Articles

Solid-Phase Synthesis of the Alkenyldiarylmethane (ADAM) Series of Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors

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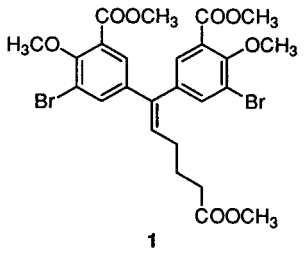
The Sonogashira and Stille cross-coupling reactions have been employed in the synthesis of several non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the alkenyldiarylmethane (ADAM) series. The synthesis has been carried out both in solution and on a solid support. In contrast to previous syntheses of NNRTIs in the ADAM series, the present strategy allows the incorporation of differently substituted aromatic rings in a stereochemically defined fashion. The most potent of the new ADAMs inhibited the cytopathic effect of HIV-1_{RF} in CEM-SS cell culture with an EC_{50} value of 20 nM.

The alkenyldiarylmethanes (ADAMs) are a class of HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs).[1−5] Certain ADAMs (e.g., ADAM **1**) have been found to inhibit of the cytopathic effect of HIV-1 in cell culture at low nanomolar concentrations.[4,5] A number of HIV-1 strains containing AZT resistance mutations have shown increased sensitivity to some of the ADAMs, indicating a possible therapeutic role for the ADAMs in combination with AZT.[4] In general, the clinical use of NNRTIs in combination with two additional anti-HIV agents decreases HIV-1 RNA levels, increases CD4 lymphocyte counts, and delays disease progression. However, several factors continue to limit the selection of NNRTIs for combination chemotherapy, including drug incompatibilities, adverse effects, the emergence of resistant viral strains, and cross-resistance.[6−10] Therefore, a need exists for additional NNRTIs that might display novel resistance mutation profiles, more favorable pharmacokinetic properties, and limited toxicities. We have therefore continued to design and synthesize additional NNRTIs in the ADAM series.



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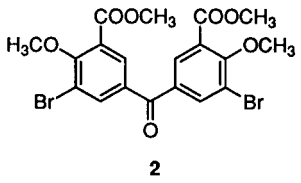

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The previously existing ADAMs (e.g., **1**) have been synthesized by attaching the alkenyl chain to a symmetrical benzophenone (e.g., **2**) by Wittig,[1−4] McMurry,[5] or Horner−Emmons[4] reactions. Since these methods proceed from the readily available, symmetrical benzophenones, they are not ideal for preparing ADAMs having nonidentical aromatic substituents. In addition, even if unsymmetrical aromatic benzophenones were used, these methods would be complicated by generation of cis and trans double bond isomers that would have to be separated and defined structurally. We have therefore sought to establish alternative syntheses of the ADAMs that would allow for the construction of compounds having nonidentical aromatic substituents with stereochemically defined relationships to the alkenyl side chain. Another goal of the present research project was to adapt the resulting solution-phase synthesis to the solid phase, which could eventually allow automation and the preparation of combinatorial libraries for lead optimization.[11−13]

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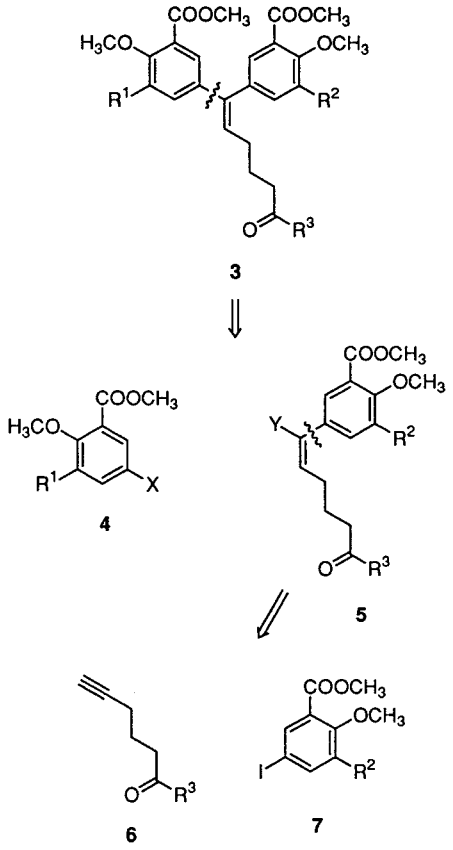
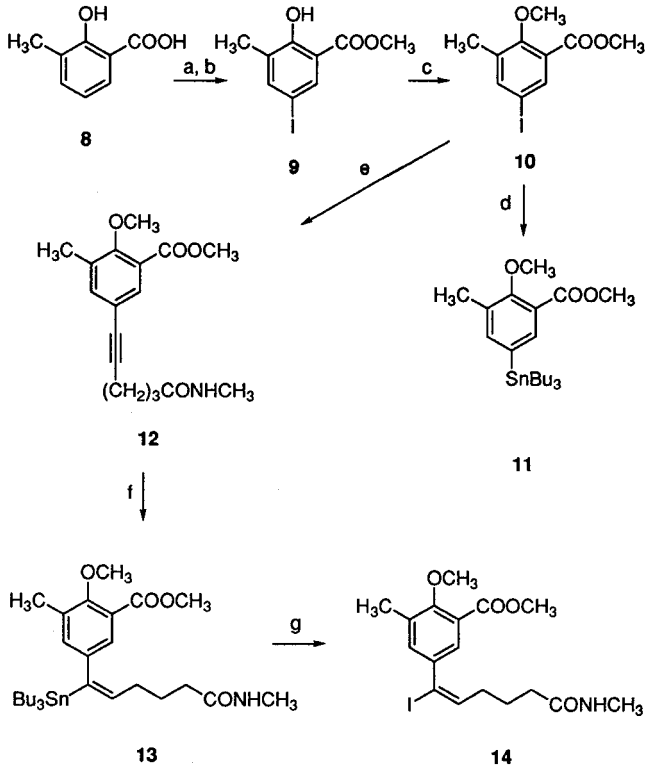
Scheme 1



Scheme 2[a]



[a] Reagents and conditions: (a) $TMSCHN_2$/MeOH, benzene, 23 °C (2 h); (b) NaI, NaOH/MeOH, 0−3 °C (3 h) then NaOCl (5.25%); (c) dimethyl sulfate, K_2CO_3, acetone, reflux (24 h); (d) $Bu_3Sn-SnBu_3$, $Pd(PPh_3)_4$, $Pd(OAc)_2$, triethylamine, 95−100 °C (3 h); (e) *N*-methyl 5-hexynoamide, $(Ph_3P)_2Pd(OAc)_2$, CuI, triethylamine, EtOAc, 23 °C (14 h); (f) Bu_3SnH, $Pd(PPh_3)_4$, THF, 23 °C (14 h); (g) I_2, CH_2Cl_2, 23 °C (20 min).

