New Alkenyldiarylmethanes with Enhanced Potencies as Anti-HIV Agents Which Act as Non-Nucleoside Reverse Transcriptase Inhibitors

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Twenty-two new alkenyldiarylmethanes (ADAMs) were synthesized and evaluated for inhibition of HIV-1 replication. The most potent compound proved to be methyl 3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexenoate (ADAM II), which displayed an EC_{50} of 13 nM for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cells. ADAM II inhibited HIV-1 reverse transcriptase with an IC_{50} of 0.3 μM but was inactive as an inhibitor of HIV-1 attachment/fusion to cells, protease, integrase, and the nucleocapsid protein. Molecular target-based and cell-based assays revealed that ADAM II acted biologically as a nonnucleoside reverse transcriptase inhibitor (NNRTI). ADAM II inhibited replication of a wide variety of laboratory, clinical, and clade-representative isolates of HIV-1 in T cell lines and cultures of peripheral blood mononuclear cells or monocyte/macrophages. Mutations that conferred resistance to ADAM II clustered at residues 101, 103, 108, 139, 179, 181, and 188, which line the nonnucleoside binding pocket of HIV-1 reverse transcriptase. However, HIV-1 NL4-3 strain expressing a mutation at residue 100 of reverse transcriptase, and an AZT-resistant virus, displayed increased sensitivity to ADAM II. Thus, ADAM II could serve as an adjunct therapy to AZT and NNRTIs that select for L100I resistance mutations.

The nonnucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) are a structurally diverse set of compounds that inhibit the enzyme by an allosteric mechanism involving binding to a site adjacent to the deoxyribonucleoside triphosphate binding site.[1] Well-known NNRTIs include hydroxyethoxymethylphenylthiothymine (HEPT),[2] tetrahydroimidazobenzodiazepinone (TIBO),[3] dipyridodiazepinone (nevirapine),[4] pyridinone,[5] bis(heteroaryl)piperazine (BHAP),[6] *tert*-butyldimethylsilylspiroaminooxathiole dioxide (TSAO),[7] and α-anilinophenylacetamide (α-APA)[8] derivatives. Although the rapid emergence of resistant viral strains has hampered the clinical development of the NNRTIs for the treatment of AIDS, several strategies have emerged for overcoming resistance, including switching to another NNRTI to which the virus has remained sensitive, using higher doses of the NNRTI against the resistant strain,[9,10] and employing combinations of agents which elicit mutations that counteract one another.[11,12] A need remains for additional NNRTIs having unique patterns of resistance mutations in order to facilitate the application of these strategies.

We recently reported a series of alkenyldiarylmethanes (ADAMs) which act as NNRTIs.[13,14] The most potent of these proved to be ADAM **1**, which displayed synergistic anti-HIV-1 activity with AZT. To improve on the lead provided by ADAM **1**, additional compounds in this series were designed to optimize parameters for space filling and hydrogen bonding in the NNRTI binding pocket. The designed compounds were prepared and evaluated as inhibitors of HIV-1 reverse transcriptase (RT) and for inhibition of the cytopathic effect of HIV in cell culture. As described below, this strategy resulted in the development of a very potent ADAM compound that demonstrated increased potency against HIV-1 strains having the L100I mutation in the RT enzyme or having multiple mutations that confer resistance to AZT. Such a compound would be an attractive adjunct to therapeutic regimens that include AZT and/or an NNRTI that loses sensitivity against the L100I mutation.

Chemistry

The design of additional ADAMs has been aided by the availability of X-ray structures of HIV-1 reverse transcriptase complexed with nevirapine,[15−17] α-APA,[17,18] TIBO,[19−21] HEPT,[17] and BHAP.[22] These structures reveal that in general the NNRTIs assume a similar "butterfly" shape and bind to the enzyme in a similar way with considerable overlap, although HEPT appears to be somewhat of an outlier to the general pattern.[17,23] This allows the construction of a hypothetical model of the binding of ADAM **1** to HIV-1 RT. The model was constructed by overlapping the structure of ADAM **1** with that of nevirapine **(2)** in the binding pocket of HIV-1 RT (Sculpt 2.0, Interactive Simulations, San Diego, CA). During this process, it was assumed that the hexenyl side chain of ADAM **1** would point in the same direction as the cyclopropyl substituent of nevirapine. The nevirapine structure was then removed, the structure of the protein "frozen", and the energy of

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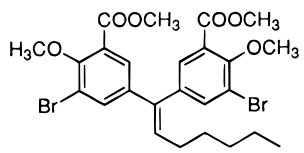

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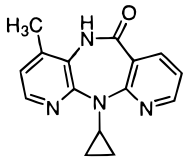

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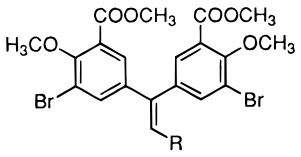
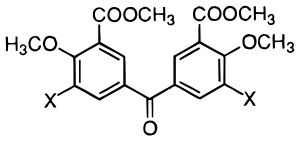
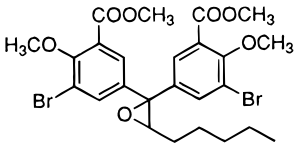


3 R = H
4 R = CH_3
5 R = OCH_3
6 R = $CH_2CH_2OTBDPS$
7 R = CH_2CH_2OH
8 R = $CH_2CH_2OSO_2CH_3$
9 R = $CH_2CH_2N_3$



10 X = Br
11 X = Cl



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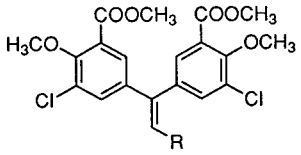
the complex minimized while allowing the ligand to move. This resulted in the hypothetical structure displayed in Figure 1. Besides being consistent with the reported structures of NNRTI−enzyme complexes, this model is also supported by our prior mutagenesis studies of the ADAM binding site of HIV-1 reverse transcriptase, in which it was determined that the resistance mutations to ADAM **1** circumscribe a well-defined binding pocket.[13,14]

In Figure 1, ADAM **1** is viewed looking down the alkenyl side chain with the end of the chain in front. According to this model, the end of the side chain is located near Glu 138 and Lys 103. Both of these residues contain side chains with functional groups capable of acting as hydrogen bond donors, assuming protonation of the acid. It might be expected that the incorporation of functional groups at the end of the alkenyl chain of the ligand which are capable of acting as hydrogen bond acceptors might allow favorable interactions with the adjacent residues. It was decided to synthesize ADAM analogues incorporating functionality at the end of the alkenyl side chain that would be capable of hydrogen bonding. Congeners of **1** were also planned in which the effect of alkenyl chain length on activity could be investigated. Various analogues were also considered in which the bromines present in **1** were replaced by chlorines, iodines, and hydrogens.

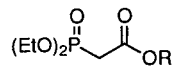
Considering first the compounds having the same substitution in the aromatic rings as in **1**, compounds **3**−**7** were prepared from the intermediate benzophenone **10**. The methylene and ethylene compounds **3** and **4** were prepared by the Wittig reactions of **10** starting from methyltriphenylphosphonium bromide and ethyltriphenylphosphonium bromide, respectively, using sodium bis(trimethylsilyl)amide as the base. The methoxyethylene congener **5** was prepared in a similar reaction employing methoxymethyl(triphenyl)phosphonium bromide. Similarly, reaction of ketone **10** with the Wittig reagent derived from [3-(*tert*-butyldiphenylsilyloxy)propyl]triphenylphosphonium bromide afforded intermediate **6**. Removal of the protecting group from **6** was accomplished with tetra-*n*-butylammonium fluoride in THF, yielding the alcohol **7**. Reaction of the alcohol **7** with mesyl chloride in the presence of triethylamine gave the mesylate **8**, which was converted to the azide **9** on treatment with sodium azide in DMF.

To convert **1** into a closely related compound bearing reactive functionality which might serve to alkylate the enzyme, the conversion of the alkene moiety to an epoxide was considered. Reaction of **1** with *m*-chloroperoxybenzoic acid in methylene chloride afforded the desired epoxide **12**.

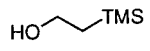
A series of dichloro ADAMs **13**−**27** bearing alkenyl substituents capable of hydrogen bonding were prepared using the substituted dichlorobenzophenone **11** as the starting material. Using the Horner−Emmons reaction, compound **28** was reacted with NaH at low temperature for 1 h to produce the corresponding anion, which was then reacted with ketone **11** to afford, after purification, ester **13** in good yield. The required reagent **28** was prepared from diethylphosphonoacetic acid **(29)** and 2-(trimethylsilyl)ethanol **(30)**. Deprotection of **13** was carried out with tetra-*n*-butylammonium fluoride in THF, affording the carboxylic acid **14** as a colorless crystalline solid. The methyl ester **15** and *tert*-butyl ester **16** were also prepared by the Horner−Emmons reaction starting from methyl diethylphosphonoacetate **(31)** and *tert*-butyl diethylphosphonoacetate **(32)**, respectively.



13 R = $COOCH_2CH_2TMS$
14 R = COOH
15 R = $COOCH_3$
16 R = $COOC(CH_3)_3$
17 R = $CH_2CH_2OTBDPS$
18 R = CH_2CH_2OH
19 R = $CH_2CH_2OSO_2CH_3$
20 R = $CH_2CH_2N_3$
21 R = $CH_2CH_2NH_2$
22 R = $CH_2CH_2CH_2COOCH_3$
23 R = $CH_2CH_2CH_2CH_2OTBDPS$
24 R = $CH_2CH_2CH_2CH_2OH$
25 R = $CH_2CH_2CH_2COOH$



28 R = CH_2CH_2TMS
29 R = H
31 R = CH_3
32 R = $C(CH_3)_3$



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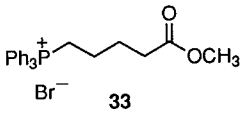

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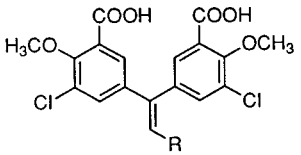


26 R = $CH_2CH_2CH_2COOH$
27 R = CH_2COOH

The Wittig reaction of the ketone **11** with the ylide derived from [3-(*tert*-butyldiphenylsilyloxy)propyl]-triphenylphosphonium bromide afforded the alkene **17**. Removal of the *tert*-butyldiphenylsilyl protecting group was accomplished with tetra-*n*-butylammonium fluoride in THF to provide the alcohol **18**. Oxidation of **18** under Jones conditions (CrO_3, sulfuric acid, acetone), followed by extraction with 3 M aqueous sodium hydroxide,

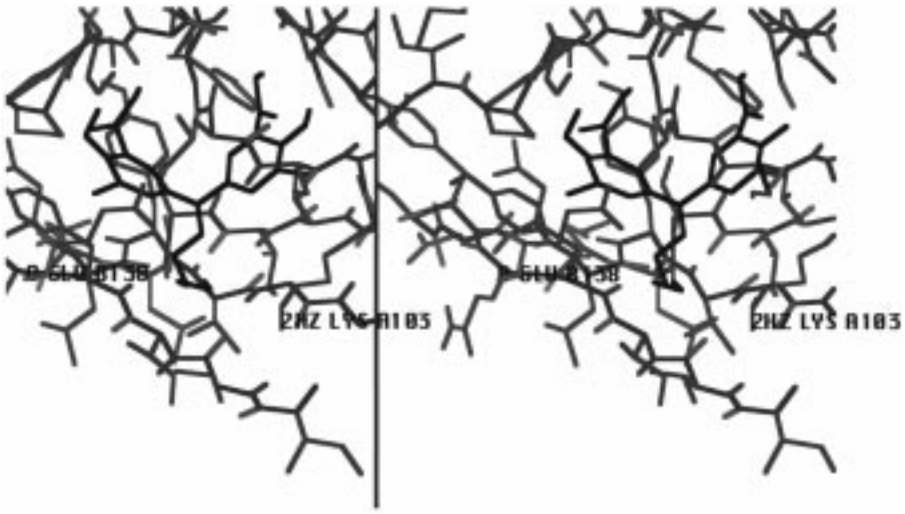


Figure 1. Hypothetical model of ADAM **1** docked in the NNRTI binding site of HIV-1 reverse transcriptase (programmed for walleyed viewing). The ligand shown in black, while the protein backbone is displayed in blue and the amino acid side chains are colored brown.

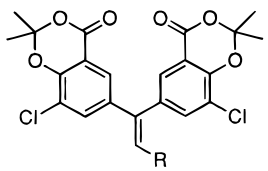
yielded the tricarboxylic acid **27**, resulting from oxidation of the primary alcohol in **18** and hydrolysis of the two ester groups during extraction. On the other hand, reaction of the alcohol **18** with methanesulfonyl chloride in dichloromethane, using triethylamine as the base, afforded the mesylate **19**. Reaction of the mesylate **19** with sodium azide in DMF gave the azide **20**.

