Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 2366–2374

Synthesis and anti-HIV activity of new alkenyldiarylmethane (ADAM) non-nucleoside reverse transcriptase inhibitors (NNRTIs) incorporating benzoxazolone and benzisoxazole rings

Bo-Liang Deng,[a] Matthew D. Cullen,[a] Zhigang Zhou,[a] Tracy L. Hartman,[b] Robert W. Buckheit Jr.,[b] Christophe Pannecouque,[c] Erik De Clercq,[c] Phillip E. Fanwick[d] and Mark Cushman[a,*]

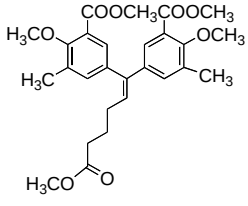
[a]*Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, IN 47907, USA*
[b]*ImQuest BioSciences, 7340 Executive Way, Suite R, Frederick, MD 21704, USA*
[c]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*
[d]*Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA*

Received 14 September 2005; revised 6 November 2005; accepted 8 November 2005
Available online 29 November 2005

Abstract—The HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) constitute a large and structurally diverse set of compounds, several of which are currently used in the treatment of AIDS. A series of novel alkenyldiarylmethanes (ADAMs) were designed and synthesized as part of an ongoing investigation to replace the metabolically labile methyl ester moieties found in the ADAM pharmacophore with stable modifications that retain the potent anti-HIV activity of the parent compounds. Unsurprisingly, the rat plasma half-lives of the new ADAMs were not improved when compared to the parent compounds, but all of the synthesized ADAMs inhibited the cytopathic effect of HIV-1 in cell culture. The most potent compound identified was (*E*)-5-[1-(3,7-dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-5-methoxycarbonyl-pent-1-enyl]-2-methoxy-3-methylbenzoic acid methyl ester (**7**), which inhibited the cytopathic effects of both HIV-1_{RF} and HIV-1_{IIIB} strains in cell cultures with EC_{50} values of 30 and 90 nM, respectively, and inhibited HIV-1 reverse transcriptase with an IC_{50} of 20 nM.

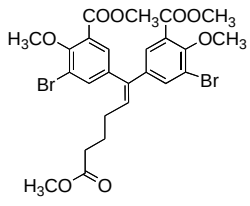
1. Introduction

The human immunodeficiency virus (HIV) is a retrovirus known to cause acquired immunodeficiency syndrome (AIDS), which is characterized by the loss of helper T-lymphocytes and heavy damage to the lymphatic tissues. A number of therapeutic agents with various mechanisms of action have been developed to combat the progression of AIDS; however, clinical treatment of the disease often leads to the emergence of drug-resistant strains due to the rapid mutability of the HIV virus.[1,2] As such, new anti-HIV agents capable of inhibiting the pathology of both wild-type and drug-resistant HIV strains are desperately needed, as is underscored by the rampant spread of the virus throughout



1

RT Inhibition IC_{50}= <1.0 μM
Inhibition of HIV-1_{IIIB} cytopathic effect on MT-4 Cells: EC_{50}= 1.0 μM

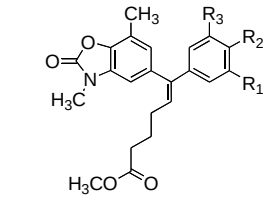


2

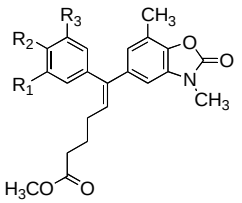
RT Inhibition IC_{50}= 0.3 μM
Inhibition of HIV-1_{IIIB} cytopathic effect on MT-4 Cells: EC_{50}= 0.3 μM

Keywords: Alkenyldiarylmethane (ADAM); Anti-HIV; Benzoxazolone; Benzisoxazole; Non-nucleoside reverse transcriptase inhibitor (NNRTI).
* Corresponding author. Tel.: +765 494 1465; fax: +765 494 6790; e-mail: cushman@pharmacy.purdue.edu

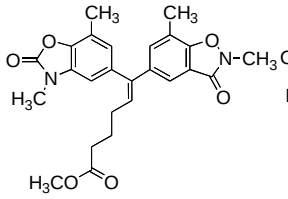
0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.11.014



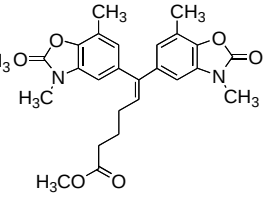
3 $R_1 = CH_3$; $R_2 = OCH_3$; $R_3 = COOCH_3$
4 $R_1 = Cl$; $R_2 = OCH_3$; $R_3 = COOCH_3$
5 $R_1 = H$; $R_2 = OCH_3$; $R_3 = COOCH_3$



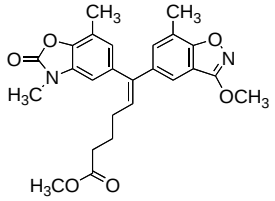
6 $R_1 = F$; $R_2 = H$; $R_3 = CF_3$
7 $R_1 = CH_3$; $R_2 = OCH_3$; $R_3 = COOCH_3$



8



9



10

Africa and the recent classification of AIDS as an epidemic by the World Health Organization (WHO) and UNIAIDS.

The alkenyldiarylmethanes (ADAMs) belong to the unique class of anti-HIV agents called non-nucleoside reverse transcriptase inhibitors (NNRTIs), that are capable of inhibiting HIV-1 reverse transcriptase (RT) through an allosteric mechanism.[3–11] Several ADAMs have been found to inhibit HIV-1_{RT} and the cytopathic effect of the virus at submicromolar concentrations (examples are ADAMs **1** and **2**). In addition, ADAMs have displayed synergistic activity with AZT and have shown enhanced activity when tested against various drug-resistant strains of HIV-1.[4,5,7,8] However, the potential therapeutic use of the ADAMs is compromised by the metabolically labile methyl ester groups present in the pharmacophore. More specifically, the ester moieties are likely to be hydrolyzed by non-specific esterases present in blood plasma,[†] resulting in the formation of the inactive carboxylic acid metabolites.[5]