The application of palladium-catalyzed reactions for C−C bond formation is particularly attractive for solid-phase combinatorial library syntheses since the conditions employed are mild and many functional groups are compatible.[14−16] The present article describes the application of the Sonogashira and Stille cross-coupling reactions for the solution and solid-phase synthesis of stereochemically defined ADAMs having differently substituted aromatic rings. As shown by the retrosynthetic analysis described in Scheme 1, Stille coupling of **4** and **5** (X and Y = I or $SnBu_3$) would be expected to result in the ADAM **3**. The coupling partner **5** (in the case of Y = $SnBu_3$) could in turn be made by the Sonogashira reaction of the alkyne **6** and the aryl iodide **7**, followed by hydrostannation. Since the Stille reaction proceeds with retention of the stereochemical integrity of the coupling partners, and hydrostannation of alkynes results in regiochemically defined cis addition to the triple bond, the stereochemistry (cis vs trans) of the side chain relative to the two aromatic rings in the final product would be predictable (i.e., the side chain would be cis to the aromatic ring containing R^2 in structure **3**).

Results and Discussion

As shown in Scheme 2, 3-methylsalicylic acid **(8)** was quantitatively converted into its methyl ester using $TMSCHN_2$ in a mixture of MeOH and benzene.[17] The methyl ester was then iodinated with sodium iodide in the presence of sodium hypochlorite as an oxidant.[18] Treatment of the iodinated ester **9** with dimethyl sulfate and potassium carbonate in refluxing acetone produced methyl 5-iodo-3-methyl-2-methoxybenzoate **10** in 90% overall yield. The tributyltin derivative **11** was synthesized by heating a mixture of iodo ester **10**, bis(tributyltin), tetrakis(triphenylphosphine)palladium, and palladium acetate in triethylamine at 95−100 °C overnight.[18] The Sonogashira reaction of *N*-methyl 5-hexynoamide with iodo ester **10** afforded alkyne **12**,[19,20] which underwent hydrostannation in the presence of $Pd(PPh_3)_4$ to afford the regio- and stereodefined vinylstannane **13** in 87% yield.[21,22] Subsequent treatment of **13** with I_2 in CH_2Cl_2 produced vinyl iodide **14**.[23]

Our initial attempts to prepare ADAM **15** (Scheme 3), by employing vinyl tributylstanne **13** and aryl iodide **10** (or the alternative combination, vinyl iodide **14** and aryl

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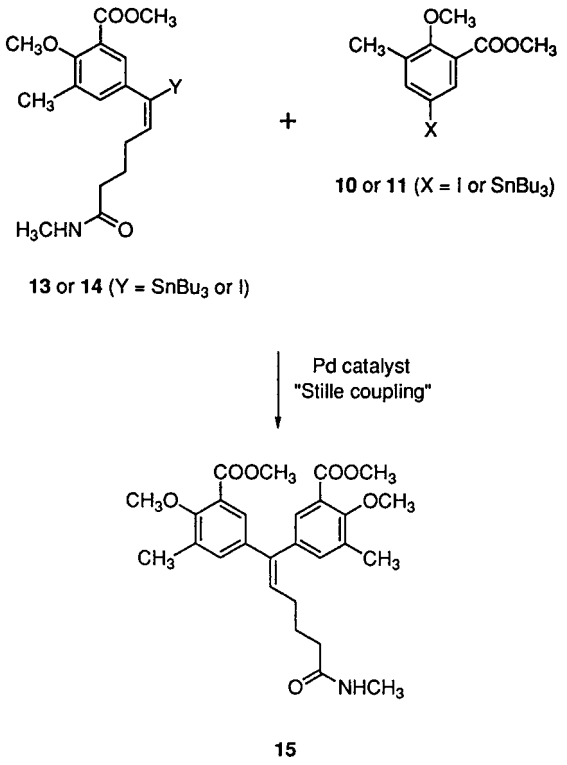
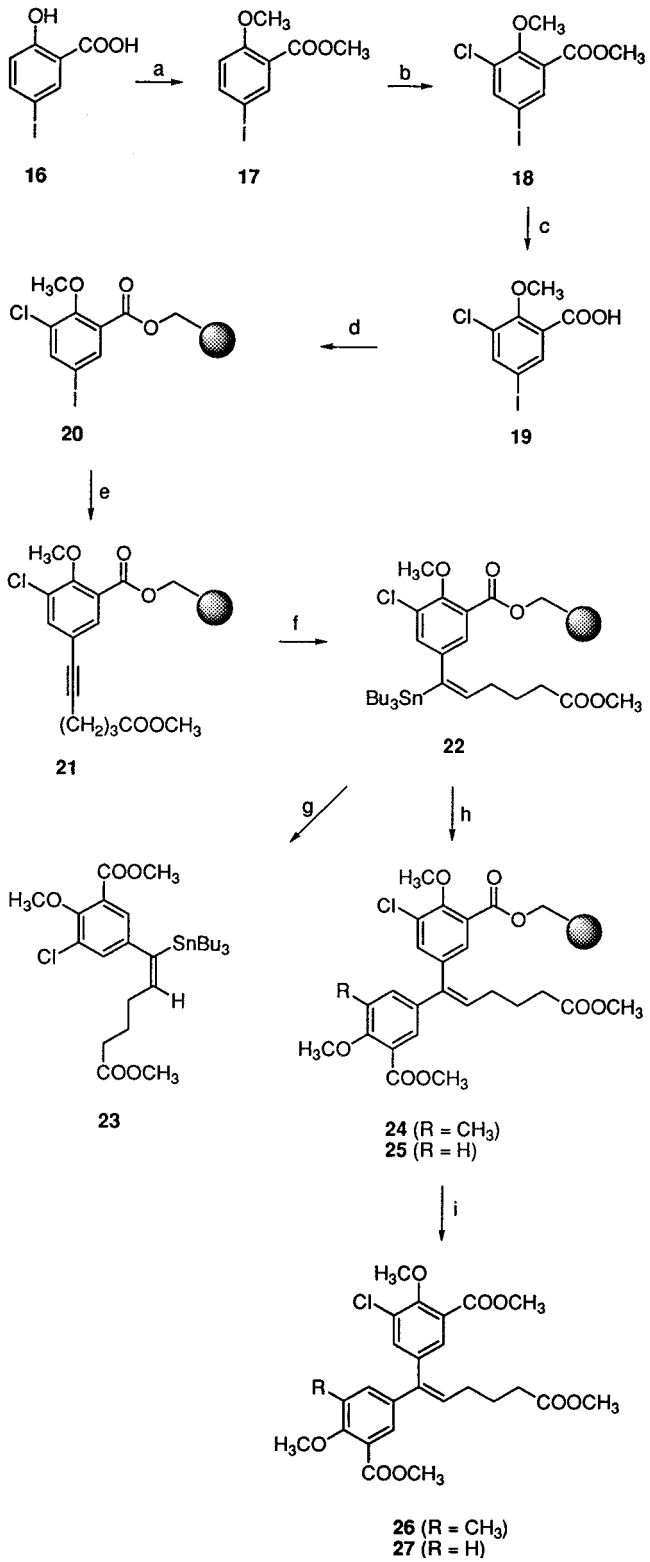
Scheme 3