Various methods were considered for the reduction of the azide **20** to the amine **21**. The usual methods for conversion of azides to amines involving lithium aluminum hydride[24] or catalytic hydrogenation[25] are obviously not suitable for accomplishing the conversion of **20** to **21** because of the other functionality in addition to the azide present in **20**. Following a protocol described by Brown and Salunkhe,[26] compound **20** was reacted with dichloroborane dimethyl sulfide complex to produce, after appropriate workup, amine **21** in low yield (15%). Dichloroborane dimethyl sulfide complex has been reported to react with olefins, but this process has been described to be slow and incomplete in the absence of trichloroborane.[27] However, it is likely that the reactivity of the borane complex with olefins is at least in part responsible for the poor yield observed in the conversion of **20** to amine **21**. Alternative methods were then considered in order to reduce **20** to **21** in acceptable yield. Zwierzak et al. reported the preparation of amines from azides using a variant of the Staudinger reaction.[28] They reacted organic azides with trialkyl phosphites, instead of the commonly used triphenylphosphine, to produce iminophosphorane intermediates, which after treatment with hydrogen chloride afforded the corresponding amine hydrochlorides. The higher reactivity of trialkyl phosphites toward azides was the main assumption for the modification. This methodology was considered to be the most adequate for the conversion of azide **20** to amine **21**, since the other reactive functionality present in **21** would not be affected by this method. In fact, the reaction of azide **20** with triethyl phosphite for 24 h, followed by hydrolysis of the intermediate iminophosphorane with dry hydrogen chloride for 48 h, afforded the amine **21** as its hydrochloride salt in 86% yield.

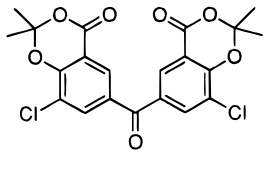
To synthesize an ADAM having a terminal methoxycarbonyl group at the end of an extended alkenyl side chain, the benzophenone **11** was subjected to a Wittig reaction with the ylide derived from the reaction of the phosphonium bromide **33** with sodium bis(trimethylsilyl)amide in THF. This resulted in the formation of the desired compound **22**, now called ADAM II.

The Wittig reaction of the ketone **11** with the ylide derived from [5-(*tert*-butyldiphenylsilanyloxy)pentyl]-triphenylphosphonium bromide[29] afforded the alkene **23**. Removal of the *tert*-butyldiphenylsilyl protecting group was accomplished with tetra-*n*-butylammonium fluoride in THF to provide the alcohol **24**. An inverse-addition Jones oxidation was used for the preparation of **25**, which involved the slow addition of a solution of alcohol **24** in acetone to chromium trioxide−aqueous sulfuric acid. Compound **22** was hydrolyzed with 10% potassium hydroxide to provide tricarboxylic acid **26**.

The effect of replacement of the methoxycarbonyl and methyl ether groups by cyclic acetonides could be readily investigated by evaluating the known alcohol **34**, prepared previously from the ketone **36**.[30] The primary alcohol was converted to the corresponding bromide **35** with carbon tetrabromide and triphenylphosphine in acetonitrile.[31,32]



34 R = CH_2CH_2OH
35 R = CH_2CH_2Br



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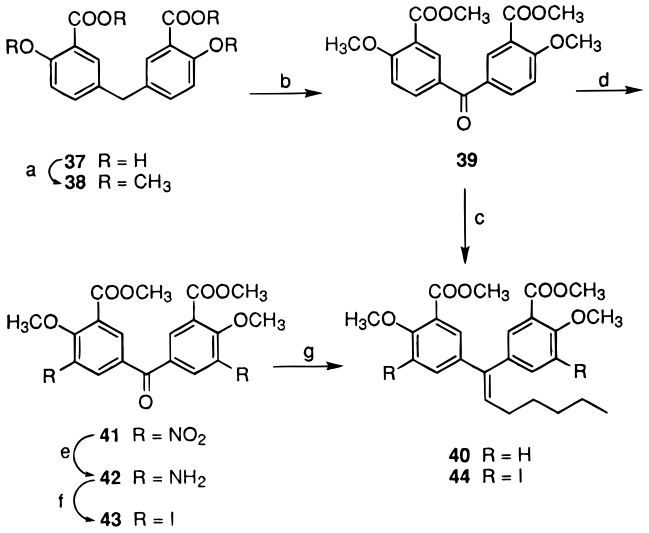
To determine the importance of the two halogen atoms for biological activity, congeners of ADAM **1** were prepared in which the halogens were replaced by iodines and by hydrogens. The syntheses of these compounds are outlined in Scheme 1. Treatment of the known diphenylmethane **37**[33] with dimethyl sulfate in refluxing acetone with potassium carbonate as the base resulted in methylation of both carboxylic acids and both phenols to afford compound **38**, which was oxidized to the benzophenone **39** with chromium trioxide in acetic

Scheme 1[a]



[a] Reagents: (a) Me_2SO_4, K_2CO_3, Me_2CO, reflux (6 h); (b) CrO_3, Ac_2O, 0 °C (1 h) and RT (12 h); (c) (1) *n*-$C_6H_{13}PPh_3Br$, $NaN(SiMe_3)_2$, THF, 0 °C (30 min), (2) add **39**, 23 °C (12 h); (d) HNO_3, Ac_2O, RT (12 h); (e) H_2, PtO_2, EtOAc; (f) (1) NaONO, HCl, H_2O, 30 min (0 °C), (2) I_2, KI, H_2O, RT (30 min); (g) (1) *n*-$C_6H_{13}PPh_3Br$, $NaN(SiMe_3)_2$, THF, 0 °C (30 min), (2) add **43**, 23 °C (12 h).

anhydride. Reaction of the ketone present in **39** with the Wittig reagent derived from *n*-hexyltriphenylphosphonium bromide gave the desired analogue **40**, in which the two bromines present in **1** have been replaced by hydrogens.

Nitration of **39** with nitric acid in acetic anhydride afforded the dinitro intermediate **41**. The two nitro groups present in **41** were reduced to amines with Adam's catalyst in ethyl acetate to provide the diamino compound **42**. Treatment of the diamine **42** with nitrous acid resulted in the conversion of both amines to diazonium groups, which were displaced by iodide in the presence of potassium iodide and iodine under aqueous conditions to provide intermediate **43**. The reaction of **43** with the ylide derived from *n*-hexyltriphenylphosphonium bromide afforded the desired analogue **44**.

Biological Results and Discussion

Twenty-two new ADAMs were tested 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. In addition, these compounds were tested as inhibitors of HIV-1 reverse transcriptase, and the resulting IC_{50} values are also listed in Table 1. The most potent of the new ADAMs, as well as the one with the highest therapeutic index, proved to be ADAM II **(22)**, which displayed an EC_{50} for prevention of the cytopathic effect of HIV-1_{RF} of 0.013 μM. This represents an approximately 700-fold increase in potency over the lead compound **1**, which was the most potent ADAM of the previously published series.[14] Perhaps more importantly, the therapeutic index increased by a factor of 162. The other new compounds which were equipotent or more potent than **1** for prevention of HIV-1 cytopathicity included the primary alcohol **7** (EC_{50} = 6.0 μM), the azide **9** (EC_{50} = 1.1 μM), the primary alcohol **18** (EC_{50} = 8.6 μM), and the azide **20** (EC_{50} = 0.27 μM). The remaining new ADAMs were inactive as inhibitors of HIV-1 cytopathicity at concentrations up to those resulting in cytotoxicity in uninfected cells.

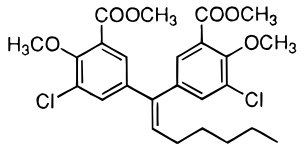
Table 1. Anti-HIV-1 Activities of ADAMs

		XTT assay		
compd	RT (IC_{50} μM)[a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
1	0.38	9.2	138	15
3	>100	NA[e]	316	
4	26	NA	10	
5	>100	NA	22	
7	31.6	6.0	>316	>52
9	94	1.1	>316	>278
12	5.6	NA	>100	
14	>100	NA	48	
15	16	NA	25	
16	>100	NA	>100	
18	>100	8.6	16.8	2
19	96	NA	6.9	
20	2.0	0.27	41.8	155
21	63	NA	15	
22	0.3	0.013	31.6	2430
24	3.6	NA	10.2	
25	>100	NA	100.4	
26	>100	180	>316	1.7
27	>100	NA	>316	
34	>100	NA	18.8	
35	>100	NA	176	
40	3.2	NA	14	
44	11	NA	>316	

[a] Inhibitory activity vs HIV-1 reverse transcriptase with rCdG 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}. [e] NA means there was no observed inhibition of HIV-1 cytopathicity up to the cytotoxic concentration in uninfected cells.

The observed increase in antiviral potency of **22** relative to **1** did not correlate with inhibition of HIV-1 RT with poly(rC)·oligo(dG) as the template primer, since **1** (IC_{50} vs RT = 0.38 μM) was essentially equipotent with ADAM II **(22)** (IC_{50} vs RT = 0.30 μM) as an enzyme inhibitor. All of the remaining new compounds displaying anti-HIV activity were less potent than **1** as inhibitors of HIV-1 RT with poly(rC)·oligo(dG) as the template primer, and some of them were significantly less active. Examples include compounds **7** (IC_{50} vs RT = 31.6 μM), **9** (IC_{50} vs RT = 94 μM), and **18** (IC_{50} vs RT > 100 μM). Conversely, analogues **12** and **40** inhibit HIV-1 RT with IC_{50} values of 5.6 and 3.2 μM, respectively, and are inactive as inhibitors of HIV-1 cytopathicity. All of these observations lead to the conclusion that there is not a strong correlation in this series between inhibition of RT in cell free systems with poly(rC)·oligo(dG) as the template primer and inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cells.

Closely related structural analogues of **1** are revealing in their inactivity as inhibitors of the cytopathic effect of HIV-1. Examples include the epoxide **12**, the dechlorinated analogue **40**, and the diiodo congener **44**. These inactive compounds emphasize the fact that there is a relatively high degree of structural specificity associated with the antiviral activity of the compounds in the series, so that even small changes in structure can result in complete loss of activity. The inactivity of **40** and **44** emphasize a significant role played by the two bromine atoms present in **1**. In this regard, the dichloro analogue **45** was reported previously to retain activity,



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although it was approximately half as potent as **1**.[14] However, the effect of bromine vs chlorine substitution is not always consistent. For example, if one considers the bromo alcohol **7** (EC_{50} 6.0 μM) vs the chloro alcohol **18** (EC_{50} 8.6 μM), they are approximately equipotent as inhibitors of viral cytopathicity. On the other hand, the chloro azide **20** (EC_{50} 0.27 μM) is 4 times more potent than the bromo azide **9** (EC_{50} 1.1 μM). The inactivity of the diiodo compound **44** as an inhibitor of HIV-1 induced cytopathicity and its lower activity as an inhibitor of RT (IC_{50} 11 μM) vs that of the corresponding dibromo compound **1** (IC_{50} 0.38 μM) may reflect a possible size limitation to activity, since the van der Waals radius of iodine (2.15 Å) is larger than that of bromine (1.95 Å).

The effect of replacement of the methoxycarbonyl and methyl ether groups by cyclic acetonides can be seen by comparison of the activities of the primary alcohols **18** and **34**. While **18** inhibited the cytopathic effect of HIV-1_{RF} with an EC_{50} of 8.6, the cyclic acetonide **34** was inactive. The corresponding primary bromide **35** was also inactive. The inactivity of **34** vs **18** could be due to the conformational restriction imposed by the six-membered ring and/or the increased steric bulk due to the *gem*-dimethyl moiety.

There appears to be an important effect of chain length of the alkenyl appendage. Both of the active azides **9** and **20** have a six-atom non-hydrogen chain length which is identical with the alkenyl chain present in ADAM **1**, and in the most active compound, ADAM II **(22)**, the seven-atom chain length is only slightly longer.

The effect of incorporation of hydrogen bonding groups at the end of the alkenyl chain seems to be variable, depending on the specific group incorporated and the length of the chain. For example, the primary alcohol **18** was effective in preventing the cytopathic effect of HIV-1_{RF} with an EC_{50} of 8.6 μM, but the corresponding amine **21** was inactive at 100 μM. Assuming the amine is protonated at physiological pH, it would be capable of acting as a hydrogen bond donor but not as a hydrogen bond acceptor at physiological pH, whereas the alcohol would be capable of functioning as both. The most effective compounds for inhibition of viral cytopathicity were ADAM II **(22)**, having a methyl ester at the terminus of the chain, and the two azides **9** and **20**. Although these compounds do have hydrogen bond accepting groups at the end of the alkenyl chain, it is difficult to attribute their greater antiviral activity to a greater affinity for the enzyme, because they are not more potent than **1** as enzyme inhibitors, at least with poly(rC)·oligo(dG) as the template primer. At this point, it is not possible to attribute the differences in activity among **1** and **9**, **20**, or **22** to any well-defined interaction with the biological system.

The methyl ester groups present in **22** were found to be essential for antiviral activity in this series. Conversion of ADAM II to the carboxylic acid **25** completely eliminated the antiviral activity. In addition, **25** did not show inhibition of HIV-1 RT. Results obtained with the tricarboxylic acid **26**, in which all three ester groups of ADAM II have been hydrolyzed, are not surprising since results in our previous series of ADAMs had indicated that hydrolysis of the esters on the aromatic rings was detrimental to the biological activity.[14] The antiviral assays of ADAM II were performed in the presence of fetal calf serum (FCS), and various tests with the compound in different levels of FCS or time in FCS have not revealed any instability. These results suggest that hydrolysis of **22** by esterases present in FCS is unlikely under the antiviral assay conditions.

Table 2. Mechanistic Evaluations

	IC_{50} (μM)	
parameter	**20**	**22**
RT rAdT	9.9	1.9
rCdG	2.0	0.3
integrase	NI_{100}[a]	NI_{100}
protease	>100[b]	>100[c]
nucleocapsid p7 protein	NI_{25}	NI_{25}

[a] NI indicates that no inhibition of activity was observed at the indicated high test concentration. [b] A 30% inhibition of HIV-1 protease was observed at a concentration of 100 μM. [c] A 17% inhibition of HIV-1 protease was observed at a concentration of 100 μM.