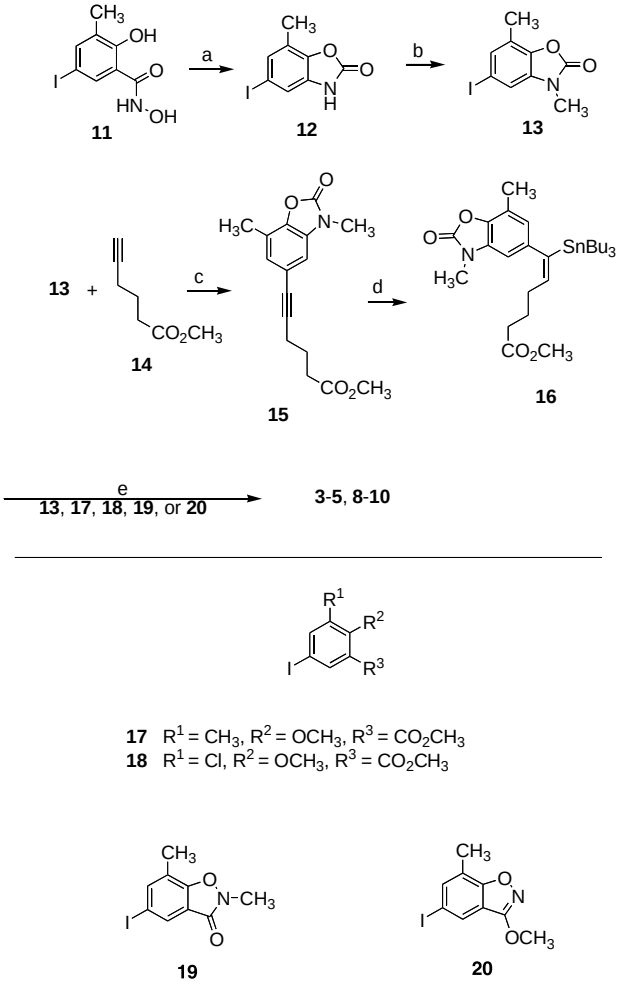
Accordingly, we have sought to investigate the replacement of these functionalities with methyl ester mimics that would hopefully display enhanced metabolic stability and retain the desirable anti-HIV-1 activity of the parent compounds, but the extremely complex SAR of the ADAMs has made achieving this goal difficult, in both practice and concept. As part of our ongoing search for metabolically stable methyl ester replacements for ADAMs, the present study was performed to investigate the replacement of the methyl ester and the adjacent methoxyl groups on the aromatic rings with fused *N*-methyloxazolidinone, *N*-methylisoxazolone, and 3-methoxy isoxazoline ring systems, that have been successfully employed in the investigations of other potential therapeutics.[12,13] In our case, the ADAMs **2**–**10** resulting from these replacements were tested for inhibition of the cytopathic effects of HIV-1 (RF and IIIB strains) and HIV-2_{ROD}, as well as inhibition of HIV-1 reverse transcriptase. The metabolic stabilities of these compounds were also investigated in rat plasma.

2. Chemistry

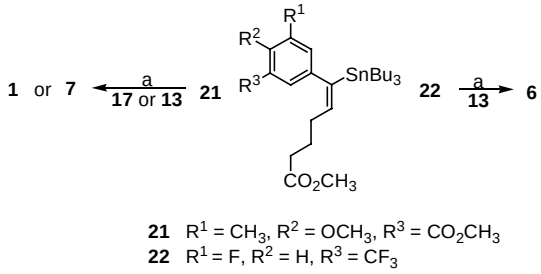
Over the course of the project, a general and practical method to synthesize alkenyldiarylmethanes has been developed using metal-catalyzed reactions (Sonogashira reaction, hydrostannation, and Stille coupling). In order to incorporate *N*-methyloxazolidinone, *N*-methylisoxazolone, and 3-methoxy isoxazoline ring systems into the ADAMs, it was necessary to synthesize aryl halide precursors that contained the heterocyclic functionalities of interest.[10,14] The syntheses of the aryl halide precursors bearing the isoxazoline and isoxazolone functionalities (compounds **19** and **20**; Scheme 1) have been previously reported.[14] Precursor **13**, bearing the oxazolidinone functionality, was synthesized as shown in Scheme 1. Treatment of the 2,*N*-dihydroxybenzamide **11** with triphenylphosphine and DEAD led to the formation of benzoxazolone **12** via a Lossen rearrangement. Syntheses of benzoxazol-2-ones that utilize the Lossen rearrangement have been widely studied and reported in the literature.[15,16] The rearrangement of **11** to the benzoxazolone was confirmed after **12** was methylated to afford product **13**, whose structure was confirmed by X-ray crystallography (Fig. 1). Other aryl halide precursors (compounds **17** and **18**) were also prepared as previously described.[14]

Having prepared all of the necessary aryl halides, we proceeded to follow our general synthetic method for synthesizing ADAMs. Sonogashira coupling of aryl iodide **13** with methyl 5-hexynoate (**14**) yielded disubstituted alkyne **15**. The hydrostannation of **15** with tri-*n*-butyltin hydride in the presence of $Pd(PPh_3)_4$ afforded the regiochemically and stereochemically defined vinylstannane **16** that would serve as a common intermediate for several target ADAMs. Stille coupling of stannane **16** with aryl iodides **13** and **17**–**20**, using $Pd(^tBu_3P)_2$ in the presence of CsF, afforded ADAMs **3**–**5** and **8**–**10**. It is worth noting that ADAM **5** was originally not a synthetic target for our investigation and was isolated as a significant byproduct from the Stille coupling of **16** and **18**. The formation of ADAM **5** can easily be rationalized as an undesirable dehaloge-

[†] The half-life of ADAM **1** in Sigma rat plasma (lot 052K7609) was determined to be 0.76 min and the half-life of ADAM **2** was 3.14 min (lot 052K7609).



Scheme 1. The strategy for synthesis of alkenyldiarylmethanes Reagents and conditions: (a) PPh_3, diethyl azodicarboxylate, THF, 0 °C; (b) CH_3I, K_2CO_3, DMSO, or CH_3I, tetrabutylammonium bromide, EtOAc–H_2O; (c) $PdCl_2(PPh_3)_2$, Cu(I)I, Et_3N, THF, room temperature; (d) Bu_3SnH, $Pd(PPh_3)_4$, THF, room temperature; (e) $Pd(^tBu_3P)_2$, CsF, toluene, reflux.



Scheme 2. Reagents and condition: (a) $Pd(P^tBu_3)_2$, toluene, reflux.

nation of ADAM **4** because the dehalogenation of aryl halides, even chlorides, commonly occurs during metal-catalyzed cross-coupling reactions.[17–19] ADAMs **1**, **6**, and **7** were synthesized from stannanes **21** and **22** (Scheme 2), whose syntheses have been previously reported.[14] Stille coupling of stannane **21** with aryl iodides **17** and **13** afforded ADAMs **1** and **7**, while coupling of stannane **22** with iodide **13** provided ADAM **6**.

3. Biological results and discussion

The ADAMs of the present series were tested for inhibition of the cytopathic effects of HIV-1_{RF} in CEM-SS cells, and both HIV-1_{IIIB} and HIV-2_{ROD} in MT-4 cells. The observed EC_{50} values are listed in Table 1, along with the cytotoxicities (CC_{50} values) in uninfected CEM-SS cells and MT-4 cells. The ADAMs were also tested for their ability to inhibit HIV-1 RT, and the IC_{50} values are included in Table 1. The previously reported biological data for ADAM **1** and current clinical AIDS therapeutics, nevirapine (**23**) and efavirenz (**24**), in our assays are included for comparison. In addition, the metabolic half-lives of ADAMs **1** and **3–10** were determined in Sigma rat plasma lot 052K7609 (data reported in Table 1).

