviral potency of **28** (4X AZT/L100I 5.5-fold resistance vs L100I alone 4.3-fold resistance; 4X AZT/Y181C 58.3-fold resistance vs Y181C alone 102-fold resistance). Results from this study demonstrate once again that this class of NNRTIs may have potential clinical utility against AZT-resistant strains of HIV-1.

Experimental Section

General. Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were obtained as follows: CI mass spectra on a Finnegan 4000 spectrometer; FAB mass spectra and EI mass spectra on a Kratos MS50 spectrometer; ^{1}H NMR spectra on Varian VXR-500S and Bruker ARX-300 spectrometers; IR spectra on a Beckman IR-33 spectrometer or a Perkin-Elmer 1600 series FTIR. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of

the calculated compositions. Silica gel used for column chromatography was 230−400 mesh.

Methyl 3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-4,4-diphenyl-3-butenoate (3). Carboxylic acid **2**[9] (0.050 g, 0.104 mmol) was dissolved in THF (2.5 mL). Excess ethereal diazomethane solution (∼0.1 mL) was added. The pale yellow solution was left at room temperature for 2 h. Acetic acid (5 drops) was then added to destroy excess diazomethane. The solvent was removed and the residue was purified by flash chromatography on silica gel (25 g; column: 2 × 19.5 cm), eluting with hexanes:ethyl acetate (3:1). Compound **3** was obtained as a colorless oil in good yield (41 mg, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, J = 2.5 Hz, 1 H), 7.47 (d, J = 2.2 Hz, 1 H), 7.32 (dd, J = 2.8 and J = 2.5 Hz, 2 H), 6.23 (t, J = 7.5 Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.12 (s, 3 H); IR (film) 2950, 1735, 1476, 1261, 1165, 997, 846 cm^{-1}; FABMS m/z 497 (MH^+), 496 (M^+), 465 ($MH^+ - CH_3OH$). Anal. ($C_{23}H_{22}Cl_2O_8$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-3-(1,3-dioxolan-2-yl)-1-propene (4). A suspension of phosphonium bromide **14** (0.833 g, 1.878 mmol) in dry THF (25 mL) was stirred at 0 °C under an argon atmosphere. A 1.0 M solution of $NaN(SiMe_3)_2$ in THF (1.94 mL, 1.937 mmol) was added dropwise. The yellow mixture was stirred at 0 °C for 30 min. A solution of ketone **13**[10] (0.500 g, 1.174 mmol) in dry THF (20 mL) was then added dropwise. The ice bath was removed and the dark brown reaction mixture was stirred at room temperature for 4.25 h. The reaction mixture was quenched with satd NH_4Cl solution (30 mL). The phases were separated and the aqueous one was extracted with Et_2O (3 × 35 mL). The combined organic extracts were washed with brine (1 × 40 mL), dried over magnesium sulfate, and filtered and the solvent was removed. Purification was achieved by flash chromatography on silica gel (55 g; column: 3 × 20 cm), eluting with hexane:ethyl acetate (2:1). Compound **4** was obtained as a yellow oil in moderate yield (0.271 g, 45.2%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, J = 2.2 Hz, 1 H), 7.49 (d, J = 2.4 Hz, 1 H), 7.40 (d, J = 2.2 Hz, 1 H), 7.30 (d, J = 2.4 Hz, 1 H), 6.12 (t, J = 7.6 Hz, 1 H), 4.96 (t, J = 4.5 Hz, 1 H), 4.00−3.86 (m, 4 H), 3.96 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 2.46 (dd, J = 4.5 and 7.6 Hz, 2 H); IR (film) 2915, 1731, 1477, 1257, 997 cm^{-1}; FABMS m/z 511 (MH^+). Anal. ($C_{24}H_{24}Cl_2O_8$) C, H.

Methyl 3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-5,5-diphenyl-4-pentenoate (5). A suspension of (methoxycarbonylpropyl)triphenylphosphonium bromide (**15**)[11] (0.25 g, 0.563 mmol) in dry THF (15 mL) was stirred at −78 °C under an argon atmosphere. A 1.0 M solution of $NaN(SiMe_3)_2$ in THF (0.53 mL, 0.528 mmol) was added dropwise. The mixture was stirred at −78 °C for 40 min. A solution of ketone **13**[10] (0.150 g, 0.352 mmol) in dry THF (6 mL) was then added dropwise. The reaction mixture was stirred at −78 °C for 12 h and then at 25 °C for 16 h. The reaction mixture was quenched with satd NH_4Cl solution (15 mL). The phases were separated and the aqueous one was extracted with Et_2O (3 × 25 mL). The combined organic extracts were washed with brine (1 × 30 mL), dried over magnesium sulfate, and filtered. The solvent was removed and the residue was flash chromatographed on silica gel (29 g; column: 2 × 25 cm), eluting with hexane:ethyl acetate (4:1). Compound **5** was obtained in good yield (0.13 g, 72%) as a yellowish oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J = 2.3 Hz, 1 H), 7.45 (d, J = 2.1 Hz, 1 H), 7.32 (d, J = 2.2 Hz, 1 H), 7.27 (d, J = 2.4 Hz, 1 H), 6.01 (t, J = 7.2 Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.66 (s, 3 H), 2.41 (m, 4 H); IR (film) 2952, 1736, 1477, 1436, 1263, 1207, 996 cm^{-1}; FABMS m/z 511 (MH^+). Anal. ($C_{24}H_{24}Cl_2O_8$) C, H.

