Synthesis, Biological Activity, and Crystal Structure of Potent Nonnucleoside Inhibitors of HIV-1 Reverse Transcriptase That Retain Activity against Mutant Forms of the Enzyme†

Marshall L. Morningstar,‡ Thomas Roth,‡ David W. Farnsworth,*,‡ Marilyn Kroeger Smith,‡,§ Karen Watson,# Robert W. Buckheit, Jr.,# Kalyan Das,|| Wanyi Zhang,|| Eddy Arnold,|| John G. Julias,⊥ Stephen H. Hughes,⊥ and Christopher J. Michejda*,‡

Molecular Aspects of Drug Design Section and Retroviral Replication Laboratory, National Cancer Institute—Frederick, P.O. Box B, Frederick, Maryland 21702, McDaniel College, Westminster, Maryland 21157, Southern Research Institute—Frederick Research Center, 431 Aviation Way, Frederick, Maryland 21701, and Center for Advanced Biotechnology and Medicine and Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey 08854

Received January 31, 2006

In an ongoing effort to develop novel and potent nonnucleoside HIV-1 reverse transcriptase (RT) inhibitors that are effective against the wild type (WT) virus and clinically observed mutants, 1,2-bis-substituted benzimidazoles were synthesized and tested. Optimization of the N1 and C2 positions of benzimidazole led to the development of 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-methylbenzimidazole (**1**) ($IC_{50} = 0.2$ μM, $EC_{50} = 0.44$ μM, and $TC_{50} \geq 100$ against WT). This paper describes how substitution on the benzimidazole ring profoundly affects activity. Substituents at the benzimidazole C4 dramatically enhanced potency, while at C5 or C6 substituents were generally detrimental or neutral to activity, respectively. A 7-methyl analogue did not inhibit HIV-1 RT. Determination of the crystal structure of **1** bound to RT provided the basis for accurate modeling of additional analogues, which were synthesized and tested. Several derivatives were nanomolar inhibitors of wild-type virus and were effective against clinically relevant HIV-1 mutants.

Introduction

The rapid global spread of human immunodeficiency virus type-1 (HIV-1[a]) and the emergence of drug resistance make the development of new drugs for the treatment of acquired immunodeficiency syndrome (AIDS) an important problem. The replicative cycle of HIV-1 provides several potential targets for chemotherapeutic intervention. One of these is the viral enzyme reverse transcriptase (RT), which catalyzes the conversion of the single-stranded viral genomic RNA into double-stranded DNA. Nucleoside analogue inhibitors of RT (NRTIs), such as 3′-azido-2′,3′-dideoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), and 2′,3′-dideoxycytidine (ddC) are commonly used in the treatment of HIV-infected patients. However, because NRTIs are substrates for host polymerases, their utility is compromise by serious side effects.[1] The nonnucleoside RT inhibitors (NNRTIs), such as *N*-11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′3′-*e*]diazepin-6-one (nevirapine, BI-RG-587),[2] α-anilinophenylacetamide (α-APA),[3] and 8-chloro-4,5,6,7-tetrahydro-5-methylimidazo-[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-one (8-Cl TIBO),[4] (−)-6-chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2*H*-3, 1-benzoxazin-one (efavirence),[5] 1-(5-methanesulphonamido)-1*H*-indol-2-yl-carbonyl)-4-[3-(isopropylamino)-2-pyridinyl]piperazine (delaviridine),[6] and more recently 4-[[4-[[4-[(1*E*)-2-cyanoethenyl]-2, 6-dimethylphenyl]-amino]-2-pyrimidinyl]amino]benzonitrile (R278474, rilpivirine) of the DAPY[7] class of NNRTIs, inhibit HIV-1 RT by binding to a site near the polymerase active site. All anti-HIV-1 drugs, including NNRTIs, select for drug resistance. In the case of the NNRTIs, resistance involves mutation of critical residues in the binding pocket.[8] However, because the NNRTI binding pocket is specific for HIV-1 RT, NNRTIs can be designed to target HIV-1 RT; thus NNRTIs are less likely to exhibit systemic toxicity than NRTIs.

Crystallographic analysis of HIV-1 RT has defined the details of binding of the NNRTIs and delineated important structural differences between unliganded RT, RT bound to nucleic acids, and RT bound to NNRTIs.[9] Although all of the NNRTIs bind in the same pocket, they are quite diverse in part because the pocket is flexible. This makes it difficult to predict a priori which inhibitors will bind tightly and how an individual resistance mutation will affect the binding of a specific NNRTI.

We previously reported the discovery of a new class of NNRTIs that has activity against wild-type RT and some important variants, the 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles[10] (BPBIs). This class of inhibitors was designed to be more flexible than analogues of 1-(2,6-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole (TZB),[11−13] a known inhibitor of wild-type (WT) HIV-1 RT. It is now generally recognized that NNRTIs that have the structural flexibility and the ability to adapt to changes in the geometry of the binding pocket caused by resistance mutations are important in discovering NNRTIs that will be effective against resistant viruses.[10,14,15] Removal of the thiazolo ring from TZB and optimization of the lead compound resulted in the development of 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-methylbenzimidazole (**1**) ($IC_{50} = 0.2$ μM against HIV-1 RT and $EC_{50} = 0.44$ μM in a cytoprotection assay[16,17] using wild-type virus). We showed that this novel NNRTI was active in cytoprotection

† This paper is dedicated to the memory of Dr. Christopher J. Michejda.

* To whom correspondence should be addressed. Fax: (301) 846-6231. Phone: (301) 846-1224. E-mail: farnswor@ncifcrf.gov.

‡ Molecular Aspects of Drug Design Section, National Cancer Institute—Frederick.

§ McDaniel College.

Southern Research Institute—Frederick Research Center.

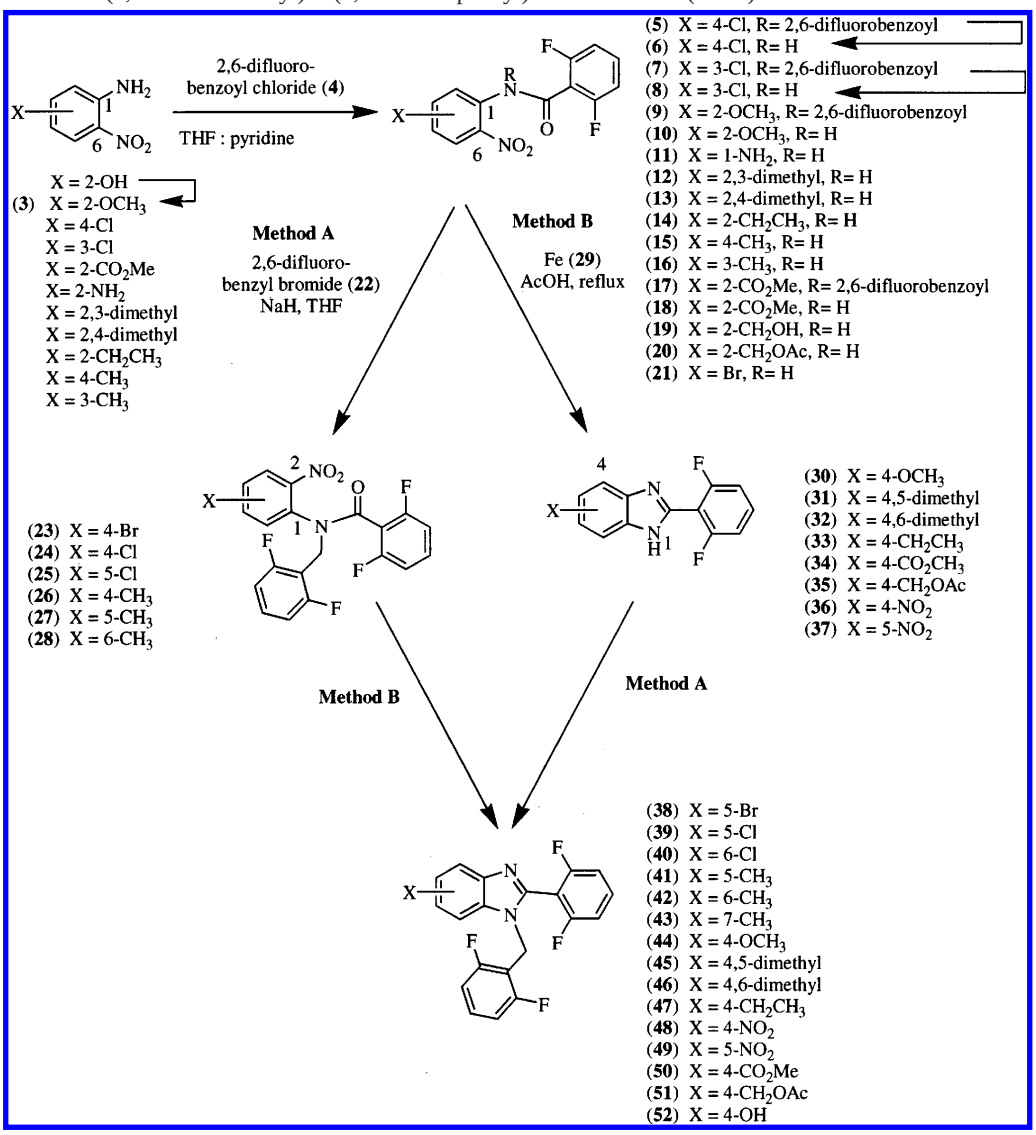
|| Rutgers University.

⊥ Retroviral Replication Laboratory, National Cancer Institute—Frederick.

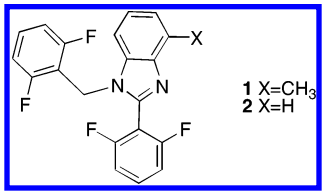
[a] Abbreviations. AIDS, acquired immunodeficiency syndrome; HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; TZB, 1-(2,6-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole; NNRTI, nonnucleoside reverse transcriptase inhibitor; BPBI, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole; WT, wild type; 8-Cl TIBO, 8-chloro-4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one; SAR, structure−activity relationship.

10.1021/jm060103d CCC: $37.00
Published on Web 07/31/2007

Scheme 1. Synthesis of 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles (BPBI)



assays against clinically relevant HIV-1 variants that have amino acid substitutions in the NNRTI binding pocket of RT.



Crystallographic analysis of compound **1** in the HIV-1 RT binding pocket was then used as a guide to synthesize analogues with better antiviral properties. The C4 desmethyl analogue (**2**) showed a consistent 3- to 4-fold better inhibitory activity against wild type and against clinically important RT variants.[10] To understand the significance of this difference in inhibitory activity, we systematically varied the substituents at the 4, 5, 6, and 7 positions of 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles. The results of these combined studies led to the discovery of derivatives with improved activity and allowed us to formulate relevant structure−activity relationships.

Chemistry

The general synthetic route to the substituted 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole derivatives is outlined in Scheme 1. A variety of substituted 6-nitroanilides are commercially available, and this starting material was used to prepare a variety of differentially substituted benzimidazole derivatives. Depending on the type and regiochemistry of the different substitutents, the desired monoacylated product could be obtained in good yields using 1.2 equiv of 2,6-difluorobenzoyl chloride. In the case of electron-withdrawing substituents, benzoylation led to a mixture of monoacylated and bis-acylated products. Variations in time, temperature, equivalents of reactants, and concentration were examined in unsuccessful efforts to produce only the desired mono-2,6-difluorobenzoyl products. These intermediate compounds were obtained in high yields by synthesizing the bis-2,6-difluorobenzoyl derivatives with excess 2,6-difluorobenzoyl chloride followed by selective base-catalyzed deacylation to the monobenzoyl product. Depending on the desired regiochemistry of the final benzimidazoles, the monoacylated 6-nitroanilides were either reductively cyclized with iron (method B) and alkylated with 2,6-difluorobenzyl bromide (method A) or alkylated with 2,6-difluorobenzyl bromide and then reductively cyclized. For instance, 2-substituted 6-nitroanilides were reductively cyclized to 4-substituted benzimidazoles. Alkylation of the benzimidazole N1 position with 2,6-difluorobenzyl bromide proceeded with >92% regiochemical purity, for a variety of 4-substituted compounds

(**30**−**36**). In the case of 5-, 6-, and 7-substituted benzimidazoles, regiochemical control was maintained by alkylation of the monoacylated derivative (method A) to *N*-(2,6-difluorobenzyl)-*N*-(2,6-difluorophenyl)nitroanilides (**23**−**28**) followed by reductive cyclization (method B).

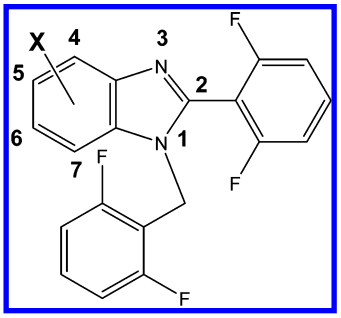
Nonreductive acid-catalyzed cyclization, in contrast, was used to synthesize 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-nitrobenzimidazole (**48**), a key intermediate for many C4 derivatives. Monoacylation of 3-nitro-1,2-phenylenediamine with 2,6-difluorobenzoyl chloride yielded a single product (**11**) that was subsequently cyclized with only acid and heat. Since cyclization of either N1 or N2 acylated product would lead to the desired 2-arylbenzimidazole, detailed regiochemical analysis was not carried out on **11**.

The 4-nitro group 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-nitrobenzimidazole (**48**) was reduced subsequently with tin(II) chloride in acetic acid to yield the 4-amino compound (**53**). Treatment of the 4-amino compound (**53**) with either HBr or HCl under Sandmeyer conditions yielded the 4-bromo (**54**) or 4-chloro (**55**) product, respectively. Alternatively, reaction of the 4-amino compound (**53**) with formaldehyde and sodium borohydride provided the 4-dimethylamino derivative (**59**). Monoacetylation of the 4-amino (**53**) with acetic anhydride gave the 4-acetamido product (**56**). Subsequent alkylation of the 4-acetamido (**56**) with methyl iodide yielded a mixture of *N*-methyl-*N*-acetamido product (**57**) and starting material. Deacylation of **57** under acidic conditions yielded the 4-methylamino compound (**58**).