To determine whether the antiviral activity of ADAM II **(22)** was indeed due to its action as an NNRTI, it was tested in a number of assays representative of important events in the replication cycle of HIV-1 (Table 2). These included, in addition to RT inhibition with poly(rA)·oligo(dT) and poly(rC)·oligo(dG) as template primers, assays for inhibition of HIV-1 attachment/fusion to target cells and the activities of HIV-1 integrase and protease enzymes. The effect of the compounds on the nucleocapsid protein was also investigated.[34] ADAM II **(22)** demonstrated no significant effect on attachment/fusion, integrase, protease, or nucleocapsid protein. However, it inhibited RT with either poly(rA)·oligo(dT) or poly(rC)·oligo(dG) as template primers. The greater sensitivity to inhibition with poly(rC)·oligo(dG) as the template/primer is characteristic of the HIV-1-specific nonnucleoside reverse transcriptase inhibitors.[3,5,35−38] Interestingly, ADAM **1**, which had an EC_{50} of 0.38 μM with poly(rC)·oligo(dG) as the template primer, did not inhibit the enzyme with poly(rA)·oligo(dT) as the template primer.[14] Thus, there exists a clear distinction between the abilities of ADAM **1** and ADAM II **(22)** to inhibit RT activity with the poly(rA)·oligo(dT) template primer system.

The in vitro IC_{50} for compound **22** vs HIV-1 reverse transcriptase 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 (13 nM). This difference is not unusual for the NNRTIs.[3,35−37] As discussed elsewhere, this discrepancy may simply reflect the differences between the in vitro assay, in which synthetic template/primer has been added, and the cellular system.[36]

In addition to the molecular target-based mechanistic assays, ADAM II **(22)** was also evaluated in a time course assay to determine the site of action of the compound during the early phase of HIV-1 replication. The profile of inhibition of ADAM II **(22)** corresponded

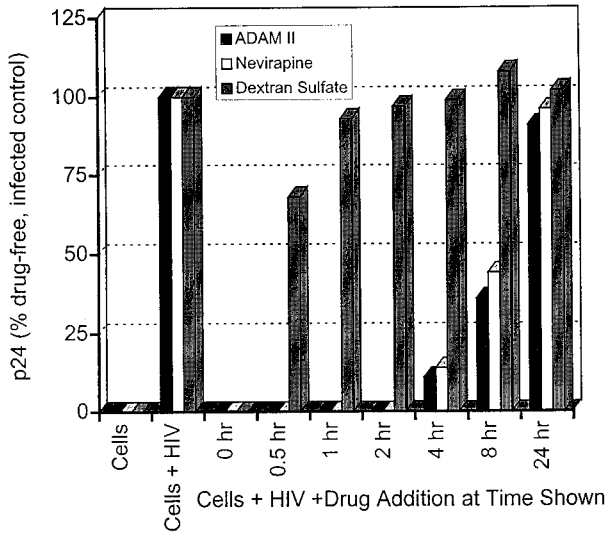

Figure 2. Effect of time of addition on ADAM II (**22**) activity. ADAM II (10 μM, solid bars), nevirapine (1 μM, open bars), or dextran sulfate (100 μg/mL, stippled bars) was added to cultures of CEM-SS cells infected with HIV-1_{IIIB} at the time of infection (0 h) or at the indicated time after initiation of infection. Infection was monitored by measurement of cell-free, virion-associated p24 in the culture fluid. Quantities of p24 are expressed as the percentage of the levels measued in the drug-free, HIV-1-infected cultures.

to that of an NNRTI (Figure 2), in which antiviral activity was diminished when the compound was added to cultures 4 h after initiation of infection. This is analogous to the effects of nevirapine (NNRTI), but unlike the action of dextran sulfate, which blocks virus attachment at the cell surface and loses its effectiveness within the first half hour after initiation of infection.[39] PCR-based analysis confirmed that ADAM II **(22)** prevented formation of proviral DNA during the early phase of infection (data not shown). ADAM II **(22)** had no effect on late phase virus production from latently HIV-1 infected U1 cells induced with TNF-α to produce virus from integrated proviral DNA (data not shown). Thus, ADAM II **(22)** acted biologically to inhibit replication by preventing reverse transcription.

The scope of the anti-HIV activity of ADAM II **(22)** was investigated in a number of laboratory-adapted strains, lymphocyte-tropic clinical isolates, clade representatives, and monocyte-tropic strains of HIV-1. Likewise, ADAM II **(22)** was tested for inhibitory activity against an array of HIV-1 strains containing mutations in the reverse transcriptase enzyme. The mutant viruses were obtained either by in vitro biological selection in the presence of various NNRTIs or by site-directed mutagenesis. To preserve continuity of data across the surfeit of virus strains, a greater amount of virus (higher MOI) was used in these competitive studies than was used in the original screening assays. For this reason, the EC_{50} was generally about 20-fold greater in these studies than in the original screen. Nevertheless, ADAM II **(22)** retained potent anti-HIV-1 activity against a wide variety of laboratory and clinical HIV-1 strains in CEM-SS, PBMC, and Mono/Mac cells (Table 3). Results from studies with the panels of viruses having defined mutations in reverse transcriptase are listed in Tables 4 and 5. Mutations conferring resistance to ADAM II **(22)** clustered within the amino acid residues comprising the NNRTI binding

Table 3. ADAM II Activity against HIV-1 Laboratory-Adapted Strains, Lymphocyte-Tropic Clinical Isolates, Clades, and Monocyte-Tropic Strains of HIV-1

virus isolate	virus type	cell type	EC_{50} (μM)
HIV-1 RF	WT-laboratory[a]	CEM-SS	0.91
HIV-1 IIIB	WT-laboratory[a]	CEM-SS	0.16
HIV-1 TEKI	WT-clinical[b]	PBMC	2.20
HIV-1 ROJO	WT-clinical[b]	PBMC	2.28
HIV-1	clade A	PBMC	1.09
HIV-1	clade B	PBMC	0.43
HIV-1	clade C	PBMC	1.04
HIV-1	clade D	PBMC	1.02
HIV-1	clade E	PBMC	0.40
HIV-1	clade F	PBMC	1.70
HIV-1 Ba-L	monocyte-tropic	Mono/Mac	0.36
HIV-1 ADA	monocyte-tropic	Mono/Mac	0.28

[a] Wild-type laboratory-adapted strain. [b] Wild-type clinical isolate.

Table 4. ADAM II Activity against a Biological Panel of Resistant Isolates in CEM-CC Cells

virus isolate	mutation in RT	EC_{50} (μM)	fold resistance
HIV-1 IIIB	wild-type	0.39	
OC[a]	L100I	0.94	2.4
TSAO/cost[b]	K101E	11.9	30
129/cost[b]	K103N	10.8	28
thiazol[c]	V108I	>50	>128
calo[d]	T139I	15	38
DPS[e]	Y181C	>50	>128
3TC	M184I	1.08	2.8
cost	Y188 H	>50	>128
HEPT[f]	*P*236L	8.08	21

[a] Oxathiin carboxanilide resistant. [b] Costatolide resistant. [c] Thiazolobenzimidazole resistant. [d] Calanolide resistant. [e] Diphenyl sulfone resistant. [f] (Hydroxyethoxy)methyl(phenylthio)thymine resistant.

Table 5. ADAM II Activity against a Site-Directed Panel of Resistant Isolates in CEM-SS Cells

virus isolate	mutation in RT	EC_{50} (μM)	fold resistance
HIV-1 NL4−3	wild-type	1.78	
	L74V	2.23	NC[a]
	A98G	0.84	NC[a]
	L100I	<0.16	S[b]
	K101E	3.05	NC[a]
	K103N	>50	>28
	V106A	12.7	7.13
	V108I	12.0	6.74
	V179D	50	28
	Y181C	>50	>28
	Y188C	10.80	6.07
	4XAZT/L100I[c]	0.27	S[b]
	4XAZT[c]	0.90	NC[a]
	4XAZT/Y181C[c]	>50	>28

[a] No significant change in sensitivity. [b] Enhanced sensitivity. [c] AZT resistant.

pocket.[15,18,19,40] Resistance mutations to the NNRTIs α-APA and BHAP are located close to the bound inhibitors,[18,22] and it therefore seems likely that the mutations conferring resistance to ADAM II **(22)** may also be located close to the bound ligand. The mutations conferring greater than 10-fold resistance to ADAM II **(22)** were located at positions 101, 103, 108, 139, 181, and 188. In contrast, the L100I mutant was significantly more sensitive to ADAM II than the corresponding wild-type virus HIV-1 NL4−3 (Table 5). However, the L100I mutation conferred slightly decreased sensitivity when introduced into the HIV-1 IIIB strain (Table 4).

The mutations conferring resistance and greater sensitivity to ADAM II **(22)** are displayed graphically

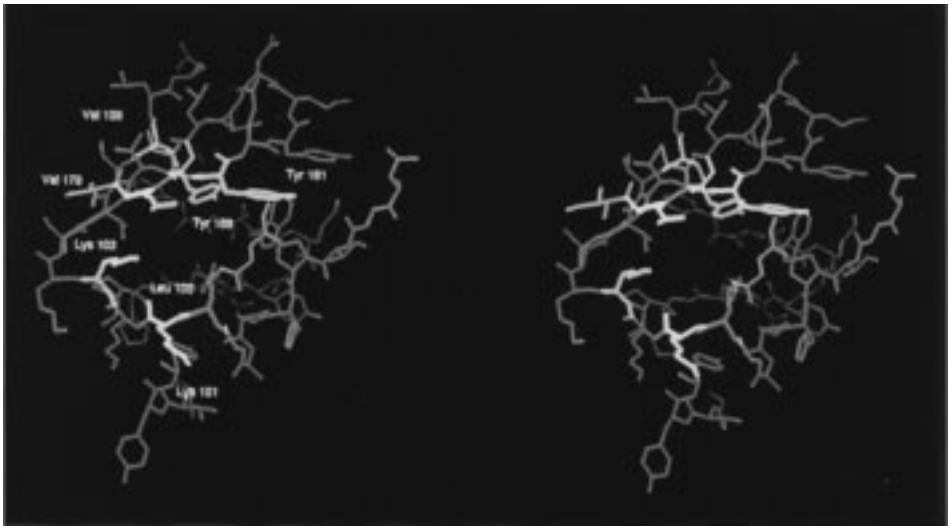

Figure 3. The non-nucleoside HIV-1 reverse transcriptase inhibitor (NNRTI) binding site. Mutation of the yellow- and red-colored residues results in resistance to known NNRTIs. Mutation of the yellow residues also results in resistance to ADAM II (**22**), while mutation of the red residue results in increased sensitivity to ADAM II (**22**).

in Figure 3, which was constructed by erasing nevirapine from the structure of the nevirapine−RT complex. This shows the amino acid residues in the p66 subunit surrounding the NNRTI binding pocket.[15] Mutation of the yellow-colored residues in Figure 3 results in resistance to ADAM II **(22)**, while mutation of the red-colored residue results in greater sensitivity to ADAM II **(22)** in the NL4-3 strain. The mutation at amino acid residue 139, which results in resistance to ADAM II **(22)**, is not shown because it is located in the p51 subunit. The cluster of yellow-colored amino acid residues shown in Figure 3 indicates the geometrical relationship of the residues that are likely to be involved in ADAM II **(22)** binding.

More impressive though was the finding that the virus containing the L100I mutation and the multiple mutations that confer resistance to AZT also displayed increased sensitivity to ADAM II **(22)**. This suggests the possible clinical utility of ADAM II **(22)** against AZT resistant strains of HIV-1 and against strains of HIV-1 that express L100I-based resistance to other NNRTIs. Ongoing crystallographic studies may lend insight into the binding interactions of ADAM II **(22)** with HIV-1 RT, while in vivo toxicology, pharmacology, and efficacy studies will be required to determine the utility of ADAM II **(22)** as a potential clinical candidate. Consistent with this finding, an isolate selected in cell culture for resistance to OC (oxathiin carboxanilide) and having an L100I mutation in RT retained sensitivity to ADAM II (EC_{50} = 0.94 μM).

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 on 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.

3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenylethene (3). Methyl(triphenyl)phosphonium bromide (132 mg, 0.36 mmol) was suspended in anhydrous THF (2 mL), the mixture was stirred under Ar in an ice bath, and sodium bis(trimethylsilyl)amide (1.0 M in THF, 0.4 mL, 0.4 mmol) was added by syringe. The mixture was stirred for 20 min, and a solution of **10**[14] (155 mg, 0.3 mmol) in anhydrous THF (3 mL) was slowly injected. The ice bath was removed, and the reaction mixture was stirred for 1 h at ambient temperature and for 1 h at 60 °C overnight at ambient temperature. The reaction was quenched with saturated ammonium chloride solution (3 mL). The yellow organic layer was separated, and the aqueous layer was extracted with benzene (2 × 5 mL). The combined organic extracts were washed with brine (2 mL) and dried (Na_2SO_4). The solvent was evaporated, and the residue was purified by flash chromatography on silica gel (6 g), eluting with hexanes−ethyl acetate (6:1) to give **3** (62 mg 42%) as a colorless solid: mp 144−145 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (m, 2 H), 7.68 (m, 2 H), 5.53 (s, 2 H), 4.01 (s, 3 H), 4.00 (s, 3 H), 3.97 (s, 3 H), 3.96 (s, 3 H); IR (KBr) 2943, 1730, 1472, 1434, 1246, 1208, 1086, 992, 797, 726 cm^{-1}; CIMS *m*/*z* (relative intensity) 516 (27), 515 (96), 514 (21), 512 (M^+, 5), 486 (10), 485 (45), 484 (21), 483 (100). Anal. ($C_{20}H_{18}Br_2O_6 \cdot 1H_2O$) C, H.