6-Bromo-3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-hexene (7). A solution of alcohol **6**[8] (0.100 g, 0.201 mmol) and carbon tetrabromide (0.133 g, 0.402 mmol) in dry acetonitrile (3.5 mL) was heated at reflux under Ar. A solution of triphenylphosphine (0.158 g, 0.603 mmol) in dry acetonitrile (5.5 mL) was added over 3 min. The reaction mixture was heated at reflux for 80 min. At this time, it was cooled and the solvent evaporated. The residue was then purified by flash chromatography on silica gel (∼30 g; column: 2 × 22 cm), eluting with hexanes:ethyl acetate (6:1). Bromide **7** was obtained as a colorless solid in excellent yield (0.107 g, 95.5%). The analytical sample was recrystallized from acetone−ethanol: mp 99−101 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.4 Hz, 1 H), 7.45 (d, J = 2.2 Hz, 1 H), 7.29 (d, J = 2.1 Hz, 1 H), 7.27 (d, J = 2.4 Hz, 1 H), 6.03 (t, J = 7.5 Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.35 (t, J = 6.6 Hz, 2 H), 2.10 (q, J = 7.5 Hz, 2 H), 1.83 (m, 2 H), 1.58 (m, 2 H); IR (film) 2949, 1731, 1477, 1266, 1207, 998, 743 cm^{-1}; FABMS m/z 559 (MH^+), 528 ($MH^+ - 31$). Anal. ($C_{24}H_{25}BrCl_2O_6$) C, H.

3′,3″,6-Trichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-hexene (8). A solution of alcohol **6** (0.100 g, 0.201 mmol) and Et_3N (0.08 mL, 0.603 mmol) in dry dichloromethane (1.5 mL) was stirred in an ice bath under Ar. Mesyl chloride (0.05 mL, 0.603 mmol) was added and the reaction mixture was stirred for 7 h, while maintaining the temperature below 10 °C. Additional mesyl chloride (0.03 mL) was added. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane (15 mL) and washed with 1.0 N HCl (2 × 20 mL), satd $NaHCO_3$ (1 × 15 mL), and brine (1 × 20 mL). The organic extracts were then dried over magnesium sulfate and filtered, and the solvent was removed. Purification by flash chromatography on silica gel (30 g; column: 2 × 20 cm), eluting with hexanes:ethyl acetate (5:1), afforded compound **8** as a white solid in moderate yield (64 mg, 61%): mp 106−107 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.3 Hz, 1 H), 7.45 (d, J = 2.2 Hz, 1 H), 7.29 (d, J = 2.2 Hz, 1 H), 7.27 (d, J = 2.3 Hz, 1 H), 6.03 (t, J = 7.5 Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.48 (t, J = 6.4 Hz, 2 H), 2.10 (q, J = 7.4 Hz, 2 H), 1.74 (m, 2 H), 1.58 (m, 2 H); IR (film) 2950, 1731, 1477, 1266, 1208, 999, 742 cm^{-1}; FABMS m/z 515 (MH^+), 483 ($MH^+ - CH_3OH$). Anal. ($C_{24}H_{25}Cl_3O_6$) C, H.

6-Azido-3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-hexene (9). A solution of alcohol **6** (0.100 g, 0.201 mmol) and Et_3N (0.15 mL, 1.005 mmol) in dry dichloromethane (1.5 mL) was stirred in an ice bath under Ar. Mesyl chloride (0.08 mL, 1.005 mmol) was added and the yellowish mixture was stirred in an ice bath for 5 h and then at room temperature overnight. The mixture was diluted with dichloromethane (20 mL) and washed with 2.0 N HCl (2 × 15 mL), satd $NaHCO_3$ (15 mL), and brine (20 mL). The organic extracts were then dried over magnesium sulfate and filtered, and the solvent was evaporated. The yellow residue was dissolved in dry DMF (2 mL) and sodium azide (0.065 g, 1.005 mmol) was added. The reaction mixture was stirred at 55 °C for 20 h. The mixture was cooled, diluted with water (20 mL), and extracted with ethyl ether (4 × 20 mL). The combined organic extracts were washed with brine (1 × 30 mL), dried over magnesium sulfate, and filtered and the solvent was removed. The crude residue was purified by flash chromatography on silica gel (∼30 g; column: 2 × 22.5 cm), eluting with hexanes:ethyl acetate (4:1). Compound **9** was obtained as a white solid in moderate yield (0.05 g, 52%): mp 70−72 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.5 Hz, 1 H), 7.45 (d, J = 2.1 Hz, 1 H), 7.29 (d, J = 2.1 Hz, 1 H), 7.27 (d, J = 2.5 Hz, 1 H), 6.02 (t, J = 7.5 Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.23 (t, J = 6.4 Hz, 2 H), 2.11 (q, J = 7.2 Hz, 2 H), 1.54 (m, 4 H); IR (film) 2949, 2096, 1735, 1477, 1263, 1207, 998, 743 cm^{-1}; FABMS m/z 522 (MH^+), 490 ($MH^+ -$ MeOH). Anal. ($C_{24}H_{25}Cl_2N_3O_6$) C, H, N.