The 4-methyl ester (**50**) was a key intermediate for a number of 4-substituted benzimidazole derivatives (**60**−**66**). Treatment of the 4-methyl ester derivative (**50**) with dimethylaluminum amine[18] resulted in the 4-nitrile derivative (**60**). The 4-nitrile (**60**) was used subsequently to prepare the 4-hydroxyamidino (**61**) and 4-amido (**62**) compounds by hydrolysis with hydroxylamine and hydrogen peroxide, respectively. Bis methylation of the 4-amido derivative (**62**) with sodium hydride and methyl iodide yielded the desired 4-(*N*,*N*-dimethyl)amido derivative (**63**). Alternatively, reaction of the 4-methyl ester derivative (**50**) with barium hydroxide or methyl lithium yielded the 4-carboxylate (**64**) and 4-isopropanol (**65**) derivatives, respectively. Hydrolysis of the 4-isopropanol derivative (**65**) with strong acid led to the 4-isopropenyl compound (**66**) in low yields. Reduction of the 4-methyl ester (**50**) to the 4-hydroxymethyl derivative (**67**) resulted in low yields under a number of reducing conditions (LAH, $NaBH_4$, borane). Consequently, the reduction step was carried out earlier in the synthesis with the precursor methyl *N*-(2,6-difluorobenzoyl)-6-nitro-2-anilidecarboxylate (**18**). Protection of the 2-hydroxymethyl-6-nitroanilide (**19**) with acetic anhydride and subsequent modification as done with the 4-methyl ester led to the desired 4-hydroxymethylbenzimidazole derivative (**67**). This product was subsequently converted to the 4-formyl (**68**), 4-chloromethyl (**69**), 4-azidomethyl (**70**), 4-aminomethyl (**71**), 4-acetamidomethyl (**72**), 4-(*N*-methyl)acetamido (**73**), 4-methoxymethyl **(74)**, and 4-cyanomethyl **(75)**.

Other compounds prepared for this series were made via synthetic modification of intermediates described in our earlier publication.[10] Bromination of 1-(2,6-difluorobenzoyl)-2-nitroanilide yielded 1-(2,6-difluorobenzoyl)-4-bromo-2-nitroanilide (**23**). This derivative was then benzylated and reductively cyclized with standard conditions to yield 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-5-bromobenzimidazole (**38**). Mononitration of 2-(2,6-difluorophenyl)benzimidazole with nitric acid at room temperature yielded 5-nitrobenzimidazole (**37**) as the only product. We subsequently found that alkylation with 2,6-difluorobenzyl bromide (**22**) occurred regiospecifically to yield the 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-5-nitrobenzimidazole (**49**).

Table 1. Enzyme Inhibition Data for Substituted Benzimidazoles[a]



compd	X	% inhibition[b] (1 μM)
	(A) 5, 6, or 7-Methyl-Substituted 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles	
2	H	50
1	4-CH_3	71
41	5-CH_3	22
42	6-CH_3	42
43	7-CH_3	1
45	4,5-diCH_3	26
46	4,6-diCH_3	37
	(B) 4, 5, or 6-Substituted 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles	
54	4-Br	78
38	5-Br	43
55	4-Cl	75
39	5-Cl	48
40	6-Cl	69
48	4-NO_2	51
49	5-NO_2	30

[a] Enzyme assays done with WT RT. [b] % inhibition is the average of two experiments done in triplicate.

Results and Discussion

An in vitro HIV-1 RT assay using rC-dG template primer, as described previously, was used as a screen for the activity of compounds **38**−**43**, **45**, **46**, **48**, **49**, **54**, **55**, and the data are presented in Table 1. This initial screen for activity was done at 1 μM (10-fold lower than used in our earlier publication).[10] As illustrated in Table 1, the level of inhibitory activity differs dramatically as the regiochemistry of substituents changes from the 4 to 7 position. Substitution at the 4 position always enhanced the inhibitory activity relative to the other sites of substitution, regardless of the type of substituent [CH_3 (**1** vs **41**−**43**), Br (**54** vs **38**), Cl (**55** vs **39** and **40**), NO_2 (**48** vs **49**)]. A combination of a C4 substituent (CH_3) with an additional substituent at the 5 or 6 position (**1** vs **45** and **1** vs **46**) reduced the inhibitory activity, suggesting a preference for monosubstitution at C4. Substitution at the 7 position with a CH_3 (**43**), however, resulted in complete loss of activity. Molecular modeling of the 7-CH_3 (**43**) vs the 4-CH_3 (**1**) suggested an alternative and possibly nonproductive conformation for the 7-CH_3 (**43**) as a consequence of steric interactions between the C7 substituent and the *N*1-2,6-difluorobenzyl group. This was confirmed by structure determination of (**1**) in complex with HIV-1 RT by X-ray crystallography (Table 2, Figure 1). Most NNRTIs bind to the hydrophobic binding pocket in a "butterfly-like" conformation.[14,15] Like a butterfly, the two wings of the inhibitor, wing 1 and wing 2, generally contain π-systems that interact with aromatic amino acids (Y181, Y188, F227, and W229) and hydrophobic amino acids (L100 and L234) through favorable π-stacking interactions. The X-ray data suggest

Table 2. X-ray Crystallographic Data and Refinement Statistics

X-ray Diffraction Data		
PDB code	2B6A	
X-ray source	CHESS F1	CHESS F1
data collection temp (K)	268	104
no. of crystals	10	1
wavelength (Å)	0.918	0.918
space group	*C*2	*C*2
cell constants		
a (Å)	227.8	223.9
b (Å)	70.2	68.8
c (Å)	106.7	103.0
β (deg	105.7	107.2
resolution range (Å)	40.0−2.5	40−2.7
no. of unique reflections	49 714	38 261
no. of observations	334 752	377 187
completeness (%)	88.2	92.0
R_{merge} (in last shell)	0.087	0.084
I/σ cutoff	$\|F\| < 1.0\sigma(\|F\|)$	$\|F\| < 1.0\sigma(\|F\|)$
Refinement Statistics		
total no. of atoms		7933
total no. of inhibitors		27
resolution range (Å)		20.0−2.65
no. of reflections		44 766
R_{free} set		2245
completeness (in last shell, 2.82−2.65 Å) (%)		94.6 (82.5)
cutoff criterion		$\|F\| < 1.00\sigma(\|F\|)$
R_{cryst}		0.226
R_{free}		0.277
rms deviation		
bond length (Å)		0.012
bond angle (deg)		1.8

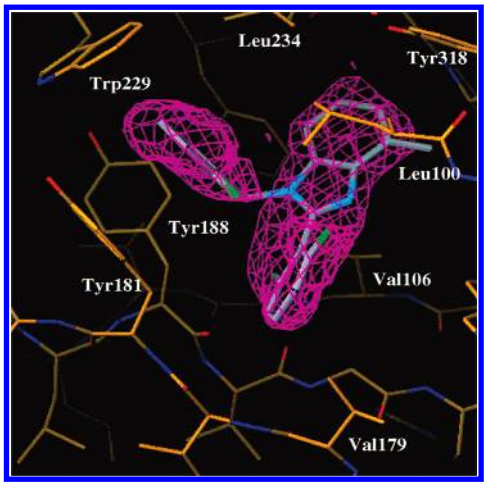


Figure 1. Electron density of 4-methyl BPBI (**1**) in the binding pocket of HIV-1 RT. The piece of electron density covering the inhibitor is from a $|F_o| - |F_c|$ map calculated prior to inclusion of the inhibitor in structure refinement.

(Figures 1−3) that a substituent in the 7 position would have unfavorable steric interactions, especially with residue F227, which would force a repositioning of aromatic wing 1 and loss of π-stacking interaction with Y188 and Y181.

The crystal structure of **1** in HIV-1 RT can explain why substituents in position 4 are particularly favorable. These substituents point into space surrounded by the backbone and side chain groups of residues 100−103 and 318. This can be seen in Figure 2, where the 4-methyl group of **1** is oriented into that space. Thus, 4-substituents that are capable of making favorable contacts, especially with the backbone atoms, would favor tighter binding of the inhibitor and, in general, be more effective against drug-resistant HIV-1 RTs.

In light of these results, analogues in Table 3 were synthesized and tested to determine whether the enhancement of potency caused by a C4 group was general for any substituent or related

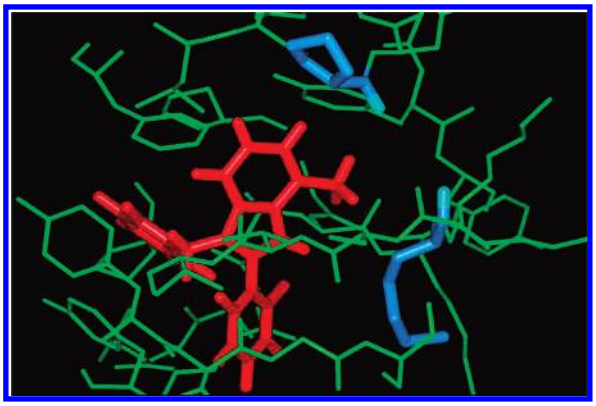


Figure 2. Orientation of 4-methyl BPBI in the binding pocket of HIV-1 RT allows for expansion of substituents in the 4 position of the benzimidazole ring.

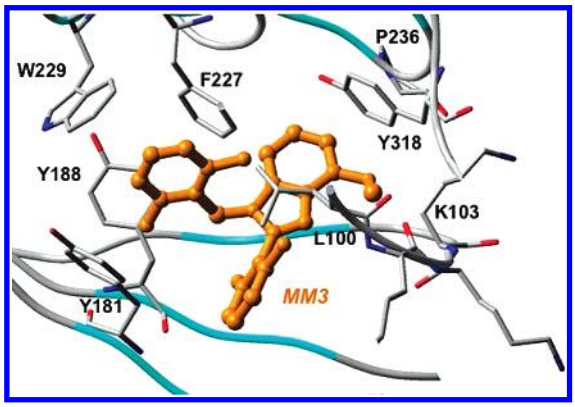
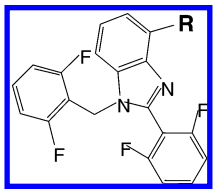


Figure 3. Orientation of 4-methoxy BPBI (**44**) in the binding pocket of HIV-1 RT.

to some chemical or structural property of the substituent. The compounds were tested for their ability to inhibit HIV-1 (NL4-3) replication.[16,17] The data are presented as concentrations to reduce the cell killing by the virus by 50% (EC_{50}). We also carried out enzymatic assays on the inhibition of HIV-1 RT by selected analogues as described previously.[10] These data are reported as IC_{50}. Our compounds exhibit relatively low toxicity (see TC_{50} values as determined in CEM cells) in comparison to their activities (EC_{50} and IC_{50} values). All data are reported in Table 3. While a variety of C4 substituents [(NH_2 (**53**), Br (**54**), Cl (**55**), CN (**60**)] showed a modest increase in potency relative to the unsubstituted (**2**) or methyl (**1**) parent compounds, the methoxyl (**44**) substituent showed a dramatic 25-fold increase in the inhibitory activity against WT RT (IC_{50} = 7.3 nM) relative to the lead compound (**1**). Preliminary X-ray crystal structure data for this analogue (Figure 3) show increased favorable interactions with binding pocket residues, thus explaining the increase in potency of this analogue. In contrast, the isosteric ethyl (**47**) and monomethylamino (**58**) compounds were significantly poorer inhibitors than the lead compound.

Analogues **50**, **56**−**59**, and **61**−**64** were synthesized and tested to determine whether an oxygen or any other type of substituent in the 4-position would enhance the potency of the compound. An *N*-methylacetamide (**57**) at the C4 position significantly enhanced inhibitory potency, while other carbonyl groups [CO_2Me (**50**), CNHNHOH (**61**), $CONH_2$ (**62**), $CON(CH_3)_2$ (**63**), and CO_2H (**64**)] were clearly detrimental to potency. Synthesis of compounds **65**−**73** and determination of each analogue's EC_{50} value focused attention on whether a hydrophobic or methylene inserted at the C4 position would have potencies similar to the those of des-methylene analogues

Table 3. Enzyme Inhibition Data for 4-Substituted 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles[a]



compd	R	IC_{50} (μM)	EC_{50} (μM)	TC_{50} (μM)
1	CH_3	0.2	0.44	>100
2	H	0.11	1.7	18.8
44	OCH_3	0.0073	0.06	44.3
57	$NAcCH_3$	0.32	0.02	>100
47	CH_2CH_3	17.5	0.82	26.1
48	NO_2	1.88	2.4	30.1
50	CO_2Me	157.8	NRVC[c]	57.7
51	CH_2OAc	ND[b]	0.059	55.8
52	OH	ND[b]	0.65	>100
53	NH_2	0.28	0.46	26.3
54	Br	1.61	0.37	24.5
55	Cl	2.63	0.44	30.4
56	NHAc	54.7	1.48	39.3
58	$NHCH_3$	4.94	1.7	27.2
59	$N(CH_3)_2$	40	2.37	>100
60	CN	ND[b]	0.43	26.7
61	CNH(NHOH)	ND[b]	0.19	23.1
62	$CONH_2$	ND[b]	4.9	>100
63	$CON(CH_3)_2$	ND[b]	NRVC[c]	80.1
64	CO_2H	ND[b]	NRVC[c]	23.5
65	isopropyl	ND[b]	6.34	27.0
66	isoprenyl	ND[b]	1.44	28.3
67	CH_2OH	ND[b]	0.023	58.1
68	CHO	ND[b]	0.12	0.82
69	CH_2Cl	ND[b]	0.18	13.4
70	CH_2N_3	ND[b]	0.045	>100
71	CH_2NH_2	ND[b]	0.037	49.3
72	CH_2NHAc	ND[b]	NRVC[c]	20.8
73	CH_2NCH_3Ac	ND[b]	0.58	20.6
74	CH_2OCH_3	ND[b]	0.06	ND[b]
75	CH_2CN	ND[b]	0.06	ND[b]
	nevirapine	ND[b]	0.035	ND[b]

[a] IC_{50} is the quantity of drug required to reduce WT RT enzyme activity by 50%. EC_{50} is the quantity of drug required to reduce cell killing of HIV-1 infected CEM cells by 50%. TC_{50} is the quantity of drug required to reduce cell viability by 50%. [b] ND: not determined. [c] NRVC: no reduction in viral cytopathic effect.