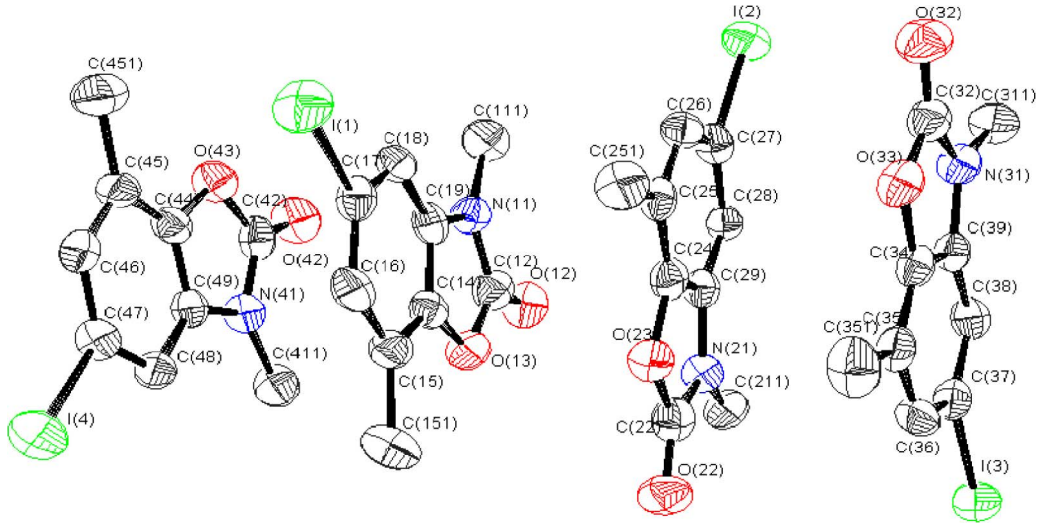
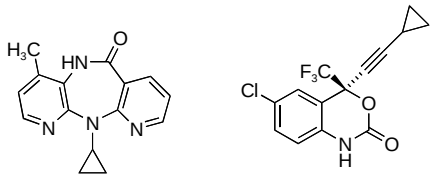


Figure 1. ORTEP drawing of compound **13** with atomic labeling scheme. Hydrogen atoms have been omitted for clarity.

Table 1. Anti-HIV activities, cytotoxicities, and metabolic stabilities of ADAM analogues

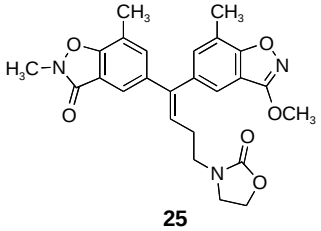
Compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]			CC_{50} (μM)[c]		Rat plasma $t_{1/2}$ (min ± SD)
		HIV-1_{RF}	HIV-1_{IIIB}	HIV-2_{ROD}	CEM-SS cells	MT-4 cells	
1	1.0	0.25	1.0	NA[d]	6.0	6.1	0.76 ± 0.04
3	0.16	0.87	1.01	>28.23	9.39	28.23	1.74 ± 0.00
4	0.93	0.53	0.25	>29.72	9.47	29.72	0.19 ± 0.01
5	0.99	3.08	4.15	>115	>13.46	115	0.34 ± 0.15
6	>100	>100	4.36	>60.03	15.0	50.95	3.06 ± 0.32
7	0.02	0.03	0.09	>16.86	5.1	16.86	1.30 ± 0.09
8	49.2	1.79	4.48	>36.52	11.1	36.52	0.46 ± 0.03
9	0.5	0.62	0.22	>32.52	31	32.52	0.59 ± 0.01
10	0.25	0.26	0.33	>7.26	2.08	7.26	1.58 ± 0.13
Nevirapine	C.N.A.[e]	0.015	0.053	NA[d]	C.N.A.[e]	15.0	NT[f]
Efavirenz	C.N.A.[e]	0.005	0.001	NA[d]	C.N.A.[e]	6.0	NT[f]

[a] Inhibitory activity versus HIV-1 reverse transcriptase with poly(rC).ologo(dG) as the template primer.
[b] EC_{50} is the 50% effective concentration for inhibition of cytopathicity of HIV-1_{RF} in CEM-SS cells, HIV-1_{IIIB} in MT-4 cells, or HIV-2_{ROD} in MT-4 cells.
[c] The CC_{50} is the 50% cytotoxic concentration for mock-infected CEM-SS cells or MT-4 cells.
[d] Not active.
[e] Data currently not available.
[f] Not tested. All data represent mean values for at least two separate experiments.



23

24



25

Using poly(rC)·oligo(dG) as the template primer, all of the analogues, except for **6** and **8**, inhibit HIV-1_{RT} with submicromolar IC_{50} values. In the cellular assays, all of the ADAMs exhibit anti-HIV-1_{RF} activity in the low micromolar to submicromolar ranges (except for ADAM **6**) and they also display anti-HIV-1_{IIIB} activity in the low micromolar and submicromolar ranges. Similar to other known NNRTIs, all of the ADAMs in this series are inactive against HIV-2. Comparing the biological data for the current series of ADAMs to those of nevirapine and efavirenz in our assays, ADAM **7** exhibited anti-HIV activity close to those of the clinically used therapeutics. The ADAMs **3**–**10** displayed a range of metabolic stabilities in rat plasma, with half-lives ranging from 0.19 to 3.06 min, which indicated all of the compounds, including potent ADAM **7** ($t_{1/2}$ = 1.30 min), are metabolically unstable. These results were not surprising because one would not expect the metabolic stability of the ADAMs to increase drastically if one or more of the metabolically labile esters were still present in the compounds; however, in the past, increases in the half-lives of some ADAMs by several minutes have been achieved simply by replacing one of the esters.[20] We have also previously synthesized ADAM **25**, in which all of the esters have been replaced with heterocyclic moieties, to show that replacing all of the three esters would result in metabolically stable ADAMs ($t_{1/2}$ = >24 h for **25**), but unfortunately, ADAM **25** exhibited very poor anti-HIV activity.[20]

In order to gain a better understanding of how potent ADAM **7** interacts with HIV-1_{RT}, a hypothetical model of the complex was constructed using GLIDE (Fig. 2).[21,22] At the outset, the GLIDE docking procedure utilized for ADAM **7** was validated by its ability to duplicate the crystal structures of the nevirapine and GCA-186 complexes with HIV-1_{RT} (PDB codes 1VRT and 1C1B, docked structures not shown). The same docking routine used to model ADAM **7** was also

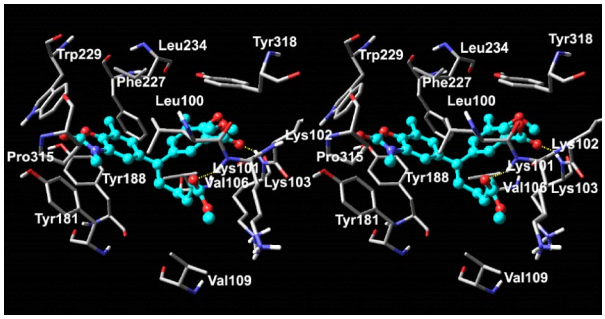


Figure 2. The modeled structure of bound ADAM **7** in the NNRTI binding pocket of HIV-1_{RT}. The ligand is displayed in a stick–ball model and the protein is displayed in a stick model. The carbons of the ligand are colored in cyan, while the carbons of the protein are colored in gray. Oxygens are in red, nitrogens are in blue, and polar hydrogens are in white for both ligand and protein. The two esters of the ligand form two hydrogen bonds with Lys101 and Lys103 (yellow dotted lines).