5′,5″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexen-1-yl *N*-Ethylcarbamate (10). A solution of alcohol **6** (0.100 g, 0.202 mmol) in dry Et_3N (3 mL) was stirred at room temperature under Ar. DMAP (10 mg, 0.082 mmol) and ethyl isocyanate (0.05 mL, 0.606 mmol) were added and the mixture stirred at room temperature for 24 h. At this time, additional ethyl isocyanate (0.05 mL) was added and stirring continued for 24 h. The mixture was diluted with ethyl acetate (50 mL) and washed with 2 N HCl (3 × 30 mL) and brine (1 × 30 mL). The organic phase was dried over

magnesium sulfate and filtered and the solvent removed. Purification was achieved by flash chromatography on silica gel (~35 g; column: 2 × 24.5 cm), eluting with hexanes:ethyl acetate (2:1 to 1:1). Carbamate **10** was obtained in excellent yield (0.11 g, 98%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.4 Hz, 1 H), 7.45 (d, J = 2.0 Hz, 1 H), 7.29 (d, J = 2.1 Hz, 1 H), 7.26 (d, J = 2.2 Hz, 1 H), 6.03 (t, J = 7.4 Hz, 1 H), 4.63 (bs, 1 H), 4.00 (m, 2 H), 3.97 (s, 3 H), 3.90 (s, 6 H), 3.88 (s, 3 H), 3.18 (m, 2 H), 2.10 (q, J = 7.2 Hz, 2 H), 1.58−1.48 (m, 4 H); IR (film) 3393, 2950, 1718, 1476, 1252, 1207, 998, 743 cm^{-1}; FABMS m/z 568 (MH^+). Anal. ($C_{27}H_{31}Cl_2NO_8$) C, H, N.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexenal (11). A solution of oxalyl chloride (0.1 mL, 1.14 mmol) in dichloromethane (4.0 mL) was put under argon and cooled to −78 °C. A solution of DMSO (0.16 mL, 2.279 mmol) in dichloromethane (3.5 mL) was added over 8 min. The mixture was stirred for 15 min, and then a solution of alcohol **6** (0.514 g, 1.036 mmol) in dichloromethane (6 mL) was added over 5 min. The reaction mixture stirred for 20 min at −78 °C. Triethylamine (0.722 mL, 5.18 mmol) was added and stirring was continued at −78 °C for 15 min. The bath was removed and the mixture was allowed to warm to room temperature. Dichloromethane (40 mL) was added followed by water (45 mL). The phases were separated and the aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, and filtered, and the solvent was removed. The residue was flash chromatographed on silica gel (~65 g; column: 3 × 26 cm), eluting with hexanes:ethyl acetate (3:1 to 2:1). Aldehyde **11** was obtained as a white solid in good yield (0.32 g, 79%; based on recovered unreacted **6**: 0.105 g): mp 102−104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (t, J = 1.4 Hz, 1 H), 7.47 (d, J = 2.6 Hz, 1 H), 7.46 (d, J = 2.9 Hz, 1 H), 7.28 (d, J = 1.9 Hz, 1 H), 7.26 (d, J = 2.4 Hz, 1 H), 6.01 (t, J = 7.4 Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 2.43 (dt, J = 7.2 Hz and J = 1.3 Hz, 2 H), 2.12 (q, J = 7.5 Hz, 2 H), 1.76 (qn, J = 7.3 Hz, 2 H); IR (film) 2950, 1735, 1477, 1263, 1208, 998, 743 cm^{-1}; FABMS m/z 495 $(MH)^+$, 463 (MH^+ − CH_3OH). Anal. ($C_{24}H_{24}Cl_2O_7$) C, H.

Methyl 3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-7,7-diphenyl-6-heptenoate (12). A slurry of $TiCl_4$·2THF complex (0.705 g, 2.112 mmol) and Zn dust (0.276 g, 4.224 mmol) in THF (20 mL) was stirred and heated under reflux for 2 h. A solution of benzophenone **13**[10] (0.300 g, 0.704 mmol) and methyl 6-oxohexanoate **(16)**[20] (0.162 g, 1.124 mmol) in THF (15 mL) was added. The black mixture was heated under reflux for 3 h. It was then cooled to room temperature and a 10% solution of K_2CO_3 (30 mL) was added. The mixture was stirred at room temperature overnight. It was filtered through a pad of Celite and washed with EtOAc (3 × 30 mL). The layers were separated and the aqueous one was extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$, and filtered, and the solvent was removed. The residue was flash chromatographed on silica gel (~35 g; column: 2 × 27 cm) eluting with hexanes:ethyl acetate (3:1) to provide **12** as a pale yellowish oil in moderate yield (0.219 g, 57.8%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J = 2.4 Hz, 1 H), 7.44 (d, J = 2.2 Hz, 1 H), 7.29 (d, J = 2.2 Hz, 1 H), 7.27 (d, J = 2.3 Hz, 1 H), 6.02 (t, J = 7.5 Hz, 1 H), 3.97 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.64 (s, 3 H), 2.26 (t, J = 7.3 Hz, 2 H), 2.09 (q, J = 7.3 Hz, 2 H), 1.65−1.54 (m, 2 H), 1.50−1.40 (m, 2 H); IR (film) 2933, 1734, 1474, 1261, 1206, 1092, 998 cm^{-1}; FABMS m/z 539 (MH^+), 507 (MH^+ − CH_3OH). Anal. ($C_{26}H_{28}Cl_2O_8$) C, H.