3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenylpropene (4). Ethyl(triphenyl)phosphonium bromide (134 mg, 0.36 mmol) was suspended in anhydrous THF (2 mL), and the mixture was stirred under Ar in an ice bath. Sodium bis(trimethylsilyl)amide (1.0 M in THF, 0.5 mL) was added by syringe. The mixture was stirred for 20 min, and a solution of **10** (155 mg, 0.3 mmol) in anhydrous THF (3 mL) was slowly injected. The ice bath was removed, and the reaction mixture was stirred for 24 h at ambient temperature. The reaction was quenched with saturated ammonium chloride solution (3 mL). The yellow organic layer was separated, and the aqueous layer was extracted with benzene (2 × 5 mL). The combined organic extracts were washed with brine (2 mL) and dried (Na_2SO_4). The solvent was evaporated and the residue purified by flash chromatography on silica gel (6 g), eluting with hexanes−ethyl acetate (6:1), to afford **4** (55 mg, 35%) as a colorless solid: mp 76−77 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54 (m, 2 H), 7.51 (m, 1 H), 7.49 (m, 1 H), 6.18 (q, J = 7.2 Hz, 1 H), 3.99 (s, 3 H), 3.93 (s, 6 H), 3.92 (s, 3 H), 1.77 (d, J = 7.2 Hz, 3 H); IR (KBr) 2950, 1732, 1475, 1286, 1263, 1207, 997, 725 cm^{-1}; CIMS *m*/*z* (rel intensity) 531 (62), 530 (29), 529 (94), 528 (23), 527 (M + 1, 57), 500 (12), 499 (53), 498 (24), 497 (100), 495 (53). Anal. ($C_{21}H_{20}Br_2O_6$) C, H.

3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1-methoxy-2,2-diphenylethene (5). Methoxymethyl(triphenyl)phosphonium bromide (257 mg, 0.75 mmol) was suspended in anhydrous THF (4 mL), the mixture was stirred

under Ar in an ice bath, and sodium bis(trimethylsilyl)amide (1.0 M in THF, 0.75 mL, 0.75 mmol) was added by syringe. The mixture was stirred for 20 min, and a solution of **10** (258 mg, 0.5 mmol) in anhydrous THF (4 mL) was slowly injected. The ice bath was removed, and the reaction mixture was stirred for 24 h at ambient temperature, 3 h at 60 °C, and overnight at ambient temperature. The reaction was quenched with saturated ammonium chloride solution (5 mL). The yellow organic layer was separated, and the aqueous layer was extracted with benzene (2 × 10 mL). The combined organic extracts were washed with brine (4 mL) and dried (Na_2SO_4). The solvent was evaporated and the residue purified by flash chromatography on silica gel (15 g), eluting with hexanes−ethyl acetate (6:1), to give compound **5** as a colorless oil (76 mg, 28%) having an R_f of 0.15 on silica gel when hexanes−ethyl acetate (6:1) was used as the solvent system: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, J = 2.1 Hz, 1 H), 7.74 (d, J = 2.1 Hz, 1 H), 7.61 (d, J = 2.2 Hz, 1 H), 7.58 (d, J = 2.2 Hz, 1 H), 6.51 (s, 1 H), 4.01 (s, 6 H), 3.99 (s, 3 H), 3.97 (s, 3 H), 3.89 (s, 3 H); IR (neat) 2949, 1733, 1636, 1475, 1436, 1289, 1255, 1208, 1124, 1081, 1049, 998 cm^{-1}; HRFABMS calcd for $C_{21}H_{21}Br_2O_7$ m/z 542.9654, found m/z 542.9661.

3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-4-[(*tert*-butyldiphenylsilyl)oxy]-1-butene (6). [3-(*tert*-Butyldiphenylsilyloxy)propyl]triphenylphosphonium bromide (595 mg, 0.93 mmol) was suspended in anhydrous THF (6 mL) and the mixture stirred under Ar on an ice bath. Sodium bis(trimethylsilyl)amide (1.0 M in THF, 1 mL) was added by syringe. The reaction mixture turned into a bright orange solution and was stirred in an ice bath for 30 min. A solution of the ketone **10** (320 mg, 0.62 mmol) dissolved in anhydrous THF (5 mL) was added, and the reaction mixture was stirred at ambient temperature for 24 h. The mixture was quenched with saturated ammonium chloride solution (10 mL), followed by ethyl acetate (10 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine (15 mL) and dried (Na_2SO_4). The solvent was evaporated and the yellow oily residue purified by flash chromatography on silica gel (20 g), eluting with hexanes−ethyl acetate (3:1), to give **6** (284 mg, 58%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.67 (d, J = 7.7 Hz, 4 H), 7.58 (m, 3 H), 7.51 (d, J = 2.1 Hz, 1 H), 7.40 (m, 6 H), 6.10 (t, J = 7.4 Hz, 1 H), 4.03 (s, 3 H), 3.98 (s, 3 H), 3.95 (s, 3 H), 3.93 (s, 3 H) 3.79 (t, J = 6.1 Hz, 2 H), 2.39 (m, 2 H), 1.09 (s, 9 H); IR (neat) 2951, 2860, 1734, 1473, 1265, 1205, 998, 704 cm^{-1}. Anal. ($C_{38}H_{40}Br_2O_7Si$) C, H.

3′,3″-Dibromo-4-hydroxy-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (7). Compound **6** (266 mg, 0.33 mmol) was dissolved in dry THF (7 mL) and the solution stirred at 0 °C under Ar. A 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (0.7 mL, 0.7 mmol) was added. The solution turned yellow and was stirred at 0 °C for 5.5 h. Brine (10 mL) was added, and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 20 mL). The extract was dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by flash column chromatography on silica gel, eluting with hexanes−ethyl acetate (3:1 to 1:1) to give **7** (129 mg, 70%) as a yellowish oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.58 (m, 3 H), 7.52 (m, 1 H), 6.14 (t, J = 7.1 Hz, 1 H), 4.00 (s, 3 H), 3.96 (s, 3 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.77 (t, J = 6.1 Hz, 2 H), 2.41 (q, J = 6.3 Hz, 2 H), 1.59 (brs, 1 H); IR (neat) 3430 (broad band), 2951, 1732, 1474, 1265, 1208, 998 cm^{-1}. Anal. ($C_{22}H_{22}Br_2O_7$) C, H.

3′,3″-Dibromo-4-methanesulfonyloxy-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (8). Compound **7** (310 mg, 0.55 mmol) and anhydrous triethylamine (0.23 mL, 1.7 mmol) were dissolved in dry dichloromethane (7 mL), and the mixture was stirred under Ar at 0 °C. Mesyl chloride (0.2 mL, 2.56 mmol) was added, and the mixture was stirred at 0 °C for 3 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed with 0.5 N HCl (2 × 20 mL), followed by saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic extract was dried ($MgSO_4$). The solvent was evaporated and the residue purified on silica gel (16 g), eluting with hexanes−ethyl acetate (3:1), to afford the mesylate **8** (270 mg, 77%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55 (m, 2 H), 7.51 (m, 2 H), 6.14 (t, J = 7.4 Hz, 1 H), 4.30 (t, J = 6.2 Hz, 2 H), 3.99 (s, 3 H), 3.93 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.04 (s, 3 H), 2.56 (q, J = 7.0 Hz, 2 H); IR (neat) 2952, 1732, 1474, 1358, 1265, 1175, 1089, 995 cm^{-1}. Anal. ($C_{23}H_{24}Br_2SO_9$) C, H.

4-Azido-3′,3″-dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (9). Compound **8** (231 mg, 0.363 mmol) was dissolved in dry *N*,*N*-dimethylformamide (5 mL). Sodium azide (120 mg, 1.82 mmol) was added, and the mixture was stirred at 35−50 °C for 3 h. The reaction mixture was allowed to reach ambient temperature, and then it was diluted with ethyl ether (42 mL). The ethereal solution was washed with water (2 × 35 mL) and brine (1 × 35 mL) and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified on silica gel (16 g), eluting with hexanes−ethyl acetate (3:1), to give **9** (132 mg, 62%) as a colorless solid: mp 69−70 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.56 (m, 3 H), 7.50 (m, 1 H), 6.05 (m, 1 H), 3.99 (m, 1 H), 3.93 (m, 3 H), 3.40 (m, 2 H), 2.41 (m, 2 H); IR (KBr) 2944, 2105, 1731, 1473, 1436, 1246, 1207, 1085, 999, 959, 806, 726 cm^{-1}. Anal. ($C_{22}H_{21}Br_2N_3O_6$) C, H.

3′,3″-Dibromo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-heptene Epoxide (12). Compound **1**[14] (67 mg, 0.116 mmol) was dissolved in dry methylene chloride (1 mL) and the solution cooled in a freezer for 5 min. 3-Chloroperoxybenzoic acid [minimum 57% (70 mg)] was dissolved in CH_2Cl_2 (0.5 mL) and injected into the solution. The mixture was allowed to reach ambient temperature and was stirred for 21 h. Solvent was evaporated, and the residue was purified by flash column chromatography on silica gel (7.5 g), eluting with hexanes−ethyl acetate (20:1 followed by 10:1), to give the epoxide **12** (43 mg, 62%) as an oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (m, 2 H), 7.67 (m, 1 H), 7.65 (m, 1 H), 3.97 (s, 3 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.35 (m, 1 H), 1.45 (m, 4 H) 1.27 (m, 5 H), 0.88 (m, 3 H); IR (neat) 2953, 1732, 1472, 1435, 1260, 1207, 1087, 999, 720 cm^{-1}. Anal. ($C_{25}H_{28}Br_2O_7$) C, H.

2-(Trimethylsilyl)ethyl 3′,3″-Dichloro-4′,4″-dimethoxy-5,5″-bis(methoxycarbonyl)-3,3-diphenylpropenoate (13). A suspension of sodium hydride (0.018 g, 0.750 mmol) in dry THF (5 mL) was stirred in an ice bath under an argon atmosphere. Phosphonoacetate **28** (0.21 mL, 0.70 mmol) was added, and the mixture was stirred at 0 °C for 1 h. The initial suspension turned into a clear solution within minutes. A solution of ketone **11** (0.200 g, 0.469 mmol) in dry THF (4 mL) was then added dropwise. The ice bath was removed, and the reaction mixture was stirred at room temperature for 30 h. The solvent was removed, and the residue was taken up in cold water (30 mL) and ethyl ether (30 mL). The layers were separated, and the aqueous one was extracted with ethyl ether (2 × 30 mL). The combined organic fractions were washed with brine (30 mL), dried over magnesium sulfate, and filtered. The solvent was removed in vacuo to give a residue. After flash chromatography on silica gel (35 g), eluting with hexanes−ethyl acetate (5:1), compound **13** (0.216 g, 81.2%) was obtained as a thick oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 2.4 Hz, 1 H), 7.53 (d, J = 2.2 Hz, 1 H), 7.39 (d, J = 3.1 Hz, 1 H), 7.38 (d, J = 2.5 Hz, 1 H), 6.31 (s, 1 H), 4.11 (m, J = 8.7 Hz, 2 H), 3.99 (s, 3 H), 3.95 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 0.89 (m, J = 8.7 Hz, 2 H), 0.01 (s, 9 H); IR (film) 2952, 1737, 1477, 1251, 1161, 997, 859, 744 cm^{-1}; FABMS m/z 568.8 (MH^+). Anal. ($C_{26}H_{30}Cl_2O_8Si$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-3,3-diphenylpropenoic Acid (14). A solution of ester **13** (0.198 g, 0.348 mmol) in dry THF (10 mL) was stirred under argon in an ice bath. A 1.0 M solution of tetra-*n*-butylammonium fluoride (0.7 mL, 0.697 mmol) was added dropwise and the reaction mixture stirred at 0 °C for 1.5 h. The mixture turned into a light yellowish solution. Brine (30 mL) was added and the mixture stirred for 10 min. A 1.0 N solution of HCl (10 mL) was then added, and the product was

extracted with ethyl ether (3 × 30 mL). The combined organic extracts were washed with brine (40 mL), dried over magnesium sulfate, and filtered, and the solvent was removed. Pure **14** (0.135 g, 83%) was obtained as a colorless crystalline solid after flash chromatography on silica gel (20 g), using chloroform−methanol−formic acid (200:10:0.1 mL) as eluant: mp 60−62 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 2.4 Hz, 1 H), 7.50 (d, J = 2.2 Hz, 1 H), 7.37 (d, J = 2.2 Hz, 1 H), 7.35 (d, J = 2.4 Hz, 1 H), 6.29 (s, 1 H), 3.97 (s, 3 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H); IR (film) 3400−2900, 2955, 1731, 1477, 1262, 1210, 1164, 994, 743 cm^{-1}; CIMS *m/z* (relative intensity) 469 (MH^+, 25), 451 ($MH^+ - 18$, 100), 437 ($MH^+ - 32$, 23). Anal. ($C_{21}H_{18}Cl_2O_8 \cdot 0.4H_2O$) C, H.