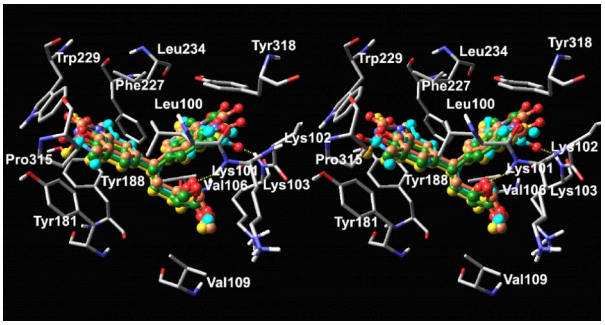


Figure 3. The docked binding structures of several ADAMs in the NNRTI binding pocket of HIV-1_{RT}. The ligands are displayed in stick–ball models and the protein is displayed in a stick model. Oxygens are in red, nitrogens are in blue, and polar hydrogens are in white for both ligand and protein. ADAM **3**, **7**, **9**, and **10** are colored in yellow, cyan, brown, and green, respectively.

used to model the other ADAM derivatives **3**, **9**, and **10**. The resulting models show that ADAM **7** adopts the typical 'butterfly' conformation that is characteristic of many non-nucleoside reverse transcriptase inhibitors (Fig. 2).[23,24] The calculated structures of the three other ADAMs (**3**, **9**, and **10**) indicate that they also bind with orientations similar to ADAM **7** (Fig. 3). These features are similar to those of the previous hypothetical molecular models obtained with ADAM **2**.[6,7] The benzoxazolone ring of ADAM **7** has an apparent π-stacking interaction with Tyr188 and/or with Tyr181. The ester carbonyl of the ligand side-chain hydrogen bonds with the backbone NH of Lys101, while the ester carbonyl on the aromatic ring of the ligand hydrogen bonds to the backbone carbonyl of Lys103. The combination of hydrophobic interactions, π-stacking, and side-chain hydrogen bonding appears to be what governs the binding of the ADAMs to RT.

4. Conclusions

In summary, the incorporation of benzoxazolone rings into the alkenyldiarylmethane system generated several compounds active against both IIIB and RF strains of HIV-1, of which ADAM **7** exhibited potencies near those of nevirapine and efavirenz. Unfortunately, the ADAMs in this series did not show enhanced metabolic stability over the parent compounds, but the information gained from this investigation can be used to design future ADAMs that are both active and metabolically stable. Future investigations will focus on synthesizing compounds in which both the esters on the aromatic rings and the side chain will be replaced with metabolically stable isosteres.

5. Experimental

5.1. Chemistry

Unless noted otherwise, NMR spectra were obtained at 300 MHz (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$ using $CHCl_3$ as the internal standard. IR spectra were recorded using a Perkin-Elmer 1600 series FT-IR. Flash chromatography was performed with 230–400 mesh silica gel. TLC was carried out on Baker-flex silica gel IB2-F plates of 0.25 mm thickness. Melting points are uncorrected. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone ketyl radical prior to use. Lyophilized rat plasma (lot 052K7609) was obtained from Sigma Chemical Co., St. Louis, MO. Microanalyses were performed at the Purdue University Microanalysis Laboratory. All yields given refer to isolated products.

5.2. General procedure for the synthesis of alkenyldiarylmethanes by the Stille cross-coupling reaction of vinylstannanes with aromatic iodides or bromides

A mixture of vinylstannane (1 equiv), iodide or bromide (1.2–1.5 equiv), cesium fluoride (3.0–4.5 equiv), and $Pd(P^tBu_3)_2$ (~10 mol%) in toluene (1 mL) under argon was stirred at different temperatures for various periods of time. The reaction mixture was cooled to room temperature, filtered through a short column of silica gel (5 g), and the column was washed with ethyl acetate. The organic solution was concentrated.

5.2.1. Methyl 4′,4″-dimethoxy-3′,3″-di(methoxycarbonyl)-5′,5″-dimethyl-6,6-diphenyl-5-hexenoate (1)[8]. The general procedure was followed using vinylstannane **21** (246 mg, 0.413 mmol), aryl iodide **17** (167 mg, 0.547 mmol), cesium fluoride (259 mg, 1.69 mmol), and $Pd(P^tBu_3)_2$ (24 mg, 0.045 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 15 h, at 60 °C for 7 h, and at 110 °C for 17 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc–hexanes (0–30%) to afford the product **1** (136 mg) as an oil in 68% yield. ^{1}H NMR[8] δ 7.44 (d, J = 2.4 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.07 (m, 2H), 5.94 (t, J = 7.5 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 3.61 (s, 3H), 2.30 (s, 3H), 2.28 (m, 2H), 2.23 (s, 3H), 2.09 (m, 2H), 1.74 (m, 2H).

5.2.2. (*Z*)-5-[1-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-5-methoxycarbonyl-pent-1-enyl]-2-methoxy-3-methylbenzoic acid methyl ester (3). The general procedure was followed using vinylstannane **16** (381 mg, 0.658 mmol), 5-iodo-2-methoxy-3-methylbenzoic acid methyl ester (**17**)[14] (245 mg, 0.800 mmol), cesium fluoride (395 mg, 2.57 mmol), and $Pd(P^tBu_3)_2$ (36 mg, 0.069 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 22 h, at 60 °C for 31.5 h, and at 110 °C for 13.5 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc–hexanes (0–30%) to afford the product **3** (202 mg) as an oil in 66% yield. IR (KBr) 2950, 1780, 1732, 1618, 1475, 1436, 1352, 1232, 1196, 1137, 1062, 1007, 880, 750, 621 cm^{-1}; ^{1}H NMR δ 7.38 (d, J = 2.4 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 6.64 (s, 1H), 6.51 (s, 1H), 5.93 (t, J = 7.5 Hz, 1H), 3.78 (s, 3H), 3.72 (s, 3H), 3.53 (s, 3H), 3.30 (s, 3H), 2.29 (s, 3H), 2.24 (t, J = 7.5 Hz, 2H), 2.17 (s, 3H), 2.05 (q, J = 7.5 Hz, 2H),

1.70 (dt, $J = 7.5$ Hz, 2H); ^{13}C NMR δ 173.48, 166.71, 157.11, 154.70, 140.57, 140.11, 137.67, 135.05, 133.36, 132.12, 131.27, 129.17, 127.17, 125.30, 124.10, 119.92, 106.71, 61.22, 51.93, 51.18, 33.14, 28.95, 27.98, 24.69, 15.86, 14.24; ESIMS *m*/*z* (rel intensity) 467.77 (MH^+, 30), 436.18 ($M-OCH_3^+$, 100). Anal. Calcd for $C_{26}H_{29}NO_7$: C, 66.80; H, 6.25; N, 3.00. Found: C, 66.41; H, 6.15; N, 3.04.

