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

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Abstract

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**) ($IC_{50} = 0.2 \mu\text{M}$, $EC_{50} = 0.44 \mu\text{M}$, 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

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

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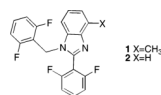
Supporting Information Available: Elemental analysis data of all new compounds described here. This material is available free of charge via the Internet at <http://pubs.acs.org>.

^aAbbreviations. 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.

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 compromised 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 (efavirenz),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]benzotrile (R278474, rilpivirine) of the DAPY⁷ 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)benzimidazoles¹⁰ (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**) (IC₅₀ = 0.2 μ M against HIV-1 RT and EC₅₀ = 0.44 μ M in a cytoprotection assay^{16,17} using wild-type virus). We showed that this novel NNRTI was active in cytoprotection 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 substituents, 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, benzylation led to a mixture of monoacylated and bisacylated 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 amine¹⁸ 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, NaBH₄, 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 regioselectively to yield the 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-5-nitrobenzimidazole (49).

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 [CH₃ (1 vs 41-43), Br (54 vs 38), Cl (55 vs 39 and 40), NO₂ (48 vs 49)]. A combination of a C4 substituent (CH₃) 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 CH₃ (43), however, resulted in complete loss of activity. Molecular modeling of the 7-CH₃ (43) vs the 4-CH₃ (1) suggested an alternative and possibly nonproductive conformation for the 7-CH₃ (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 (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 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% (EC₅₀). 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 IC₅₀. Our compounds exhibit relatively low toxicity (see TC₅₀ values as determined in CEM cells) in comparison to their activities (EC₅₀ and IC₅₀ values). All data are reported in Table 3. While a variety of C4 substituents [(NH₂ (**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 (IC₅₀ = 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 [CO₂Me (**50**), CNHNHOH (**61**), CONH₂ (**62**), CON-(CH₃)₂ (**63**), and CO₂H (**64**)] were clearly detrimental to potency. Synthesis of compounds **65-73** and determination of each analogue's EC₅₀ 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 (**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 substituents 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 EC₅₀ 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 crystal-lographic 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 ($\Delta G_{\text{binding}}$).¹⁹ The data in Table 5 show that in general the modeled free energies correspond to the experimental values (less than ± 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 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. ¹H NMR spectra were recorded on a Varian 200 or 300 MHz spectrometer with Me₄Si 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 substituents and a single prime to the protons on the

aromatic substituents 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): 1H NMR (300 MHz, CD_2Cl_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): 1H NMR (300 MHz, CD_2Cl_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): 1H 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): 1H 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): $^1\text{H NMR}$ (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, $\text{H}_{4'}$), 7.26 (dd, $J = 2.3$, 9.0 Hz, 1H, H_4), 7.08 (m, 1H, $\text{H}_{3',5'}$).

***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): $^1\text{H NMR}$ (300 MHz, CD_2Cl_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, $\text{H}_{4',4'}$), 7.15 (dd, $J = 1.4$, 8.4 Hz, 1H, H_3), 6.87 (m, 4H, $\text{H}_{3',5',3',5'}$), 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: $^1\text{H NMR}$ (200 MHz, CD_2Cl_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, $\text{H}_{3',5'}$), 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: $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) 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, $\text{H}_{3',5'}$), 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: $^1\text{H NMR}$ (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, $\text{H}_{4'}$), 7.28 (d, $J = 8.4$ Hz, 1H, H_5), 7.05 (m, 2H, $\text{H}_{3',5'}$), 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: $^1\text{H NMR}$ (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, $\text{H}_{4'}$), 7.42 (d, $J = 2.6$ Hz, 1H, H_3), 7.04 (m, 2H, $\text{H}_{3',5'}$), 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: $^1\text{H NMR}$ (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, $\text{H}_{4'}$), 7.45 (dd, $J = 7.9$,

8.1 Hz, 1H, H₄), 7.06 (m, 2H, H_{3',5'}), 2.81 (q, *J* = 7.6 Hz, 2H, CH₂), 1.29 (t, *J* = 7.6 Hz, 3H, CH₃).

***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: ¹H NMR (300 MHz, CD₂Cl₂) δ 10.64 (s, 1H, NH), 8.75 (d, *J* = 8.6, 1H, H₆), 8.06 (s, 1H, H₃), 7.52 (m, 1H, H_{4'}), 7.48 (d, *J* = 8.6, 1H, H₅), 7.06 (m, 2H, H_{3',5'}), 2.42 (s, 3H, CH₃).

***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: ¹H NMR (300 MHz, CD₂Cl₂) δ 10.85 (s, 1H, NH), 8.75 (s, 1H, H₆), 8.16 (d, *J* = 8.6, 1H, H₃), 7.50 (m, 1H, H_{4'}), 7.48 (d, *J* = 8.6, 1H, H₄), 7.06 (m, 2H, H_{3',5'}), 2.50 (s, 3H, CH₃).

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 suspended 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: ¹H NMR (200 MHz, DMSO-*d*₆) δ 8.41 (dd, *J* = 1.5, 8.2 Hz, 1H, H₅), 8.29 (dd, *J* = 1.5, 8.0 Hz, 1H, H₃), 7.86 (dd, *J* = 8.0, 8.2 Hz, 1H, H₄), 7.55 (m, 2H, H_{4',4'}), 7.13 (m, 4H, H_{3',5',3',5'}), 3.86 (s, 3H, CO₂Me).