(**53**−**57**). These results indicated that bulkier alkyl substituents at C4 decreased activity. However, compounds **51, 67**−**71**, **74**, and **75** showed excellent activity against the WT virus. This was especially true for the hydroxymethylene derivative **67**, the acetoxymethylene derivative **51**, the azidomethylene derivative **70**, the aminomethylene derivative **71**, the methoxymethylene derivative **74**, and the cyanomethylene derivative **75**. These compounds were the most active inhibitors in our panel along with the 4-*N*-(acetyl)methyl derivative **57** and the 4-methoxy derivative **44**. As determined by crystallography, to be effective, the substituent can point toward the main chain of amino acids 100−103 or toward the Pro236. However, it is clear that bulky substituents can cause steric hindrance with the 100−103 loop unless the binding pocket is significantly enlarged. Small substitutents bearing heteroatoms that can make hydrogen bond interactions with backbone residues of the 100−103 loop should enhance binding, as predicted by molecular modeling.[19]

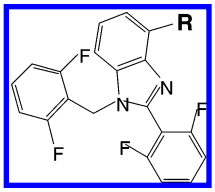
In addition to obtaining EC_{50} values against WT virus, we tested some of the compounds against MT-4 cells infected with HIV-1 variants containing NNRTI-resistant RTs (Table 4). The 4-methoxy compound **44**, which is an order of magnitude more active against the WT virus than the prototypic compound **1**, was however ineffective against most of the NNRTI-resistant viruses, especially the K103N mutant. This mutation is one of the most commonly observed in patients treated with NNRTIs. In fact, most of our inhibitors failed against that mutation, with the dramatic exception of compound **51**. This derivative was more active against the virus carrying the K103N mutation than against the WT virus. However, compound **51** was inactive against other NNRTI-resistant viruses, specifically Y181C and V106A. One derivative, compound **57**, showed excellent activity against the V106A mutation and was only moderately effective against Y181C. It appears that mutations of tyrosine at the 181 position are particularly deleterious to the binding of this class of inhibitors. The reason for this is apparent in the crystallographic structure of **1** in complex with HIV-1 RT, where the difluorobenzyl moiety of the inhibitor is π-stacked with tyrosine 181. Interestingly, compound **51** effectively inhibits the Y188C mutant, which is a frequently observed variant in viruses treated with NNRTIs. The other analogues in Table 3, including the cyanomethylene derivative **75** that showed excellent activity against WT RT, were much less active against the NNRTI-resistant viruses.

It should be noted that the acetoxymethylene derivative **51** can be hydrolyzed to the hydroxymethylene analogue **67**. Indeed, **51** is a substrate for porcine liver esterase (data not shown). However, the observed spectrum of activities of **51** against the HIV-1 RT variants is distinctly different from that of **67**. This suggests that the intact acetoxymethylene group is responsible for the overall activity of **51.** Likewise, it could be argued that the potent inhibitors such as **51**, the chloromethylene derivative **69**, and the azidomethylene derivative **70**, which are potential electrophiles, react covalently with nucleophilic residues in the binding pocket. While a proteomic analysis of possible covalent modifications of the protein was not carried out, the markedly different mutation profiles for these derivatives (Table 4) argue against the importance of this reaction.

The experimental activities of some of the 4-substituted derivatives were compared with the computationally derived binding energies ($\Delta G_{binding}$).[19] The data in Table 5 show that in general the modeled free energies correspond to the experimental values (less than $\pm$1 kcal). This increases our confidence that the computational method can be used to predict which compounds will be the most active in advance of their syntheses.

In summary, the following conclusions can be drawn about the structure−activity relationship involved in the variation of the benzimidazole moiety of **1**. The structure−activity data clearly show that substituents on C4 of the benzimidazole nucleus are generally beneficial for activity against HIV-1 RT. However, there are limitations. Compounds that have a carbonyl group or larger group substituted at the C4 position are generally inactive. The best activity is obtained with small heteroatom-bearing substituents, which presumably act as hydrogen-bond acceptors. The structure−activity data are fully supported by the X-ray crystallographic data for HIV-1 RT bound to 4-methyl BPBI. The level of activity of the compounds in the present study against the various variant forms of RT was more difficult to predict. The most active derivatives, such as compound **51**, are apparently able to make significant interactions with the RT backbone so that the variations in the binding pocket caused by NNRTI-resistant mutations can be reasonably well tolerated. It is possible that the ability to inhibit the different HIV-1 virus isolates tested reflects the flexible nature of the 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole system to better compensate for the different structural changes arising as a consequence of amino acid changes in the RT binding pocket. Thus, while no single compound in our series is able to

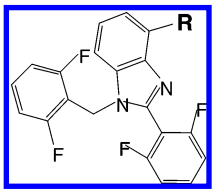
Table 4. Cross-Resistance Profile of NNRTI with Resistant HIV-1 Isolates As Determined by Cytopathic Cell Killing Assay[a]



	R group														
isolate	CH_3 **1**	OCH_3 **44**	Cl **55**	Br **54**	NH_2 **53**	Et **47**	NHAc **56**	$NAcCH_3$ **57**	CH_2N_3 **70**	CH_2OAc **51**	CH_2OH **67**	CH_2NH_2 **71**	CH_2Cl **69**	CH_2CN **75**	NVP[b]
NL4-3 (WT)	0.5	0.06	0.5	0.5	0.4	0.9	1.97	0.04	0.045	0.062	0.048	1.36	0.16	0.06	0.1
L100I	0.3	0.16	0.3	0.5	1.2	2.48	3.25	1.66	0.08	0.068	0.02	ND[c]	0.08	ND[c]	0.72
K101E	16.7	2.82	>20	>20	>20	>50	ND[c]	0.64	0.6	0.025	5	1.49	0.46	ND[c]	1.1
K103N	8.1	>50	10.2	>20	3.1	>50	>100	>10	1.48	0.027	13.6	>50	3.34	4.2	1.09
V108I	2.8	2.82	3.2	4.2	3.3	>50	ND[c]	>10	0.026	0.013	0.023	2.51	0.21	ND[c]	0.2
Y181C	6	0.26	8.4	10.7	9.2	7.18	>100	0.4	0.28	2.32	0.72	12.8	2.38	2.2	4.9
Y188C	2.3	ND[c]	3.4	3.7	2	ND[c]	ND[c]	0.54	0.22	0.033	0.17	11.6	0.57	ND[c]	0.1
V106A	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	0.05	0.61	4.5	5.9	9.23	4.7	ND[c]	2.36

[a] Antiviral data are reported as the quantity of drug required to reduce cell killing or virus production by 50% (EC_{50}). All values reported in μM. [b] NVP: nevirapine. [c] ND: not determined.

Table 5. Comparison of Experimental and Calculated Binding Energies for Several BPBI Analogues[a]



isolate	EC_{50} CH_2OAc	ΔG_{expt}[b] CH_2OAc	ΔG_{calc}[c] CH_2OAc	EC_{50} CH_2CN	ΔG_{expt}[b] CH_2CN	ΔG_{calc}[c] CH_2CN	EC_{50} CH_2N_3	ΔG_{expt}[b] CH_2N_3	ΔG_{calc}[c] CH_2N_3
NL4-3 (WT)	0.062	−10.24	−10.29	0.06	−10.24	−9.48	0.045	−10.43	−10.6
L100I	0.068	−10.28	−10.43	ND[d]	ND[d]	−9.99	0.08		
K101E	0.025						0.6		
K103N	0.027			4.2	−7.67	−7.48	1.48	−8.27	−7.85
V108I	0.013						0.026		
Y181C	2.32	−8	−8.15	2.2	−8.09	−7.77	0.28		
Y188C	0.033						0.22		
V106A	4.5	−7.59	−6.63	ND[d]	ND[d]	−6.97	0.61		

[a] All values reported in μM. [b] $\Delta G_{expt} = -RT \ln EC_{50}$. [c] See ref 19. [d] ND: not determined.

effectively block the replication of viruses carrying all of the common NNRTI-resistant mutations in RT, each of the mutations can be inhibited efficiently by at least one of the active compounds. The reported systematic studies on the BPBI series of compounds can be used in designing improved NNRTIs and may help in understanding the role of NNRTI-resistant mutations.

Experimental Section

General. Where analyses are indicated by symbols of the elements, results were within 0.4% of the theoretical values. Elemental analyses were determined by Atlantic Microlab, Inc., Norcross, GA. Melting points were determined on an Electrothermal apparatus using the supplied, stem-corrected thermometer and are as read. ^{1}H NMR spectra were recorded on a Varian 200 or 300 MHz spectrometer with Me_4Si as the internal standard. Yields were not optimized. Merck silica gel, 230−400 mesh, was used for flash chromatography. Priming nomenclature in the identification of the protons refers in the case of a double prime to the aromatic protons on the N1 benzyl substitutents and a single prime to the protons on the aromatic substitutents on the C2 aromatic in the final benzimidazoles or the N1 benzoates in the *N*-(2,6-difluorobenzoyl)-*N*-(2,6-difluorobenzyl)nitroanilide derivatives.

2-Methoxyl-6-nitroaniline (3). To 2-amino-3-nitrophenol (2.00 g, 12.98 mmol) dissolved in acetone (20 mL) were added K_2CO_3 (2.15 g) and methyl iodide (1.00 mL). After being stirred overnight, the mixture was concentrated, redissolved in ethyl acetate, washed with water, $NaHSO_4$ (10% solution), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography, eluting with 1:4 ethyl acetate/hexane (1.79 g, 10.65 mmol, 82% yield of white crystals): ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.69 (dd, J = 1.4, 8.9 Hz, 1H, H_5), 6.92 (dd, J = 1.4, 7.8 Hz, 1H, H_3), 6.62 (dd, J = 8.9, 7.8 Hz, 1H, H_4), 6.42 (br, 2H, NH_2), 3.91 (s, 3H, OCH_3).

***N,N*-Bis-(2,6-difluorobenzoyl)-4-chloro-6-nitroanilide (5).** To 4-chloro-2-nitroaniline (1.05 g, 6.08 mmol) dissolved in THF/pyridine (1:1) (20 mL) were added 2,6-difluorobenzoyl chloride (**4**) (1.9 mL, 15.1 mmol, 250 M %) and a second addition (0.6 mL, 4.77 mmol, 78 M %) after 6 h. After being stirred overnight at room temperature, the mixture was concentrated to dryness. The residue was redissolved in CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was recrystallized from ethyl acetate (2.67 g, 5.90 mmol, 97% yield of white crystals): ^{1}H NMR (300 MHz, CD_2C1_2) δ 8.15 (d, J = 2.4 Hz, 1H, H_3), 7.67 (dd, J = 2.4, 8.5 Hz, 1H, H_5), 7.51 (d, J = 8.5 Hz, 1H, H_6), 7.36 (m, 2H, $H_{4',4'}$), 6.89 (m, 4H, $H_{3',5',3',5'}$).

***N*-(2,6-Difluorobenzoyl)-4-chloro-6-nitroanilide (6).** To **5** (1.00 g, 4.42 mmol) dissolved in methanol/dioxane (1:1) (40 mL) was added NaOH (solid) (0.27 g, 6.75 mmol, 150 M %). After the mixture was stirred for 30 min at room temperature, the reaction was quenched with $NaHSO_4$, diluted with CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried

(Na_2SO_4), filtered, and concentrated. The product (1.28 g, 4.09 mmol, 93% yield) was recrystallized from diethyl ether/hexane (3:1): ^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.72 (s, 1H, NH), 8.91 (d, J = 9.1 Hz, 1H, H_6), 8.27 (d, J = 2.5 Hz, 1H, H_3), 7.71 (dd, J = 9.1, 2.5 Hz, 1H, H_5), 7.52 (m, 1H, $H_{4'}$), 7.08 (m, 2H, $H_{3',5'}$).

***N,N*-Bis-(2,6-difluorobenzoyl)-5-chloro-3-nitroanilide (7).** To 5-chloro-2-nitroaniline (1.02 g, 5.91 mmol) dissolved in pyridine/THF (1:1) (20 mL) was added **4** (1.50 mL, 11.9 mmol, 200 M %). After being stirred overnight at room temperature, the mixture was concentrated to dryness. The residue was redissolved in CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was recrystallized from ethyl acetate (2.50 g, 5.52 mmol, 93% yield of white crystals): ^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.12 (dd, J = 8.5, 1.1 Hz, 1H, H_3), 7.58 (AB, J = 2.4, 1.1 Hz, 1H, H_6), 7.57 (AB, J = 2.4, 8.4 Hz, 1H, H_4), 7.36 (m, 2H, $H_{4',4'}$), 6.89 (m, 4H, $H_{3',5',3',5'}$).

***N*-(2,6-Difluorobenzoyl)-5-chloro-2-nitroanilide (8).** To **7** (1.00 g, 2.20 mmol) dissolved in methanol/dioxane (1:1) (20 mL) was added NaOH (solid) (92 mg, 2.30 mmol, 105 M %). After the mixture was stirred for 30 min at room temperature, additional NaOH (92 mg, 2.30 mmol, 105 M %) was added. After an additional 15 min, the reaction was quenched with $NaHSO_4$, diluted with CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was recrystallized from diethyl ether/hexane (3:1) (0.55 g, 1.85 mmol, 84% yield of white crystals): ^{1}H NMR (200 MHz, CD_2Cl_2) δ 10.91 (s, 1H, NH), 9.04 (d, J = 2.3 Hz, 1H, H_6), 8.23 (d, J = 9.0 Hz, 1H, H_3), 7.52 (cm, 1H, $H_{4'}$), 7.26 (dd, J = 2.3, 9.0 Hz, 1H, H_4), 7.08 (m, 1H, $H_{3'5'}$).

***N,N*-Bis-(2,6-difluorobenzoyl)-2-methoxyl-6-nitroanilide (9).** To **3** (13.75 g, 81.8 mmol) dissolved in THF/pyridine (1:1) (300 mL) was added **4** (22.0 mL, 174.4 mmol, 215 M %). After being stirred overnight at room temperature, the mixture was concentrated to dryness. The residue was suspended in water and filtered, and the filtered solid was resuspended in hot methanol and filtered (33.7 g, 75.2 mmol, 92% yield of white crystals): ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.70 (dd, J = 1.4, 8.4 Hz, 1H, H_5), 7.48 (dd, J = 8.4, 8.4 Hz, 1H, H_4), 7.35 (2H, m, $H_{4',4'}$), 7.15 (dd, J = 1.4, 8.4 Hz, 1H, H_3), 6.87 (m, 4H, $H_{3',5',3',5'}$), 3.85 (s, 3H, OCH_3).