Methyl 3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-3,3-diphenylpropenoate (15). A suspension of sodium hydride (0.012 g, 0.493 mmol) in dry THF (5 mL) was stirred in an ice bath under an argon atmosphere. Methyl diethylphosphonoacetate **(31)** (0.09 mL, 0.469 mmol) was added, and the mixture was stirred at 0 °C for 1 h. The initial suspension turned into a clear solution within minutes. A solution of ketone **11** (0.100 g, 0.235 mmol) in dry THF (5 mL) was added dropwise. The ice bath was removed, and the reaction mixture was stirred at room temperature for 30 h. Cold water (15 mL) was added, and the mixture was stirred for 10 min. The product was extracted with ethyl ether (3 × 25 mL). The combined organic fractions were washed with brine (30 mL), dried over magnesium sulfate, and filtered. The solvent was removed in vacuo to give a residue. After purification by flash chromatography on silica gel (35 g), eluting with hexanes−ethyl acetate (2:1), compound **15** (0.0948 g, 83.8%) was obtained as a thick colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 2.4 Hz, 1 H), 7.53 (d, J = 2.2 Hz, 1 H), 7.37 (d, J = 2.2 Hz, 1 H), 7.36 (d, J = 2.4 Hz, 1 H), 6.32 (s, 1 H), 3.99 (s, 3 H), 3.94 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.63 (s, 3 H); IR (film) 2951, 1731, 1477, 1435, 1264, 1164, 996 cm^{-1}; CIMS *m/z* (relative intensity) 483 (MH^+, 30) and 451 ($MH^+ - CH_3OH$, 100). Anal. ($C_{22}H_{20}Cl_2O_8$) C, H.

***tert*-Butyl 3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-3,3-diphenylpropenoate (16).** *tert*-Butyl diethylphosphonoacetate **(32)** (0.44 mL, 1.872 mmol) was dissolved in dry THF (15 mL), and the mixture was stirred under argon in an ice bath. A 1.0 M solution of sodium bis(trimethylsilyl)amide (2.0 mL, 2.01 mmol) was added, and the solution was stirred at 0 °C for 1 h. At this time, a solution of the ketone **11**[41] (0.400 g, 0.936 mmol) in dry THF (12 mL) was added dropwise, and the resulting mixture was stirred at room temperature for 24 h. The solvent was evaporated and the residue partitioned between ethyl ether (40 mL) and water (60 mL). The layers were separated, and the aqueous one was extracted with ethyl ether (2 × 40 mL). The combined organic extracts were dried over magnesium sulfate and filtered, and the solvent was removed in vacuo to give a yellowish oil. Purification by flash chromatography (silica gel, 30 g), eluting with hexanes−ethyl acetate (3:1), afforded pure **16** (0.229 g, 46.6%) as a white solid: mp 114−115.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, J = 2.4 Hz, 1 H), 7.51 (d, J = 2.3 Hz, 1 H), 7.37 (d, J = 2.2 Hz, 1 H), 7.35 (d, J = 2.4 Hz, 1 H), 6.22 (s, 3 H), 3.98 (s, 3 H), 3.94 (s, 3 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 1.29 (s, 3 H); IR (film) 2952, 1731, 1479, 1368, 1258, 1095, 998, 848, 744 cm^{-1}; CIMS *m/z* (relative intensity) 451 ($MH^+ - C_4H_8$, 100), 469 ($MH^+ - C_4H_9OH$, 92), 470 ($MH^+ - C_4H_9O$, 64). Anal. ($C_{25}H_{26}Cl_2O_8$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-4-[(*tert*-butyldiphenylsilyl)oxy]-1-butene (17). [3-(*tert*-Butyldiphenylsilyloxy)propyl]triphenylphosphonium bromide (0.45 g, 0.702 mmol) was suspended in dry THF (5 mL) and stirred under argon. The suspension was cooled in an ice bath. A 1.0 M solution of NaHMDS (0.75 mL, 0.75 mmol) in THF was added dropwise. The reaction mixture turned into a bright orange solution and was stirred in the ice bath for 30 min. A solution of the ketone **11** (0.200 g, 0.468 mmol) in dry THF (2 mL) was added. The solution was stirred at room temperature for 24 h. A saturated solution of ammonium chloride (10 mL) was then added, followed by ethyl acetate (10 mL). The phases were separated, and the aqueous one was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine (40 mL), dried over magnesium sulfate, and filtered, and the solvent was evaporated to give a dark brown oil. Purification by flash chromatography (silica gel, 30 g, 3 × 14 cm column), eluting with hexanes−ethyl acetate (3:1), provided **17** (0.26 g, 76%) as a yellowish oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (dd, J = 1.4 Hz, 4 H), 7.49 (d, J = 2.3 Hz, 1 H), 7.46 (d, J = 2.1 Hz, 1 H), 7.31 (d, J = 2.0 Hz, 1 H), 7.28 (d, J = 2.3 Hz, 1 H), 7.34 (m, 6 H), 6.10 (t, J = 7.4 Hz, 1 H), 3.99 (s, 3 H), 3.94 (s, 3 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.75 (t, J = 6.1 Hz, 2 H), 2.36 (t, J = 6.3 Hz, 1 H), 2.34 (t, J = 6.4 Hz, 1 H), 1.05 (s, 9 H); IR (film) 3050, 2951, 1737, 1475, 1428, 1270, 1202, 1093, 999, 910, 702 cm^{-1}. Anal. ($C_{38}H_{40}Cl_2O_7Si$) C, H.

3′,3″-Dichloro-4-hydroxy-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)- 1,1-diphenyl-1-butene (18). Silyl ether **17** (0.2 g, 0.282 mmol) was dissolved in dry THF (6 mL) and stirred at 0 °C under argon. A 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (0.6 mL, 0.6 mmol) was added, and the initially yellow solution turned into an orange solution which was stirred at 0 °C for 5.5 h. Brine (10 mL) was added, and the phases were separated. The aqueous one was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine (40 mL), dried over magnesium sulfate and filtered, and the solvent was evaporated to give an orange oil. Purification by flash chromatography (silica gel, 20 g, 2 × 14 cm column) using hexanes−ethyl acetate (1:1) as the eluant afforded **18**[14] (94.5 mg, 71.4%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 2.4 Hz, 1 H), 7.50 (d, J = 2.1 Hz, 1 H), 7.37 (d, J = 2.1 Hz, 1 H), 7.31 (d, J = 1.8 Hz, 1 H), 6.13 (t, J = 7.4 Hz, 1 H), 3.99 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.75 (t, J = 6.3 Hz, 2 H), 2.38 (q, J = 13.4 and J = 6.7 Hz, 1 H), 1.66 (bs, 1 H); IR (film) 3542, 2951, 2874, 1732, 1477, 1428, 1268, 1210, 1093, 998, 866, 743 cm^{-1}.

3′,3″-Dichloro-4-methanesulfonyloxy-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (19). A solution of alcohol **18** (0.700 g, 1.491 mmol) and triethylamine (0.62 mL, 4.473 mmol) in dry dichloromethane (20 mL) was stirred under argon at 0 °C. Mesyl chloride (0.35 mL, 4.473 mmol) was added, and the mixture was stirred at 0 °C for 3 h. The reaction mixture was then diluted with dichloromethane (30 mL) and washed with 0.5 N HCl (2 × 40 mL), followed by saturated $NaHCO_3$ (40 mL) and brine (40 mL). The organic extract was dried over magnesium sulfate and filtered, and the solvent was removed in vacuo to produce a thick yellow oil (0.77 g). Purification of a fraction of this oil (0.557 g) by flash chromatography on silica gel (25 g) afforded pure **19** (0.521 g, 88.5%) as a thick oil, which slowly crystallized. The analytical sample was obtained by recrystallization from chloroform−methanol: mp 83−85 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J = 2.4 Hz, 1 H), 7.47 (d, J = 2.2 Hz, 1 H), 7.31 (d, J = 2.1 Hz, 1 H), 7.29 (d, J = 2.3 Hz, 1 H), 6.04 (t, J = 7.4 Hz, 1 H), 4.27 (t, J = 6.3 Hz, 2 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.00 (s, 3 H), 2.53 (q, J = 6.4 Hz and J = 7.2 Hz, 2 H); IR (film) 2952, 1731, 1479, 1359, 1269, 1174, 995 cm^{-1}; CIMS *m/z* (relative intensity) 547 (MH^+, 46), 515 (100). Anal. ($C_{23}H_{24}Cl_2O_9S$) C, H.

4-Azido-3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (20). Mesylate **19** (0.215 g, 0.393 mmol) was dissolved in dry DMF (5 mL). Sodium azide (0.13 g, 1.965 mmol) was added, and the mixture was stirred at 35−50 °C for 3 h. The mixture was allowed to cool at room temperature, and then it was diluted with ether (45 mL). The ethereal solution was washed with water (2 × 40 mL) and brine (40 mL) and dried over magnesium sulfate. After filtration and evaporation of the solvent in vacuo, a yellowish oil was obtained. Purification by flash chromatography on silica gel (16 g), eluting with hexanes−ethyl acetate (3:1), afforded pure **20** (0.148 g, 76.1%) as an oil which slowly crystallized. The analytical sample was obtained by recrystallization from chloroform−pentane: mp 70−72 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (t, J = 2.1 Hz, 2 H), 7.34 (d, J = 2.2

Hz, 1 H), 7.30 (d, J = 2.4 Hz, 1 H), 6.05 (t, J = 7.4 Hz, 1 H), 3.99 (s, 3 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.39 (t, J = 6.6 Hz, 2 H), 2.39 (q, J = 6.7 Hz and J = 7.2 Hz, 2 H); IR (film) 2952, 2098, 1734, 1477, 1267, 997, 742 cm^{-1}; CIMS m/z (relative intensity) 494 (MH^+, 100), 496 (70). Anal. ($C_{22}H_{21}Cl_2N_3O_6 \cdot 0.5H_2O$) C, H.

4-Amino-3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (21). A solution of azide **20** (0.228 g, 0.462 mmol) and $P(OEt)_3$ (0.24 mL, 1.387 mmol) in benzene (6 mL) was stirred under argon at room temperature for 24 h. The reaction mixture was then saturated with dry HCl for 10 min and stirred for 48 h at room temperature. The solvent was removed, and dry ethyl ether (15 mL) was added. The solution was placed in the freezer for 48 h. The precipitated amine hydrochloride was separated by filtration, washed with cold ethyl ether, and dried under high vacuum overnight to provide **21** (0.200 g, 86%) as a white solid. The analytical sample was recrystallized from ethanol−ethyl ether−pentane: mp 156−158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (t, J = 2.3 Hz, 2 H), 7.47 (d, J = 2.2 Hz, 1 H), 7.32 (d, J = 2.2 Hz, 1 H), 7.29 (d, J = 2.4 Hz, 1 H), 6.06 (t, J = 7.4 Hz, 1 H), 3.96 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 2.81 (t, J = 6.6 Hz, 2 H), 2.25 (q, J = 7.1 Hz, 2 H), 1.90 (bs, 2 H, exchangeable with D_2O); IR (film) 2951, 1731, 1476, 1435, 1261, 1208, 997, 742 cm^{-1}; CIMS m/z (relative intensity) 467 (M^+, 90), 466 ($M^+ - 1$, 100). Anal. ($C_{22}H_{23}Cl_2NO_6 \cdot HCl$) C, H, N.