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 CH₂Cl₂, washed with NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, evaporated, and recrystallized from methanol, yielding 18.31 g (54.5 mmol, 92% yield) of a white solid: ¹H NMR (200 MHz, CD₂Cl₂) δ 10.81 (br, 1H, NH), 8.22 (dd, *J* = 1.7, 8.1 Hz, 1H, H₅), 8.15 (dd, *J* = 1.7, 8.1 Hz, 1H, H₃), 7.50 (m, 1H, H_{4'}), 7.42 (dd, *J* = 8.1, 8.1 Hz, 1H, H₄), 7.05 (m, 2H, H_{3',5'}), 3.94 (s, 3H, CO₂Me).

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, 5.26 mmol, 40 M %) at 2 h. After 4 h, the mixture was concentrated and the residue was redissolved in EtOAc, washed with NaHSO₄ (10% solution), NaHCO₃ (saturated aqueous), and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and evaporated. The product was purified by flash chromatograph, eluting with 4% MeOH/CH₂Cl₂ and recrystallizing from CH₂Cl₂, yielding 2.47 g (8.01 mmol, 60% yield) of a white solid: ¹H NMR (200 MHz, CD₂Cl₂) δ 7.92 (dd, *J* = 1.4, 8.2 Hz, 1H, H₅), 7.79 (dd, *J* = 1.4, 7.8 Hz, 1H, H₃), 7.54–7.43 (m, 2H, H_{4',4'}), 7.06 (m, 2H, H_{3',5'}), 4.74 (s, 2H, CH₂O).

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 anhydride (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 NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and evaporated. The product was purified by flash chromatography eluting with 4% MeOH/CH₂Cl₂ and titration from Et₂O, yielding 5.09 g (14.5 mmol, 75% yield) of a white solid: ¹H NMR (200 MHz, CD₂Cl₂) δ 9.16 (br, 1H, NH), 7.99 (dd, *J* = 1.5, 8.3 Hz, 1H, H₅), 7.79 (dd, *J* = 1.5, 7.7 Hz, 1H, H₃), 7.50 (m, 1H, H_{4'}), 7.49 (dd, *J* = 7.7, 8.3 Hz, 1H, H₄), 7.06 (m, 2H, H_{3',5'}), 5.20 (s, 2H, CH₂O), 2.09 (s, 3H, OAc).

N-(2,6-Difluorobenzoyl)-4-bromo-2-nitroanilide (**21**)

To *N*-(2,6-difluorobenzoyl)-2-nitroanilide¹ (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 NaHCO₃ (saturated aqueous). The solution was extracted with CH₂Cl₂, and the organic extract was washed with NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and concentrated. The product was recrystallized from ethyl acetate (1.55 g, 4.34 mmol, 50% yield of yellow crystals): ¹H NMR (200 MHz, CD₂Cl₂) δ 10.72 (br s, 1H, NH), 8.85 (d, *J* = 9.1 Hz, 1H, H₅), 8.42 (d, *J* = 2.4 Hz, 1H, H₃), 7.84 (ddd, *J* = 0.5, 2.4, 9.1 Hz, 1H, H₆), 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 CH₂Cl₂, washed with NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), 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): ¹H NMR (200 MHz, DMSO-*d*₆) (rotamers) δ 8.30 (d, *J* = 2.3 Hz, 1H, H₃, rotamer 1), 8.25 (d, *J* = 2.3 Hz, 1H, H₃, rotamer 2), 8.02 (dd, *J* = 2.3, 8.5 Hz, 1H, H₅, rotamer 1), 7.85 (dd, *J* = 2.3, 8.5 Hz, 1H, H₅, 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, CH₂PhF₂, rotamer 2), 4.99 (br, 1H, CH₂PhF₂, rotamer 1), 4.87 (br, 1H, CH₂PhF₂, rotamer 1), 4.86 (d, *J* = 14.3 Hz, 1H, CH₂-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: ¹H NMR (300 MHz, CD₂Cl₂) (rotamers) δ 8.02 (d, *J* = 2.4 Hz, 1H, H₃, rotamer 1), 7.95 (d, *J* = 2.4 Hz, 1H, H₃, rotamer 2), 7.57 (dd, *J* = 8.6, 2.4 Hz, 1H, H₅, rotamer 1), 7.50 (m, 1H, H_{4'}, rotamer 1), 7.30 (dd, *J* = 8.4, 2.4 Hz, 1H, H₅, 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, CH₂PhF₂, rotamer 2), 4.94 (br s, 2H, CH₂-PhF₂, rotamer 1), 4.82 (d, *J* = 14.4 Hz, 1H, CH₂PhF₂, 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: ¹H NMR (200 MHz, DMSO-*d*₆) (rotamers) δ 8.08 (d, *J* = 9.4 Hz, 1H, H₆, rotamer 1), 8.03 (d, *J* = 8.8 Hz, 1H, H₆, 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 Hz, 1H, CH₂PhF₂, rotamer 2), 5.12 (br, 1H, CH₂PhF₂, rotamer 1), 4.99 (d, *J* = 14.7 Hz, 1H, CH₂PhF₂, rotamer 2), 4.87 (br, 1H, CH₂PhF₂, 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: ¹H NMR (300 MHz, DMSO-*d*₆) (rotamers) δ 7.89 (dd, *J* = 2.0, 0.9 Hz, 1H, H₃ rotamer 1), 7.87 (dd, *J* = 2.0, 0.8 Hz, 1H, H₃ rotamer 2), 7.66 (m, 1H, H_{4'} rotamer 1), 7.56 (ddd, *J* = 8.2, 2.0, 0.9 Hz, 1H, H₅ rotamer 1), 7.43 (m, 1H, H_{4'} rotamer 2), 7.37–7.27 (m, 5H, H₅ rotamer 2, H_{3',5'} rotamer 1, H_{4'} rotamers 1 and 2) 7.21 (d, *J* = 8.2 Hz, 1H, H₆ 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, H₆ rotamer 2), 5.71 (d, *J* = 14.4 Hz, 1H, CH₂PhF₂ rotamer 2), 4.97 (d, *J* = 14.4 Hz, 1H, CH₂PhF₂ rotamer 1), 4.83 (d, *J* = 14.4 Hz, 1H, CH₂PhF₂ rotamer 1), 4.78 (d, *J* = 14.4 Hz, 1H, CH₂PhF₂ rotamer 2), 2.41 (s, 3H, CH₃ rotamer 1), 2.27 (s, 3H, CH₃ 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: ¹H NMR (200 MHz, CD₃OD) (rotamers) δ 7.92 (d, *J* = 8.5 Hz, 1H, H₆, rotamer 1), 7.89 (d, *J* = 8.5 Hz, 1H, H₆, 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, CH₂PhF₂, rotamer 2), 4.98 (br, 2H, CH₂PhF₂, rotamer 1), 4.88 (d, *J* = 14.3 Hz, 1H, CH₂PhF₂, rotamer 2), 2.35 (s, 3H, CH₃, rotamer 1), 2.35 (s, 3H, CH₃, rotamer 2).