***N*-(2,6-Difluorobenzoyl)-2-methoxyl-6-nitroanilide (10).** To **9** (33.7 g, 75.2 mmol) dissolved in pyridine (500 mL) was added hydrazine (4.50 mL, 92.6 mmol, 120 M %). After being stirred overnight at room temperature, the mixture was concentrated, and the residue was redissolved in CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, evaporated, and recrystallized from methanol, yielding 22.39 g (72.6 mmol, 96% yield) of a white solid: ^{1}H NMR (200 MHz, CD_2C1_2) δ 8.82 (br, 1H, NH), 7.51 (dd, J = 1.6, 8.2 Hz, 1H, H_5), 7.45 (m, 2H, $H_{4'}$), 7.35 (dd, J = 8.2, 8.3 Hz, 1H, H_4), 7.12 (dd, J = 8.3, 1.6 Hz, 1H, H_3), 7.01 (m, 2H, $H_{3',5'}$), 3.92 (s, 3H, OMe).

***N*-(2,6-Difluorobenzoyl)-2-amino-3-nitroanilide (11).** 3-Nitro-1,2-phenylenediamine (13.3 g, 86.85 mmol) and **4** (15.33 g, 10.95 mL, 86.85 mmol) gave, after being stirred overnight and recrystallization from ethyl acetate/hexane (1:1), 12 g (41 mmol, 48% yield) of yellow crystals: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.23 (s, 1H, NH), 7.99 (dd, J = 8.7, 1.4 Hz, 1H, H_4), 7.71 (dd, J = 7.6 Hz, 1.4 Hz, 1H, H_6), 7.62 (m, 1H, $H_{4'}$), 7.28 (m, 2H, $H_{3',5'}$), 6.91 (s, 2H, NH_2), 6.76 (dd, J = 8.7, 7.6 Hz, 1H, H_5).

***N*-(2,6-Difluorobenzoyl)-2,3-dimethyl-6-nitroanilide (12).** 2,3-Dimethyl-6-nitroaniline (2.00 g, 12.04 mmol) and **4** (1.50 mL, 13.93 mmol, 115 M %) and a second addition of **4** (0.50 mL, 4.64 mmol, 40 M %) at 3 h gave, after 5 h and recrystallization from ethyl acetate/hexane (1:4), 2.71 g (8.85 mmol, 74% yield) of yellow crystals: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.67 (s, 1H, NH), 7.81-(d, J = 8.4 Hz, 1H, H_6), 7.48 (m, 1H, $H_{4'}$), 7.28 (d, J = 8.4 Hz, 1H, H_5), 7.05 (m, 2H, $H_{3',5'}$), 2.44 (s, 3H, CH_3), 2.31 (s, 3H, CH_3).

***N*-(2,6-Difluorobenzoyl)-2,4-dimethyl-6-nitroanilide (13).** 4,6-Dimethyl-2-nitroaniline (4.25 g, 25.6 mmol) and **4** (4.25 mL, 33.80 mmol, 130 M %) gave, after 5 h and recrystallization from ethyl acetate/hexane (1:1), 7.07 g (23.1 mmol, 90% yield) of white crystals: ^{1}H NMR (200 MHz, CD_2Cl_2) δ 8.41 (br, 1H, NH), 7.68 (br d, J = 2.6 Hz, 1H, H_5), 7.48 (m, 1H, $H_{4'}$), 7.42 (d, J = 2.6 Hz, 1H, H_3), 7.04 (m, 2H, $H_{3',5'}$), 2.42 (s, 3H, CH_3), 2.39 (s, 3H, CH_3).

***N*-(2,6-Difluorobenzoyl)-2-ethyl-6-nitroanilide (14).** 2-Ethyl-6-nitroaniline (3.10 g, 18.65 mmol) and **4** (3.50 mL, 27.83 mmol, 150 M %) gave, after being stirred overnight at room temperature and flash chromatography with 2% methanol/CH_2Cl_2 and recrystallization from ethyl acetate/hexane (1:4), 2.34 g (7.64 mmol, 41% yield) of a white solid: ^{1}H NMR (200 MHz, CD_2Cl_2) δ 8.25 (br, 1H, NH), 7.85 (dd, J = 1.7, 8.1 Hz, 1H, H_5), 7.66 (dd, J = 1.7, 7.9 Hz, 1H, H_3), 7.49 (m, 1H, $H_{4'}$), 7.45 (dd, J = 7.9, 8.1 Hz, 1H, H_4), 7.06 (m, 2H, $H_{3',5'}$), 2.81 (q, J = 7.6 Hz, 2H, CH_2), 1.29 (t, J = 7.6 Hz, 3H, CH_3).

***N*-(2,6-Difluorobenzoyl)-4-methyl-2-nitroanilide (15).** 4-Methyl-2-nitroaniline (4.95 g, 32.5 mmol) and **4** (4.50 mL, 41.8 mmol, 130 M %) gave, after recrystallization from ethyl acetate, 6.69 g (22.9 mmol, 70% yield) of yellow crystals: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.64 (s, 1H, NH), 8.75 (d, J = 8.6, 1H, H_6), 8.06 (s, 1H, H_3), 7.52 (m, 1H, $H_{4'}$), 7.48 (d, J = 8.6, 1H, H_5), 7.06 (m, 2H, $H_{3',5'}$), 2.42 (s, 3H, CH_3).

***N*-(2,6-Difluorobenzoyl)-5-methyl-2-nitroanilide (16).** 5-Methyl-2-nitroaniline (4.95 g, 32.5 mmol) and **4** (4.50 mL, 41.8 mmol, 130 M %) gave, after recrystallization from ethyl acetate, 8.71 g (29.8 mmol, 92% yield) of yellow crystals: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.85 (s, 1H, NH), 8.75 (s, 1H, H_6), 8.16 (d, J = 8.6, 1H, H_3), 7.50 (m, 1H, $H_{4'}$), 7.48 (d, J = 8.6, 1H, H_4), 7.06 (m, 2H, $H_{3',5'}$), 2.50 (s, 3H, CH_3).

Methyl *N,N*-Bis(2,6-difluorobenzoyl)-6-nitro-2-anilidecarboxylate (17). To methyl 2-amino-6-nitrobenzoate (12.75 g, 65.0 mmol) dissolved in THF/pyridine (1:1) (300 mL) was added **4** (18.0 mL, 142.7 mmol, 220 M %). After a second addition at 7 h of **4** (8.0 mL, 64 mmol, 100 M %) and after being stirred overnight at room temperature, the mixture was concentrated. The residue was suspened in water and filtered. The filtered solid was then suspended in boiling methanol and filtered yielding 28.26 g (59.3 mmol, 91% yield) of a white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.41 (dd, J = 1.5, 8.2 Hz, 1H, H_5), 8.29 (dd, J = 1.5, 8.0 Hz, 1H, H_3), 7.86 (dd, J = 8.0, 8.2 Hz, 1H, H_4), 7.55 (m, 2H, $H_{4',4'}$), 7.13 (m, 4H, $H_{3',5',3',5'}$), 3.86 (s, 3H, CO_2Me).

Methyl *N*-(2,6-Difluorobenzoyl)-6-nitro-2-anilidecarboxylate (18). To **17** (28.20 g, 59.2 mmol) suspended in pyridine (300 mL) was added hydrazine (3.0 mL, 61.7 mmol, 105 M %). After 6 h at room temperature, the mixture was concentrated and the residue was redissolved in CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, evaporated, and recrystallized from methanol, yielding 18.31 g (54.5 mmol, 92% yield) of a white solid: ^{1}H NMR (200 MHz, CD_2C1_2) δ 10.81 (br, 1H, NH), 8.22 (dd, J = 1.7, 8.1 Hz, 1H, H_5), 8.15 (dd, J = 1.7, 8.1 Hz, 1H, H_3), 7.50 (m, 1H, $H_{4'}$), 7.42 (dd, J = 8.1, 8.1 Hz, 1H, H_4), 7.05 (m, 2H, $H_{3',5'}$), 3.94 (s, 3H, CO_2Me).

2,6-Difluorobenzoic Acid (2-Hydroxymethyl)-6-nitroanilide (19). To **18** (4.50 g, 13.4 mmol) dissolved in THF (50 mL) at 0 °C was added lithium aluminum hydride (0.50 g, 13.2 mmol, 100 M %). After 30 min, the mixture was allowed to warm to room temperature followed by a second addition of lithium aluminum hydride (0.20 g, 5.26mmol, 40 M %) at 2 h. After 4 h, the mixture was concentrated and the residue was redissolved in EtOAc, washed with $NaHSO_4$ (10% solution), $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and evaporated. The product was purified by flash chromatograph, eluting with 4% MeOH/CH_2Cl_2 and recrystallizing from CH_2Cl_2, yielding 2.47 g (8.01 mmol, 60% yield) of a white solid: ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.92 (dd, J = 1.4, 8.2 Hz, 1H, H_5), 7.79 (dd, J = 1.4, 7.8 Hz, 1H, H_3), 7.54−7.43 (m, 2H, $H_{4'}$,H_4), 7.06 (m, 2H, $H_{3',5'}$), 4.74 (s, 2H, CH_2O).

2,6-Difluorobenzoic Acid (2-Acetoxymethyl)-6-nitroanilide (20). To **19** (6.00 g, 19.5 mmol) dissolved in THF (85 mL) and triethylamine (4.00 mL) was added acetic anhydide (4.00 mL, 42.4 mmol, 220 M %). After being stirred overnight at room temperature, the mixture was concentrated and the residue was redissolved in

EtOAc, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and evaporated. The product was purified by flash chromatography eluting with 4% MeOH/CH_2Cl_2 and titration from Et_2O, yielding 5.09 g (14.5 mmol, 75% yield) of a white solid: ^{1}H NMR (200 MHz, CD_2C1_2) δ 9.16 (br, 1H, NH), 7.99 (dd, J = 1.5, 8.3 Hz, 1H, H_5), 7.79 (dd, J = 1.5, 7.7 Hz, 1H, H_3), 7.50 (m, 1H, $H_{4'}$), 7.49 (dd, J = 7.7, 8.3 Hz, 1H, H_4), 7.06 (m, 2H, $H_{3',5'}$), 5.20 (s, 2H, CH_2O), 2.09 (s, 3H, OAc).

***N*-(2,6-Difluorobenzoyl)-4-bromo-2-nitroanilide (21).** To *N*-(2,6-difluorobenzoyl)-2-nitroanilide[1] (1.20 g, 8.69 mmol) suspended in 10 mL of pyridine/THF (1:1) was added bromine (0.5 mL) dissolved in acetic acid (0.5 mL). After the mixture was stirred for 1 h at room temperature, the reaction was quenched with $NaHCO_3$ (saturated aqueous). The solution was extracted with CH_2Cl_2. and the organic extract was washed with NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was recrystallized from ethyl acetate (1.55 g, 4.34 mmol, 50% yield of yellow crystals): ^{1}H NMR (200 MHz, CD_2Cl_2) δ 10.72 (br s, 1H, NH), 8.85 (d, J = 9.1 Hz, 1H, H_5), 8.42 (d, J = 2.4 Hz, 1H, H_3), 7.84 (ddd, J = 0.5, 2.4, 9.1 Hz, 1H, H_6), 7.52 (m, 1H, $H_{4'}$), 7.07 (m, 2H, $H_{3',5'}$).

Method A: *N*-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-4-bromo-2-nitroanilide (23). To **21** (0.26 g, 0.73 mmol) and 2,6-difluorobenzyl bromide (**22**) (0.27 g, 1.30 mmol, 180 M %) dissolved in THF (2 mL) was added NaH (0.15 g, 500 M %). After 6 h, the reaction was quenched with methanol and concentrated. The residue was redissolved in CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with ethyl acetate/hexane (1:4) and recrystallization from diethyl ether/hexane (3:1) (0.26 g, 0.54 mmol, 74% yield of white crystals): ^{1}H NMR (200 MHz, DMSO-d_6) (rotamers) δ 8.30 (d, J = 2.3 Hz, 1H, H_3, rotamer 1), 8.25 (d, J = 2.3 Hz, 1H, H_3, rotamer 2), 8.02 (dd, J = 2.3, 8.5 Hz, 1H, H_5, rotamer 1), 7.85 (dd, J = 2.3, 8.5 Hz, 1H, H_5, rotamer 2), 7.68 (m, 1H, $H_{4'}$, rotamer 1), 7.54−7.27 (m, 5H, $H_{6,4'}$ rotamer 1 and $H_{6,4',4'}$ rotamer 2), 7.16−6.94 (m, $H_{3',5',3',5'}$ rotamers 1 and 2), 5.63 (d, J = 14.3 Hz, 1H, CH_2PhF_2, rotamer 2), 4.99 (br, 1H, CH_2PhF_2, rotamer 1), 4.87 (br, 1H, CH_2PhF_2, rotamer 1), 4.86 (d, J = 14.3 Hz, 1H, CH_2-Ph, rotamer 2).

The following compounds were prepared by method A.

***N*-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-4-chloro-2-nitroanilide (24). 6** (600 mg, 1.92 mmol) and **22** (478 mg, 2.3 mmol) gave, after recrystallization from diethyl ether/hexane (3:1), 620 mg (1.41 mmol, 74%) of white crystals: ^{1}H NMR (300 MHz, CD_2Cl_2) (rotamers) δ 8.02 (d, J = 2.4 Hz, 1H, H_3, rotamer 1), 7.95 (d, J = 2.4 Hz, 1H, H_3, rotamer 2), 7.57 (dd, J = 8.6, 2.4 Hz, 1H, H_5, rotamer 1), 7.50 (m, 1H, $H_{4'}$, rotamer 1), 7.30 (dd, J = 8.4, 2.4 Hz, 1H, H_5, rotamer 2), 7.35−7.03 (m, 5H, $H_{4'}$ rotamer 2 and $H_{6,4'}$ rotamers 1 and 2), 6.94−6.68 (m, 8H, $H_{3',5',3',5'}$, rotamers 1 and 2), 5.84 (d, J = 14.4, 1H, CH_2PhF_2, rotamer 2), 4.94 (br s, 2H, CH_2-PhF_2, rotamer 1), 4.82 (d, J = 14.4 Hz, 1H, CH_2PhF_2, rotamer 2).