5′,5″-Dichloro-4′,4″-dimethoxy-3′,3″-bis(methoxycarbonyl)-1,1-diphenyl-5-[(2-ethyl-(1,3)-dioxolan-2-yl)-1-pentene (18). A slurry of $TiCl_4$·2THF complex (2.28 g, 6.822 mmol) and zinc dust (0.892 g, 13.644 mmol) in THF (60 mL) was put under Ar and heated under reflux for 2 h. A solution of ketone **13**[10] (0.97 g, 2.274 mmol) and aldehyde **17**[21,22] (0.470 g, 2.729 mmol) in THF (45 mL) was added. The black mixture was heated under reflux for 1.5 h. It was allowed to cool at room temperature, and a 10% solution of K_2CO_3 (50 mL) was added. The resulting mixture was stirred at room temperature overnight. The mixture was filtered through a pad of Celite and washed with ethyl acetate (3 × 35 mL) and water (50 mL). The layers were separated and the aqueous one was extracted with ethyl acetate (3 × 35 mL). The combined organic extracts were washed 10% K_2CO_3 (50 mL) and brine (50 mL). The organic extracts were dried over $MgSO_4$ and filtered. The residue was purified by column chromatography on silica gel (~170 g; column: 5 × 23.5 cm), eluting with hexanes:ethyl acetate (3:1). Compound **18** was obtained as a white solid in good yield (0.972 g, 75.5%): mp 74−76 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.4 Hz, 1 H), 7.44 (d, J = 2.2 Hz, 1 H), 7.29 (d, J = 2.1 Hz, 1 H), 7.26 (d, J = 2.3 Hz, 1 H), 6.03 (t, J = 7.4 Hz, 1 H), 3.97 (s, 3 H), 3.90 (s, 4 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 2.08 (q, J = 7.2 Hz, 2 H), 1.62−1.45 (m, 6 H), 0.87 (t, J = 7.4 Hz, 3 H); IR (film) 2951, 2878, 1736, 1476, 1264, 1206, 999, 742 cm^{-1}; FABMS m/z 567 (MH^+), 536 (MH^+ − OCH_3). Anal. ($C_{28}H_{32}Cl_2O_8$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-6-oxo-1-octene (19). A solution of ketal **18** (0.080 g, 0.141 mmol) and pyridinium *p*-toluenesulfonate (0.012 g, 0.048 mmol) in wet acetone (2 mL containing 3 drops of water) was heated under reflux for 2.25 h. The mixture was allowed to cool and the solvent removed. The residue was diluted with ethyl ether (15 mL) and washed with satd $NaHCO_3$ (15 mL) and brine (15 mL). After drying over $MgSO_4$, the solvent was removed and the residue was purified by flash chromatography on silica gel (~30 g; column: 2 × 17.5 cm), eluting with hexanes:ethyl acetate (3:1). Compound **19** was obtained in moderate yield (0.046 g, 62.5%) as a thick colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.4 Hz, 1 H), 7.44 (d, J = 2.2 Hz, 1 H), 7.29 (d, J = 2.2 Hz, 1 H), 7.26 (d, J = 2.4 Hz, 1 H), 6.02 (t, J = 7.4 Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 2.38 (t, J = 7.3 Hz, 2 H), 2.37 (q, J = 7.3 Hz, 2 H), 2.07 (q, J = 7.5 Hz, 2 H), 1.70 (qn, J = 7.4 Hz, 2 H), 1.00 (t, J = 7.4 Hz, 3 H); IR (film) 2950, 1734, 1716, 1476, 1287, 1262, 998 cm^{-1}; FABMS m/z 523 (MH^+), 491 (MH^+ − CH_3OH). Anal. ($C_{26}H_{28}Cl_2O_7$) C, H.

3′,3″-Dichloro-5′,5″-dihydroxymethyl-4′,4″-dimethoxy-1,1-diphenyl-5-(2-ethyl-(1,3)-dioxolan-2-yl)-1-pentene (20). A solution of ester **18** (0.171 g, 0.302 mmol) in dichloromethane (5 mL) was cooled to −78 °C and put under Ar. A 1.0 M solution of DIBAL-H in toluene (1.26 mL, 1.26 mmol) was added. The mixture was stirred at −78 °C for 4 h. A concentrated aqueous solution of Rochelle salt (potassium sodium tartrate, 15 mL) was added and the mixture warmed to room temperature over 3 h. The mixture was diluted with dichloromethane (15 mL) and the phases were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$, and filtered, and the solvent was removed. The residue was purified by flash chromatography on silica gel (~30 g; column: 2 × 24 cm), eluting with hexanes: ethyl acetate (1:1). Compound **20** was obtained as a colorless oil in very good yield (0.144 g, 93.5%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (d, J = 2.2 Hz, 1 H), 7.08 (d, J = 2.0 Hz, 1 H), 7.07 (d, J = 2.3 Hz, 1 H), 7.03 (d, J = 2.1 Hz, 1 H), 5.98 (t, J = 7.5 Hz, 1 H), 4.70 (s, 2 H), 4.64 (s, 2 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.87 (s, 4 H), 2.05 (q, J = 7.3 Hz, 2 H), 1.61−1.43 (m, 6 H), 0.85 (t, J = 7.5 Hz, 3 H); IR (film) 3421, 2938, 2879, 1479, 1231, 1002, 873 cm^{-1}; FABMS m/z 512 (M^+ + 2), 511 (MH^+). Anal. ($C_{26}H_{32}Cl_2O_8$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′-formyl-5″-hydroxymethyl-1,1-diphenyl-5-(2-ethyl-(1,3)-dioxolan-2-yl)-1-pentene (21). This compound eluted in the first fractions during the chromatographic purification of **20**. Compound **21** was obtained as a colorless oil in low yield (0.010 g, 6%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.35 (s, 0.5 H), 10.30 (s, 0.5 H), 7.56 (d, J = 2.5 Hz, 1 H), 7.39 (d, J = 2.4 Hz, 1 H), 7.05 (m, 2 H), 6.04 (dt, J = 7.3 and 7.2 Hz, 1 H), 4.71 (s, 1 H), 4.65 (s, 1 H), 4.04 (s, 2 H), 3.97 (s, 1 H), 3.93 (s, 1 H), 3.89 (s, 4 H), 3.87 (s, 2 H), 2.07 (m, 2 H), 1.61−1.44 (m, 6 H), 0.86 (t, J = 7.5 Hz, 3 H); IR

(film) 3421, 2938, 2879, 1479, 1231, 1002, 873 cm^{-1}; FABMS *m/z* 510 ($M^+ + 2$), 508 (M^+). Anal. ($C_{26}H_{30}Cl_2O_8$) C, H.