tributylstanne **11**) in the presence of $Pd(PPh_3)_4$, were unsuccessful because of the low yield of the coupling reaction.[24] In general, densely substituted stannanes reacts poorly, and their coupling must be carefully optimized. Considering the Stille coupling catalytic cycle, a better combination of catalyst, ligand, additive, and solvent might be beneficial to the cross-coupling reaction.[25] It has been shown that ligands with donicity toward Pd(II) lower than that of PPh_3 (i.e., $AsPh_3$) can lead to major (up to 1000-fold) rate enhancement in the transmetalation. Besides the ligand, the addition of fluoride might lead to more efficient coupling, perhaps by facilitating transmetalation from tin to palladium, since tin is fluorophilic.[26−28] The cross-coupling reactions of organosilicon compounds are known to be facilitated by fluoride.[29−32] Under the "optimized" conditions ($Pd_2(dba)_3$/$AsPh_3$/CsF/DMF), Stille cross-coupling of vinyl tributylstannane **13** and aryl ioide **10** (or vinyl iodide **14** and aryl tributylstannane **11**) afforded **15** in 74% (or 80%) isolated yield. The reactions proceeded exclusively with the desired ipso substitution, and none of the undesired cine substitution products were detected in the reaction mixtures.

Scheme 4[a]



[a] Reactions and conditions: (a) Dimethyl sulfate, K_2CO_3, acetone, reflux (24 h); (b) SO_2Cl_2, CH_2Cl_2, 50 °C (14 h); (c) aq. NaOH/MeOH, 60 °C (3 h); (d) DCC, HOBt/DMF, 23 °C (24 h); (e) methyl 5-hexynoate, $Pd(OAc)_2$, CuI, Et_3N, DMF, 23 °C (12 h); (f) Bu_3SnH, $Pd(PPh_3)_4$, THF, 23 °C (4 h); (g) Et_3N, MeOH (15:85, v/v), 50 °C (16 h); (h) **10** or **17**, $Pd_2(dba)_3$, $AsPh_3$, CsF/DMF, 80 °C (12 h); (i) NaOMe, MeOH, THF (1:2), reflux (20 h).

Having explored the performance of the above reaction sequence in solution, we went on to apply it on a solid support. The building block **19** was prepared through a three-step synthesis (Scheme 4). Methylation of both the

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Table 1. Anti-HIV-1 Activities of ADAMs

compd	RT IC_{50} rCdG[a] (μM)	EC_{50} MTS assay[b] (μM)	CC_{50} MTS assay[c] (μM)	TI[d]
AZT		0.005	>1.0	>201
15	0.804	2.26	17.10	7
26	0.516	0.020	1.46	73
27	0.587	0.080	2.17	27
28	0.300	0.013	31.6	2430

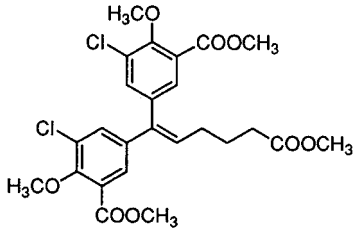
[a] Inhibitory activity vs HIV-1 reverse transcriptase with rCdG as the template:primer. [b] The EC_{50} is the 50% inhibitory concentration for cytopathicity of HIV-1_{RF} in CEM-SS cells. [c] The CC_{50} is the 50% cytotoxic concentration for mock-infected CEM cells. [d] The TI is the therapeutical index, which is the CC_{50} divided by the EC_{50}.