***N*-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorobenzyl)-2-methyl-6-nitroanilide (28)**

2,6-Difluorobenzoyl-2-methyl-6-nitroanilide¹ (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: ¹H NMR (300 MHz, CD₂Cl₂) δ 7.82 (dd, *J* = 8.0, 1.5 Hz, 1H, H₅), 7.52 (m, 1H, H_{4'}), 7.51 (dd, *J* = 7.9, 1.6 Hz, 1H, H₃), 7.42 (t, *J* = 7.9 Hz, 1H, H₄), 7.25 (m, 1H, H_{4'}), 7.12 (m, 2H, H_{3',5'}), 6.74 (m, 2H, H_{3',5'}), 4.80 (s, 2H, CH₂), 2.17 (s, 3H, CH₃).

Method B: 2-(2,6-Difluorophenyl)-4-methoxybenzimidazole (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 CH₂-Cl₂, washed with NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, evaporated, purified by flash chromatography with 2% methanol/CH₂Cl₂, and recrystallized from methanol to yield 16.08 g (61.8 mmol, 86% yield) of white crystals: ¹H NMR (300 MHz, CD₂Cl₂) 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, OCH₃).

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: $^1\text{H NMR}$ (300 MHz, CD_2Cl_2) δ 7.49–7.37 (m, 2H, $\text{H}_{4',7}$), 7.16–7.07 (m, 3H, $\text{H}_{3',5',6}$), 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: $^1\text{H NMR}$ (200 MHz, $\text{CD}_2\text{-Cl}_2$) δ 9.69 (br, 1H, NH), 7.43 (m, 1H, $\text{H}_{4'}$), 7.26 (br s, H_5), 7.09 (m, 2H, $\text{H}_{3',5'}$), 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: $^1\text{H NMR}$ (200 MHz, CD_2Cl_2) δ 9.78 (br, 1H, NH), 7.46 (br, 1H, H_5), 7.45 (m, 1H, $\text{H}_{4'}$), 7.23 (dd, $J = 7.4, 7.9$ Hz, H_6), 7.17–7.04 (m, 3H, $\text{H}_{3',5',7}$), 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: $^1\text{H NMR}$ (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, $\text{H}_{4'}$), 7.37 (dd, $J = 7.7, 8.1$ Hz, H_6), 7.13 (m, 2H, $\text{H}_{3',5'}$), 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 reflux, 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: $^1\text{H NMR}$ (200 MHz, CD_2Cl_2) δ 10.64 and 9.97_(rotamers) (br, 1H, NH), 7.83 (m, 1H), 7.45 (m, 1H, $\text{H}_{4'}$), 7.29 (m, 2H), 7.13 (m, 2H, $\text{H}_{3',5'}$), 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%): $^1\text{H NMR}$ (300 MHz, CD_2Cl_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, $\text{H}_{4'}$), 7.45 (dd, $J = 8.0, 8.2$ Hz, 1H, H_6), 7.13 (m, 2H, $\text{H}_{3',5'}$).

2-(2,6-Difluorophenyl)-5-nitrobenzimidazole (37)

To 2-(2,6-difluorophenyl)benzimidazole¹ (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): $^1\text{H NMR}$ (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, $\text{H}_{4'}$), 7.24 (m, 2H, $\text{H}_{3',5'}$).