3′,3″-Dichloro-5′,5″-dihydroxymethyl-4′,4″-dimethoxy-6-oxo-1,1-diphenyl-1-octene (22). A solution of ketal **20** (0.070 g, 0.137 mmol) and pyridinium *p*-toluenesulfonate (0.012 g, 0.048 mmol) in wet acetone (2 mL containing 3 drops of water) was heated under reflux for 2.5 h. The mixture was allowed to cool and the solvent removed. The residue was diluted with ethyl ether (25 mL) and washed with satd $NaHCO_3$ (15 mL) and brine (15 mL). After drying over $MgSO_4$, the solvent was removed and the residue was purified by flash chromatography on silica gel (~30 g; column: 2 × 24.5 cm), eluting with hexanes:ethyl acetate (1:1). Compound **22** was obtained in moderate yield (0.04 g, 64%) as a colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, J = 2.0 Hz, 1 H), 7.11 (d, J = 2.2 Hz, 1 H), 7.07 (d, J = 2.2 Hz, 1 H), 7.01 (d, J = 2.1 Hz, 1 H), 5.94 (t, J = 7.6 Hz, 1 H), 4.73 (s, 2 H), 4.63 (s, 2 H), 3.92 (s, 3 H), 3.85 (s, 3 H), 2.39 (t, J = 6.8 Hz, 2 H), 2.35 (q, J = 7.3 Hz, 2 H), 2.00 (q, J = 7.7 Hz, 2 H), 1.69 (qn, J = 6.9 Hz, 2 H), 0.97 (t, J = 7.2 Hz, 3 H); IR (film) 3600−3200, 2936, 1705, 1478, 1231, 1113, 1001, 870 cm^{-1}; FABMS *m/z* 449 ($MH^+ - H_2O$). Anal. ($C_{24}H_{28}Cl_2O_5$) C, H.

3′,3″-Dichloro-5′,5″-diformyl-4′,4″-dimethoxy-1,1-diphenyl-5-(2-ethyl-(1,3)-dioxolan-2-yl)-1-pentene (23). A solution of oxalyl chloride (0.1 mL, 1.147 mmol) in dry CH_2Cl_2 (2.0 mL) was cooled to −78 °C and put under Ar. A solution of DMSO (0.16 mL, 2.295 mmol) in CH_2Cl_2 (3.0 mL) was added over 8 min, and the mixture was then stirred for 15 min at −78 °C. A solution of alcohol **20** (0.234 g, 0.459 mmol) in CH_2Cl_2 (4.0 mL) was added over 5 min. The mixture was stirred for 20 min. Et_3N (0.72 mL, 5.187 mmol) was added slowly; the mixture was stirred for 15 min at −78 °C and then allowed to warm to room temperature. The mixture was diluted with CH_2Cl_2 (30 mL) and washed with water (30 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine (35 mL), dried over $MgSO_4$, and filtered. The solvent was removed, and the residue was purified by flash chromatography on silica gel (~75 g; column: 3 × 30 cm), eluting with hexanes:ethyl acetate (3:1). Compound **23** was obtained as a yellowish oil in moderate yield (0.14 g, 60%): ^1H NMR (300 MHz, $CDCl_3$) δ 10.36 (s, 1 H), 10.31 (s, 1 H), 7.51 (d, J = 2.2 Hz, 1 H), 7.50 (d, J = 2.1 Hz, 1 H), 7.39 (d, J = 2.3 Hz, 1 H), 7.38 (d, J = 2.2 Hz, 1 H), 6.07 (t, J = 7.4 Hz, 1 H), 4.06 (s, 3 H), 3.98 (s, 3 H), 3.90 (s, 4 H), 2.09 (q, J = 7.1 Hz, 2 H), 1.62−1.44 (m, 6 H), 0.87 (t, J = 7.4 Hz, 3 H); IR (film) 2937, 2877, 1693, 1476, 1241, 994 cm^{-1}; FABMS *m/z* 507 (MH^+). Anal. ($C_{26}H_{28}Cl_2O_6$) C, H.

3′,3″-Dichloro-5′,5″-diformyl-4′,4″-dimethoxy-6-oxo-1,1-diphenyl-1-octene (24). A solution of ketal **23** (0.140 g, 0.277 mmol) and pyridinium *p*-toluenesulfonate (0.024 g, 0.97 mmol) in wet acetone (4 mL containing 6 drops of water) was heated under reflux for 8 h. The mixture was allowed to cool and the solvent removed. The residue was diluted with ethyl ether (40 mL) and washed with satd $NaHCO_3$ (25 mL) and brine (25 mL). After drying over $MgSO_4$, the solvent was removed and the residue was purified by flash chromatography on silica gel (~28 g; column: 2 × 22 cm), eluting with hexanes:ethyl acetate (3:1). Compound **24** was obtained in moderate yield (0.08 g, 65%) as a colorless thick oil: ^1H NMR (300 MHz, $CDCl_3$) δ 10.36 (s, 1 H), 10.31 (s, 1 H), 7.50 (d, J = 2.4 Hz, 1 H), 7.48 (d, J = 2.3 Hz, 1 H), 7.38 (d, J = 2.1 Hz, 1 H), 7.37 (d, J = 2.0 Hz, 1 H), 6.05 (t, J = 7.4 Hz, 1 H), 4.05 (s, 3 H), 3.98 (s, 3 H), 2.37 (t, J = 7.2 Hz, 2 H), 2.36 (q, J = 7.2 Hz, 2 H), 2.07 (q, J = 7.5 Hz, 2 H), 1.70 (qn, J = 7.4 Hz, 2 H), 1.00 (t, J = 7.4 Hz, 3 H); IR (film) 2936, 2873, 1694, 1592, 1476, 1242, 993 cm^{-1}; FABMS *m/z* 463 (MH^+). Anal. ($C_{24}H_{24}Cl_2O_5$) C, H.