phenol and carboxylic acid groups of **16** using dimethyl sulfate/K_2CO_3 in acetone gave methyl 5-iodo-2-methoxybenzoate **17**, which was reacted with SO_2Cl_2 to result in complete chlorination of the aromatic ring at the C3 position. The resulting ester **18** was then hydrolyzed in aqueous NaOH to afford the key intermediate **19** in 95% yield. Coupling of **19** with hydroxymethylpolystyrene resin (Wang resin) under standard DCC coupling conditions (DCC, 3 equiv; HOBt, 3 equiv in DMF) afforded the desired support-bound ester **20**.[33] It was found that a double-coupling protocol consistently provided much higher loading of the acid onto the solid support [based on elemental analysis (I or Cl) of the modified resin]. Palladium-catalyzed Sonogashira reaction of **20** with 5-hexynoate afforded the resin-bound alkyne **21**.[14,15] The IR spectrum (KBr) of the derivatized resin **21** revealed a new absorption at 2232 cm^{-1} (internal carbon−carbon triple bond), indicating that the desired Sonogashira coupling had occurred. The resin-bound alkyne **21** was then treated with Bu_3SnH in the presence of $Pd(PPh_3)_4$ to afford **22**. The overall efficiency of Sonogashira and hydrostannation reactions was determined to be 86% on the basis of the yield of **23** obtained by basic cleavage (transesterfication, Et_3N/MeOH 15:85, v/v). The Stille coupling of **22** with methyl 3-methyl-5-iodo-2-methoxybenzoate **(10)** or methyl 5-iodo-2-methoxybenzoate **(17)** using $Pd_2(dba)_3$, $AsPh_3$, and CsF in DMF afforded **24** and **25**.[16] Cleavage of **24** and **25** with sodium methoxide in methanol and THF (40:60, v/v) afforded ADAMs **26** and **27**, respectively.[14] ^1H NMR analysis of crude products cleaved from the resin showed purities ranging from 75% to 85%. Both **26** and **27** were further purified by silica gel flash chromatography (EtOAc/hexanes 1:3, v/v).

To evaluate the anti-HIV activities of ADAMs **15**, **26**, and **27**, all three compounds were tested for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS lymphocytes, and the resulting EC_{50} values are displayed in Table 1. In addition, these compounds were tested for inhibition of HIV-1 reverse transcriptase with poly(rC)·oligo(dG) as the template primer, and the IC_{50} values are also reported in Table 1. The cytotoxicities of the compounds in uninfected CEM-SS cells were also investigated, and the 50% cytotoxic concentrations (CC_{50} values) are also shown.

It is clear from examination of the numbers in Table 1 that there is not an exact correlation between the IC_{50} values for inhibition of HIV-1 reverse transcriptase and inhibition of the cytopathic effect of the virus. For example, for compound **15**, the IC_{50} of 0.804 μM for enzyme inhibition is less than the EC_{50} of 2.26 μM for inhibition of the viral cytopathic effect, whereas for ADAMs **26** and **27**, the IC_{50} values of 0.516 and 0.587 μM are significantly greater than the EC_{50} values of 0.020 and 0.080 μM. However, our prior examination of the ADAMs in cells containing mutant HIV-1 reverse transcriptase leave little doubt that the cellular effects of these compounds as inhibitors of cell killing by the virus are in fact due to inhibition of reverse transcriptase.[3−5] Moreover, the lack of correlation of the enzyme IC_{50} values determined with poly(rC)·oligo(dT) as the template primer and the EC_{50} values is not unusual for the non-nucleoside reverse transcriptase inhibitors.[34−37] The discrepancy may simply reflect the difference between the enzyme assay, in which a synthetic template/primer is used, and the cellular system, in which the native viral RNA serves as the template/primer.[36]

Examination of the antiviral activities of compounds **26**, **27**, and the previously synthesized compound **28**[4] allows an assignment of the effect of variation of the R group as shown in structures **26** and **27**. The relative anti-HIV potencies are Cl > CH_3 > H.



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In conclusion, we have devised a new synthesis of ADAMs that allows the incorporation of nonidentical aryl groups in a stereochemically defined fashion. The synthesis can be performed both in solution and on a solid support. The greater flexibility of the present route should allow the future synthesis of non-nucleoside reverse transcriptase inhibitors in the ADAM series having optimized biological activities.

Experimental Section

General. Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. The proton nuclear magnetic resonance spectra (^1H NMR) of compounds **18**, **19**, **26**, and **27** were recorded on a 500 MHz spectrometer, and the ^1H NMR spectra of the remaining compounds were determined at 300 MHz. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard. The indicated J_{SnH} must be considered as an approximate mean value of $J(^{117}SnH)$ and $J(^{119}SnH)$. The ^{13}C NMR spectra were recorded at 75 MHz. As a rule, mass spectra of alkenyltributylstannanes are characterized by the presence of an important peak (often the

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base peak) at $M^+ - 57$, which corresponds to the loss of an *n*-butyl fragment. The M^+ peak was, in the case of **23**, not detected. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within 0.4% of the calculated compositions. Silica gel used for column chromatography was 230−400 mesh.

Methyl 2-Hydroxy-5-iodo-3-methylbenzoate (9).[38] $TMSCHN_2$ (9 mL of 2 M solution in hexanes) was added to a stirred mixture of 3-methyl salicylic acid **(8)** (2.28 g, 15 mmol) in methanol (20 mL) and benzene (20 mL) at room temperature. The mixture was stirred for 2 h and concentrated to afford the corresponding methyl ester[39] quantitatively. The methyl ester (2.49 g, 15 mmol) was dissolved in methanol (40 mL). Sodium iodide (2.25 g, 15 mmol) and sodium hydroxide (0.60 g, 15 mmol) were added, and the solution was cooled to 0 °C. Aqueous sodium hypochlorite (22.0 g, 5.25% NaOCl) was added dropwise. The brown mixture was stirred for 3 h at 0−3 °C and then treated with 10% aqueous sodium thiosulfate. The pH of the mixture was adjusted to 6−7 using 1 N HCl. Ether (100 mL) was added, and the layers were separated. The ether layer was washed with brine and dried over anhydrous sodium sulfate. After the ether was evaporated, the crude solid was crystallized from a mixture of acetone and hexanes (1:1, v/v) to afford **9** as a yellow solid (4.0 g, 91.3%): mp 82−84 °C; ^{1}H NMR ($CDCl_3$) δ 10.94 (s, 1 H), 7.95 (d, J = 2.1 Hz, 1 H), 7.56 (d, J = 1.53 Hz, 1 H), 3.92 (s, 3 H), 2.19 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.67, 159.69, 144.33, 135.61, 129.44, 113.56, 79.59, 52.43, 15.24; CIMS *m*/*z* 293 (MH^+).