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 NaHCO₃ (saturated aqueous). The organic solution was collected and washed with NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% methanol/CH₂Cl₂ and then recrystallization from 3:1 diethyl ether/hexane (0.14 g, 0.33 mmol, 62% yield of white crystals): mp 155–156 °C; ¹H NMR (300 MHz, CD₂Cl₂) δ 8.11 (dd, *J* = 0.6, 1.9 Hz, 1H, H₄), 7.56 (cm, 1H, H_{4'}), 7.41 (AB, *J* = 1.9, 8.7 Hz, 1H, H₇), 7.40 (AB, *J* = 0.6, 8.7 Hz, 1H, H₆), 7.26 (cm, 1H, H_{4'}), 7.10 (cm, 2H, H_{3',5'}), 6.83 (cm, 2H, H_{3',5'}), 5.35 (s, 2H, CH₂PhF₂). Anal. (C₂₀H₁₁ BrF₄N₂•³/4H₂O) 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; ¹H NMR (300 MHz, CD₂Cl₂) δ 7.77 (d, *J* = 1.95 Hz, H₄), 7.55 (m, 1H, H_{4'}), 7.42 (d, *J* = 8.7 Hz, 1H, H₇), 7.27 (dd, *J* = 8.7, 1.95 Hz, 1H, H₆), 7.26 (m, 1H, H_{4'}), 7.09 (m, 2H, H_{3',5'}), 6.82 (m, 2H, H_{3',5'}), 5.34 (s, 2H, CH₂PhF₂). Anal. (C₂₀H₁₁ClF₄N₂) 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/CH₂Cl₂ and recrystallization from 3:1 diethyl ether/hexane, 0.43 g (1.10 mmol, 85% yield) of white crystals: mp 152–153 °C; ¹H NMR (300 MHz, CD₂Cl₂) δ 7.73 (dd, *J* = 0.9, 8.6 Hz, 1H, H₄), 7.56 (m, 1H, H_{4'}), 7.51 (d, *J* = 1.9 Hz, 1H, H₇), 7.29 (dd, *J* = 1.9, 8.6 Hz, 1H, H₅), 7.27 (m, 1H, H_{4'}), 7.09 (m, 2H, H_{3',5'}), 6.84 (m, 2H, H_{3',5'}), 5.33 (s, 2H, CH₂PhF₂). Anal. (C₂₀H₁₁ClF₄N₂) 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; ¹H NMR (300 MHz, CD₂Cl₂) δ 7.60–7.49 (cm, 2H, H_{4,4'}), 7.37 (d, *J* = 8.4 Hz, 1H, H₇), 7.24 (m, 2H, H_{4'}), 7.14 (m, 1H, H₆), 7.09 (m, 2H, H_{3',5'}), 6.81 (m, 2H, H_{3',5'}), 5.34 (s, 2H, CH₂PhF₂), 2.47 (s, 3H, CH₃). Anal. (C₂₁H₁₄F₄N₂) 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; ¹H NMR (300 MHz, CD₂Cl₂) δ 7.64 (cm, 1H, H₄), 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, CH₂PhF₂), 2.48 (s, 3H, CH₃). Anal. (C₂₁H₁₄F₄N₂) 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; ¹H NMR (300 MHz, CD₂Cl₂) δ 7.62 (d, *J* = 8.2 Hz, 1H, H₄), 7.45 (m, 1H, H_{4'}), 7.18 (m, 1H, H_{4'}), 7.17 (dd, *J* = 7.3, 8.2 Hz, 1H, H₅), 7.08 (d, *J* = 7.3 Hz, 1H, H₆), 6.95 (m, 2H, H_{3',5'}), 6.70 (m, 2H, H_{3',5'}), 5.64 (s, 2H, CH₂PhF₂), 2.74 (s, 3H, CH₃). Anal. (C₂₁H₁₄F₄N₂) C, H, N.