5.2.3. (*E*)-3-Chloro-5-[1-(3,7-dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-5-methoxycarbonyl-pent-1-enyl]-2-methoxybenzoic acid methyl ester (4). The general procedure was followed using vinylstannane **16** (352 mg, 0.608 mmol), aryl iodide **18**[14] (226 mg, 0.815 mmol), cesium fluoride (350 mg, 2.28 mmol), and $Pd(P^tBu_3)_2$ (34 mg, 0.064 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 17.5 h, at 60 °C for 24.5 h, and then at 110 °C for 24 h. The residue was purified by column chromatography on silica gel (15 g), eluting with EtOAc–hexanes (0–30%) to afford the product **4** (54.7 mg) as an oil in 18% yield and **5** (35 mg) as solid in 13% yield. Spectra of **4**: ^{1}H NMR δ 7.48 (d, $J = 1.8$ Hz, 1H), 7.26 (d, $J = 2.1$ Hz, 1H), 6.66 (s, 1H), 6.52 (s, 1H), 6.00 (t, $J = 7.2$ Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.58 (s, 3H), 2.34 (s, 3H), 2.26 (t, $J = 7.2$ Hz, 2H), 2.09 (q, $J = 7.5$ Hz, 2H), 1.74 (dt, $J = 7.5$ Hz, 2H); ESIMS *m*/*z* (rel intensity) 487.67/489.73 (MH^+, 57/25). Anal. Calcd for $C_{25}H_{26}ClNO_7$: C, 61.54; H, 5.37; Cl, 7.27; N, 2.87. Found: C, 61.91; H, 5.48; Cl, 6.94; N, 2.75.

5.2.4. (*Z*)-5-[1-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-5-methoxycarbonyl-pent-1-enyl]-2-methoxy-benzoic acid methyl ester (5). Compound **5** was obtained as described above: mp 134–135 °C. IR (KBr) 2949, 1778, 1732, 1618, 1499, 1463, 1436, 1353, 1306, 1266, 1192, 1157, 1083, 1028, 750 cm^{-1}; ^{1}H NMR δ 7.62 (d, $J = 2.4$ Hz, 1 H), 7.19 (dd, $J = 2.4$ Hz and 8.7 Hz, 1H), 6.83 (d, $J = 8.7$ Hz, 1H), 6.69 (s, 1H), 6.53 (s, 1H), 5.96 (t, $J = 7.5$ Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.59 (s, 3H), 3.35 (s, 3H), 2.35 (s, 3H), 2.27 (t, $J = 7.5$ Hz, 2H), 2.10 (q, $J = 7.5$ Hz, 2H), 1.75 (dt, $J = 7.5$ Hz, 2H); ^{13}C NMR δ 173.76, 166.78, 158.08, 154.97, 140.54, 140.31, 135.39, 134.70, 132.08, 131.43, 129.85, 128.43, 125.57, 120.20, 119.87, 111.69, 106.86, 56.07, 52.08, 51.44, 33.40, 29.16, 28.18, 24.95, 14.47; ESIMS *m*/*z* (rel intensity) 453.78 (MH^+, 21), 476.02 (MNa^+, 17). Anal. Calcd for $C_{25}H_{27}NO_7$: C, 66.21; H, 6.00; N, 3.09. Found: C, 65.92; H, 5.80; N, 3.13.

5.2.5. (*Z*)-6-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-6-(3-fluoro-5-trifluoromethylphenyl)-hex-5-enoic acid methyl ester (6). The general procedure was followed using vinylstannane **22** (325 mg, 0.561 mmol), aryl iodide **13** (245 mg, 0.847 mmol), cesium fluoride (275 mg, 1.792 mmol), and $Pd(P^tBu_3)_2$ (30 mg, 0.053 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 19 h, at 60 °C for 24 h, and then at 110 °C for 9 h. The residue was purified by column chromatography on silica gel (25 g), eluting with EtOAc–hexanes (0–5%) to afford the product **6** (98 mg) as an oil in 39% yield. IR (KBr) 2952, 1778, 1737, 1560, 1438, 1316, 1214, 1169, 1129, 936, 878, 750 cm^{-1}; ^{1}H NMR δ 7.29 (d, $J = 7.8$ Hz, 2H), 7.20 (s, 1H), 7.05 (d, $J = 8.4$ Hz, 1H), 6.68 (s, 1H), 6.54 (s, 1H), 6.03 (t, $J = 7.5$ Hz, 1H), 3.62 (s, 3H), 3.33 (s, 3H), 2.31 (s, 3H), 2.30 (t, $J = 7.5$ Hz, 2H), 2.16–2.08 (q, $J = 7.2$–7.8 Hz, 2H), 1.84–1.74 (m, 2H); ^{13}C NMR δ 173.57, 163.98, 160.67, 154.89, 143.21, 140.76, 139.89, 137.63, 131.49, 130.88, 123.57, 122.32, 120.37, 120.17, 111.88, 104.40, 51.48, 33.30, 29.05, 28.16, 24.78, 14.39; ESIMS *m*/*z* (rel intensity) 452.26 (MH^+, 67). Anal. Calcd for $C_{23}H_{21}F_4NO_4$: C, 61.20; H, 4.69; F, 16.83; N, 3.10. Found: C, 60.83; H, 4.62; F, 16.51; N, 3.05.

5.2.6. (*E*)-5-[1-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-5-methoxycarbonyl-pent-1-enyl]-2-methoxy-3-methyl-benzoic acid methyl ester (7). The general procedure was followed using vinylstannane **21** (342 mg, 0.574 mmol), aryl iodide **13** (227 mg, 0.785 mmol), cesium fluoride (315 mg, 2.05 mmol), and $Pd(P^tBu_3)_2$ (34 mg, 0.065 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 10 h, at 50 °C for 24 h, and then at 100 °C for 14.5 h. The residue was purified by column chromatography on silica gel (25 g), eluting with EtOAc–hexanes (0–30%) to afford the product **7** (195 mg) as a white solid in 73% yield: mp 120–120.5 °C. IR (KBr) 2950, 1732, 1548, 1496, 1436, 1395, 1318, 1255, 1203, 1141, 1009, 910, 765 cm^{-1}; ^{1}H NMR δ 7.36 (d, $J = 2.1$ Hz, 1H), 7.05 (d, $J = 2.0$ Hz, 1H), 6.68 (s, 1H), 6.53 (d, $J = 1.5$ Hz, 1H), 5.89 (t, $J = 7.5$ Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H), 3.56 (s, 3H), 3.27 (s, 3H), 2.256 (s, 3H), 2.255 (t, $J = 7.5$ Hz, 3H), 2.247 (s, 3H), 2.13–2.05 (q, $J = 7.4$ Hz, 2H), 1.77–1.68 (m, 2H); ^{13}C NMR δ 173.66, 166.61, 157.28, 154.85, 140.78, 140.35, 138.67, 136.14, 134.90, 132.61, 131.13, 130.11, 129.43, 124.18, 123.45, 119.70, 104.48, 61.36, 52.05, 51.32, 33.30, 29.00, 28.05, 16.01, 14.29; ESIMS *m*/*z* (rel intensity) 467.97 (MH^+, 95). Anal. Calcd for $C_{26}H_{29}NO_7$: C, 66.80; H, 6.25; N, 3.00. Found: C, 66.87; H, 6.32; N, 2.99.