Methyl 3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenylhexanoate (25). Methyl ester **1**[7,8] (0.052 g, 0.099 mmol) was dissolved in ethyl acetate (6 mL) and hydrogenated over PtO_2 (26 mg) for 1 h at room temperature. The catalyst was filtered off and washed with ethyl acetate, and the filtrate was evaporated in vacuo. The residue was purified by flash chromatography on silica gel (~40 g; column: 2 × 29 cm), eluting with hexanes:ethyl acetate (4:1). Compound **25** was obtained as a colorless oil in moderate yield (0.038 g, 73%): ^1H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J = 2.2 Hz, 2 H), 7.32 (d, J = 2.2 Hz, 2 H) 3.90 (s, 6 H), 3.88 (s, 6 H), 3.78 (t, J = 7.8 Hz, 1 H), 3.62 (s, 3 H), 2.26 (t, J = 7.4 Hz, 2 H), 1.97 (q, J = 7.8 Hz, 2 H), 1.64 (qn, J = 7.6 Hz, 2 H), 1.28−1.18 (m, 2 H); IR (film) 2950, 2865, 1736, 1475, 1258, 1198, 999, 738 cm^{-1}; FABMS *m/z* 527 (MH^+), 495 (MH^+ − MeOH). Anal. ($C_{25}H_{28}Cl_2O_8$) C, H.

Methyl 3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexenoate (28). A slurry of $TiCl_4$·2THF complex (0.315 g, 0.945 mmol) and zinc dust (0.123 g, 1.89 mmol) in THF (10 mL) was put under Ar and heated under reflux for 2 h. A solution of ketone **26**[6] (0.162 g, 0.315 mmol) and aldehyde **27**[24] (0.082 g, 0.63 mmol) in THF (10 mL) was added. The black mixture was heated under reflux for 1.5 h. It was allowed to cool at room temperature, and 1.0 N HCl (20 mL) was added. The resulting mixture was stirred for 2 h. The layers were separated and the aqueous one was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$ and filtered, and the solvent was removed. Purification was achieved by flash chromatography on silica gel (~40 g; column: 2 × 29 cm), eluting with hexane:ethyl acetate (3:1). Compound **28** was obtained as a pale yellow oil in low yield (0.044 g, 22.8%): ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 2.4 Hz, 1 H), 7.48 (d, J = 2.2 Hz, 1 H), 7.45 (d, J = 2.2 Hz, 1 H), 7.44 (d, J = 2.3 Hz, 1 H), 6.00 (t, J = 7.5 Hz, 1 H), 3.96 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.61 (s, 3 H), 2.28 (t, J = 7.4 Hz, 2 H), 2.11 (q, J = 7.5 Hz, 2 H), 1.75 (qn, J = 7.4 Hz, 2 H); IR (film) 2950, 1736, 1473, 1434, 1258, 1207, 996 cm^{-1}; FABMS *m/z* 613 (MH^+), 581 (MH^+ − CH_3OH). Anal. ($C_{25}H_{26}Br_2O_8$) C, H.

3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-dimethoxycarbonyl-1,1,3-triphenyl-1-propene (30). $TiCl_4$·2THF (0.970 g, 2.91 mmol) and zinc dust (0.369 g, 5.81 mmol) were mixed in THF (11 mL). The slurry was heated at reflux for 45 min under nitrogen. [3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-di(methoxycarbonyl)diphenyl]methanone (**26**)[6] (0.283 g, 0.584 mmol) and hydrocinnamaldehyde (**29**) (90% technical grade; 0.130 g, 0.872 mmol) were taken up in THF (11 mL) and transferred to the low-valent titanium slurry via cannula under nitrogen pressure. The resulting reaction mixture was heated at reflux for 45 min and then poured at once onto a column of silica gel (25 g). The product was eluted from the column with EtOAc and the solution concentrated on a rotary evaporator to give the crude product as a yellow oil. The product was further purified by flash column chromatography on silica gel (30 g) using a gradient of 0−12% EtOAc in hexanes as the eluent. Concentration of the fractions containing the product afforded the pure product as a colorless oil (0.26 g, 76%): ^1H NMR (300 MHz, C_6D_6) δ 7.67 (d, J = 2.2 Hz, 1 H), 7.54 (d, J = 2.2 Hz, 1 H), 7.50 (d, J = 2.1 Hz, 1 H), 7.24 (d, J = 2.2 Hz, 1 H), 7.13−6.92 (m, 5 H), 5.70 (t, J = 7.7 Hz, 1 H), 3.74 (s, 3 H), 3.71 (s, 3 H), 3.34 (s, 3 H), 3.31 (s, 3 H), 2.41 (t, J = 6.9 Hz, 2 H), 2.12 (m, 2 H); IR (neat on NaCl) 2950, 1733, 1595, 1474, 1436, 1423, 1400, 1263, 1208, 1164, 1089, 996, 892, 794, 721, 701 cm^{-1}. Anal. ($C_{28}H_{26}Br_2O_6$) C, H.