The following compounds were prepared by method A.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-methoxybenzimidazole (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/CH₂Cl₂ and recrystallization from diethyl ether/hexane (3:1), 1.68 g (4.35 mmol, 91% yield) of white crystals: mp 154–155 °C; ¹H NMR (200 MHz, CD₂C1₂) δ 7.52 (m, 1H, H_{4'}), 7.25 (m, 1H, H_{4'}), 7.19 (dd, *J* = 7.8, 1.0 Hz, 1H, H₅), 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, H₇), 5.33 (s, 2H, CH₂PhF₂), 4.00 (s, 3H, OCH₃). Anal. (C₂₁H₁₄F₄N₂O) 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/CH₂Cl₂ and recrystallization from ethyl acetate/hexane (1:1), 0.38 g (0.81 mmol, 83% yield) of white crystals: mp 176–177 °C; ¹H NMR (300 MHz, CD₃OD) δ 7.63 (cm, 1H, H_{4'}), 7.34 (cm, 1H, H₆), 7.30 (cm, 1H, H_{4'}), 7.16 (cm, 1H, H₇), 7.13 (cm, 2H, H_{3',5'}), 6.85 (cm, 2H, H_{3',5'}), 5.40 (s, 2H, CH₂PhF₂), 2.54 (s, 3H, CH₃), 2.40 (s, 3H, CH₃). Anal. (C₂₂H₁₆F₄N₂) 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/CH₂Cl₂ and recrystallization from hexane, 0.68 g (1.77 mmol, 91% yield) of white crystals: mp 153–154 °C; ¹H NMR (200 MHz, CD₂Cl₂) δ 7.51 (cm, 1H, H_{4'}), 7.23 (m, 1H, H_{4'}), 7.11 (m, 1H, H₅), 7.05 (cm, 2H, H_{3',5'}), 6.93 (m, 1H, H₇), 6.80 (cm, 2H, H_{3',5'}), 5.29 (s, 2H, CH₂PhF₂), 2.58 (s, 3H, CH₃), 2.44 (s, 3H, CH₃). Anal. (C₂₂H₁₆F₄N₂) 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/CH₂Cl₂ and recrystallization from diethyl ether/hexane (3:1), 0.79 g (2.06 mmol, 76% yield) of white crystals: mp 165–166 °C; ¹H NMR (200 MHz, CD₂C1₂) δ 7.53 (m, 1H, H_{4'}), 7.32 (d, *J* = 8.2 Hz, 1H, H₅), 7.29–7.16 (m, 2H, H_{6,4'}), 7.14–7.01 (m, 1H, H₇), 7.07 (m, 2H, H_{3',5'}), 6.81 (m, 2H, H_{3',5'}), 5.33 (s, 2H, CH₂-PHF₂), 3.07 (q, *J* = 7.6 Hz, 2H, CH₂), 1.35 (t, *J* = 7.6 Hz, 3H, CH₃). Anal. (C₂₂H₁₆F₄N₂•¹/₅H₂O) 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; ¹H NMR (300 MHz, CD₂C1₂) δ 8.13 (dd, *J* = 8.1, 0.9 Hz, 1H, H₅), 7.86 (dd, *J* = 8.1, 0.9 Hz, 1H, H₇), 7.59 (m, 1H, H_{4'}), 7.43 (dd, *J* = 8.1 Hz, H₆), 7.28 (m, 1H, H_{4'}), 7.12 (m, 2H, H_{3',5'}), 6.84 (m, 2H, H_{3',5'}), 5.44 (s, 2H, CH₂). Anal. (C₂₀H₁₁F₄N₃) 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; ¹H NMR (300 MHz, CD₂Cl₂) δ 8.69 (dd, *J* = 0.5, 2.2 Hz, 1H, H₄), 8.23 (dd, *J* = 2.2, 9.0 Hz, 1H, H₆), 7.59 (dd, *J* = 0.5, 9.0 Hz, 1H, H₇), 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, CH₂PhF₂). Anal. (C₂₀H₁₁F₄N₃O₂) 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/CH₂Cl₂ and recrystallization from diethyl ether, 17.63 g (42.55 mmol, 76% yield) of white crystals: mp 184–186 °C; ¹H NMR (200 MHz, CD₂C1₂) δ 7.94 (dd, *J* = 1.1, 7.6 Hz, 1H, H₅), 7.72 (dd, *J* = 1.1, 8.2 Hz, 1H, H₇), 7.56 (m, 1H, H_{4'}), 7.37 (dd, *J* = 7.6, 8.2 Hz, 1H, H₆), 7.26 (m, 1H, H_{4'}), 7.09 (m, 2H, H_{3',5'}), 6.82 (m, 2H, H_{3',5'}), 5.39 (s, 2H, CH₂PhF₂), 3.95 (s, 3H, CO₂CH₃). Anal. (C₂₂H₁₄F₄N₂O₂) 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/CH₂Cl₂ and recrystallization from diethyl ether, 5.94 g (13.9 mmol, 76% yield) of white crystals: mp 126–127 °C; ¹H NMR (300 MHz, CD₂C1₂) δ 7.54 (m, 1H, H_{4'}), 7.48 (m, 1H, H₆), 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, CH₂PhF₂), 5.36 (s, 2H, CH₂O), 2.08 (s, 3H, OAc). Anal. (C₂₃H₁₆F₄N₂O₂) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-hydroxybenzimidazole (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 NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% methanol/CH₂Cl₂ and recrystallization from diethyl ether, yielding 0.25 g (0.67 mmol, 86% yield): mp 257–259 °C; ¹H NMR (200 MHz, CD₂C1₂) δ 7.57 (m, 1H, H_{4'}), 7.25 (m, 1H, H_{4'}), 7.17 (dd, *J* = 7.9, 8.3 Hz, 1H, H₆), 7.12 (m, 2H, H_{3',5'}), 6.99 (d, *J* = 8.3 Hz, 1H, H₇), 6.82 (m, 2H, H_{3',5'}), 6.73 (dd, *J* = 7.9, 1.0 Hz, H₅), 5.35 (s, 2H, CH₂-PhF₂). Anal. (C₂₀H₁₂F₄N₂O) 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 SnCl₄•2H₂O (9.80 g) dissolved in HCl (concentrated) (10 mL). After being stirred for 30 min at room temperature, the mixture was concentrated. The residue was diluted with CH₂Cl₂, washed with water, NaHCO₃ (saturated aqueous), and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, concentrated, and recrystallized from methanol (2.23 g, 5.99 mmol, 90% yield): mp 178–179 °C; ¹H NMR (300 MHz, CD₂C1₂) 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, H₆), 6.82 (d, *J* = 8.1 Hz, H₇), 6.81 (m, 2H, H_{3',5'}), 6.52 (dd, *J* = 7.7, 0.9 Hz, 1H, H₅), 5.29 (s, 2H, CH₂), 4.42 (s, 2H, NH₂). Anal. (C₂₀H₁₃F₄N₃) 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 NaNO₂ (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 NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, evaporated, purified by flash chromatography eluting with 2% methanol in CH₂Cl₂, and recrystallized with diethyl ether/hexane (3:1) (267 mg, 0.61 mmol, 75% of white powder): mp 147–148 °C; ¹H NMR (300 MHz, CD₂-C1₂) δ 7.56 (m, 1H, H_{4'}), 7.49 (dd, *J* = 7.7, 0.87 Hz, 1H, H₅), 7.47 (d, *J* = 7.9 Hz, H₇), 7.26 (m, 1H, H_{4'}), 7.18 (dd, *J* = 8.2, 7.7 Hz, 1H, H₆), 7.09 (m, 2H, H_{3',5'}), 6.82 (m, 2H, H_{3',5'}), 5.35 (s, 2H, CH₂). Anal. (C₂₀H₁₁BrF₄N₂•¼H₂O) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-chlorobenzimidazole (55)