***N*-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-5-chloro-2-nitroanilide (25). 8** (1.95 g, 6.24 mmol), **22** (1.40 g, 6.76 mmol, 110 M %), and additional **22** (1.30 g, 100 M % and 0.75 g, 60 M %, respectively at 2 and 5 h) gave, after 8 h and purification by flash chromatography eluting with ethyl acetate/hexane (1:4), 2.06 g (4.69 mmol, 75% yield) of white crystals: ^{1}H NMR (200 MHz, DMSO-d_6) (rotamers) δ 8.08 (d, J = 9.4 Hz, 1H, H_6, rotamer 1), 8.03 (d, J = 8.8 Hz, 1H, H_6, rotamer 2), 7.79−6.91 (m, 16H, $H_{3,4,3',4',5',3',\ 4',5'}$ rotamers 1 and 2), 5.55 (d, J = 14.7, 1H, CH_2PhF_2, rotamer 2), 5.12 (br, 1H, CH_2PhF_2, rotamer 1), 4.99 (d, J = 14.7 Hz, 1H, CH_2PhF_2, rotamer 2), 4.87 (br, 1H, CH_2PhF_2, rotamer 1).

***N*-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-4-methyl-2-nitroanilide (26). 15** (2.00 g, 6.84 mmol) and **22** (2.12 g, 10.2 mmol, 150 M %) gave, after 3 h and recrystallization from diethyl ether/hexane (3:1), 2.80 g (6.69 mmol, 98% yield) of white crystals: ^{1}H NMR (300 MHz, DMSO-d_6) (rotamers) δ 7.89 (dd, J = 2.0, 0.9 Hz, 1H, H_3 rotamer 1), 7.87 (dd, J = 2.0, 0.8 Hz, 1H, H_3 rotamer 2), 7.66 (m, 1H, $H_{4'}$ rotamer 1), 7.56 (ddd, J = 8.2, 2.0, 0.9 Hz, 1H, H_5 rotamer 1), 7.43 (m, 1H, $H_{4'}$ rotamer 2), 7.37−7.27 (m, 5H, H_5 rotamer 2, $H_{3',5'}$ rotamer 1, $H_{4'}$ rotamers 1 and 2) 7.21 (d, J = 8.2 Hz, 1H, H_6 rotamer 1), 7.07 (m, 2H, $H_{3',5'}$ rotamer 2), 7.01−6.90 (m, 4H, $H_{3',5'}$ rotamers 1 and 2), 6.83 (br d, J = 7.7 Hz, 1H, H_6 rotamer 2), 5.71 (d, J = 14.4 Hz, 1H,CH_2PhF_2 rotamer 2), 4.97 (d, J = 14.4 Hz, 1H, CH_2PhF_2 rotamer 1), 4.83 (d, J = 14.4 Hz, 1H,CH_2PhF_2 rotamer 1), 4.78 (d, J = 14.4 Hz, 1H, CH_2PhF_2 rotamer 2), 2.41 (s, 3H, CH_3 rotamer 1), 2.27 (s, 3H, CH_3 rotamer 2).

***N*-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-5-methyl-2-nitroanilide (27). 16** (2.00 g, 6.84 mmol) and **22** (2.12 g, 10.2 mmol, 150 M %) gave, after 3 h and recrystallization from diethyl ether/hexane (3:1), 1.92 g (4.59 mmol, 67% yield) of white crystals: ^{1}H NMR (200 MHz, CD_3OD) (rotamers) δ 7.92 (d, J = 8.5 Hz, 1H, H_6, rotamer 1), 7.89 (d, J = 8.5, 1H, H_6, rotamer 2), 7.60 (m, 1H, $H_{4'}$, rotamer 1), 7.44−6.72 (m, 15H, $H_{3,4,3',5',3',4',5'}$ rotamers 1 and $H_{3,4,3',4',5',3',4',5'}$ rotamer 2), 5.86 (d, J = 14.3 Hz, 1H, CH_2PhF_2, rotamer 2), 4.98 (br, 2H, CH_2PhF_2, rotamer 1), 4.88 (d, J = 14.3 Hz, 1H, CH_2PhF_2, rotamer 2), 2.35 (s, 3H, CH_3, rotamer 1), 2.35 (s, 3H, CH_3, rotamer 2).

***N*-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-2-methyl-6-nitroanilide (28).** 2,6-Difluorobenzoyl-2-methyl-6-nitroanilide[1] (450 mg, 1.54 mmol) and **22** (351 mg, 1.69 mmol) gave, after recrystallization from diethyl ether/methanol, 490 mg (1.17 mmol, 76% yield) of colorless crystals: ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.82 (dd, J = 8.0, 1.5 Hz, 1H, H_5), 7.52 (m, 1H, $H_{4'}$), 7.51 (dd, J = 7.9, 1.6 Hz, 1H, H_3), 7.42 (t, J = 7.9 Hz, 1H, H_4), 7.25 (m, 1H, $H_{4'}$), 7.12 (m, 2H, $H_{3',5'}$), 6.74 (m, 2H, $H_{3',5'}$), 4.80 (s, 2H, CH_2), 2.17 (s, 3H, CH_3).

Method B: 2-(2,6-Difluorophenyl)-4-methoxylbenzimidazole (30). To **10** (22.04 g, 71.5 mmol) suspended in acetic acid (250 mL) was added iron powder (**29**) (22.0 g). After 1 h at reflux, the mixture was concentrated and the residue was redissolved in CH_2-Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, evaporated, purified by flash chromatography with 2% methanol/CH_2Cl_2, and recrystallized from methanol to yield 16.08 g (61.8 mmol, 86% yield) of white crystals: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.07 (br d, J = 36 Hz, 1H, NH), 7.40 (cm, 1H), 7.29−7.00 (cm, 4H), 6.75 (dd, J = 8.0, 13.8 Hz, 1H), 4.00 (d, J = 4.2 Hz, 3H, OCH_3).

The following compounds were prepared by method B.

2-(2,6-Difluorophenyl)-4,5-dimethylbenzimidazole (31). 12 (1.40 g, 4.57 mmol) and **29** (1.05 g) gave, after 1 h at reflux and recrystallization from ethyl acetate, 1.07 g (4.14 mmol, 91% yield) of white crystals: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.49−7.37 (m, 2H, $H_{4',7}$), 7.16−7.07 (m, 3H, $H_{3',5',6}$), 2.54 (br s, 3H, CH_3), 2.42 (s, 3H, CH_3).

2-(2,6-Difluorophenyl)-4,6-dimethylbenzimidazole (32). 13 (4.55 g, 14.9 mmol) and **29** (3.45 g) gave, after 0.5 h at reflux and recrystallization from diethyl ether/hexane (3:1), 3.35 g (12.97 mmol, 87% yield) of white crystals: ^{1}H NMR (200 MHz, CD_2-Cl_2) δ 9.69 (br, 1H, NH), 7.43 (m, 1H, $H_{4'}$), 7.26 (br s, H_5), 7.09 (m, 2H, $H_{3',5'}$), 6.95 (br s, 1H, H_7), 2.59 (s, 3H, CH_3), 2.45 (s, 3H, CH_3).

2-(2,6-Difluorophenyl)-4-ethylbenzimidazole (33). 14 (2.00 g, 6.53 mmol) and **29** (2.00 g) gave, after 0.5 h at reflux, flash chromatography with 2% methanol/CH_2Cl_2, and recrystallization from CH_2Cl_2, 1.65 g (6.39 mmol, 98% yield) of white crystals: ^{1}H NMR (200 MHz, CD_2Cl_2) δ 9.78 (br, 1H, NH), 7.46 (br, 1H, H_5), 7.45 (m, 1H, $H_{4'}$), 7.23 (dd, J = 7.4, 7.9 Hz, H_6), 7.17−7.04 (m, 3H, $H_{3',5',7}$), 3.03 (br, 2H, CH_2), 1.39 (t, J = 7.7 Hz, 3H, CH_3).

Methyl 2-(2,6-Difluorophenyl)benzimidazole-4-carboxylate (34). 18 (30.38 g, 90.35 mmol) and **29** (30.80 g) gave, after 0.5 h at reflux and recrystallization from EtOAc, 18.47 g (64.07 mmol, 71% yield) of white crystals: ^{1}H NMR (200 MHz, CD_2Cl_2) δ 10.92 (br, 1H, NH), 8.06 (d, J = 8.1 Hz, 1H, H_5), 7.97 (d, J = 7.7 Hz, 1H, H_7), 7.49 (m, 1H, $H_{4'}$), 7.37 (dd, J = 7.7,8.1 Hz, H_6), 7.13 (m, 2H, $H_{3',5'}$), 4.01 (s, 3H, CO_2CH_3).

2-(2,6-Difluorophenyl)-4-acetoxymethylbenzimidazole (35). 20 (2.82 g, 8.05 mmol) and **29** (2.50 g) gave, after 0.5 h at relux, flash chromatography with 2% methanol/CH_2Cl_2, and titration from 3:1 Et_2O/hexane, 1.83 g (6.05 mmol, 75% yield) of white crystals:

^{1}H NMR (200 MHz, CD_2Cl_2) δ 10.64 and 9.97$_{(rotamers)}$ (br, 1H, NH), 7.83 (m, 1H), 7.45 (m, 1H, $H_{4'}$), 7.29 (m, 2H), 7.13 (m, 2H, $H_{3',5'}$), 5.61 and 5.46$_{(rotamers)}$ (s, 2H, CH_2O), 2.11 (s, 3H, Ac).

2-(2,6-Difluorophenyl)-4-nitrobenzimidazole (36). 11 (11.73 g, 40.0 mmol) was dissolved in 150 mL of acetic acid and heated to reflux. After being heated overnight, the reaction mixture was cooled to room temperature, concentrated, redissolved in CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and evaporated. The residue was purified by flash chromatography eluting with ethyl acetate/hexane (1:1) and recrystallization from ethyl acetate/hexane (1:4), yielding 7.90 g (28.7 mmol, 72%): ^{1}H NMR (300 MHz, CD_2C1_2) δ 11.00 (s, 1H, NH), 8.23 (dd, J = 8.2, 0.8 Hz, 1H, H_5), 8.21 (dd, J = 8.0, 0.8 Hz, 1H, H_7), 7.54 (m, 1H, $H_{4'}$), 7.45 (dd, J = 8.0, 8.2 Hz, 1H, H_6), 7.13 (m, 2H, $H_{3',5'}$).

2-(2,6-Difluorophenyl)-5-nitrobenzimidazole (37). To 2-(2,6-difluorophenyl)benzimidazole[1] (2.00 g, 8.70 mmol) dissolved in H_2SO_4 (5.0 mL) was added HNO_3 (5.0 mL). After 2 h at room temperature the reaction was quenched with ice (50 mL), filtered, and washed with water, yielding a white solid (1.92 g, 80% yield): ^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.60 (d, J = 2.2 Hz, 1H, H_4), 8.25 (dd, J = 2.2, 8.9 Hz, 1H, H_7), 7.78 (d, J = 8.9 Hz, 1H, H_6), 7.65 (m, 1H, $H_{4'}$), 7.24 (m, 2H, $H_{3',5'}$).

The following compounds were prepared by method B.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-5-bromobenzimidazole (38). To **23** (0.26 g, 0.54 mmol) dissolved in glacial acetic acid (5 mL) was added **29** (0.55 g). After 30 min, the mixture was concentrated to dryness, diluted with ethyl acetate, and adjusted to pH 7 with $NaHCO_3$ (saturated aqueous). The organic solution was collected and washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% methanol/CH_2Cl_2 and then recrystallization from 3:1 diethyl ether/hexane (0.14 g, 0.33 mmol, 62% yield of white crystals): mp 155−156 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.11 (dd, J = 0.6, 1.9 Hz, 1H, H_4), 7.56 (cm, 1H, $H_{4'}$), 7.41 (AB, J = 1.9, 8.7 Hz, 1H, H_7), 7.40 (AB, J = 0.6, 8.7 Hz, 1H, H_6), 7.26 (cm, 1H, $H_{4'}$), 7.10 (cm, 2H, $H_{3',5'}$), 6.83 (cm, 2H, $H_{3',5'}$), 5.35 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{11}BrF_4N_2 \cdot {}^3/_4H_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-5-chlorobenzimidazole (39). 24 (620 mg, 1.41 mmol) and **29** (200 mg) after 3 h gave 250 mg (0.64 mmol, 45%) of colorless crystals: mp 136−137 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.77 (d, J = 1.95 Hz, H_4), 7.55 (m, 1H, $H_{4'}$), 7.42 (d, J = 8.7 Hz, 1H, H_7), 7.27 (dd, J = 8.7, 1.95 Hz, 1H, H_6), 7.26 (m, 1H, $H_{4'}$), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.34 (s, 2H, CH_2PHF_2). Anal. ($C_{20}H_{11}ClF_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-6-chlorobenzimidazole (40). 25 (0.57 g, 1.30 mmol) and **29** (0.43 g) gave, after 1 h and purification by flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from 3:1 diethyl ether/hexane, 0.43 g (1.10 mmol, 85% yield) of white crystals: mp 152−153 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.73 (dd, J = 0.9, 8.6 Hz, 1H, H_4), 7.56 (m, 1H, $H_{4'}$), 7.51 (d, J = 1.9 Hz, 1H, H_7), 7.29 (dd, J = 1.9, 8.6 Hz, 1H, H_5), 7.27 (m, 1H, $H_{4'}$), 7.09 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3',5'}$), 5.33 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{11}ClF_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-5-methylbenzimidazole (41). 26 (1.55 g, 3.71 mmol) and **29** (0.79 g) gave, after recrystallization from 3:1 diethyl ether/hexane, 0.85 g (2.30 mmol, 62% yield) of white crystals: mp 147−149 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.60−7.49 (cm, 2H, $H_{4,4'}$), 7.37 (d, J = 8.4 Hz, 1H, H_7), 7.24 (m, 2H, H $_{4'}$), 7.14 (m, 1H, H_6), 7.09 (m, 2H, $H_{3',5'}$), 6.81 (m, 2H, $H_{3',5'}$), 5.34 (s, 2H, CH_2PhF_2), 2.47 (s, 3H, CH_3). Anal. ($C_{21}H_{14}F_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-6-methylbenzimidazole (42). 27 (1.56 g, 3.73 mmol) and **29** (0.79 g) gave, after recrystallization from 3:1 diethyl ether/hexane, 0.89 g (2.41 mmol, 64% yield) of white crystals: mp 172−173 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.64 (cm, 1H, H_4), 7.52 (m, 1H, $H_{4'}$), 7.30−7.19 (m, 2H, $H_{7,\ 4'}$), 7.14−7.03 (m, 3H, $H_{5,\ 3',5'}$), 6.81 (m, 2H, $H_{3',5'}$), 5.33 (s, 2H, CH_2PhF_2), 2.48 (s, 3H, CH_3). Anal. ($C_{21}H_{14}F_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-7-methylbenzimidazole (43). 28 (300 mg, 0.72 mmol) and **29** (50 mg) gave, after recrystallization from ethyl acetate, 149 mg (0.15 mmol, 56% yield) of colorless crystals: mp 177−178 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.62 (d, J = 8.2 Hz, 1H, H_4), 7.45 (m, 1H, $H_{4'}$), 7.18 (m, 1H, $H_{4'}$), 7.17 (dd, J = 7.3, 8.2 Hz, 1H, H_5), 7.08 (d, J = 7.3 Hz, 1H, H_6), 6.95 (m, 2H, H $_{3',5'}$), 6.70 (m, 2H, $H_{3',5'}$), 5.64 (s, 2H, CH_2PHF_2), 2.74 (s, 3H, CH_3). Anal. ($C_{21}H_{14}F_4N_2$) C, H, N.