(3,3′-Dicarboxy-5,5′-difluoro-4,4′-dihydroxydiphenyl)-methane (32). 3-Fluorosalicylic acid (**31**)[27] (748 mg, 4.72 mmol) was taken up in methanol (3.5 mL) and water (0.6 mL). The mixture was cooled to −78 °C and stirred vigorously while concentrated sulfuric acid (8.3 mL) was added dropwise. The dry ice/acetone bath was exchanged for an ice bath and stirring was continued for 1 h at 0 °C. The reaction mixture was again cooled to −78 °C and an aqueous 37% solution of formaldehyde (2 mL) was added dropwise over 5 min. The mixture was warmed to 0 °C and stirred for 5 h. The ice bath was removed and the solution was allowed to warm to room temperature and stir overnight. The reaction mixture was poured over crushed ice (50 g) and the precipitate was collected by vacuum filtration on a Buchner funnel. The solid was dried first at room temperature overnight and then in a vacuum desiccator to afford the product as a white solid (743 mg, 95%). An analytical sample was obtained by recrystallization from

acetone−heptane (2:1): mp 288 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 7.61 (m, 2 H), 7.37 (dd, J = 11.8 and 2.2 Hz, 2 H), 3.98 (s, 2 H); IR (Kbr pellet) 3400−2500, 1665, 1622, 1485, 1448, 1274, 1187, 995, 809, 735 cm^{-1}; FABMS m/z 325 (MH^+), 324 (M^+), 307 ($MH^+ - H_2O$). Anal. ($C_{15}H_{10}F_2O_6$) C, H.

[3,3′-Difluoro-4,4′-dimethoxy-5,5′-bis(methoxycarbonyl)diphenyl]methane (33). A 250-mL three-necked, round-bottomed flask was equipped with a mechanical stirrer, a 10-mL pressure equalizing dropping funnel, and a reflux condenser fitted with a drying tube. (3,3′-Dicarboxy-5,5′-difluoro-4,4′-dihydroxydiphenyl)methane (**32**) (2.86 g, 8.77 mmol) was added to the flask and taken up in acetone (90 mL) with stirring. Potassium carbonate (9.68 g, 70 mmol) was added to the stirring mixture, and the reaction mixture was heated at reflux. The dropping funnel was charged with dimethyl sulfate (4.60 mL, 48.5 mmol), which was added dropwise to the vigorously stirring reaction mixture. The mixture was stirred vigorously under reflux for 20 h. The mixture was filtered, and the inorganic salts were washed with methylene chloride (4 × 5 mL). The solvents were removed in vacuo, water (10 mL) was added, and the mixture was extracted with methylene chloride (3 × 5 mL). The combined organic extracts were washed with water, dried over sodium sulfate, and evaporated in vacuo to yield the crude product as a yellow oil. The product was purified by flash column chromatography on silica gel (100 g) using 25% ethyl acetate in hexanes as the eluent. The fractions containing pure product were concentrated to yield the product as a crystallizing oil (2.65 g, 79%). An analytical sample was obtained by recrystallization from hexane and diethyl ether: mp 48 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33 (m, 2 H), 7.00 (dd, J = 11.6, 1.6 Hz, 2 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.90 (s, 6 H), 3.90 (s, 2 H); IR (Kbr pellet) 2944, 2838, 1722, 1493, 1435, 1321, 1281, 1202, 1019, 799, 766 cm^{-1}; CIMS m/z 381 (MH^+), 349 (MH^+ − MeOH). Anal. ($C_{19}H_{18}F_2O_6$) C, H.

3,3′-Difluoro-4,4′-dimethoxy-5,5′-bis(methoxycarbonyl-)diphenyl Ketone (34). [3,3′-Difluoro-4,4′-dimethoxy-5,5′-bis-(methoxycarbonyl)diphenyl]methane (**33**) (1.3 g, 3.58 mmol) was taken up in acetic anhydride (30 mL) with stirring. The mixture was cooled in an ice bath, and chromic anhydride (1.44 g, 14.3 mmol) was added in small portions over 10 min. The resulting reaction mixture was warmed to room temperature and stirred for 20 h. The inorganic salts were filtered off and washed with methylene chloride (5 × 10 mL). The solvent was removed in vacuo yielding the crude product as a dark green solid. The product was purified by flash column chromatography on silica gel (25 g) using methylene chloride as the eluent. The fractions containing the pure product were concentrated on a rotary evaporator yielding the pure product as a white solid (1.09 g, 81%): mp 90−91 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.92 (dd, J = 2.2 and 1.4 Hz, 2 H), 7.68 (dd, J = 11.6 and 2.2 Hz, 2 H), 4.10 (s, 3 H), 4.09 (s, 3 H), 3.91 (s, 6 H); IR (Kbr pellet) 3079, 2959, 1736, 1713, 1664, 1609, 1495, 1425, 1272, 1207, 993, 835, 759 cm^{-1}; CIMS m/z 395 (MH^+), 363 (MH^+ − MeOH). Anal. ($C_{19}H_{16}F_2O_6$) C, H.