Methyl 3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenylhexenoate (22). (4-Methoxycarbonylbutyl)triphenylphosphonium bromide **(33)**[42] (0.321 g, 0.704 mmol) was stirred in dry THF (15 mL) under argon at −78 °C. A 1.0 M solution of $NaN(SiMe_3)_2$ in THF (0.78 mL, 0.78 mmol) was then added, and the yellow solution was stirred in a dry ice−acetone bath for 1 h. A −78 °C solution of ketone **11** (0.200 g, 0.469 mmol) in dry THF (5 mL) was added, and the reaction mixture was stirred at −78 °C for 12 h and then at room temperature for 12 h. Saturated NH_4Cl (25 mL) was added and the mixture stirred for 15 min. The layers were separated, and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine (40 mL), dried over magnesium sulfate and filtered, and the solvent was removed in vacuo to give a thick orange residue. Purification was achieved by flash chromatography (silica gel, 23 g, 2 × 20.1 cm column), eluting with hexane−ethyl acetate (3:1), to provide **22** (0.110 g, 44.6%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, J = 2.3 Hz, 1 H), 7.48 (d, J = 2.2 Hz, 1 H), 7.33 (d, J = 2.2 Hz, 1 H), 7.30 (d, J = 2.3 Hz, 1 H), 6.05 (t, J = 7.5 Hz, 1 H), 4.01 (s, 3 H), 3.95 (s, 3 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.65 (s, 3 H), 2.32 (t, J = 7.4 Hz, 2 H), 2.15 (q, J = 7.4 Hz, 2 H), 1.80 (m, J = 7.3 Hz, 2 H); IR (film) 2951, 1736, 1477, 1261, 1208, 1093, 999, 743 cm^{-1}; FABMS m/z (relative intensity) 525 (MH^+, 30), 509 ($M^+ - CH_3$, 28), 493 ($M^+ - OCH_3$, 100). Anal. ($C_{25}H_{26}Cl_2O_8$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-6-[(*tert*-butyldiphenylsilyl)oxy]-1-hexene (23). A suspension of [5-(*tert*-butyldiphenylsilanyloxy)pentyl]triphenylphosphonium bromide[29] (8.66 g, 13.00 mmol) in dry THF (150 mL) was stirred at −78 °C under an argon atmosphere. A 1.0 M solution of $NaN(TMS)_2$ in THF (13.9 mL, 13.9 mmol) was added dropwise. The mixture turned into a bright orange solution and was stirred at −78 °C for 45 min. A solution of benzophenone **11** (3.69 g, 8.66 mmol) in dry THF (50 mL) was then added dropwise. The reaction mixture was stirred at 0 °C for 1.5 h. The reaction mixture was then quenched with saturated NH_4Cl solution (70 mL). The phases were separated, and the aqueous one was extracted with Et_2O (3 × 50 mL). The combined organic extracts were washed with brine (1 × 70 mL), dried over magnesium sulfate, and filtered. The solvent was removed, and the residue was chromatographed on silica gel (200 g, column: 5 cm × 10.5 in.), eluting with hexane−ethyl acetate (6:1) to hexane−ethyl acetate (5:1). Compound **23** was obtained in moderate yield (4.00 g, 63% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64−7.60 (m, 4 H), 7.46 (d, J = 2.4 Hz, 1 H), 7.43 (d, J = 2.2 Hz, 1 H), 7.39−7.31 (m, 6 H), 7.27 (dd, J = 2.4 Hz, 2 H), 6.01 (t, J = 7.5 Hz, 1 H), 3.96 (s, 3 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.61 (t, J = 5.7 Hz, 1 H), 2.02 (q, J = 7.1 Hz, 2 H), 1.51 (m, 4 H), 1.01 (s, 9 H): IR (film): 2934, 1736, 1477, 1260, 1093, 998, 702 cm^{-1}. FABMS m/z (relative intensity) 703 ($MH^+ - CH_3OH$, 7) and 677 ($M^+ - C_4H_9$, 35). Anal. ($C_{40}H_{44}Cl_2O_7Si$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-hexen-6-ol (24). Silyl ether **23** (3.905 g, 5.318 mmol) was dissolved in dry THF (50 mL) and stirred at 0 °C under an argon atmosphere. A 1.0 M solution of TBAF in THF (10.6 mL, 10.63 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h. The ice bath was then removed, and the mixture was stirred at 25 °C for 2 h. Brine (60 mL) was added to quench the reaction. The phases were separated, and the aqueous one was extracted with Et_2O (3 × 50 mL). The organic extracts were combined and washed with brine (1 × 70 mL), dried over $MgSO_4$, and filtered. Purification by flash chromatography on silica gel (150 g, column: 5 cm × 17.5 in.), eluting with hexanes−ethyl acetate (1:1), followed by ethyl acetate−hexanes (2:1), afforded compound **24** as a white solid in good yield (75%): mp 94−96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 2.3 Hz, 1 H), 7.46 (d, J = 2.1 Hz, 1 H), 7.30 (d, J = 2.2 Hz, 1 H), 7.28 (d, J = 2.2 Hz, 1 H), 6.05 (t, J = 7.5 Hz, 1 H), 3.98 (s, 3 H), 3.91 (s, 6 H), 3.89 (s, 3 H), 3.60 (t, J = 5.7 Hz, 1 H), 2.11 (q, J = 7.0 Hz, 2 H), 1.61−1.50 (m, 4 H); IR (film) 3500−3200, 2936, 1735, 1477, 1258, 998, 743 cm^{-1}. Anal. ($C_{24}H_{26}Cl_2O_7$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-6,6-diphenyl-5-hexenoic Acid (25). A solution of chromium trioxide (0.101 g, 1.01 mmol) and 0.75 N sulfuric acid (1.6 mL, 2.323 mmol) was stirred in an ice bath. A solution of alcohol **24** (0.100 g, 0.202 mmol) in acetone (10 mL) was added over 6 h, while the temperature was maintained below 5 °C. After the addition was finished, the reaction mixture was stirred at 25 °C for 2 h. It was left overnight without stirring. The solvent was removed, and the residue was diluted with water (5 mL). The mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with water (1 × 25 mL) and brine (1 × 25 mL), dried over magnesium sulfate, and filtered. The solvents were removed, and the residue was purified by flash chromatography on silica gel (30 g, column: 2 cm × 21 cm), eluting with hexanes−ethyl acetate (1:1). Compound **24** was obtained as a colorless oil in moderate yield (44 mg, 42.8%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.3 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 = 2.3 Hz, 1 H), 6.02 (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), 2.32 (t, J = 7.3 Hz, 2 H), 2.14 (q, J = 7.5 Hz, 2 H), 1.76 (qn, J = 7.4 Hz, 2 H); IR (film) 3400−2500, 2951, 1732, 1477, 1263, 1209, 998. 742 cm^{-1}; FABMS m/z (relative intensity): 511 (MH^+, 20) and 479 ($MH^+ - CH_3OH$, 50). Anal. ($C_{24}H_{24}Cl_2O_8$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(carboxy)-6,6-diphenyl-5-hexenoic Acid (26). Compound **22** (0.403 g, 0.796 mmol) was dissolved in MeOH (6 mL), and 10% aqueous KOH (10 mL) was added. The mixture was heated at 60 °C for 24 h. The solvent was removed, and the aqueous residue was diluted with water (10 mL) and washed with ethyl acetate (15 mL). It was then acidified with concentrated HCl and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with brine (1 × 20 mL), dried over magnesium sulfate, and filtered. After evaporation of the solvent, compound **26** was obtained as an off-white solid (0.34 g, 91.7%): mp 203−206 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.58 (dd, J = 2.4 and J = 2.1 Hz, 2 H), 7.52 (d, J = 2.4 Hz, 1 H), 7.50 (d, J = 2.3 Hz, 1 H), 6.28 (t, J = 7.5 Hz, 1 H), 3.95 (s, 3 H), 3.89 (s, 3 H), 2.30 (t, J = 7.2 Hz, 2 H), 2.20 (q, J = 7.4 Hz, 2 H), 1.78 (qn, J = 7.3 Hz, 2 H); IR (film) 3500−2500, 2930, 1708, 1477, 1256, 998 cm^{-1}; FABMS m/z (relative intensity) 483 (MH^+, 10) and 465 ($MH^+ - H_2O$, 25). Anal. ($C_{22}H_{20}Cl_2O_8$) C, H.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(carboxy)-4,4-

diphenyl-3-butenoic Acid (27). Chromium trioxide (0.64 g, 6.39 mmol) was dissolved in 1.5 M sulfuric acid (9.3 mL, 13.9 mmol), and the solution was stirred in an ice bath. A solution of alcohol **18** (0.50 g, 1.06 mmol) in acetone (15 mL) was then added and the ice bath removed. The reaction mixture was stirred at room temperature for 7 h. Ethyl ether (50 mL) was added, and the phases were separated. The organic phase was washed with water (3 × 40 mL) and then extracted with 3 M sodium hydroxide (3 × 20 mL). The combined aqueous extracts were acidified with concentrated hydrochloric acid, and the cloudy solution was kept in the refrigerator overnight. Separation of the precipitated solid by filtration, followed by washing with water, afforded a yellowish solid (0.131 g), which was purified by recrystallization from ethyl ether−dichloromethane to yield **27** (63 mg, 12.2%) as a pale yellow solid: mp 206−207 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.65 (d, J = 2.3 Hz, 1 H), 7.62 (d, J = 2.1 Hz, 1 H), 7.57 (d, J = 2.1 Hz, 1 H), 7.52 (d, J = 2.3 Hz, 1 H), 6.42 (t, J = 7.4 Hz, 1 H), 3.97 (s, 6 H), 3.91 (s, 6 H), 3.20 (d, J = 7.4 Hz, 2 H); IR (film) 3400−2800, 2938, 1702, 1478, 1259, 996, 708 cm^{-1}; FABMS *m/z* (relative intensity) 454 (M^+, 45), 437 (M^+ - 17, 100). Anal. ($C_{20}H_{16}Cl_2O_8$·0.4H_2O) C, H.

(2-Trimethylsilyl)ethyl Diethylphosphonoacetate (28).[43] BOP-Cl (2.53 g, 9.95 mmol) was added to a solution of diethylphosphonoacetic acid **(29)** (1.6 mL, 10 mmol), 2-(trimethylsilyl)ethanol **(30)** (1.57 mL, 10.9 mmol), and triethylamine (2.77 mL, 19.9 mmol) in dry dichloromethane (25 mL). The initial suspension turned into a clear solution within minutes, which was stirred at room temperature under Ar for 1.2 h. A 5% aqueous solution of sodium carbonate (60 mL) was added, and the phases were separated. The organic phase was diluted with dichloromethane (2 × 30 mL) and washed with water (50 mL) and brine (50 mL). The organic phase was dried over magnesium sulfate and filtered and the solvent evaporated to give a liquid residue (6.89 g). Purification by flash chromatography (silica gel, 135 g, 5 × 20.3 cm column), eluting with hexanes−ethyl acetate (2:1), afforded **28** as a colorless liquid (2.47 g, 84%): bp 120−122 °C/0.05 mmHg (lit.[43] bp 140−145 °C/0.1 mmHg); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.18 (m, J = 8.6 Hz, 2 H), 4.14 (m, J = 7.2 Hz, 4 H), 2.94 (s, 1 H), 2.87 (s, 1 H), 1.31 (t, J = 7.1 Hz, 6 H), 0.98 (m, J = 8.7 Hz, 2 H), 0.01 (s, 9 H); IR (film) 2981, 2954, 2904, 1737, 1268, 1027, 969, 838 cm^{-1}. Anal. ($C_{11}H_{25}O_5PSi$) C, H.

1-Bromo-4,4-bis[8′,8″-dichloro-2′,2′,2″,2″-tetramethyl-4′,4″-dioxo-6′,6″-(1,3-benzodioxyl)]-3-butene (35). A solution of alcohol **34**[30] (0.106 g, 0.215 mmol) and carbon tetrabromide (0.09 g, 0.271 mmol) in dry acetonitrile (8.5 mL) was stirred under argon. A solution of triphenylphosphine (0.08 g, 0.305 mmol) in dry acetonitrile (1.5 mL) was added dropwise. The reaction mixture was stirred at reflux for 20 h. The solvent was removed in vacuo, and the resulting oil was extracted with ethyl ether (5 × 5 mL). The solvent was evaporated from the combined organic extracts to give a thick solid. Purification by flash chromatography on silica gel (25 g), eluting with hexanes−ethyl acetate (5:1), afforded the product **35** (66 mg, 56%) as a yellowish solid: mp 175−178 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (d, J = 2.0 Hz, 1 H), 7.69 (d, J = 1.8 Hz, 1 H), 7.45 (d, J = 2.2 Hz, 1 H), 7.44 (d, J = 2.1 Hz, 1 H), 6.09 (t, J = 7.2 Hz, 1 H), 3.45 (t, J = 6.5 Hz, 2 H), 2.70 (q, J = 6.7 Hz, 2 H), 1.84 (s, 6 H), 1.80 (s, 6 H); IR (film) 2999, 1745, 1607, 1483, 1283, 1199, 1063, 874, 756 cm^{-1}; CIMS *m/z* (relative intensity) 555 (MH^+, 55), 557 (MH^+ + 2, 100), 559 (MH^+ + 4, 40). Anal. ($C_{24}H_{21}BrCl_2O_6$) C, H.

4,4′-Dimethoxy-3,3′-bis(methoxycarbonyl)diphenylmethane (38). A suspension of 3,3′-dicarboxy-4,4′-dihydroxydiphenylmethane **(37)**[33] (0.576 g, 2 mmol), dimethyl sulfate (2 mL, 12 mmol), and potassium carbonate (2.0 g) in acetone (20 mL) was heated at reflux for 6 h. The solid mass was separated by filtration and washed with acetone. Evaporation of the acetone gave the product **38** (0.51 g, 80%) as a brown oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (d, J = 1.8 Hz, 2 H), 7.25 (dd, J = 1.8, 8.6 Hz, 2 H), 6.90 (d, J = 8.5 Hz, 2 H), 3.90 (s, 2 H), 3.87 (s, 6 H). Anal. ($C_{19}H_{20}O_6$) C, H.

4,4′-Dimethoxy-3,3′-bis(methoxycarbonyl)benzophenone (39). A solution of intermediate **38** (0.5 g, 1.8 mmol) in acetic anhydride (25 mL) was cooled to 0 °C in an ice bath containing NaCl. Solid chromium(VI) oxide (3 g, 30 mmol) was added slowly to the solution at 0 °C. After complete addition, the mixture was stirred at 0 °C for 1 h and then room temperature for 12 h. The resulting viscous paste was broken up with ethyl acetate and partitioned between 1 N HCl (200 mL) and ethyl acetate (200 mL). The organic layer was washed with 1 N HCl (100 mL) and brine (100 mL) and dried over Na_2SO_4, and the solvent was removed under reduced pressure. Trituration of the solid in 1 N HCl and filtering gave the product **39** (0.12 g, 22%) as an off white solid: mp 142−144 °C; ^{1}H NMR (acetone-d_6) δ 8.14 (d, J = 2.2 Hz, 2 H), 7.96 (dd, J = 2.2, 8.7 Hz, 1 H), 7.30 (d, J = 8.7 Hz, 1 H), 3.99 (s, 3 H), 3.84 (s, 3 H); FTIR (KBr) 2953, 2849, 1735, 1710, 1650, 1603, 1502, 1438, 1406, 1274, 1152, 1081, 1010, 950 cm^{-1}. Anal. ($C_{19}H_{18}O_7$) C, H.