Methyl 5-Iodo-2-methoxy-3-methyl-benzoate (10). Compound **9** (3.0 g, 10.27 mmol) was dissolved in acetone (50 mL). Anhydrous K_2CO_3 (4.2 g, 30.47 mmol) and dimethyl sulfate (3.88 mL, 41.08 mmol) were added. The reaction mixture was vigorously stirred under reflux for 24 h and then filtered, and the inorganic salts were washed with acetone (45 mL). The solvent was evaporated, and the yellow residue was washed with water (3 × 100 mL) to remove excess dimethyl sulfate and crystallized from acetone to afford **10** as white crystals (3.1 g, 99%): mp 55−57 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 2.28 Hz, 1 H), 7.65 (d, J = 2.20 Hz, 1 H), 3.90 (s, 3 H), 3.80 (s, 3 H), 2.27 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 165.21, 158.26, 143.43, 137.55, 135.34, 126.32, 86.54, 61.49, 52.27, 15.60; EIMS *m*/*z* 306 (M^+); HRMS calcd for $C_{10}H_{12}IO_3$ (MH^+) 306.9831, found 306.9828. Anal. Calcd for $C_{10}H_{11}IO_3$: C, 39.34; H, 3.61. Found: C, 39.01; H, 3.50.

Methyl 5-(Tri-*n*-butylstannyl)-2-methoxy-3-methylbenzoate (11). Compound **10** (612 mg, 2.0 mmol) was dissolved in triethylamine (20 mL). Palladium(II) acetate (34 mg, 0.148 mmol) and tetrakis(triphenylphosphine)palladium(0) (86 mg, 0.074 mmol) were added, followed by bis(tri-*n*-butyltin) (1.75 mL, 3.45 mmol). The mixture was heated at 95−100 °C for 3 h. The black reaction mixture was filtered, and the black solid was washed with triethylamine (3 × 10 mL). The filtrate was evaporated under reduced pressure. The oily residue was purified by flash chromatography on silica gel (120 g, column 5 cm × 31 cm), eluting with a mixture of ethyl acetate and hexanes (1:5, v/v), to afford a colorless oil (600 mg, 64%): ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1 H), 7.39 (s, 1 H), 3.92 (s, 3 H), 3.82 (s, 3 H), 2.31 (s, 3 H), 1.62−1.27 (m, 16 H), 1.05 (t, J = 8.11 Hz, 2 H), 0.92 (t, J = 7.30 Hz, 3 H), 0.89 (t, J = 7.24 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 167.44, 158.17, 143.01, 136.56, 131.72, 124.01, 118.33, 61.29, 51.99, 29.02, 28.90, 27.19, 26.51, 16.29, 15.88, 13.52, 13.45, 9.52; ESI *m*/*z* 469 (MH^+); HRMS calcd for $C_{22}H_{39}SnO_3$ (MH^+) 469.1915, found 469.1934. Anal. Calcd for $C_{22}H_{38}SnO_3$: C, 56.41; H, 8.12. Found: C, 56.22; H, 7.90.

***N*-Methyl-6-[4-methoxy-5-(methoxycarbonyl)-3-methylphenyl]hex-5-ynoamide (12).** 5-Hexynoic acid (1.0 g, 8.92 mmol) was dissolved in dry THF (15 mL). Oxalyl chloride (1.17 mL, 13.38 mmol) was added, followed by a catalytic amount of DMF (1 drop). The mixture was stirred for 45 min at room temperature. Excess solvent and oxalyl chloride were removed under reduced pressure. The resulting residue was dissolved in dry THF (10 mL) and cooled to 0 °C. Methylamine (10 mL of 2 M methylamine in THF) and triethylamine (0.9 g, 8.92 mmol) were added. The mixture was stirred for 2 h and then washed with water (2 × 5 mL). The organic solvent was evaporated, and the crude residue was further purified by flash chromatography on silica gel (40 g, column 1.5 cm × 31 cm; eluent, ethyl acetate) to give *N*-methyl-5-hexynoamide[40] as a light yellow oil (0.81 g, 73%): ^{1}H NMR ($CDCl_3$) δ 5.72 (br, 1 H), 2.79 (d, J = 4.47 Hz, 3 H), 2.30 (t, J = 7.34 Hz, 2 H), 2.23 (td, J = 6.88 and 2.24 Hz, 2 H), 1.95 (t, J = 2.23 Hz, 1 H), 1.84 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 172.76, 83.42, 68.99, 34.80, 26.15, 24.04, 17.70. The amide (375 mg, 3.0 mmol), methyl 5-iodo-2-methoxy-3-methylbenzoate **4** (875 mg, 2.73 mmol), $Pd(Ph_3P)_2(OAc)_2$ (145 mg, 0.194 mmol), copper(I) iodide (104 mg, 0.546 mmol), and triethylamine (791 mg, 7.84 mmol) were stirred in ethyl acetate (25 mL) at room temperature for 14 h under argon. The mixture was filtered through a pad of Celite, and the Celite was washed with a mixture of ethyl acetate and hexanes (20 mL, 1:1, v/v). The combined filtrates were evaporated, and the brown residue was purified by flash chromatography (silica gel 45 g, column 1.5 cm × 31 cm), eluting with ethyl acetate/hexanes (1:1, v/v), to afford a colorless oil (800 mg, 80%): ^{1}H NMR ($CDCl_3$) δ 7.65 (d, J = 2.04 Hz, 1 H), 7.35 (d, J = 2.04 Hz, 1 H), 5.63 (br, 1 H), 3.92 (s, 3 H), 3.80 (s, 3 H), 2.81 (d, J = 4.92 Hz, 3 H), 2.44 (t, J = 6.79 Hz, 2 H), 2.34 (t, J = 7.41 Hz, 2 H), 2.26 (s, 3 H), 1.91 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 172.82, 166.14, 157.78, 137.71, 132.85, 132.23, 131.87, 128.48, 128.33, 124.40, 119.01, 88.96, 61.46, 52.13, 35.05, 26.15, 24.35, 18.72, 15.76; CIMS *m*/*z* 304 (MH^+); HRMS calcd for $C_{17}H_{22}NO_4$ (MH^+) 304.1549, found 304.1534. Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.33; H, 6.93. Found: C, 67.12; H, 6.87.