5.2.7. (*Z*)-6-(2,7-Dimethyl-3-oxo-2,3-dihydro-benzo[*d*]isoxazol-5-yl)-6-(3,7-dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-hex-5-enoic acid methyl ester (8). The general procedure was followed using vinylstannane **16** (370 mg, 0.640 mmol), 5-iodo-2,7-dimethyl-benzo[*d*]isoxazol-3-one (**19**)[14] (231 mg, 0.80 mmol), cesium fluoride (404 mg, 2.63 mmol), and $Pd(P^tBu_3)_2$ (42 mg, 0.08 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 22.5 h, at 70 °C for 24.5 h, and then at 110 °C for 27 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc–hexanes (0–50%) to afford the product **8** (101 mg) as a solid in 35% yield: mp 152.5–154 °C. IR (KBr) 2949, 1778, 1736, 1690, 1617, 1492, 1470, 1355, 1298, 1249, 1224, 1169, 1062, 1032, 880, 854, 771, 750, 619 cm^{-1}; ^{1}H NMR δ 7.27 (s, 1H), 7.19 (s, 1H), 6.60 (s, 1H), 6.45 (s, 1H), 5.92 (t, $J = 7.5$ Hz, 1H), 3.55 (s, 3H), 3.51 (s, 3H), 3.26 (s, 3H), 2.26 (s, 3H), 2.24 (s, 3H), 2.20 (t, $J = 7.5$ Hz, 2H), 2.03 (q, $J = 7.5$ Hz, 2H), 1.67 (dt, $J = 7.5$ Hz, 2H); ^{13}C NMR δ 173.64, 162.97, 158.02, 154.87, 140.83, 140.34, 138.59, 135.28, 132.80, 131.46, 129.47, 125.48, 120.27, 119.80, 115.74, 106.79, 51.38, 33.29, 32.54, 29.13, 28.11, 24.82, 14.40, 14.05;

ESIMS *m/z* (rel intensity) 473.03 (MNa^+, 100). Anal. Calcd for $C_{25}H_{26}N_2O_6$: C, 66.65; H, 5.82; N, 6.22. Found: C, 66.39; H, 5.94; N, 5.98.

5.2.8. 6,6-Bis-(3,7-dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-hex-5-enoic acid methyl ester (9). The general procedure was followed using vinylstannane **16** (371 mg, 0.642 mmol), 5-iodo-3,7-dimethyl-3*H*-benzoxazol-2-one (**13**) (226 mg, 0.782 mmol), cesium fluoride (334 mg, 2.18 mmol), and $Pd(P^tBu_3)_2$ (35 mg, 0.067 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 22.5 h, at 60 °C for 24.5 h, and then at 110 °C for 27 h. The residue was purified by column chromatography on silica gel (15 g), eluting with EtOAc–hexanes (0–50%) to afford the product **9** (133 mg) as a solid in 46% yield: mp 163.5–165 °C. IR (KBr) 2948, 1772, 1734, 1638, 1618, 1494, 1469, 1358, 1302, 1165, 1064, 880, 749, 625 cm^{-1}; ^1H NMR δ 6.70 (s, 1H), 6.68 (s, 1H), 6.56 (d, J = 1.5 Hz, 1H), 6.54 (d, J = 0.9 Hz, 1H), 5.94 (t, J = 7.5 Hz, 1H), 3.57 (s, 3H), 3.33 (s, 3H), 3.27 (s, 3H), 2.33 (s, 3H), 2.27 (t, J = 7.5 Hz, 2H), 2.25 (s, 3H), 2.09 (q, J = 7.5 Hz, 2H), 1.74 (dt, J = 7.5 Hz, 2H); ^{13}C NMR δ 173.64, 154.85, 141.45, 140.38, 140.23, 138.83, 135.48, 131.36, 131.18, 129.26, 125.45, 123.37, 120.07, 119.72, 106.81, 104.40, 51.35, 33.30, 29.13, 28.07, 24.84, 20.87, 14.37, 14.31; ESIMS *m/z* (rel intensity) 450.96 (MH^+, 100). Anal. Calcd for $C_{25}H_{26}N_2O_6$: C, 66.65; H, 5.82; N, 6.22. Found: C, 66.27; H, 5.95; N, 6.17.

5.2.9. (*Z*)-6-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-6-(3-methoxy-7-methyl-benzo[*d*]isoxazol-5-yl)-hex-5-enoic acid methyl ester (10). The general procedure was followed using vinylstannane **16** (374 mg, 0.647 mmol), 5-iodo-3-methoxy-7-methylbenzo[*d*]isoxazole (**20**)[14] (229 mg, 0.792 mmol), cesium fluoride (355 mg, 2.31 mmol), and $Pd(P^tBu_3)_2$ (36 mg, 0.069 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 22 h, at 60 °C for 31 h, and then at 110 °C for 16.5 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc–hexanes (0–65%) to afford the product **10** (114 mg) as solid in 46% yield: mp 54–55 °C. IR (KBr) 2947, 1778, 1736, 1617, 1548, 1495, 1458, 1357, 1297, 1208, 1167, 1062, 878, 750, 690, 619 cm^{-1}; ^1H NMR δ 7.17 (s, 1H), 7.12 (s, 1H), 6.69 (s, 1H), 6.54 (s, 1H), 5.97 (t, J = 7.5 Hz, 1H), 4.06 (s, 3H), 3.55 (s, 3H), 3.32 (s, 3H), 2.39 (s, 3H), 2.33 (s, 3H), 2.27 (t, J = 7.5 Hz, 2H), 2.10 (q, J = 7.5 Hz, 2H), 1.74 (dt, J = 7.5 Hz, 2H); ^{13}C NMR δ 173.62, 167.19, 162.51, 154.82, 141.17, 140.23, 138.42, 135.56, 131.39, 130.18, 129.20, 125.45, 120.25, 120.10, 116.35, 113.44, 106.81, 57.14, 51.33, 33.27, 29.13, 28.07, 24.84, 14.51, 14.36; ESIMS *m/z* (rel intensity) 451.13 (MH^+, 38), 473.02 (MNa^+, 45). Anal. Calcd for $C_{25}H_{26}N_2O_6$: C, 66.52; H, 5.82; N, 6.22. Found: 66.46; H, 5.83; N, 5.96.

5.3. 5-Iodo-7-methyl-3*H*-benzoxazol-2-one (12)

Diethyl azodicarboxylate (1.27 mL, 7.90 mmol) was added dropwise to a solution of 2,*N*-dihydroxy-5-iodo-3-methylbenzamide (**11**)[14] (1.55 g, 5.28 mmol) and PPh_3 (2.09 g, 7.90 mmol) in THF (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 45 min and at room temperature for 2.5 h. The mixture was quenched with a 1:1 mixture of methanol/acetic acid (2.0 mL) and then concentrated. The residue was purified by column chromatography on silica gel (30 g) using EtOAc–hexanes (0–10%) to afford the product **12** (879 mg) as white crystals in 60% yield: mp 248–250 °C. IR (KBr) 3153, 3040, 2977, 2922, 1865, 1790, 1722, 1640, 1615, 1480, 1438, 1385, 1370, 1319, 1296, 1158, 1099, 1067, 943, 932, 836, 736 cm^{-1}; ^1H NMR (acetone-d_6) δ 7.31 (s, 1H), 7.28 (s, 1H), 2.81 (s, 1H), 2.30 (s, 3H); negative ion ESIMS *m/z* (rel intensity) 274.18 [$(M-H^+)^-$, 100]. Anal. Calcd for $C_8H_6INO_2$: C, 34.93; H, 2.20; I, 46.14; N, 5.09. Found: C, 35.22; H, 2.19; I, 46.25; N, 5.18.