The following compounds were prepared by method A.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-methoxylbenzimidazole (44). 30 (1.25 g, 4.80 mmol) and **22** (1.30 g, 6.28 mmol, 130 M %) gave, after flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from diethyl ether/hexane (3:1), 1.68 g (4.35 mmol, 91% yield) of white crystals: mp 154−155 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.52 (m, 1H, $H_{4'}$), 7.25 (m, 1H, $H_{4'}$), 7.19 (dd, J = 7.8, 1.0 Hz, 1H, H_5), 7.13−7.00 (m, 3H, $H_{3',5',6}$), 6.81 (m, 2H, $H_{3',5'}$), 6.72 (dd, J = 7.8, 1.0 Hz, H_7), 5.33 (s, 2H, CH_2PhF_2), 4.00 (s, 3H, OCH_3). Anal. ($C_{21}H_{14}F_4N_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4,5-dimethylbenzimidazole (45). 31 (0.25 g, 0.97 mmol) and **22** (0.44 g, 2.12 mmol, 220 M %) gave, after flash chromatography eluting with 4% methanol/CH_2Cl_2 and recrystallization from ethyl acetate/hexane (1:1), 0.38 g (0.81 mmol, 83% yield) of white crystals: mp 176−177 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.63 (cm, 1H, $H_{4'}$), 7.34 (cm, 1H, H_6), 7.30 (cm, 1H, $H_{4'}$), 7.16 (cm, 1H, H_7), 7.13 (cm, 2H, $H_{3',5'}$), 6.85 (cm, 2H, $H_{3',5'}$), 5.40 (s, 2H, CH_2PhF_2), 2.54 (s, 3H, CH_3), 2.40 (s, 3H, CH_3). Anal. ($C_{22}H_{16}F_4N_2$) C, H, N

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4,6-dimethylbenzimidazole (46). 32 (0.50 g, 1.94 mmol) and **22** (0.52 g, 2.51 mmol, 130 M %) gave, after flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from hexane, 0.68 g (1.77 mmol, 91% yield) of white crystals: mp 153−154 °C; ^{1}H NMR (200 MHz, CD_2Cl_2) δ 7.51 (cm, 1H, $H_{4'}$), 7.23 (m, 1H, $H_{4'}$) 7.11 (m, 1H, H_5), 7.05 (cm, 2H, $H_{3',5'}$), 6.93 (m, 1H, H_7), 6.80 (cm, 2H, $H_{3',5'}$), 5.29 (s, 2H, CH_2PhF_2), 2.58 (s, 3H, CH_3), 2.44 (s, 3H, CH_3). Anal. ($C_{22}H_{16}F_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-ethylbenzimidazole (47). 33 (0.70 g, 2.71 mmol) and **22** (0.72 g, 3.48 mmol, 130 M %) gave, after flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from diethyl ether/hexane (3:1), 0.79 g (2.06 mmol, 76% yield) of white crystals: mp 165−166 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.53 (m, 1H, $H_{4'}$), 7.32 (d, J = 8.2 Hz, 1H, H_5), 7.29−7.16 (m, 2H, $H_{6,4'}$), 7.14−7.01 (m, 1H, H_7), 7.07 (m, 2H, $H_{3',5'}$), 6.81 (m, 2H, $H_{3',5'}$), 5.33 (s, 2H, CH_2-PHF_2), 3.07 (q, J = 7.6 Hz, 2H, CH_2), 1.35 (t, J = 7.6 Hz, 3H, CH_3). Anal. ($C_{22}H_{16}F_4N_2 \cdot {}^1/_5H_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-nitrobenzimidazole (48). 36 (7.7 g, 28 mmol) and **22** (6.95 g, 33.6 mmol) gave, after recrystallization from diethyl ether/hexane (3:1), 9.8 g (24.4 mmol, 87%) of white crystals: mp 168−170 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 8.13 (dd, J = 8.1, 0.9 Hz, 1H, H_5), 7.86 (dd, J = 8.1, 0.9 Hz, 1H, H_7), 7.59 (m, 1H, $H_{4'}$), 7.43 (dd, J = 8.1 Hz, H_6), 7.28 (m, 1H, $H_{4'}$), 7.12 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3',5'}$), 5.44 (s, 2H, CH_2). Anal. ($C_{20}H_{11}F_4N_3$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-5-nitrobenzimidazole (49). 37 (0.91 g, 3.31 mmol), **22** (1.08 g, 5.22 mmol, 160 M %), and a second addition of **22** (0.47 g, 2.27 mmol, 70 M %) after 1 h gave, after flash chromatography eluting with ethyl acetate/hexane (1:4), 1.09 g (2.71 mmol, 82% yield) of white crystals: mp 168−169 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.69 (dd, J = 0.5, 2.2 Hz, 1H, H_4), 8.23 (dd, J = 2.2, 9.0 Hz, 1H, H_6), 7.59 (dd, J = 0.5, 9.0 Hz, 1H, H_7), 7.59 (m, 1H, $H_{4'}$), 7.28 (m, 1H, $H_{4'}$), 7.12 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3',5'}$), 5.44 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{11}F_4N_3O_2$) C, H, N.

Methyl 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carboxylate (50). 34 (16.15 g, 56.0 mmol) and **22** (14.67 g, 70.86 mmol, 125 M %) gave, after flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from diethyl ether,

17.63 g (42.55 mmol, 76% yield) of white crystals: mp 184−186 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.94 (dd, J = 1.1, 7.6 Hz, 1H, H_5), 7.72 (dd, J = 1.1, 8.2 Hz, 1H, H_7), 7.56 (m, 1H, $H_{4'}$), 7.37 (dd, J = 7.6, 8.2 Hz, 1H, H_6), 7.26 (m, 1H, $H_{4'}$), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.39 (s, 2H, CH_2PhF_2), 3.95 (s, 3H, CO_2CH_3). Anal. ($C_{22}H_{14}F_4N_2O_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-acetoxymethylbenzimidazole (51). 35 (3.55 g, 11.74 mmol) and **22** (3.40 g, 16.4 mmol, 140 M %) gave, after flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from diethyl ether, 5.94 g (13.9 mmol, 76% yield) of white crystals: mp 126−127 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.54 (m, 1H, $H_{4'}$), 7.48 (m, 1H, H_6), 7.33−7.27 (m, 2H, $H_{5,7}$), 7.25 (m, 1H, $H_{4'}$), 7.08 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.54 (s, 2H, CH_2PhF_2), 5.36 (s, 2H, CH_2O), 2.08 (s, 3H, OAc). Anal. ($C_{23}H_{16}F_4N_2O_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-hydroxylbenzimidazole (52). To **44** (0.30 g, 0.78 mmol) and hexadecyltrimethylammonium bromide (0.30 g) dissolved in acetic acid (9.0 mL) was added HBr (1.0 mL). After 7 h at reflux, the mixture was concentrated and the residue was diluted with EtOAc, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from diethyl ether, yielding 0.25 g (0.67 mmol, 86% yield): mp 257−259 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.57 (m, 1H, $H_{4'}$), 7.25 (m, 1H, $H_{4'}$), 7.17 (dd, J = 7.9, 8.3 Hz, 1H, H_6), 7.12 (m, 2H, $H_{3',5'}$), 6.99 (d, J = 8.3 Hz, 1H, H_7), 6.82 (m, 2H, $H_{3',5'}$), 6.73 (dd, J = 7.9, 1.0 Hz, H_5), 5.35 (s, 2H, CH_2PhF_2). Anal. ($C_{20}H_{12}F_4N_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-aminobenzimidazole (53). To **48** (2.50 g, 6.63 mmol) dissolved in acetic acid (25 mL) was added $SnC1_2 \cdot 2H_2O$ (9.80 g) dissolved in HC1 (concentrated) (10 mL). After being stirred for 30 min at room temperature, the mixture was concentrated. The residue was diluted with CH_2Cl_2, washed with water, $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, concentrated, and recrystallized from methanol (2.23 g, 5.99 mmol, 90% yield): mp 178−179 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.52 (m, 1H, $H_{4'}$), 7.23 (m, 1H, $H_{4'}$), 7.07 (m, 2H, $H_{3',5'}$), 7.06 (dd, J = 8.1, 7.7 Hz, 1H, H_6), 6.82 (d, J = 8.1 Hz, H_7), 6.81 (m, 2H, $H_{3',5'}$), 6.52 (dd, J = 7.7, 0.9 Hz, 1H, H_5), 5.29 (s, 2H, CH_2), 4.42 (s, 2H, NH_2). Anal. ($C_{20}H_{13}F_4N_3$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-bromobenzimidazole (54). To **53** (300 mg, 0.81 mmol) suspended in 48% HBr (3 mL) at 0 °C was slowly added $NaNO_2$ (88 mg, 1.26 mmol, 160 M %) in water (1.0 mL). After being stirred for 30 min at 0−5 °C, the mixture was added to CuBr (140 mg, 0.98 mmol, 120 M %) dissolved in 48% HBr (1 mL). After 30 min at room temperature, water (60 mL) was added and the pH adjusted to 7 with NaOH (solid). The mixture was extracted with ethyl acetate, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, evaporated, purified by flash chromatography eluting with 2% methanol in CH_2Cl_2, and recrystallized with diethyl ether/hexane (3:1) (267 mg, 0.61 mmol, 75% of white powder): mp 147−148 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.56 (m, 1H, $H_{4'}$), 7.49 (dd, J = 7.7, 0.87 Hz, 1H, H_5), 7.47 (d, J = 7.9 Hz, H_7), 7.26 (m, 1H, $H_{4'}$), 7.18 (dd, J = 8.2, 7.7 Hz, 1H, H_6), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.35 (s, 2H, CH_2). Anal. ($C_{20}H_{11}BrF_4N_2 \cdot {}^1/_4H_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-chlorobenzimidazole (55). To **53** (800 mg, 2.15 mmol) dissolved in 37% HC1 (7 mL) and water (5 mL) at 0 °C was slowly added $NaNO_2$ (193 mg, 2.8 mmol) in water (1.5 mL). After 30 min at 0−5 °C, the mixture was added to CuC1 (256 mg, 2.6 mmol) in HCl (concentrated) (2 mL) at 0 °C. After rising to room temperature over 40 min, the pH was adjusted to pH 5, diluted with water (80 mL), extracted with ethyl acetate, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by gravity chromatography eluting with hexane/acetone (2:1) and recrystallized from acetone/hexane (350 mg of yellow crystals, 0.90 mmol, 42%): mp 163−164 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.56 (m, 1H, $H_{4'}$), 7.43 (dd, J = 8.0, 1.1 Hz, 1H, H_7), 7.31 (dd, J = 7.8, 1.1 Hz, 1H, H_5), 7.26 (m, 1H, $H_{4'}$), 7.24 (dd, J = 8.0, 7.8 Hz, 1H, H_6), 7.09 (m, 2H, $H_{3',5'}$), 6.83 (m, 2H, $H_{3',5'}$), 5.36 (s, 2H, CH_2). Anal. ($C_{20}H_{11}ClF_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-acetamidobenzimidazole (56). To **53** (0.30 g, 0.81 mmol) dissolved in THF (3 mL) was added acetic anhydride (100 mL, 1.06 mmol). After 3 h, additional acetic anhydride (20 mL, 0.21 mmol) was added. After 5 h, the mixture was concentrated to dryness, diluted with ethyl acetate, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was recrystallized from diethyl ether/hexane (3:1) (0.32 g, 0.77 mmol, 95% yield of white crystals): mp 194−195 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 8.49 (1H, br, NH), 8.20 (d, J = 7.8 Hz, 1H, H_5), 7.56 (m, 1H, $H_{4'}$), 7.26 (t, J = 7.9 Hz, 1H, H_6), 7.26 (m, 1H, $H_{4'}$), 7.19 (d, J = 7.9 Hz, 1H, H_7), 7.10 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.34 (s, 2H, CH_2PhF_2), 2.21 (s, 3H, Ac). Anal. ($C_{22}H_{15}F_4N_3O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(*N*-methylacetamido)benzimidazole (57). To **56** (2.44 g, 5.90 mmol) and methyl iodide (0.60 mL, 9.69 mmol) dissolved in THF (30 mL) was added excess NaH. After being stirred overnight, the solution was concentrated to dryness, diluted with CH_2Cl_2, washed with water, $NaHSO_4$ (10% solution), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography eluting with 2% methanol in CH_2Cl_2 (0.89 g, 2.08 mmol, and 1.07 g recovered starting material, 63% yield): mp 155−156 °C; ^{1}H NMR (200 MHz, CD_2Cl_2) δ 7.55 (m, 1H, $H_{4'}$), 7.48 (br d, J = 7.4 Hz, 1H, H_5), 7.31 (dd, J = 7.4, 7.7 Hz, 1H, H_6), 7.27 (m, 1H, $H_{4'}$), 7.14 (dd, J = 1.1, 7.7 Hz, 1H, H_7), 7.09 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3',5'}$), 5.38 (s, 2H, CH_2PhF_2), 3.34 (s, 3H, NCH_3), 1.83 (s, 3H, NAc). Anal. ($C_{23}H_{17}F_4N_3O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(*N*-methylamino)benzimidazole (58). To **57** (0.50 g, 1.17 mmol) suspended in water (9.0 mL) was added HCl (1.0 mL). After 3 h at reflux, the solution was concentrated to dryness, diluted with ethyl acetate, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography eluting with 2% methanol in CH_2Cl_2 (0.35 g, 78% yield): mp 194−195 °C; ^{1}H NMR (200 MHz, CD_2Cl_2) δ 7.52 (m, 1H, $H_{4'}$), 7.23 (m, 1H, $H_{4'}$), 7.13 (dd, J = 8.3, 8.0 Hz, 1H, H_6), 7.07 (m, 2H, $H_{3',5'}$), 6.80 (m, 2H, $H_{3',5'}$), 6.76 (d, J = 8.3 Hz, 1H, H_5), 6.36 (d, J = 8.0 Hz, 1H, H_7), 5.29 (s, 2H, CH_2PhF_2), 2.96 (s, 3H, NCH_3). Anal. ($C_{21}H_{15}F_4N_3$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(*N*,*N*-dimethylamino)benzimidazole (59). To a mixture of 3 M H_2SO_4 (0.80 mL) and 37% H_2CO (0.50 mL) was added a slurry of **53** (0.37 g, 1.0 mmol) and sodium borohydride (0.27 g). After the addition was complete, the mixture was concentrated to dryness, diluted with ethyl acetate, washed with Na_2CO_3 (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, concentrated, and recrystallized from diethyl ether/hexane (3:1) (0.34 g, 0.85 mmol, 85% yield of white crystals): mp 155−156 °C; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.51 (m, 1H, $H_{4'}$), 7.23 (m, 1H, $H_{4'}$), 7.13 (dd, J = 8.0 Hz, 1H, H_6), 7.06 (m, 2H, $H_{3',5'}$), 6.90 (d, J = 8.0 Hz, 1H, H_5), 6.80 (m, 2H, $H_{3',5'}$), 6.48 (d, J = 8.0 Hz, 1H, H_7), 5.30 (s, 2H, CH_2PhF_2), 3.18 (s, 6H, $N(CH_3)_2$). Anal. ($C_{22}H_{17}F_4N_3$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carbonitrile (60). To **50** (1.02 g, 2.46 mmol) suspended in xylene (60 mL) was added freshly prepared 1.0 M $AlMe_2NH_2$ (20 mL). After 3 h at reflux, the mixture was concentrated. The residue was redissolved in CH_2Cl_2, washed with $NaHSO_4$ (10% solution), $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from diethyl ether/hexane (3:1) to give 1.72 g (4.51 mmol, 61% yield) of white crystals: mp 156−157 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.76 (dd, J = 1.0, 8.6 Hz, 1H, H_5), 7.63 (dd, J = 1.0, 7.6 Hz, 1H, H_7), 7.58 (m, 1H, $H_{4'}$), 7.37 (dd, J = 7.6, 8.6 Hz, 1H, H_6), 7.28 (m, 1H, $H_{4'}$), 7.11 (m, 2H, $H_{3',5'}$), 6.83 (m, 2H, $H_{3',5'}$), 5.40 (s, 2H, CH_2PhF_2). Anal. ($C_{21}H_{11}F_4N_3$) C, H, N.