Methyl 5-[(1*E*)-5-(*N*-Methylcarbamoyl)-1-(tributylstannyl)pent-1-enyl]-2-methoxy-3-methylbenzoate (13). Bu_3SnH (1.3 mL, 4.85 mmol) was added dropwise to a stirred solution of alkyne **12** (1.0 g, 3.3 mmol) and $(Ph_3P)_4Pd$ (109.4 mg, 0.095 mmol) in dry THF (25 mL) at room temperature. The reaction mixture was stirred for 40 min under argon. The solvent was evaporated, and the brown residue was purified by flash chromatography on silica gel (100 g, EtOAc/hexanes 2:3, v/v) to afford a light brown oil (1.70 g, 86.7%): ^{1}H NMR ($CDCl_3$) δ 7.21 (d, J = 2.28 Hz, 1 H), 6.92 (d, J = 2.07 Hz, 1 H), 5.75 (t, J = 6.96 Hz, 1 H, $^3J_{SnH}$ = 33 Hz), 5.40 (br, 1 H), 3.95 (s, 3 H), 3.84 (s, 3 H), 2.74 (d, J = 4.89 Hz, 3 H), 2.32 (s, 3 H), 2.11 (t, J = 7.39 Hz, 2 H), 2.08 (m, 2 H), 1.73 (m, 2 H), 1.23−1.51 (m, 18 H), 0.90 (t, J = 7.0 Hz, 9 H); ^{13}C NMR ($CDCl_3$) δ 173.30, 166.99, 155.58, 144.86, 141.48, 140.13, 133.60, 132.16, 127.25, 123.84, 61.37, 51.97, 35.82, 29.26, 28.84, 27.14, 26.06, 25.55, 16.00, 13.53, 9.85; ESI *m*/*z* 594/596 (MH^+). Anal. Calcd for $C_{29}H_{49}NSnO_4.CH_3COOC_2H_5$: C, 58.15; H, 8.37. Found: C, 57.86; H, 8.16.

Methyl 5-[(1*E*)-1-Iodo-5-(*N*-methylcarbamoyl)pent-1-enyl]-2-methoxy-3-methylbenzoate (14). The vinyl tributylstannane **13** (594 mg, 1.0 mmol) was dissolved in dry CH_2Cl_2 (20 mL). Finely divided I_2 (305 mg, 1.2 mmol) was added, and the mixture was stirred vigorously at room temperature for 20 min. Saturated aqueous $Na_2S_2O_3$ (15 mL) was added, the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 ×10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on silica gel (30 g, EtOAc as eluent) to afford a brown oil (310 mg, 71.9%): ^{1}H NMR ($CDCl_3$) δ 7.51 (d, J = 2.31 Hz, 1 H), 7.23 (d, J = 2.01 Hz, 1 H), 6.43 (t, J = 7.60 Hz, 1 H), 5.50 (br, 1 H), 3.91 (s, 3 H), 3.82 (s, 3 H), 2.71 (d, J = 4.87 Hz, 3 H), 2.29 (s, 3 H), 2.08 (t, J = 7.48 Hz, 2 H), 2.10 (dt, J = 7.34 and 7.33 Hz, 2 H), 1.71 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 172.75, 166.32, 157.78, 142.98, 136.81, 135.08, 132.85, 129.12, 124.08, 93.59, 61.43, 52.22, 35.37, 31.36, 26.12, 24.77, 16.03; CIMS *m*/*z* 432 (MH^+); HRMS calcd for $C_{17}H_{22}NIO_4$ 432.0672, found 432.0671. Anal. Calcd for $C_{17}H_{22}NIO_4$: C, 47.33; H, 5.10; N, 3.25; I, 29.47. Found: C, 47.52; H, 5.22; N, 3.21; I, 29.36.

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Methyl 3,3′-Dimethyl-4,4′-dimethoxy-5,5′-bis(methoxycarbonyl)-6,6-diphenylhexenoamide (15). Method A. Under an atmosphere of argon, a solution of iodo ester **10** (60 mg, 0.2 mmol) in dry THF (5 mL) and a solution of $AsPh_3$ (24.5 mg, 0.08 mmol) in dry THF (5 mL) were added in turn to a two-necked flask charged with $Pd_2(dba)_3$ (18.3 mg, 0.02 mmol) and CsF (67 mg, 0.44 mmol). The organostannane **13** (120 mg, 0.2 mmol in 5 mL of dry THF) was then added by syringe, and the flask was placed in an oil bath (70−80 °C) and stirred for 14 h under argon. The reaction mixture was cooled to room temperature, diluted with Et_2O, and filtered through a pad of Celite. The Celite was washed throughly with Et_2O, and the combined filtrates were concentrated by rotary evaporation. The product was purified by flash chromatography on silica gel (30 g), eluting with ethyl acetate, to afford the desired ADAM-amide **15** (71 mg, 74%): ^{1}H NMR ($CDCl_3$) δ 7.49 (d, J = 2.12 Hz, 1 H), 7.44 (d, J = 1.75 Hz, 1 H), 7.13 (m, 2 H), 6.01 (t, J = 7.38 Hz, 1 H), 3.96 (s, 3 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.79 (s, 3 H), 2.78 (d, J = 5.02 Hz, 3 H), 2.35 (s, 3 H), 2.28 (s, 3 H), 2.21 (t, J = 7.55 Hz, 2 H), 2.16 (dt, J = 7.60 and 6.11 Hz, 2 H), 1.76−1.85 (m, 2 H); CIMS m/z 484 $(MH)^+$; HRMS calcd for $C_{27}H_{33}NO_7$ 483.2257, found 483.2263. Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.08; H, 6.83. Found: C, 66.97; H, 6.79.