5.4. 5-Iodo-3,7-dimethyl-3*H*-benzoxazol-2-one (13)

Method I: Compound **12** (105 mg, 0.38 mmol) was dissolved in ethyl acetate (8 mL), and then tetrabutylammonium iodide (15 mg, 0.04 mmol) was added, followed by addition of a solution of potassium carbonate (193 mg, 1.40 mmol) in water (3 mL). Iodomethane (0.06 mL, 0.9637 mmol) was added dropwise. The resulting mixture was stirred at room temperature for one day. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (2× 15 mL). The combined organic solution was washed with brine (2× 50 mL), dried over anhydrous sodium sulfate, and concentrated to afford a residue. The residue was purified by column chromatography on silica gel (15 g) using ethyl acetate in hexanes (0–10%) to afford the product **13** (92 mg) in 84% yield.

Method II: Iodomethane (0.05 mL, 0.795 mmol) was added to a mixture of compound **12** (98 mg, 0.35 mmol) and potassium carbonate (148 mg, 1.07 mmol) in DMSO (5 mL). The resulting mixture was stirred at room temperature for one day. Water (10 mL) was added to quench the reaction. The mixture was extracted with ethyl acetate (3× 15 mL). The organic solution was washed with brine (2× 50 mL), dried over anhydrous sodium sulfate, and concentrated to afford a residue. The residue was purified by column chromatography on silica gel using ethyl acetate in hexanes to afford the product **13** (84 mg) as a white solid in 82% yield. The product was recrystallized from ethyl acetate and hexanes to afford crystals for X-ray crystallography: mp 100–101 °C. IR (film) 2924, 1775, 1639, 1602, 1486, 1360, 1292, 1247, 1210, 1176, 1060, 1026, 882, 838, 746, 624, 666 cm^{-1}; ^1H NMR δ 7.28 (s, 1H), 7.09 (s, 1H), 3.34 (s, 3H), 2.31 (s, 3H); ^{13}C NMR δ 154.10, 140.86, 132.75, 132.56, 122.58, 114.33, 85.87, 28.24, 14.06; ESIMS *m/z* (rel intensity) 290.07 (MH^+, 100). Anal. Calcd for $C_9H_8INO_2$: C, 37.39; H, 2.79; I, 43.90; N, 4.85. Found: C, 37.69; H, 2.75; I, 43.62; N, 4.59.

5.5. Determination of the structure of 5-iodo-3,7-dimethyl-3*H*-benzoxazol-2-one (13) by X-ray crystallography

A colorless plate of $C_9H_8INO_2$ having approximate dimensions of $0.30 \times 0.30 \times 0.10$ mm was crystallized from ethyl acetate–hexanes and mounted on a glass fiber

in a random orientation. Preliminary examination and data collection were performed by Mo K_α radiation ($\lambda = 0.71073$ Å) on a Nonius KappaCCD equipped with a graphite crystal, incident beam monochromator. The orthorhombic cell parameters and calculated volume are: $a = 13.7861(4)$, $b = 16.5709(4)$, $c = 34.1035(10)$ Å, and $V = 7790.9(4)$ Å^3. For $Z = 32$ and FW = 289.07 the calculated density is 1.97 g/cm^3. The space group was *P*bca (# 61). The data were collected at a temperature of 150(1) K.

5.6. 6-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-hex-5-ynoic acid methyl ester (15)

5-Iodo-3,7-dimethyl-3*H*-benzoxazol-2-one (**13**) (2.335 g, 8.077 mmol) and hex-5-ynoic acid methyl ester (**14**) (1.019 g, 8.074 mmol) were dissolved in THF (15 mL) at room temperature. Triethylamine (3.0 mL, 21.52 mmol), $Pd(PPh_3)_2Cl_2$ (285 mg, 0.4 mmol), and Cu(I)I (154 mg, 8.076 mmol) were added. After the resulting mixture was stirred at room temperature for 23 h, water (50 mL) was added to quench the reaction. The mixture was concentrated to remove the organic solvents, and the residue was extracted with ethyl acetate (3× 50 mL). The combined organic solution was washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (40 g), eluting with EtOAc–hexanes (0–30%) to afford the product **15** (1.729 g) as a white solid in 75% yield: mp 97–98 °C. IR (KBr) 2950, 1779, 1735, 1619, 1468, 1374, 1332, 1302, 1211, 1158, 1061, 880, 749, 681, 630 cm^{-1}; ^{1}H NMR δ 6.99 (s, 1H), 6.80 (s, 1H), 3.67 (s, 3H), 3.35 (s, 3H), 2.49 (t, $J = 7.5$ Hz, 2H), 2.46 (t, $J = 6.9$ Hz, 2H), 2.31 (s, 3H), 1.91 (m, 2H); ^{13}C NMR δ 173.45, 154.64, 140.60, 131.12, 127.81, 120.27, 119.04, 108.48, 88.16, 80.70, 51.54, 32.79, 28.08, 23.76, 18.80, 14.14; ESIMS *m*/*z* (rel intensity) 309.82 (MNa^+, 5), 596.62 ($2M+Na^+$, 100). Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.96; N, 4.88. Found: C, 66.91; H, 5.98; N, 4.81.

5.7. 6-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-6-(tributylstannanyl)-hex-5-enoic acid methyl ester (16)

6-(3,7-Dimethyl-2-oxo-2,3-dihydro-benzoxazol-5-yl)-hex-5-ynoic acid methyl ester (**15**) (1.70 g, 5.92 mmol) was dissolved in THF (310 mL) and then tetrakis(triphenylphosphine)palladium (68 mg, 0.059 mmol) was added. The mixture was cooled to 0 °C, degassed by gently bubbling argon for 20 min, and then tributyltin hydride (2.5 mL, 9.02 mmol) was added dropwise over 30 min. The mixture was stirred at 0 °C for 60 min and at room temperature for 5 h, and then concentrated to yield a residue. The residue was purified by column chromatography on silica gel (60 g), using hexanes and EtOAc–hexanes (5%) to afford the vinylstannane **16** (3.07 g) as oil in 90% yield. IR (KBr) 2954, 2926, 2851, 1781, 1740, 1616, 1491, 1459, 1374, 1294, 1206, 1158, 1064, 1027, 879, 751, 686 cm^{-1}; ^{1}H NMR δ 6.42 (s, 1H), 6.29 (s, 1H), 5.70 (t, $J = 6.9$ Hz, 1H), 3.58 (s, 3H), 3.34 (s, 3H), 2.31 (s, 3H), 2.23 (t, $J = 7.5$ Hz, 2H), 3.02 (q, $J = 7.2$–7.5 Hz, 2H), 1.71–1.63 (m, 2H), 1.49–1.36 (m, 6H), 1.30–1.18 (m, 6H), 0.86–0.78 (m, 9H); ^{13}C NMR δ 173.92, 155.00, 145.98, 141.17, 140.87, 138.83, 131.16, 122.43, 119.90, 103.82, 51.43, 33.40, 29.36, 28.97, 28.07, 27.28, 24.84, 14.51, 13.67, 9.90; ESIMS *m*/*z* (rel intensity) 600.23 (MNa^+, 22), 602.08 (MNa^+, 20). Anal. Calcd for $C_{28}H_{45}NO_4Sn$: C, 58.15; H, 7.84; N, 2.42; Sn, 20.52. Found: 58.18; H, 8.00; N, 2.63; Sn, 20.43.