3′,3″-Difluoro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-heptene (35). $TiCl_4$·2THF (2.05 g, 6.15 mmol) and zinc dust (0.804 g, 12.3 mmol) were heated at reflux in THF (20 mL) under an argon atmosphere for 1 h. 3,3′-Difluro-4,4′-dimethoxy-5,5′-bis(methoxycarbonyl)diphenyl ketone (**34**) (0.486 g, 1.23 mmol) and hexanal (0.22 mL, 1.85 mmol) were taken up in THF (20 mL) and added in one portion via cannula under argon pressure to the refluxing mixture. TLC (50% EtOAc/hexanes) showed the reaction to be complete in 30 min. The reaction mixture was cooled to ambient temperature and silica gel (20 mL) was added to the reaction mixture. The slurry was transferred to a chromatography column containing silica gel (40 g), and the inorganic salts were filtered off by elution of the product mixture with EtOAc. The eluent was concentrated in vacuo to give the crude product. Purification of the crude product by flash column chromatography on silica gel (10 g) using a gradient of 0−4% EtOAc in hexanes as the eluent yielded the pure product as a slightly yellow oil (0.373 g, 66%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 2 H), 7.02 (m, 2 H), 6.07 (t, J = 7.5 Hz, 1 H), 4.03 (s, 3 H), 3.96 (s, 3 H), 3.90 (s, 6 H), 2.08 (m, 2 H), 1.43 (m, 2 H), 1.26 (m, 4 H), 0.87 (t, J = 6.9 Hz, 3 H); IR (film) 2953, 2858, 1732, 1613, 1574, 1493, 1436, 1420, 1373, 1260, 1201, 1168, 1112, 1004, 884, 793, 774, 677, 650 cm^{-1}; CIMS m/z 463 (MH^+), 462 (M^+), 431 (MH^+ − MeOH). Anal. ($C_{25}H_{28}F_2O_6$) C, H.

Methyl 3′,3″-Difluoro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexenoate (36). $TiCl_4$·2THF (1.64 g g, 4.9 mmol) and zinc dust (0.0.641 g, 9.8 mmol) were heated at reflux in THF (16 mL) under an argon atmosphere for 1 h. Benzophenone **34** (0.387 g, 0.98 mmol) and methyl 5-oxopentanoate (**27**)[24] (0.255 g, 2.0 mmol) were taken up in THF (16 mL) and added in one portion via cannula under argon pressure to the refluxing mixture. TLC (50% EtOAc/hexanes) showed the reaction to be complete in 45 min. The reaction mixture was cooled to ambient temperature and silica gel (10 g) was added to the reaction mixture. The slurry was transferred to a chromatography column containing silica gel (40 g), and the inorganic salts were filtered off by elution of the product mixture with EtOAc. The eluent was removed in vacuo to give the crude product. Purification of the crude product was accomplished by flash column chromatography on silica gel (10 g) using a gradient of 0−18% EtOAc in hexanes as the eluent. Evaporation of the eluent yielded the pure product as a slightly yellow oil which crystallized on cooling in a freezer (−10 °C) to give a slightly yellow solid (0.22 g, 46%): mp 54−56 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, J = 2.3 Hz, 1 H), 7.31 (d, J = 2.1 Hz, 1 H), 7.03 (dd, J_{H-H} = 2.1 Hz, J_{H-F} = 9.7 Hz, 1 H), 6.99 (dd, J_{H-H} = 2.3 Hz, J_{H-F} = 11.2 Hz, 1 H), 6.04 (t, J = 7.4 Hz, 1 H), 4.15 (s, 3 H), 4.04 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.64 (s, 3 H), 2.31 (t, J = 7.4 Hz, 2 H), 2.14 (dt app. q, J = 7.4 Hz, 2 H), 1.78 (m, 2 H); ^{19}F NMR (282 MHz, $CDCl_3$, relative to TFA as external standard) δ −13.5 (d, J = 11.3 Hz, 1 F), −13.9 (d, J = 12.2 Hz, 1 F); IR (Kbr pellet) 2956, 1734, 1708, 1492, 1438, 1347, 1307, 1267, 1199, 993, 878, 788, 773 cm^{-1}; CIMS m/z 493 (MH^+), 461 (MH^+ − MeOH). Anal. ($C_{25}H_{26}F_2O_8$) C, H.

In Vitro Anti-HIV Assay. Anti-HIV screening of test compounds against various viral isolates and cell lines was performed as previously described.[47] This cell-based microtiter assay quantitates the drug-induced protection from the cytopathic effect of HIV-1. Data are presented as the percent control of XTT values for the uninfected, drug-free control. EC_{50} values reflect the drug concentration that provides 50% protection from the cytopathic effect of HIV-1 in infected cultures, while the CC_{50} reflects the concentration of drug that causes 50% cell death in the uninfected cultures. XTT-based results were confirmed by measurement of cell-free supernatant RT and p24 levels. All XTT cytoprotection data were derived from triplicate tests on each plate, with two separate sister plates. Thus, the EC_{50} value from each plate represents the average of triplicates, and the two EC_{50} values from sister plates were averaged. The variation from the mean averaged less than 10%.

Screening Against HIV-1 Viruses Containing NNRTI Resistance Mutations. These studies were performed as previously described.[48]

Assay for Inhibition of HIV-1 RT. The effects of the compounds on the HIV-1 RT enzyme were performed using purified p66/51 RT (a kind gift of S. Hughes, ABL-Basic Research Program, NCI−FCRDC). Inhibition of reverse transcription was measured by the level of incorporation of [^{32}P]-TTP into the poly(rA)-oligo(dT) (rAdT) or [^{32}P]GTP into the poly(rC)-oligo(dG) (rCdG) homopolymer template primer system.[6]

Acknowledgment. This investigation was made possible by Grant RO1-AI-43637, awarded by the National Institutes of Health, DHHS. A.C.-G. thanks Consejo Nacional de Ciencia y Tecnologia (Mexico) for a scholarship and Cytel Corp. for financial support.

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JM990343B