4′,4″-Dimethoxy-3′,3″-bis(methoxycarbonyl)-1,1-diphenyl-1-heptene (40). *n*-Hexyltriphenylphosphonium bromide (0.427 g, 1 mmol) was dried by azeotropic distillation from a benzene solution and then stirred in dry THF (10 mL) under nitrogen atmosphere. Sodium bis(trimethylsilyl)amide (1 M in THF, 1 mL, 1 mmol) was added, and the ylide produced was stirred under nitrogen at 0 °C for 30 min. Intermediate **39** (0.276 g, 0.77 mmol) was added as a solution in THF (10 mL) under nitrogen. The mixture was stirred at room temperature overnight and partitioned between 1 N HCl (100 mL) and ethyl acetate (100 mL). The organic layer was dried over sodium sulfate and evaporated to afford an oil which was chromatographed on silica gel (50 g), eluting with hexanes−ethyl acetate (4:1) to give the product **40** (0.15 g, 46%) as an oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (d, J = 2.3 Hz, 1 H), 7.56 (d, J = 2.2 Hz, 1 H), 7.20 (dt, J = 2.3 Hz, 1 H), 6.94 (d, J = 8.6 Hz, 1 H), 6.82 (d, J = 8.7 H, 1 H), 5.96 (t, J = 7.5 Hz, 1 H), 3.90 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 6 H), 2.05 (q, J = 7.3 Hz, 2 H), 1.39 (t, J = 7.2 Hz, 2 H), 1.22 (m, J = 1.9 Hz, 2 H), 0.83 (t, J = 6.7 Hz, 3 H); IR (neat) 2927, 2853, 1732, 1606, 1500, 1435, 1263 cm^{-1}. Anal. ($C_{25}H_{30}O_6$) C, H.

4,4′-Dimethoxy-3,3′-bis(methoxycarbonyl)-5,5′-dinitrobenzophenone (41). A solution of compound **39** (1.07 g, 3 mmol) in acetic anhydride (30 mL) was cooled to 0 °C. Nitric acid (90%, 20 mL) was added dropwise, and the solution was stirred overnight while warming to room temperature. The orange solution was poured onto ice and water and extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with 5% KOH solution (3 × 50 mL), dried over $MgSO_4$, and evaporated. The oil (1.1 g) was flash chromatographed on silica gel (250 g), eluting with hexanes−ethyl acetate (10:2), to afford **41** (0.41 g, 32%) as a solid: mp 96−98 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.55 (s, 2 H), 8.41 (s, 2 H), 4.1 (s, 6 H), 3.99 (s, 6 H). Anal. ($C_{19}H_{16}H_2O_{11}$) C, H, N.

3,3′-Diamino-5,5′-bis(methoxycarbonyl)-4,4′-dimethoxybenzophenone (42). The dinitro compound **41** (0.33 g, 0.9 mmol) was hydrogenated at atmospheric pressure over platinum oxide (0.2 g, 0.08 mmol) in ethyl acetate (50 mL). After TLC (silica gel, hexanes−acetone, 10:2) had shown that all the starting material was consumed, the catalyst was removed by filtration and the solvent was removed at reduced pressure to give an oil. The oil was flash chromatographed on silica gel (30 g) using ethyl acetate. Evaporation of the solvent gave the product **42** (0.24 g, 80%) as a glassy solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55 (d, J = 2.1 Hz, 2 H), 7.31 (d, J = 2.1 Hz, 2 H), 3.91 (s, 6 H), 3.89 (s, 6 H); IR (KBr) 3369, 2945, 2837, 1717, 1616 cm^{-1}. Anal. ($C_{19}H_{20}H_2O_7$) C, H, N.

3,3′-Diiodo-4,4′-dimethoxy-5,5′-bis(methoxycarbonyl)-benzophenone (43). A suspension of compound **42** (0.57 g, 1.4 mmol) in water (10 mL) was cooled to 0 °C. Concentrated HCl (0.6 mL) was added dropwise to give a yellow solution. After 10 min, sodium nitrite (0.22 g, 3.2 mmol) dissolved in water (2 mL) was added and the solution was stirred for 30 min at 0 °C. The solution was then poured into a solution of iodine (1 g, 3.9 mmol) and potassium iodide (1.0 g, 5.9 mmol) in water (100 mL). The solution was stirred at room temperature for 30 min. Extraction with ethyl acetate (200 mL),

washing with 10% sodium hydrosulfate solution (100 mL), drying over $MgSO_4$, and evaporation of the solvent gave the crude diiodide (0.6 g). Recrystallization from hexanes−methylene chloride gave the pure product **43** (0.54 g, 63%) as a solid: mp 160−161 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, J = 2.5 Hz, 2 H), 8.27 (d, J = 1.86 Hz, 2 H), 4.07 (s, 6 H), 3.95 (s, 6 H); IR (neat) 3078, 2954, 1734, 1670, 1608, 1540 cm^{-1}. Anal. ($C_{19}H_{16}O_7I_2$) C, H.

3′,3″-Diiodo-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-heptene (44). $NaN(TMS)_2$ (1 mL, 1 mmol) was added to an ice cold suspension of hexyltriphenylphosphonium bromide (0.427 g, 1 mmol) in THF (20 mL), and the solution was stirred for 30 min at 0 °C. A solution of the benzophenone **43** (0.2 g, 0.33 mmol) in THF (5 mL) was added to the preformed ylide, and the solution was stirred at room temperature overnight. The mixture was partitioned between 1 N HCl (100 mL) and ethyl acetate (100 mL). The organic layer was evaporated and flash chromatographed on silica gel (50.0 g), eluting with hexanes−ethyl acetate (5:1). The fractions which contained the product were pooled, evaporated, and rechromatographed on silica gel (20 g), eluting with hexanes−ethyl acetate (5:1), to give the product **44** (0.022 g, 9%) as an oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.69 (s, 2 H), 7.54 (d, J = 2.0 Hz,1 H), 7.52 (d, J = 2 Hz, 1 H), 6.01 (t, J = 7.6 Hz, 1 H), 3.93 (s, 3 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.85 (s, 3 H), 2.05 (q, J = 7.3 Hz, 2 H), 1.42 (m, 2 H), 1.25 (m, 2 H), 0.85 (t, J = 6.7 Hz, 3 H); IR (neat) 2953, 2929, 1742, 1738, 1731, 1713 cm^{-1}; HRFABMS calcd for $C_{25}H_{28}I_2O_6$ m/z 677.9975 (M^+), found m/z 677.9954. Anal. ($C_{25}H_{28}I_2O_6$·0.5EtOAc) 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.[39] 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 reverse transcriptase 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%.

Mechanistic Assays. The effects of inhibitors on the in vitro activity of purified RT (kind gift of Steve Hughes, NCI-FCRDC, Frederick, MD) were determined by measurement of incorporation of [^{32}P]TTP into the poly(rA)·oligo(dT) (rAdT) homopolymer or [^{32}P]GTP into the poly(rC)·oligo(dG) (rCdG) template primer systems. After termination of reactions with ice cold 10% trichloroacetate, samples were filtered through a GF/C membrane under vacuum. Filters were then washed with ethanol, and the level of radiolabel incorporation was quantitated by Cernkov counting on a Packard Matrix 9600 direct beta counter. 3′-Azido-2′,3′-dideoxythymidine-5′-triphosphate (AZTTP) and nevirapine served as positive controls for inhibition of RT.

To determine if compounds affected the HIV-1 nucleocapsid protein zinc fingers, fluorescence measurements of the Trp^{37} residue in the C-terminal zinc finger of the HIV-1 nucleocapsid protein were performed as previously described.[34,44] Recombinant nucleocapsid protein was prepared at 20 μg/mL in 10 mM sodium phosphate buffer (pH 7.0), treated with 25 μM of test compound, then after indicated time intervals the samples were diluted 1/10 in 10 mM sodium phosphate buffer (pH 7.0), and the fluorescence intensity was measured. The excitation and emission wavelengths utilized with the Shimadzu RF5000 spectrofluorimeter were 280 and 351 nm, respectively. The analytical procedure employed to determine the reagent-induced inhibition of HIV-1 protease activity has been previously described.[45] Recombinant HIV-1 protease (Bachem BioScience Inc., King of Prussia, PA) and the substrate (Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-NH_2, Multiple Peptide Systems, San Diego, CA) were utilized to determine the concentration of test compound required to inhibit protease activity by 50% (IC_{50}). Briefly, HIV-1 protease (14.2 nM final) was mixed with various concentrations of test compounds in 250 mM potassium phosphate buffer, pH 6.5, 2.5% (v/v) glycerol, 0.5 mM dithiothreitol, 0.5 mM EDTA, and 375 mM ammonium sulfate, after which the substrate was added (30 nmol) and the reaction incubated at 37 °C for 30 min. Reactions were terminated by the addition of 20 μL of a mixture of 8 M guanidine-HCl to 10% trifluoroacetic acid (8:1), and the reaction products were separated by reverse-phase HPLC on a Nova-Pak C-18 column. Absorbance was measured at 206 nm, peak areas were quantitated, and the percentage conversion to product was used to calculate the percentage of control cleavage in the presence of inhibitors. The 3′-cleavage and integration activities of purified HIV-1 integrase were quantitated as previously described.[46]

The attachment/fusion assay was performed as described by Ciminale[47] with modification. Briefly, HIV-1 envelope-expressing, Tat-producing HL2/3 cells and CXCR-4 expressing, LTR-β-Gal-containing MAGI cells (obtained from the AIDS Research and Reference Program, National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD) were preincubated separately with test compound for 1 h at 37 °C, followed by admixture of the two cell lines at a cell ratio of 1:1. Incubations were then continued for 16 h. The cells were then fixed and stained for the expression of β-Gal with indolyl-β-D-galactopyranoside (X-Gal) as described previously.[48] The number of blue cells (indicating completion of attachment and fusion of membranes) were counted by light microscopy.

Time Course Experiments. To identify the stage(s) of HIV infection affected by ADAM II **(22)**, the compound was evaluated in a high MOI acute phase time of addition assay. CEM-SS cells (10^5) were preincubated with HIV_{IIIB} (MOI = 1.0) at 0−4 °C for 1 h to allow attachment of virus to cells but not fusion or reverse transcription. Samples were then washed three times with ice cold media to remove unbound virus, after which the samples were rapidly warmed to 37 °C (at time zero, t_o), allowing the infectious cycle to proceed. ADAM II **(22)** was added to samples at t_o or at various times after warming to 37 °C (t = 0.5, 1, 2, 4, 8, or 24 h postwarming). Dextran sulfate (100 μg/mL) and nevirapine (1 μM) served as controls for inhibitors of virus attachment and reverse transcriptase, respectively. After a 24 h incubation, 5×10^3 cells from each sample were placed in culture and analyzed after 48 additional h for virion production by p24 antigen capture, as described previously.[39]

Screening Against HIV-1 Viruses Containing NNRTI Resistance Mutations. These studies were performed as previously described.[49]

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References

(1) De Clercq, E. What can be Expected from Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) in the Treatment of Human Immunodeficiency Virus Type 1 (HIV-1) Infections? *Med. Virol.* **1996**, *6*, 97−117.

(2) Baba, M.; Tanaka, H.; De Clercq, E.; Pauwels, R.; Balzarini, J. M. R.; Schols, D.; Nakashima, H.; Perno, C.-F.; Walker, R. T.; Miyasaka, T. Highly Specific Inhibition of Human Immunodeficiency Virus Type 1 by a Novel 6-Substituted Acyclouridine Derivative. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1375−1381.

(3) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and Selective Inhibition of HIV-1 Replication *In Vitro* by a Novel Series of TIBO Derivatives. *Nature* **1990**, *343*, 470−474.

(4) Merluzzi, V. J.; Hargrave, K. D.; Labidia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C. K.; Eckner, K.; Hattox, S.; Adams, J.; Roshthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, R. A.; Sullivan, J. L. Inhibition of HIV-1 Replication by a Non-nucleoside Reverse Transcriptase Inhibitor. *Science* **1990**, *250*, 1411−1413.

(5) Goldman, M. E.; Nunberg, J. H.; O'Brien, J. A.; Quintero, J. C.; Schleif, W. A.; Freund, K. F.; Gaul, S. L.; Saari, W. S.; Wai, J. S.; Hoffman, J. M.; Anderson, P. S.; Hupe, D. J.; Emini, E. A.; Stern, A. M. Pyridinone Derivatives: Specific Human Immunodeficiency Virus Type 1 Reverse Transcriptase Inhibitors with Antiviral Activity. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6863−6867.

(6) Romero, D. L.; Busso, M.; Tan, C. K.; Reusser, F.; Palmer, J. R.; Poppe, S. M.; Aristoff, P. A.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G. Nonnucleoside Reverse Transcriptase Inhibitors that Potently and Specifically Block Human Immunodeficiency Virus Type 1 Replication. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8806−8810.

(7) Balzarini, J.; Pérez-Pérez, M. J.; San-Félix, A.; Schols, D.; Perno, C.-F.; Vandamme, A.-M.; Camarasa, M.-J.; De Clercq, E. 2′, 5′-Bis-*O*-(*tert*-butyldimethylsilyl)-3′-spiro-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)pyrimidine (TSAO) Nucleoside Analogs: Highly Selective Inhibitors of Human Immunodeficiency Virus Type 1 that Are Targeted at the Viral Reverse Transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4392−4396.

(8) Pauwels, R.; Andries, K.; Debyser, Z.; Van Daele, P.; Schols, D.; Vandamme, A.-M.; Stoffels, P.; De Vreese, K.; Woestenborghs, R.; Janssen, C. G. M.; Anné, J.; Cauwenbergh, G.; Desmyter, J.; Heykants, J.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and Highly Selective HIV-1 Inhibition by a New Series of α-Anilinophenylacetamide (α-APA) Derivatives Targeted at HIV-1 Reverse Transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *28*, 1839−1842.

(9) Havlir, D.; Cheeseman, S. H.; McLaughlin, M.; Murphy, R.; Erice, A.; Spector, S. A.; Greenough, T. C.; Sullivan, J. L.; Hall, D.; Myers, M.; Lamson, M.; Richman, D. D. High-Dose Nevirapine: Safety, Pharmacokinetics, and Antiviral Effect in Patients with Human Immunodeficiency Virus Infection. *J. Infect. Dis.* **1995**, *171*, 537−545.