To **53** (800 mg, 2.15 mmol) dissolved in 37% HCl (7 mL) and water (5 mL) at 0 °C was slowly added NaNO₂ (193 mg, 2.8 mmol) in water (1.5 mL). After 30 min at 0–5 °C, the mixture was added to CuCl (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 (Na₂SO₄), 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; ¹H NMR (300 MHz, CD₂Cl₂) δ 7.56 (m, 1H, H_{4'}), 7.43 (dd, *J* = 8.0, 1.1 Hz, 1H, H₇), 7.31 (dd, *J* = 7.8, 1.1 Hz, 1H, H₅), 7.26 (m, 1H, H_{4'}), 7.24 (dd, *J* = 8.0, 7.8 Hz, 1H, H₆), 7.09 (m, 2H, H_{3',5'}), 6.83 (m, 2H, H_{3',5'}), 5.36 (s, 2H, CH₂). Anal. (C₂₀H₁₁ClF₄N₂) 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 NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), 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; ¹H NMR (300 MHz, CD₂Cl₂) δ 8.49 (1H, br, NH), 8.20 (d, *J* = 7.8 Hz, 1H, H₅), 7.56 (m, 1H, H_{4'}), 7.26 (t, *J* = 7.9 Hz, 1H, H₆), 7.26 (m, 1H, H_{4'}), 7.19 (d, *J* = 7.9 Hz, 1H, H₇), 7.10 (m, 2H, H_{3',5'}), 6.82 (m, 2H, H_{3',5'}), 5.34 (s, 2H, CH₂PhF₂), 2.21 (s, 3H, Ac). Anal. (C₂₂H₁₅F₄N₃O) 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 CH₂Cl₂, washed with water, NaHSO₄ (10% solution), and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, concentrated, and purified by flash chromatography eluting with 2% methanol in CH₂Cl₂ (0.89 g, 2.08 mmol, and 1.07 g recovered starting material, 63% yield): mp 155–156 °C; ¹H NMR (200 MHz, CD₂Cl₂) δ 7.55 (m, 1H, H_{4'}), 7.48 (br d, *J* = 7.4 Hz, 1H, H₅), 7.31 (dd, *J* = 7.4, 7.7 Hz, 1H, H₆), 7.27 (m, 1H, H_{4'}), 7.14 (dd, *J* = 1.1, 7.7 Hz, 1H, H₇), 7.09 (m, 2H, H_{3',5'}), 6.84 (m, 2H, H_{3',5'}), 5.38 (s, 2H, CH₂PhF₂), 3.34 (s, 3H, NCH₃), 1.83 (s, 3H, NAc). Anal. (C₂₃H₁₇F₄N₃O) 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 NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, concentrated, and purified by flash chromatography eluting with 2% methanol in CH₂Cl₂ (0.35 g, 78% yield): mp 194–195 °C; ¹H NMR (200 MHz, CD₂Cl₂) δ 7.52 (m, 1H, H_{4'}), 7.23 (m, 1H, H_{4'}), 7.13 (dd, *J* = 8.3, 8.0 Hz, 1H, H₆), 7.07 (m, 2H, H_{3',5'}), 6.80 (m, 2H, H_{3',5'}), 6.76 (d, *J* = 8.3 Hz, 1H, H₅), 6.36 (d, *J* = 8.0 Hz, 1H, H₇), 5.29 (s, 2H, CH₂PhF₂), 2.96 (s, 3H, NCH₃). Anal. (C₂₁H₁₅F₄N₃) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(*N,N*-dimethylamino)benzimidazole (59)