***N*-Hydroxy 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carboxamidine (61).** To **60** (0.60 g, 1.57 mmol) dissolved in ethanol (15 mL) and triethylamine (0.5 mL) was added hydroxylamine (0.14 g, 130 M %). After 24 h at reflux, the mixture was concentrated. The residue was redissolved in CH_2Cl_2, washed with $NaHSO_4$ (10% solution), $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 4% methanol/CH_2Cl_2 and recrystallization from methanol to give 0.57 g (1.37 mmol, 87% yield) of white crystals: mp 214−216 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.84 (dd, J = 1.1, 7.7 Hz, 1H, H_5), 7.57 (m, 1H, $H_{4'}$), 7.54 (dd, J = 1.1, 8.2 Hz, 1H, H_7), 7.30 (dd, J = 7.7, 8.2 Hz, 1H, H_6), 7.28 (m, 1H, $H_{4'}$), 7.11 (m, 2H, $H_{3',5'}$), 6.83 (m, 2H, $H_{3',5'}$), 6.61 (br, 2H, NH_2), 5.39 (s, 2H, CH_2PhF_2). Anal. ($C_{21}H_{14}F_4N_4O\cdot{}^1/_4H_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carboxylic Acid Amide (62). To **60** (1.10 g, 2.88 mmol) dissolved in ethanol (100 mL) and 3 M Na_2CO_3 (10 mL) was added 30% hydrogen peroxide (10 mL). After being stirred overnight at room temperature, the mixture was concentrated and redissolved in EtOAc, washed with $NaHSO_4$ (10% solution), $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% methanol/CH_2Cl_2 and recrystallization from methanol, yielding 0.83 g (2.08 mmol, 72% yield) of white crystals: mp 260−261 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 8.10 (dd, J = 1.1, 7.7 Hz, 1H, H_5), 7.71 (br d, J = 8.1 Hz, 1H, H_7), 7.59 (m, 1H, $H_{4'}$), 7.43 (dd, J = 7.7, 8.1 Hz, 1H, H_6), 7.28 (m, 1H, $H_{4'}$), 7.13 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3',5'}$), 5.43 (s, 2H, CH_2PhF_2), 1.92 (s, 2H, NH_2). Anal. ($C_{21}H_{13}F_4N_3O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carboxylic Acid Dimethylamide (63). To **62** (1.10 g, 2.88 mmol) and methyl iodide (0.12 mL) suspended in THF (5 mL) was added sodium hydride (0.15 g). After being stirred overnight at room temperature, the mixture was diluted with EtOAc, washed with $NaHSO_4$ (10% solution), $NaHCO_3$ (saturated aqueous), and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 5% methanol/CH_2Cl_2, yielding 0.20 g (0.47 mmol, 90% yield) of white crystals: mp 213−214 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.59−7.43 (m, 2H, $H_{4',5}$), 7.36−7.20 (m, 3H, $H_{6.7,4'}$), 7.08 (m, 2H, $H_{3',5'}$), 6.83 (m, 2H, $H_{3',5'}$), 5.37 (s, 2H, CH_2PhF_2), 3.12 (s, 3H, CH_3), 2.88 (s, 3H, CH_3). Anal. ($C_{23}H_{17}F_4N_3O\cdot{}^1/_4H_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole 4-Carboxylate (64). To **50** (1.02 g, 2.46 mmol) dissolved in methanol (20 mL) was added barrium hydroxide (1.20 g). After 2 h, acetic acid was added and the mixture concentrated. The residue was redissolved in CH_2Cl_2, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 8% methanol/CH_2Cl_2 and recrystallization from diethyl ether/hexane (3:1), yielding 0.71 g (1.77 mmol, 72% yield) of white crystals: mp 178−180 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 8.06 (d, J = 7.8 Hz, 1H, H_5), 7.76 (d, J = 8.1 Hz, 1H, H_7), 7.62 (m, 1H, $H_{4'}$), 7.47 (dd, J = 7.8, 8.1 Hz, 1H, H_6), 7.29 (m, 1H, $H_{4'}$), 7.14 (m, 2H, $H_{3',5'}$), 6.85 (m, 2H, $H_{3',5'}$), 5.45 (s, 2H, CH_2PhF_2). Anal. ($C_{21}H_{12}F_4N_2O_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-benzimidazoyl-2-propanol (65). To **50** (1.02 g, 2.46 mmol) suspended in THF (5 mL) was added 1.4 M methyllithium (0.95 mL). After 0.5 h, the mixture was diluted with EtOAc, washed with $NaHSO_4$ (10% solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 1:1 ethyl acetate/hexane and recrystallization from hexane, yielding 0.11 g (0.27 mmol, 55% yield) of white crystals: mp 149−150 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.55 (m, 1H, $H_{4'}$), 7.48 (d, J = 8.1 Hz, 1H, H_5), 7.25 (m, 2H, $H_{6,4'}$), 7.16 (dd, J = 1.1, 7.6 Hz, 1H, H_7), 7.10 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.82 (s, 1H, OH), 5.37 (s, 2H, CH_2PhF_2), 1.67 (s, 6H, CH_3). Anal. ($C_{23}H_{18}F_4N_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-isopropenylbenzimidazole (66). 65 (0.45 g, 1.09 mmol) was added to H_2SO_4 (1.00 mL). After 15 min at room temperature, the mixture was diluted with EtOAc, washed with $NaHCO_3$ (saturated aqueous) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 1% MeOH/CH_2Cl_2 and recrystallization from diethyl ether/hexane (3:1), yielding 0.11 g (0.28 mmol, 30% yield) of white crystals: mp 156−158 °C; ^{1}H NMR (200 MHz, CD_2C1_2) δ 7.53 (m, 1H, $H_{4'}$), 7.44−7.16 (cm, 4H, $H_{4',5,6,7}$), 7.07 (m, 2H, $H_{3',5'}$), 6.81 (m, 2H, $H_{3',5'}$), 6.03 (dq, J = 0.8, 2.4 Hz, 1H, vinyl), 5.37 (s, 2H, CH_2PhF_2), 5.36 (dq, J = 1.5, 2.4 Hz, 1H, vinyl), 2.33 (dd, J = 0.8, 1.5 Hz, 3H, CH_3). Anal. ($C_{23}H_{16}F_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-hydroxymethylbenzimidazole (67). To **51** (1.45 g, 3.38 mmol) dissolved in methanol (19 mL) and water (1.0 mL) was added K_2CO_3 (0.40 g). After 30 min, the mixture was concentrated, redissolved in EtOAc, washed with $NaHSO_4$ (10% solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, concentrated, and recrystallized from diethyl ether/hexane (3:1), yielding 1.25 g (3.24 mmol, 96% yield) of white crystals: mp 157−158 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.54 (m, 1H, $H_{4'}$), 7.43 (d, J = 7.9 Hz, 1H, H_7), 7.30−7.22 (m, 2H, $H_{6,4'}$), 7.18 (d, J = 7.5 Hz, 1H, H_5), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.37 (s, 2H, CH_2PhF_2), 5.06 (d, J = 5.4 Hz, 2H, CH_2O), 3.51 (t, J = 5.4 Hz, 1H, OH). Anal. ($C_{21}H_{14}F_4N_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carbaldehyde (68). To CrO_3 (1.26 g) dissolved in pyridine (1.0 mL) and CH_2Cl_2 (9.0 mL) was added **67** (0.49 g, 1.27 mmol) dissolved in pyridine/CH_2Cl_2 (1:1) (2 mL). After being stirred for 6 h, the mixture was filtered. The filtrate was dilitued with CH_2Cl_2, washed with $NaHCO_3$ (saturated solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, concentrated. The product was purified by flash chromatography eluting with 1% methanol/CH_2Cl_2 and recrystallized from diethyl ether/hexane (3:1), yielding 0.38 g (0.99 mmol, 78% yield) of white crystals: mp 151−153 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 10.82 (d, J = 0.6 Hz, 1H, CHO), 7.83 (dd, J = 7.5, 1.1 Hz, 1H, H7), 7.78 (dd, J = 8.1, 1.1 Hz, H5), 7.58 (m, 1H, $H_{4'}$), 7.44 (ddd, J = 8.1, 7.5, 0.6 Hz, 1H, H_6), 7.27 (m, 1H, $H_{4'}$), 7.11 (m, 2H, $H_{3',5'}$), 6.84 (m, 2H, $H_{3',5'}$), 5.42 (s, 2H, CH_2PhF_2). Anal. ($C_{21}H_{12}F_4N_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-chloromethylbenzimidazole (69). To **67** (0.54 g, 1.40 mmol) dissolved in $CHCl_3$ (3.0 mL) was added $SOCl_2$ (0.15 mL, 2.06 mmol, 150 M %). After 3 h at room temperature, the mixture was diluted with $CHCl_3$, washed with $NaHCO_3$ (saturated solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 1% MeOH/CH_2Cl_2 and recrystallized from diethyl ether/hexane (3:1), yielding 0.52 g (1.28 mmol, 92% yield) of white crystals: mp 143−144 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.55 (m, 1H, $H_{4'}$), 7.48 (dd, J = 7.7, 1.1 Hz, 1H, H_7), 7.36 (dd, J = 7.6 Hz, 1.1 Hz, 1H, H_5), 7.30 (dd, J = 7.7, 7.6 Hz, 1H, H_6), 7.25 (m, 1H, $H_{4'}$), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.36 (s, 2H, CH_2PhF_2), 5.07 (s, 2H, CH_2Cl). Anal. ($C_{21}H_{13}ClF_4N_2$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(azidomethylene)benzimidazole (70). To **69** (1.31 g, 3.23 mmol) dissolved in DMF (10 mL) was added sodium azide (0.68 g, 10.5 mmol, 325 M %). After 1 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ (saturated solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, concentrated, and recrystallized from diethyl ether/hexane (3:1), yielding 1.23 g (2.99 mmol, 93% yield) of white crystals: mp 150−152 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.55 (m, 1H, $H_{4'}$), 7.50 (dd, J = 7.7, 1.6 Hz, 1H, H_7), 7.32 (m, 1H, H_6), 7.30 (m, 2H, $H_{5,4'}$), 7.09 (m, 2H, $H_{3',5'}$), 6.82 (m, 2H, $H_{3',5'}$), 5.37 (s, 2H, CH_2PhF_2), 4.81 (s, 2H, CH_2N_3). Anal. ($C_{21}H_{13}F_4N_5$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(aminomethylene)benzimidazole (71). To **70** (0.55 g, 1.34 mmol) dissolved in acetic acid (10 mL) was added zinc (0.55 g, 6.27 mmol, 630 M %). After 1 h at room temperature, the mixture was concentrated,

redissolved with EtOAc, washed with $NaHCO_3$ (saturated solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 4% MeOH/CH_2Cl_2 increasing to 100% MeOH, yielding 0.16 g (0.42 mmol, 31% yield) of white crystals: mp 257−260 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.59 (m, 1H, $H_{4'}$), 7.55 (m, 1H, H_7), 7.35 (m, 2H, $H_{5,6}$), 7.27 (m, 1H, $H_{4'}$), 7.12 (m, 2H, $H_{3',5'}$), 6.83 (m, 2H, $H_{3',5'}$), 5.40 (s, 2H, CH_2PhF_2), 4.49 (s, 2H, CH_2N). Anal. ($C_{21}H_{15}F_4N_3$) C, H, N.