(10) Balzarini, J.; Karlsson, A.; Péres-Péres, M.-J.; Camarasa, M.-J.; De Clercq, E. Knocking-out Concentrations of HIV-1-Specific Inhibitors Completely Suppress HIV-1 Infection and Prevent the Emergence of Drug-resistant Virus. *Virology* **1993**, *196*, 576−585.

(11) De Clercq, E. HIV Resistance to Reverse Transcriptase Inhibitors. *Biochem. Pharmacol.* **1994**, *47*, 155−169.

(12) De Clercq, E. Resistance of Human Immunodeficiency Virus Type 1 (HIV-1) to Non-nucleoside HIV-1-Specific Reverse Transcriptase Inhibitors. *Int. J. Immunother.* **1994**, *10*, 145−158.

(13) Cushman, M.; Golebiewski, M.; Buckheit, R. W. J.; Graham, L.; Rice, W. G. Synthesis and Biological Evaluation of an Alkenyldiarylmethane (ADAM) which Acts as a Novel Non-nucleoside HIV-1 Reverse Transcriptase Inhibitor. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2713−2716.

(14) Cushman, M.; Golebiewski, W. M.; Graham, L.; Turpin, J. A.; Rice, W. G.; Fliakas-Boltz, V.; Buckheit, R. W. J. Synthesis and Biological Evaluation of Certain Alkenyldiarylmethanes as Anti-HIV-1 Agents Which Act as Non-Nucleoside Reverse Transcriptase Inhibitors. *J. Med. Chem.* **1996**, *39*, 3217−3227.

(15) Kohlstaedt, L. A.; Wang, J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. Crystal Structure at 3.5 Å Resolution of HIV-1 Reverse Transcriptase Complexed with an Inhibitor. *Science* **1992**, *256*, 1783−1790.

(16) Smerdon, S. J.; Jäger, J.; Wang, J.; Kohlstaedt, L. A.; Chirino, A. J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. Structure of the Binding Site for Nonnucleoside Inhibitors of the Reverse Transcriptase of Human Immunodeficiency Type I. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3911−3915.

(17) Ren, J.; Esnouf, R.; Garman, E.; Somers, D.; Ross, C.; Kirby, I.; Keeling, J.; Darby, G.; Jones, Y.; Stuart, D.; Stammers, D. High-Resolution Sturctures of HIV-1 RT from Four RT-Inhibitor Complexes. *Sruct. Biol.* **1995**, *2*, 293−302.

(18) Ding, J.; Das, K.; Tantillo, C.; Zhang, W.; Clark, A. D. J.; Jessen, S.; Lu, X.; Hsiou, Y.; Jacobo-Molina, A.; Andries, K.; Pauwels, R.; Moereels, H.; Koymans, L.; Janssen, P. A. J.; Smith, R. H. J.; Koepke, M. K.; Michejda, C. J.; Hughes, S. H.; Arnold, E. Structure of HIV-1 Reverse Transcriptase in a Complex with the Non-nucleoside Inhibitor α-APA R 95845 at 2.8 Å Resolution. *Structure* **1995**, *3*, 365−379.

(19) Ding, J.; Das, K.; Moereels, H.; Koymans, L.; Andries, K.; Janssen, P. A.; Hughes, S. H.; Arnold, E. Structure of HIV-1 RT/TIBO R 86183 Complex Reveals Similarity in the Binding of Diverse Nonnucleoside Inhibitors. *Nature Struct. Biol.* **1995**, *2*, 407−415.

(20) Ren, J.; Esnouf, R.; Hopkins, A.; Ross, C.; Jones, Y.; Stammers, D.; Stuart, D. The structure of HIV-1 Reverse Transcriptase Complexed with 9-Chloro-TIBO: Lessons for Inhibitor Design. *Structure* **1995**, *3*, 915−926.

(21) Das, K.; Ding, J.; Hsiou, Y.; Clark, A. D. J.; Moereels, H.; Koymans, L.; Adries, K.; Pauwels, R.; Janssen, P. A. J.; Boyer, P. L.; Clark, P.; Smith, R. H. J.; Smith, M. B. K.; Michejda, C. J.; Hughes, S. H.; Arnold, E. Crystal Structure of 8-Cl and 9-Cl TIBO Complexed with Wild-type HIV-1 RT and 8-Cl TIBO Complexed with the Tyr181Cys HIV-1 RT Drug-resistant Mutant. *J. Mol. Biol.* **1996**, *264*, 1085−1100.

(22) Esnouf, R. M.; Ren, J.; Hopkins, A. L.; Ross, C. K.; Jones, E. Y.; Stammers, D. K.; Stuart, D. I. Unique Features in the Structure of the Complex between HIV-1 Reverse Transcriptase and the bis(heteroaryl)piperazine (BHAP) U-90152 Explain Resistance Mutations for the Nonnucleoside Inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3984−3989.

(23) Schäfer, W.; Friebe, W.-G.; Leinert, W.; Mertens, A.; Poll, T.; von der Saal, W.; Zilch, H.; Nuber, H.; Ziegler, M. L. Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase: Molecular Modeling and X-ray Structure Investigations. *J. Med. Chem.* **1993**, *36*, 726−732.

(24) Bose, A. K.; Kistner, J. F.; Farber, L. A Convenient Synthesis of Axial Amines. *J. Org. Chem.* **1962**, *27*, 2925−2926.

(25) Secrist, J. A., III; Logue, M. W. Amine Hydrochlorides by Reduction in the Presence of Chloroform. *J. Org. Chem.* **1972**, *37*, 335−336.

(26) Salunkhe, A. M.; Brown, H. C. Dichloroborane-Dimethyl Sulfide, A Highly Selective Reducing Agent for Reduction of Organic Azides to Amines. *Tetrahedron Lett.* **1995**, *36*, 7987−7990.

(27) Ravindran, N.; Brown, H. C. Monochloroborane-Methyl Sulfide, $H_2BCl{\cdot}S(CH_3)_2$, and Dichloroborane-Methyl Sulfide, $HBCl_2{\cdot}S(CH_3)_2$, as New Stable Hydroborating Agents with High Regiospecificity. *J. Org. Chem.* **1977**, *42*, 2533−2534.

(28) Koziara, A.; Osowska-Pacewicka, K.; Zawadzki, S.; Zwierzak, A. One-Pot Transformation of Alkyl Bromides into Primary Amines via the Staudinger Reaction. *Synthesis* **1985**, 202−204.

(29) Cravatt, B. F.; Lerner, R. A.; Boger, D. L. Structure Determination of an Endogenous Sleep-Inducing Lipid, *cis*-9-Octadecenamide (Oleamide): A Synthetic Approach to the Chemical Analysis of Trace Quantities of a Natural Product. *J. Am. Chem. Soc.* **1996**, *118*, 580−590.

(30) Keyes, R. F.; Golebiewski, W. M.; Cushman, M. Correlation of Anti-HIV Potency with Lipophilicity in a Series of Cosalane Analogs Having Normal Alkenyl and Phosphodiester Chains as Cholestane Replacements. *J. Med. Chem.* **1996**, *39*, 508−514.

(31) Kocienski, P. J.; Cernigliaro, G.; Feldstein, G. A Synthesis of (±)-Methyl *n*-Tetradeca-*trans*-2,2,5-trienoate, an Allenic Ester Produced by the Male Dried Bean Beetle *Acanthoscelodes obtectus* (Say). *J. Org. Chem.* **1977**, *42*, 353−355.

(32) Hooz, J.; Giliani, H. H. S. A Rapid, Mild Procedure for the Preparation of Alkyl Chlorides and Bromides. *Can. J. Chem.* **1968**, *46*, 86−87.

(33) Cushman, M.; Kanamathareddy, S. Synthesis of the Covalent Hydrate of the Incorrectly Assumed Structure of Aurintricarboxylic Acid (ATA). *Tetrahedron* **1990**, *46*, 1491−1498.

(34) Rice, W. G.; Supko, J. G.; Malspeis, L.; Clanton, D. J.; Bu, M.; Graham, L.; Schaeffer, C. A.; Turpin, J. A.; Domagala, J.; Gogliotti, R.; Bader, J. P.; Halliday, S. M.; Coren, L.; Arthur, L. O.; Henderson, L. E.; Buckheit, R. W. J. Novel Inhibitors of HIV-1 p7NC Zinc Fingers as Candidates for the Treatment of AIDS. *Science* **1995**, *270*, 1194−1197.

(35) Baba, M.; De Clercq, E.; Tanaka, H.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Umezu, K.; Nakashima, H.; Mori, S.; Shigeta, S.; Walker, R. T.; Miyasaka, T. Potent and Selective Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) by 5-Ethyl-6-phenylthiouracil Derivatives through Their Interaction with the HIV-1 Reverse Transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2356−2360.

(36) Debyser, Z.; Pauwels, R.; Andries, K.; Desmyter, J.; Kukla, M.; Janssen, P. A. J.; De Clercq, E. An Antiviral Target on Reverse Transcriptase of Human Immunodeficiency Virus Type 1 Revealed by Tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione Derivatives. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1451−1455.

(37) Balzarini, J.; Pérez-Pérez, M.-J.; San-Félix, A.; Camarasa, M.-J.; Bathurst, I. C.; Barr, P. J.; De Clercq, E. Kinetics of Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) Reverse Transcriptase Analogue [2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)thymine (TSAO-T). *J. Biol. Chem.* **1992**, *267*, 11831.

(38) Tramontano, E.; Cheng, Y.-c. HIV-1 Reverse Transcriptase Inhibition by a Dipyridodiazepinone Derivative: BI-RG-587. *Biochem. Pharmacol.* **1992**, *43*, 1371−1376.

(39) Rice, W. G.; Bader, J. P. Discovery and *in Vitro* Development of AIDS Antiviral Drugs as Biopharmaceuticals. *Adv. Pharmacol. (San Diego)* **1995**, *6*, 389−438.

(40) Tantillo, C.; Ding, J.; Jacobo-Molina, A.; Nanni, R. G.; Boyer, P. L.; Hughes, S. H.; Pauwels, R.; Andries, K.; Janssen, P. A.; Arnold, E. Locations of Anti-AIDS Drug Binding Sites and Resistance Mutations in the Three-dimensional Structure of HIV-1 Reverse Transcriptase. *J. Mol. Biol.* **1994**, *243*, 369−387.

(41) Cushman, M.; Golebiewski, W. M.; McMahon, J. B.; Buckheit, R. W. J.; Clanton, D. J.; Weislow, O.; Haugwitz, R. D.; Bader, J.; Graham, L.; Rice, W. G. Design, Synthesis, and Biological Evaluation of Cosalane, a Novel anti-HIV Agent which Inhibits Multiple Features of Virus Replication. *J. Med. Chem.* **1994**, *37*, 3040−3050.

(42) Bestmann, H. J.; Koschatzky, K. H.; Schätzke, W.; Süb, J.; Vostrowsky, O. Pheromones, XXXII. Synthons for the Preparation of Bisolefinic Lepidoptera Pharomones. *Justus Liebigs Ann. Chem.* **1981**, 1705−1720.

(43) Taylor, E. C.; Davies, H. M. L. Synthesis of Fused 1,2-Diazetidinones via an Intramolecular Horner-Emmons Reaction. *J. Org. Chem.* **1986**, *51*, 1537−1540.

(44) Rice, W. G.; Turpin, J. A.; Schaeffer, C. A.; Graham, L.; Clanton, D.; Buckheit, R. W. J.; Zaharevitz, D.; Summers, M. F.; Wallqvist, A.; Covell, D. G. Evaluation of Selected Chemotypes in Coupled Cellular and Molecular Target-Based Screens Identifies Novel HIV-1 Zinc Finger Inhibitors. *J. Med. Chem.* **1996**, *39*, 3606−3616.

(45) Rice, W. G.; Schaeffer, C. A.; Graham, L.; Bu, M.; McDougal, J. S.; Orloff, S. L.; Villiger, F.; Young, M.; Oroszlan, S.; Fesen, M. R.; Pommier, Y.; Mendelev, J.; Kun, E. The Site of Action of 3-Nitrosobenzamide on the Infectivity Process of Human Immunodeficiency Virus in Human Lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9721−9724.

(46) Fesen, M. R.; Kohn, K. W.; Leteurtre, F.; Pommier, Y. Inhibitors of Human Immunodeficiency Integrase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2399−2403.

(47) Ciminale, V.; Felber, B. K.; Campbell, M.; Pavlakis, G. N. A Bioassay for HIV-1- Based on Env-CD4 Interaction. *AIDS Res. Hum. Retrovir.* **1990**, *6*, 1281−1287.

(48) Kimpton, J.; Emerman, M. Detection of Replication-competent and Pseudotypes of Human Immunodeficiency Virus with a Sensitive Cell Line on the Basis of Activation of an Integrated *β*-Galactosidase Gene. *J. Virol.* **1992**, *66*, 2232−2239.

(49) Buckheit, R. W. J.; Kinjerski, T. L.; Fliakas-Boltz, V.; Russell, J. D.; Stup, T. L.; Pallansch, L. A.; Brouwer, W. G.; Dao, D. C.; Harrison, W. A.; Schultz, R. J.; Bader, J. P.; Yang, S. S. Structure-Activity and Cross-Resistance Evaluations of a Series of Human Immunodeficiency Virus Type 1-Specific Compounds Related to Oxathiin Carboxanilide. *Antimicrob. Agents Chemother.* **1995**, *39*, 2718−2727.

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