Method B. Under an atmosphere of argon, a solution of vinyl iodide **14** (54.2 mg, 0.13 mmol) in dry THF (3 mL) and a solution of $AsPh_3$ (15.9 mg, 0.05 mmol) in dry THF (3 mL) were added in turn to a two-necked flask charged with $Pd_2(dba)_3$ (11.9 mg, 0.01 mmol) and CsF (43.6 mg, 0.29 mmol). The organostannane **11** (61.9 mg, 0.13 mmol) in dry THF (4 mL) was then added by syringe, and the flask was placed in an oil bath (70−80 °C) and stirred overnight under argon. The reaction mixture was cooled to room temperature, diluted with Et_2O, and filtered through a pad of Celite. The Celite was washed thoroughly with Et_2O, and the combined filtrates were concentrated by rotary evaporation. The product was purified by flash chromatography on silica gel (25 g), eluting with ethyl acetate, to afford the desired ADAM-amide **15** (47 mg, 84%).

Methyl 3-Chloro-5-iodo-2-methoxybenzoate (18). To a solution of methyl 5-iodo-2-methoxybenzoate **17**[41] (2.92 g, 10 mmol) in CH_2Cl_2 (10 mL) was gradually added SO_2Cl_2 (5.4 g, 40 mmol). The reaction mixture was heated at 50 °C for 14 h and then cooled to room temperature. It was poured into a large amount of water (200 mL) and extracted with CH_2Cl_2 (3 × 40 mL). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and evaporated in vacuo to afford a white solid (3.20 g, 98%). A small amount of the sample was further purified by flash chromatography on silica gel (10 g, hexanes as eluent): mp 41−43 °C; ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 2.12 Hz, 1 H), 7.84 (d, J = 2.08 Hz, 1 H), 3.90 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 164.25, 156.14, 142.05, 138.42, 130.79, 128.33, 86.15, 62.00, 52.63. Anal. Calcd for $C_9H_8IClO_3 \cdot 0.6\ H_2O$: C, 32.05; H, 2.75. Found: C, 31.90; H, 2.35.

3-Chloro-5-iodo-2-methoxybenzoic Acid (19). A 1 N NaOH solution (10 mL) was added to a MeOH solution (8 mL) containing benzoate **18** (0.9 g, 2.76 mmol). The mixture was heated at 60 °C for 3 h and then acidified with concentrated HCl solution (the pH was adjusted to 1−2). The white precipitate was filtered and recrystallized from acetone to afford compound **19** (0.82 g, 95%): mp 189−191 °C (dec); ^{1}H NMR (CD_3COCD_3) δ 8.04 (d, J = 2.24 Hz, 1 H), 7.98 (d, J = 2.13 Hz, 1 H), 3.90 (s, 3 H); ^{13}C NMR (CD_3COCD_3) δ 169.14, 160.87, 146.72, 143.67, 134.32, 91.00, 66.60; CIMS m/z 313. Anal. Calcd for $C_8H_6IClO_3$: C, 30.72; H, 1.92; Cl, 11.36; I, 40.64. Found: C, 30.42; H, 1.68; Cl, 11.08; I, 40.33.

Esterification of Wang Resin with 3-Chloro-5-iodo-2-methoxybenzoic Acid (19). Solid DCC (630 mg, 3.05 mmol) and HOBt (621 mg, 3.05 mmol) were added to a suspension of Wang resin (1.21 mmol −OH, 1 g) in a mixture of dry DMF and pyridine (30 mL, 9:1 v/v). 3-Chloro-5-iodo-2-methoxybenzoic acid **(19)** (406 mg, 1.30 mmol) was added, and the mixture was gently stirred at room temperature for 24 h. The suspension was filtered, and the resin was washed with pyridine (25 mL) and DMF, CH_2Cl_2, and MeOH (3 × 25 mL each) and then dried under reduced pressure over P_2O_5. Elemental analysis of the resin **20** (first cycle): I, 3.21; Cl, 0.93. This procedure was repeated again on this resin to afford **20**. IR (KBr) 1726.3 cm^{-1}, 1492.2 cm^{-1}. Elemental analysis of the resin **20** (second cycle): I, 12.70; Cl, 3.80.

Pd(0)-Catalyzed Sonogashira Coupling to Afford Resin 21. The resin **20** (950 mg) was suspended in a mixture of dry DMF and THF (30 mL, 5:1, v/v). Methyl 5-hexynoate (434 mg, 3.44 mmol), $Pd(OAc)_2$ (21 mg, 0.092 mmol), PPh_3 (60.3 mg, 0.23 mmol), CuI (44 mg, 0.23 mmol), and triethylamine (337 mg, 3.34 mmol) were sequentially added to a round-bottomed reaction flask containing the resin **20**. The reaction flask was then stoppered, and the reaction mixture was stirred overnight under argon. The solvent and excess reactants were then removed by filtration, and the remaining light brown solid support was washed with DMF, THF, MeOH, and CH_2Cl_2 (3 × 25 mL of each) and dried in vacuo. IR (KBr) 2232 cm^{-1}; 1731.4 cm^{-1}.

Hydrostannation of Resin-Bound Alkyne 21 to Yield Resin 22. $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) was added to a degassed suspension of polymer-bound alkyne **21** (0.83 g, ∼1 mmol) in a mixture of dry THF and DMF (30 mL, 2:1 v/v). The mixture was stirred for 5 min, and Bu_3SnH (0.41 mL, 1.5 mmol) was then slowly added via syringe over 10 min. The reaction mixture was allowed to stir at room temperature for 4 h under argon, transferred to a glass filter, and thoroughly washed with CH_2Cl_2, MeOH, DMF, and then CH_2Cl_2 (3 × 25 mL each) to afford the desired solid support **22**.