To a mixture of 3 M H₂SO₄ (0.80 mL) and 37% H₂CO (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 Na₂CO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), 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; $^1\text{H NMR}$ (300 MHz, CD_2Cl_2) δ 7.51 (m, 1H, $\text{H}_{4'}$), 7.23 (m, 1H, $\text{H}_{4'}$), 7.13 (dd, $J = 8.0$ Hz, 1H, H_6), 7.06 (m, 2H, $\text{H}_{3',5'}$), 6.90 (d, $J = 8.0$ Hz, 1H, H_5), 6.80 (m, 2H, $\text{H}_{3',5'}$), 6.48 (d, $J = 8.0$ Hz, 1H, H_7), 5.30 (s, 2H, CH_2PhF_2), 3.18 (s, 6H, $\text{N}(\text{CH}_3)_2$). Anal. ($\text{C}_{22}\text{H}_{17}\text{F}_4\text{N}_3$) 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; $^1\text{H NMR}$ (200 MHz, CD_2Cl_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, $\text{H}_{4'}$), 7.37 (dd, $J = 7.6, 8.6$ Hz, 1H, H_6), 7.28 (m, 1H, $\text{H}_{4'}$), 7.11 (m, 2H, $\text{H}_{3',5'}$), 6.83 (m, 2H, $\text{H}_{3',5'}$), 5.40 (s, 2H, CH_2PhF_2). Anal. ($\text{C}_{21}\text{H}_{11}\text{F}_4\text{N}_3$) C, H, N.

N-Hydroxy 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carboxamide (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; $^1\text{H NMR}$ (200 MHz, CD_2Cl_2) δ 7.84 (dd, $J = 1.1, 7.7$ Hz, 1H, H_5), 7.57 (m, 1H, $\text{H}_{4'}$), 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, $\text{H}_{4'}$), 7.11 (m, 2H, $\text{H}_{3',5'}$), 6.83 (m, 2H, $\text{H}_{3',5'}$), 6.61 (br, 2H, NH_2), 5.39 (s, 2H, $\text{CH}_2\text{-PhF}_2$). Anal. ($\text{C}_{21}\text{H}_{14}\text{F}_4\text{N}_4\text{O}\cdot\frac{1}{4}\text{H}_2\text{O}$) 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; $^1\text{H NMR}$ (200 MHz, CD_2Cl_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, $\text{H}_{4'}$), 7.43 (dd, $J = 7.7, 8.1$ Hz, 1H, H_6), 7.28 (m, 1H, $\text{H}_{4'}$), 7.13 (m, 2H, $\text{H}_{3',5'}$), 6.84 (m, 2H, $\text{H}_{3',5'}$), 5.43 (s, 2H, CH_2PhF_2), 1.92 (s, 2H, NH_2). Anal. ($\text{C}_{21}\text{H}_{13}\text{F}_4\text{N}_3\text{O}$) 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; $^1\text{H NMR}$ (300 MHz, CD_2Cl_2) δ 7.59–7.43 (m, 2H, $\text{H}_{4',5}$), 7.36–7.20 (m, 3H, $\text{H}_{6,7,4'}$), 7.08 (m, 2H, $\text{H}_{3',5'}$), 6.83

(m, 2H, H_{3',5'}), 5.37 (s, 2H, CH₂PhF₂), 3.12 (s, 3H, CH₃), 2.88 (s, 3H, CH₃). Anal. (C₂₃H₁₇F₄N₃O•¼H₂O) 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 CH₂Cl₂, washed with NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and concentrated. The product was purified by flash chromatography eluting with 8% methanol/CH₂Cl₂ and recrystallization from diethyl ether/hexane (3:1), yielding 0.71 g (1.77 mmol, 72% yield) of white crystals: mp 178–180 °C; ¹H NMR (200 MHz, CD₂C1₂) δ 8.06 (d, *J* = 7.8 Hz, 1H, H₅), 7.76 (d, *J* = 8.1 Hz, 1H, H₇), 7.62 (m, 1H, H_{4'}), 7.47 (dd, *J* = 7.8, 8.1 Hz, 1H, H₆), 7.29 (m, 1H, H_{4'}), 7.14 (m, 2H, H_{3',5'}), 6.85 (m, 2H, H_{3',5'}), 5.45 (s, 2H, CH₂PhF₂). Anal. (C₂₁H₁₂F₄N₂O₂) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-benzimidazolyl-2-propanol (65)

To **50** (1.02 g, 2.46 mmol) suspended in THF (5 mL) was added 1.4 M methylolithium (0.95 mL). After 0.5 h, the mixture was diluted with EtOAc, washed with NaHSO₄ (10% solution) and NaCl (saturated aqueous), dried (Na₂SO₄), 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; ¹H NMR (200 MHz, CD₂C1₂) δ 7.55 (m, 1H, H_{4'}), 7.48 (d, *J* = 8.1 Hz, 1H, H₅), 7.25 (m, 2H, H_{6,4'}), 7.16 (dd, *J* = 1.1, 7.6 Hz, 1H, H₇), 7.10 (m, 2H, H_{3',5'}), 6.82 (m, 2H, H_{3',5'}), 5.82 (s, 1H, OH), 5.37 (s, 2H, CH₂PhF₂), 1.67 (s, 6H, CH₃). Anal. (C₂₃H₁₈F₄N₂O) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-isopropenylbenzimidazole (66)