***N*-(1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(*N*-acetylaminomethylene)benzimidazole (72).** To **71** (0.28 g, 0.73 mmol) dissolved in THF (10 mL) and triethylamine (0.2 mL) was added acetic anhydride (0.20 mL, 2.12 mmol, 300 M %). After 1.5 h at room temperature, the mixture was concentrated, redissolved with EtOAc, washed with $NaHCO_3$ (saturated solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 4% MeOH/CH_2Cl_2 increasing to 8% MeOH/CH_2Cl_2, yielding 0.20 g (0.47 mmol, 65% yield) of white crystals: mp 178−179 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.56 (m, 1H, $H_{4'}$), 7.42 (m, 1H, H_7), 7.30−7.18 (m, 3H, $H_{5,6,4'}$), 7.10 (m, 2H, $H_{3',5'}$), 6.90 (br, 1H, NH), 6.82 (m, 2H, $H_{3',5'}$), 5.37 (s, 2H, CH_2PhF_2), 4.79 (d, J = 5.9 Hz, 2H, CH_2N), 1.92 (s, 3H, NAc). Anal. ($C_{23}H_{17}F_4N_3O\cdot{}^1/_2H_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(*N*-acetyl-*N*-methylaminomethylene)benzimidazole (73). To **72** (0.25 g, 0.58 mmol) and methyl iodide (0.065 mL, 1.04 mmol, 180 M %) dissolved in THF (10 mL) was added sodium hydride (50 mg, 1.25 mmol, 215 M %). After being stirred overnight at room temperature, the mixture was diluted with EtOAc, washed with $NaHSO_4$ (10% solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% MeOH/CH_2Cl_2 increasing to 4% MeOH/CH_2Cl_2, yielding 0.24 g (0.54 mmol, 94% yield) of white crystals: mp 171−172 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.55 (m, 1H, $H_{4'}$), 7.44 and 7.40 (rotamers) (d, J = 8.3 Hz, 1H, H_7), 7.32−7.20 (m, 2H), 7.15−7.04 (m, 3H), 6.82 (m, 2H, $H_{3',5'}$), 5.37 and 5.35 (rotamers) (s, 2H, CH_2PhF_2), 5.00 and 4.98 (rotamers) (s, 2H, CH_2N), 3.04 and 2.96 (rotamers) (s, 3H, NCH_3), 2.15 and 2.11 (rotamers) (s, 3H, NAc). Anal.($C_{24}H_{19}F_4N_3O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(methoxymethylene)benzimidazole (74). To **67** (0.20 g, 0.52 mmol) dissolved in THF (2 mL) was added methyl iodide (40 μL, 0.64 mmol, 120 M %) and sodium hydride (20 mg). After 2 h at room temperature, additional methyl iodide (20 μL, 0.32 mmol, 60 M %) was added. After 3 h, the mixture was diluted with EtOAc, washed with $NaHCO_3$ (saturated solution) and NaCl (saturated aqueous), dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% MeOH/CH_2Cl_2, yielding 0.10 g (0.25 mmol, 48% yield) of white crystals: mp 122−124 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.53 (m, 1H, $H_{4'}$), 7.43 (dd, J = 7.1, 2.2 Hz, 1H, H_7), 7.34−7.28 (m, 2H, $H_{5,6}$), 7.24 (m, 1H, H_4), 7.08 (m, 2H, $H_{3',5'}$), 6.81 (m, 2H, $H_{3',5'}$), 5.35 (s, 2H, CH_2PhF_2), 4.90 (s, 2H, CH_2O), 3.45 (s, 3H, OCH_3). Anal. ($C_{22}H_{15}F_4N_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(cyanomethylene)benzimidazole (75). To **69** (0.125 g, 0.309 mmol) partially dissolved in acetonitrile (0.7 mL) were added 18-crown-6 (0.006 g, 0.022 mmol, 7 M %) and potassium cyanide (0.203 g, 0.312 mmol, 101 M %). This was vigorously stirred at room temperature for 24 h, and the mixture was diluted with CH_2Cl_2, washed with H_2O, dried over Na_2SO_4, filtered, and concentrated. The product was purified by chromatography eluting with (1:1) hexane/ethyl acetate, yielding 0.109 g (0.275 mmol, 89% yield) of white crystals: mp 124−127 °C; ^{1}H NMR (300 MHz, CD_2C1_2) δ 7.55 (m, 1H, H_4), 7.50 (dd, J = 7.7, 1.6 Hz, 1H, H_7), 7.32 (m, 1H, H_6), 7.28 (m, 2H, $H_{5,4}$), 7.11 (m, 2H, $H_{3',5'}$), 6.83 (m, 2H, $H_{3',5'}$), 5.38 (s, 2H, CH_2PhF_2), 4.2 (s, 2H, CH_2CN). Anal. ($C_{22}H_{15}F_4N_3$) C, H, N.

Structure Determination. Compound **1** was crystallized as described previously.[21] The diffraction data sets (Table 2) for the complex were collected using synchrotron radiation sources at the Cornell High Energy Synchrotron Source (CHESS) F1 beam line. One data set was collected from a cryocooled crystal, and the other was from 10 crystals at 268 K. The data were processed and scaled using Denzo and Scalepack.[22] The previously reported HIV-1 RT/TIBO structure (PDB code 1HNV) was used as a template in obtaining molecular replacement solutions. Multiple crystal form averaging[21] was carried out to improve the electron density to which the initial model was fitted using the graphics program O.[23] Cycles of model building and structure refinement were carried out using O and XPLOR 3.1, respectively. The data set collected from crystals at 268 K was used to refine the final model. The final cycles of structure refinement was carried out using CNS 1.1[24] The structure was refined at 2.65 Å resolution to an R and R_{free} of 0.226 and 0.277, respectively (Table 2). The coordinates and structure factor are deposited in Protein Data Bank (PDB code 2B6A).

Molecular Modeling. Computer modeling of RT complexed with 4-methyl BPBI was based on crystallographic data. A representative model of the binding site was constructed as described previously.[19] Calculations using this model were carried out using the MCPRO (Monte Carlo calculations of proteins) software program.[20] Visualization of final structures was carried out using the Insight software program.

Biological Assays. The reverse transcriptase inhibition experiments were carried out as described previously.[10]

Acknowledgment. This research was supported [in part] by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. E.A. is grateful to NIH MERIT Award AI 27690 and Grant P01 GM 56671 for support of RT structural studies. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsements by the U.S. Government. We thank the personnel at the Cornell High Energy Synchrotron Source for their help in providing X-ray diffraction data collection resources. We also thank the Biophysics Resource in the Structural Biophysics Laboratory, NCI—Frederick, for assistance with LC−MS studies.

Supporting Information Available: Elemental analysis data of all new compounds described here. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) De Clercq, E. Highlights in the development of new antiviral agents. *Mini-Rev. Med. Chem.* **2002**, *2*, 163−175.

(2) Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih Ch.-K.; Eckner, K.; Hattox, S.; Adams, J.; Rosenthal, A. S.; Franes, R.; Eckner, R.; Koup, R. A.; Sullivan, J. L. Inhibition of HIV-1 replication by a nonnucleoside reverse transcriptase inhibitor. *Science* **1990**, *250*, 1411−1413.

(3) Pauwels, R.; Andries, K.; Debyser, Z.; Van Daele, P.; Schols, D.; Stoffels, P.; De Vreese, K.; Woestenborghs, R.; Vandamme, A.; Janssen, C. G. M.; Anne, J.; Cauwenbergh, G.; Desmyter, J.; Hekants, J.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and highly selective human immunodeficiency virus type 1 (HIV-1) inhibition by a series of α-anilinophenylacetamide derivatives targeted at HIV-1 reverse transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1711−1715.

(4) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenbogs, 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.

(5) Young, S. D.; Britcher, S. F.; Tran, L. O.; Payne, L. S.; Lumma, W. C.; Lyle, T. A.; Huff, J. R.; Anderson, P. S.; Olsen, D. B.; Carroll, S. S.; Pettibone, D. J.; O'Brien, J. A.; Ball, R. G.; Balani, S. K.; Lin, J. H.; Chen, I.-W.; Schleif, W. A.; Sardane, V. V.; Long, W. J.; Byrnes, V. W.; Emini, E. A. L-743,726 (DMP-266): a novel, highly potent nonnucleoside inhibitor of the human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agnents Chemother*. **1995**, *39*, 2602−2605.

(6) Romero, D. L.; Morge, R. A.; Biles, C.; Berrios-Pena, N.; May, P. D.; Palmer, J. R.; Johnson, P. D.; Smith, H. W.; Busso, M.; Tan, C.-K.; Voorman, R. L.; Reusser, F.; Althaus, I. W.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G.; Aristoff, P. A. Discovery, synthesis, and bioactivity of bis(heteroaryl)piperazines. 1. A novel class of non-nucleoside HIV-1 reverse transcriptase inhibitors. *J. Med. Chem.* **1994**, *37*, 999−1014.

(7) Janssen, P. A. J.; Lewi, P. J.; Arnold, E.; Daeyaert, F.; de Jonge, M.; Heeres, J.; Koymans, L.; Vinkers, M.; Guillemont, J.; Pasquier, E.; Kukla, M.; Ludovici, D.; Andries, K.; de Bethune, M.-P.; Pauwels, R.; Das, K.; Clark, A. D., Jr.; Frenkel, Y. V.; Hughes, S. H.; Medaer, B.; De Knaep, F.; Bohets, H.; De Clerck, F.; Lampo, A.; Williams, P.; Stoffels, P. In search of a novel anti-HIV drug: multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1*E*)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile (R278474, rilpivirine). *J. Med. Chem.* **2005**, *48*, 1901−1909.

(8) Campiani, G.; Ramunno, A.; Maga, G.; Nacci, V.; Fattorusso, C.; Catalanotti, B.; Morelli, E.; Novellino, E. Non-nucleoside HIV-1 reverse transcriptase (RT) inhibitors: past, present, and future perspectives. *Curr. Pharm. Des.* **2002**, *8*, 615−657.

(9) Das, K.; Clark, A. D., Jr.; Lewi, P. J.; Heeres, J.; de Jonge, M. R.; Koymans, L. M. H.; Vinkers, H. M.; Daeyaert, F.; Ludovici, D. W.; Kukla, M. J.; De Corte, B.; Kavash, R. W.; Ho, C. Y.; Ye, H.; Lichtenstein, M. A.; Andries, K.; Pauwels, R.; de Bethune, M.-P.; Boyer, P. L.; Clark, P.; Hughes, S. H.; Janssen, P. A. J.; Arnold E. Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. *J. Med. Chem.* **2004**, *47*, 2550−2560.

(10) Roth, T.; Morningstar, M. L.; Boyer, P. L.; Hughes, S. H.; Buckheit, R. W.; Michejda, C. J. Synthesis and biological activity of novel nonnucleoside inhibitors of HIV-1 reverse transcriptase. 2-Aryl-substituted benzimidazoles. *J. Med. Chem.* **1997**, *40*, 4199−4207.

(11) Chimirri, A.; Grasso, S.; Monforte, A.-M.; Monforte, P.; Zappala, M. Anti-HIV agents. I. Synthesis and in vitro anti-HIV evaluation of novel 1H,3H-thiazolo[3,4-*a*]benzimidazoles. *Farmaco* **1991**, *46*, 817−823.

(12) Buckheit, R. W.; Hollingshead, M. G.; Germany-Decker, J.; White, E. L.; McMahon, J. B.; Allen, L. B.; Ross, L. J.; Decker, W. D.; Westbrook, L.; Shannon, W. M.; Weislow, O.; Bader, J. P.; Boyd, M. R. Thiazolobenzimidazole: biological and biochemical anti-retroviral activity of a new nonnucleoside reverse transcriptase inhibitor. *Antiviral Res.* **1993**, *21*, 247−265.

(13) El Dareer, S. M.; Tillery, K. F.; Rose, L. M.; Posey, C. F.; Struck, R. F.; Stiller, S. W.; Hill, D. L. Metabolism and disposition of a thiazolobenzimidazole active against human immunodeficiency virus-1. *Drug Metab. Dispos.* **1993**, *21*, 231−235.

(14) Das, K.; Lewi, P. J.; Hughes, S. H.; Arnold, E. Crystallography and the design of anti-AIDS drugs: conformational flexibility and positional adaptability are important in the design of non-nucleoside HIV-1 reverse transcriptase inhibitors. *Prog. Biophys. Mol. Biol.* **2005**, *88*, 209−231.

(15) Ding, J.; Das, K.; Moereels, H.; Koymans, L.; Andries, K.; Janssen, P. A.; Hughes, S. H.; Anold, E. Structure of HIV-1 RT/TIBO R86183 complex reveals similarity in the binding of diverse nonnucleoside inhibitors. *Nat. Struct. Biol.* **1995**, *2*, 407−415.

(16) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New soluble-formazan assay for HIV-1 cytopathic effects: Application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. *J. Natl. Cancer Inst.* **1989**, *81*, 557−586.

(17) Yang, S.; Fliakas-Boltz, V.; Bader, J. P.; Buckheit, R. W. Characteristics of a group of nonnucleoside reverse transcriptase inhibitors with structural diversity and potent anti-human immunodeficiency virus activity. *Leukemia* **1995**, Suppl. 1, S75−S85.

(18) Wood, J. L.; Khartri, N. A.; Weinreb, S. M. A direct conversion of esters to nitriles. *Tetrahedron Lett.* **1979**, *51*, 4907−4910.

(19) Kroeger Smith, M. B.; Hose, B. M.; Hawkins, A.; Lipchock, J.; Farnsworth, D. W.; Rizzo, R. C.; Tirado-Rives, J.; Arnold, E.; Zhang, W.; Hughes, S. H.; Jorgensen, W. L.; Michejda, C. J.; Smith, R. H., Jr. Molecular modeling calculations of HIV-1 reverse transcriptase nonnucleoside inhibitors: Correlation of binding energy with biological activity for novel 2-aryl substituted benzimidazole analogues. *J. Med. Chem.* **2003**, *46*, 1940−1947.

(20) Jorgensen, W. L. *MCPRO*, version 2.0; Yale University: New Haven, CT.

(21) Das, K.; Ding, J.; Hsiou, Y.; Clark, A. D., Jr.; Moereels, H.; Koymans, L.; Andrias, K.; Pauwels, R.; Janssen, P. A.; Boyer, P. L.; Clark, P.; Smith, R. H., Jr.; Kroeger Smith, M. B.; 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) Otwinowski, Z.; Minor, W. DENZO and SCALEPACK. In *The International Union of Crystallography: Crystallography of Biological Macromolecules*; Rossmann, M. G., Arnold, E., Eds.; Kluwer Academic: Boston, MA, 2001; Vol. F, pp 226−235.

(23) Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr.* **1991**, *A47*, 110−119.

(24) Brunger, A. T.; Adams, P. D.; Rice, L. M. Recent developments for the efficient crystallographic refinement of macromolecular structures. *Curr. Opin. Struct. Biol.* **1998**, *8*, 606−611.

JM060103D