Cleavage of Compound 23 from Solid Support 22. The cleavage cocktail was prepared by degassing a solution of MeOH (17 mL), THF (5 mL), and Et_3N (3.0 mL) with argon. This solution was added to the dried resin **22** (100 mg), and the mixture was heated at 50 °C under argon for 16 h at room temperature. The resin was filtered and washed with MeOH (2 mL). The resin was subjected to this procedure a total of six times. The combined filtrates were evaporated to afford compound **23** (64 mg): ^{1}H NMR ($CDCl_3$) δ 7.16 (d, J = 2.17 Hz, 1 H), 7.02 (d, J = 2.18 Hz, 1 H), 5.68 (t, J = 6.95 Hz, 1 H, $^3J_{SnH}$ = 31.1 Hz), 3.86 (s, 3 H), 3.85 (s, 3 H), 3.56 (s, 3 H), 2.19 (t, J = 7.55 Hz, 2 H), 1.99 (dt, J = 7.04 and 7.33 Hz, 2 H), 1.61 (m, 2 H), 1.14−1.45 (m, 18 H), 0.90 (t, J = 7.0 Hz, 9 H). Anal. Calcd for $C_{28}H_{45}ClSnO_5$: C, 54.61; H, 7.37; Cl, 5.76. Found: C, 54.43; H, 7.31; Cl, 5.68.

Pd(0)-Catalyzed Stille Coupling to Afford Resins 24 and 25. A solution of $AsPh_3$ (24.5 mg, 0.08 mmol) in THF (5 mL) was added to a degassed suspension of polymer-bound vinylstannane **22** (400 mg) in anhydrous DMF (15 mL) containing $Pd_2(dba)_3$ (19.6 mg, 0.0214 mmol) and CsF (67 mg, 0.44 mmol). After 5 min, aryl iodide **10** (391 mg, 1.28 mmol) in anhydrous DMF (10 mL) was added, and the reaction mixture was heated at 80 °C overnight under argon. The reaction mixture was then transferred to a fritted syringe and thoroughly washed with DMF, CH_2Cl_2, EtOH, and CH_2Cl_2 (3 × 25 mL each) to afford solid support **24**, which was dried under reduced pressure over P_2O_5. A similar procedure was employed to prepare solid support **25** via Stille coupling of aryl iodide **17** with resin-bound vinylstannane **22**.

Cleavage of ADAMs 26 and 27 from Resins 24 and 25. The appropriate resin **24** or **25** (200 mg) was added to a degassed MeOH/THF solution (60 mL, 1:2 v/v) containing NaOMe (167 mg, 3 mmol). The reaction mixture was heated under reflux for 20 h. The resin was filtered and washed with MeOH (10 mL), and the filtrate was evaporated under reduced pressure to afford crude ADAM **26** (73 mg) or **27** (92 mg) as a light yellow liquid. Both compounds were further purified by flash chromatography on silica gel (EtOAc/hexanes 1:3, v/v).

Methyl (5*Z*)-6-[3-Chloro-4-methoxy-5-(methoxycarbonyl)phenyl]-6-[4-methoxy-5-(methoxycarbonyl)-3-methylphenyl]hex-5-enoate (26). Yield 54.7 mg; ^{1}H NMR ($CDCl_3$) δ 7.62 (d, J = 2.40 Hz, 1 H), 7.56 (d, J = 2.06 Hz, 1 H), 7.37 (d, J = 2.30 Hz, 1 H), 7.34 (d, J = 2.20 Hz, 1 H), 5.93 (t, J = 7.46 Hz, 1 H), 3.92 (s, 3 H), 3.87 (s, 3 H), 3.83 (s, 3 H), 3.73 (s, 3 H), 3.57 (s, 3 H), 2.24 (t, J = 7.32 Hz, 2 H), 2.19 (s, 3 H),

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2.06 (dt, $J = 7.58$ and 7.43 Hz, 2 H), 1.68 (m, 2 H). Anal. Calcd for $C_{26}H_{29}ClO_8$: C, 61.84; H, 5.79; Cl, 7.02. Found: C, 62.13; H, 5.77; Cl, 6.83.

Methyl (5*Z*)-6-[3-Chloro-4-methoxy-5-(methoxycarbonyl)phenyl]-6-[4-methoxy-3-(methoxycarbonyl)phenyl]-hex-5-enoate (27). Yield 79.0 mg; ^{1}H NMR ($CDCl_3$) δ 7.63 (d, $J = 2.38$ Hz, 1 H), 7.52 (d, $J = 2.46$ Hz, 1 H), 7.30 (d, $J = 2.12$ Hz, 1 H), 7.15 (dd, $J = 8.59$ and 2.34 Hz, 1 H), 6.84 (d, $J = 8.79$ Hz, 1 H), 5.97 (t, $J = 7.47$ Hz, 1 H), 3.96 (s, 3 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.86 (s, 3 H), 3.61 (s, 3 H), 2.29 (t, $J = 7.07$ Hz, 2 H), 2.12 (dt, $J = 7.43$ and 7.23 Hz, 2 H), 1.79 (m, 2 H); EIMS *m*/*z* 490 (M^+). Anal. Calcd for $C_{25}H_{27}ClO_8$: C, 61.10; H, 5.50; Cl; 7.24. Found: C, 61.28; H, 5.49; Cl, 7.12.

HIV Cytoprotection Assay. Anti-HIV screening of test compounds was performed as previously described[42,43] with minor changes. Briefly, the assays involve the killing of T4 lymphoid cells (CEM-SS cell line) by HIV-1 and inhibition of cell killing by active compounds. Experimental antiviral compounds were diluted as appropriate and added to a 96-well microtiter plate. Cells were infected in the well at a multiplicity of infection (MOI) of approximately 0.2, and viability was assayed at 6 days after infection by reduction of the CellTiter 96 Reagent (Promega, Madison, WI). This reagent contains the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS) and the electron-coupling agent phenazine ethosulfate in a colorless stable solution, which upon reduction by viable cells forms a colored solution with absorbance at 490 nm. Data are presented as the percent control of MTS 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. MTS-based results were confirmed by measurement of cell-free supernatant RT and p24 levels. All MTS 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%.

Assay for Inhibition of HIV-1 RT. The effects of the compounds on the HIV-1 RT enzyme were performed using recombinant purified p66/51 RT (a kind gift of S. Hughes, NCI-FCRDC). Inhibition of reverse transcription was measured by the level of incorporation of [^{32}P]GTP into the poly(rC)·oligo(dG) (rCdG) homopolymer template primer system.[3]

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