5.8. RT inhibition assay

Inhibition of purified recombinant reverse transcriptase enzyme was measured by the incorporation of [^{32}P]GTP into poly(rC)/oligo(dG) (rCdG) homopolymer template primers as previously described.[4,25]

5.9. In vitro antiviral assays

Evaluation of the antiviral activity of compounds against HIV-1_{RF} infection in CEM-SS cells was performed using the MTS cytoprotection assay as previously described.[26] Evaluation of the antiviral activity of the compounds against HIV-1 strain IIIB and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described.[26,27]

5.10. In vitro hydrolytic stability study in rat plasma

The alkenyldiarylmethanes **1** and **3**–**10** (with 1,1-diphenylethylene or benzophenone as internal standards) were tested for their hydrolytic stability, utilizing rat plasma and in vitro methods as previously described.[11]

5.11. Molecular modeling procedure

The coordinates of crystal complexes of RT/**23** (nevirapine, PDB code 1VRT)[28] and RT/GCA-186 (GCA-186, PDB code 1C1B)[29] were downloaded from the PDB Databank. The protein and ligand coordinates were prepared with the GLIDE[21,22] program by adding hydrogen atoms and partial charges were calculated using the OPLS force field.[30] Multi-step minimization was applied to relax hydrogens, side chains, and the whole molecule before the docking simulation was performed. 'Keep pose per ligand,' 'scoring window,' and 'poses for minimization' were increased for 10 times from their default values to 50,000, 1000, and 4000, respectively. All other default settings were used in the docking. During energy minimization, a 10 Å sphere surrounding the ligand was allowed to remain flexible (hot radius), and the remainder of the protein structure was kept rigid.

Acknowledgments

This investigation was made possible by Grant RO1-AI-43637, awarded by the National Institutes of Health, DHHS. This research was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number C06-14499 from the National Center for Research Resources of the National Institutes of Health.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.11.014.

References and notes

1. De Clercq, E. *Biochem. Biophys. Acta* **2002**, *1587*, 258–275.
2. De Clercq, E. *Chem. Biodivers.* **2004**, *1*, 44–64.
3. Cushman, M.; Golebiewski, M.; Buckheit, R. W., Jr.; Graham, L.; Rice, W. G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2713–2716.
4. Cushman, M.; Golebiewski, W. M.; Graham, L.; Turpin, J. A.; Rice, W. G.; Fliakas-Boltz, V.; Buckheit, R. W., Jr. *J. Med. Chem.* **1996**, *39*, 3217–3227.
5. Cushman, M.; Casimiro-Garcia, A.; Hejchman, E.; Ruell, J. A.; Huang, M.; Schaeffer, C. A.; Williamson, K.; Rice, W. G., ; Buckheit, R. W., Jr. *J. Med. Chem.* **1998**, *41*, 2076–2089.
6. Cushman, M.; Casimiro-Garcia, A.; Williamson, K.; Rice, W. G. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 195–198.
7. Casimiro-Garcia, A.; Micklatcher, M.; Turpin, J. A.; Stup, T. L.; Watson, K.; Buckheit, R. W.; Cushman, M. *J. Med. Chem.* **1999**, *42*, 4861–4874.
8. Xu, G.; Micklatcher, M.; Silvestri, M.; Hartman, T. L.; Burrier, J.; Osterling, M. C.; Wargo, H.; Turpin, J. A.; Buckheit, R. W., Jr.; Cushman, M. *J. Med. Chem.* **2001**, *44*, 4092–4113.
9. Xu, G.; Loftus, T. L.; Wargo, H.; Turpin, J. A.; Buckheit, R. W.; Cushman, M. *J. Org. Chem.* **2001**, *66*, 5958–5964.
10. Xu, G.; Hartman, T. L.; Wargo, H.; Turpin, J. A.; Buckheit, R. W., Jr.; Cushman, M. *Bioorg. Med. Chem.* **2002**, *10*, 283–290.
11. Silvestri, M. A.; Nagarajan, M.; De Clercq, E.; Pannecouque, C.; Cushman, M. *J. Med. Chem.* **2004**, 3149–3162.
12. Shi, G. Q.; Dropinski, J. F.; McKeever, B. M.; Xu, S. H.; Becker, J. W.; Berger, J. P.; MacNaul, K. L.; Elbrecht, A.; Zhou, G. C.; Doebber, T. W.; Wang, P. R.; Chao, Y. S.; Forrest, M.; Heck, J. V.; Moller, D. E.; Jones, A. B. *J. Med. Chem.* **2005**, *48*, 4457–4468.
13. Lenz, S. M.; Meier, E.; Pedersen, H.; Frederiksen, K.; BØgesØ, K. P.; Krogsgaard-Larsen, P. *Eur. J. Med. Chem.* **1995**, *30*, 263–270.
14. Deng, B.-L.; Hartman, T. L.; Buckheit, J. R. W.; Pannecouque, C.; De Clercq, E.; Fanwick, P. E.; Cushman, M. *J. Med. Chem.* **2005**, *48*, 6140–6155.
15. Shi, G. *Tetrahedron Lett.* **2000**, 2295–2298.
16. Sheradsk, T.; Grisaru-Auramovici, S. *Tetrahedron Lett.* **1978**, 2325–2326.
17. Peters, D.; Hornfeldt, A. B.; Gronowitz, S. *J. Heterocycl. Chem.* **1991**, *28*, 1629–1631.
18. Navarro, O.; Kaur, H.; Mahjoor, P.; Nolan, S. P. *J. Org. Chem.* **2004**, *69*, 3173–3180.
19. Handy, S. T.; Bregman, H.; Lewis, J.; Zhang, X.; Y., Z. *Tetrahedron Lett.* **2003**, *44*, 427–430.
20. Unpublished data from the Cushman Lab.
21. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739–1749.
22. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750–1759.
23. Schäfer, W.; Friebe, W.-G.; Leinert, W.; Mertens, A.; Poll, T.; von der Saal, W.; Zilch, H.; Nuber, H.; Ziegler, M. L. *J. Med. Chem.* **1993**, *36*, 726–732.
24. Ding, J.; Das, K.; Moereels, H.; Koymans, L.; Andries, K.; Janssen, P. A.; Hughes, S. H.; Arnold, E. *Nat. Struct. Biol.* **1995**, *2*, 407–415.
25. Buckheit, R. W., Jr.; Fliakas-Boltz, V.; Decker, W. D.; Robertson, J. L.; Stup, T. L.; Pyle, C. A.; White, E. L.; McMahon, J. B.; Currens, M. J.; Boyd, M. R.; Bader, J. P. *Antiviral Res.* **1995**, *26*, 117–132.
26. Rice, W. G.; Bader, J. P. *Adv. Pharmacol. (San Diego)* **1995**, *33*, 389–438.
27. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309–321.
28. Ren, J.; Esnouf, R.; Garman, E.; Somers, D.; Ross, C.; Kirby, I.; Keeling, J.; Darby, G.; Jones, Y.; Stuart, D.; Stammers, D. *Nat. Struct. Biol.* **1995**, *2*, 293–302.
29. Hopkins, A. L.; Ren, J.; Tanaka, H.; Baba, M.; Okamato, M.; Stuart, D. I.; Stammers, D. K. *J. Med. Chem.* **1999**, *42*, 4500–4505.
30. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225–11236.