65 (0.45 g, 1.09 mmol) was added to H₂SO₄ (1.00 mL). After 15 min at room temperature, the mixture was diluted with EtOAc, washed with NaHCO₃ (saturated aqueous) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and concentrated. The product was purified by flash chromatography eluting with 1% MeOH/CH₂Cl₂ and recrystallization from diethyl ether/hexane (3:1), yielding 0.11 g (0.28 mmol, 30% yield) of white crystals: mp 156–158 °C; ¹H NMR (200 MHz, CD₂C1₂) 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, CH₂-PhF₂), 5.36 (dq, *J* = 1.5, 2.4 Hz, 1H, vinyl), 2.33 (dd, *J* = 0.8, 1.5 Hz, 3H, CH₃). Anal. (C₂₃H₁₆F₄N₂) 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 K₂CO₃ (0.40 g). After 30 min, the mixture was concentrated, redissolved in EtOAc, washed with NaHSO₄ (10% solution) and NaCl (saturated aqueous), dried (Na₂SO₄), 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; ¹H NMR (300 MHz, CD₂C1₂) δ 7.54 (m, 1H, H_{4'}), 7.43 (d, *J* = 7.9 Hz, 1H, H₇), 7.30–7.22 (m, 2H, H_{6,4'}), 7.18 (d, *J* = 7.5 Hz, 1H, H₅), 7.09 (m, 2H, H_{3',5'}), 6.82 (m, 2H, H_{3',5'}), 5.37 (s, 2H, CH₂PhF₂), 5.06 (d, *J* = 5.4 Hz, 2H, CH₂O), 3.51 (t, *J* = 5.4 Hz, 1H, OH). Anal. (C₂₁H₁₄F₄N₂O) C, H, N.

1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole-4-carbaldehyde (68)

To CrO₃ (1.26 g) dissolved in pyridine (1.0 mL) and CH₂Cl₂ (9.0 mL) was added **67** (0.49 g, 1.27 mmol) dissolved in pyridine/CH₂Cl₂ (1:1) (2 mL). After being stirred for 6 h, the mixture was filtered. The filtrate was diluted with CH₂-Cl₂, washed with NaHCO₃ (saturated solution) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, concentrated. The product was purified by flash chromatography eluting with 1% methanol/CH₂-Cl₂ and

recrystallized from diethyl ether/hexane (3:1), yielding 0.38 g (0.99 mmol, 78% yield) of white crystals: mp 151–153 °C; $^1\text{H NMR}$ (300 MHz, CD_2Cl_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, H₆), 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. ($\text{C}_{21}\text{H}_{12}\text{F}_4\text{N}_2\text{O}$) 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% $\text{MeOH}/\text{CH}_2\text{Cl}_2$ and recrystallized from diethyl ether/hexane (3:1), yielding 0.52 g (1.28 mmol, 92% yield) of white crystals: mp 143–144 °C; $^1\text{H NMR}$ (300 MHz, CD_2Cl_2) δ 7.55 (m, 1H, H_{4'}), 7.48 (dd, $J = 7.7, 1.1$ Hz, 1H, H₇), 7.36 (dd, $J = 7.6$ Hz, 1.1 Hz, 1H, H₅), 7.30 (dd, $J = 7.7, 7.6$ Hz, 1H, H₆), 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. ($\text{C}_{21}\text{H}_{13}\text{ClF}_4\text{N}_2$) 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; $^1\text{H NMR}$ (300 MHz, CD_2Cl_2) δ 7.55 (m, 1H, H_{4'}), 7.50 (dd, $J = 7.7, 1.6$ Hz, 1H, H₇), 7.32 (m, 1H, H₆), 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. ($\text{C}_{21}\text{H}_{13}\text{F}_4\text{N}_5$) 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% $\text{MeOH}/\text{CH}_2\text{Cl}_2$ increasing to 100% MeOH , yielding 0.16 g (0.42 mmol, 31% yield) of white crystals: mp 257–260 °C; $^1\text{H NMR}$ (300 MHz, CD_2Cl_2) δ 7.59 (m, 1H, H_{4'}), 7.55 (m, 1H, H₇), 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. ($\text{C}_{21}\text{H}_{15}\text{F}_4\text{N}_3$) C, H, N.

N-(1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)-4-(*N*-acetylamino)methylene)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% $\text{MeOH}/\text{CH}_2\text{Cl}_2$ increasing to 8% $\text{MeOH}/\text{CH}_2\text{Cl}_2$, yielding 0.20 g (0.47 mmol, 65% yield) of white crystals: mp 178–179 °C; $^1\text{H NMR}$ (300 MHz, CD_2Cl_2) δ 7.56 (m, 1H, H_{4'}), 7.42 (m, 1H, H₇), 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. ($\text{C}_{23}\text{H}_{17}\text{F}_4\text{N}_3\text{O}\cdot\frac{1}{2}\text{H}_2\text{O}$) 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 NaHSO₄ (10% solution) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% MeOH/CH₂Cl₂ increasing to 4% MeOH/CH₂Cl₂, yielding 0.24 g (0.54 mmol, 94% yield) of white crystals: mp 171–172 °C; ¹H NMR (300 MHz, CD₂C1₂) δ 7.55 (m, 1H, H_{4'}), 7.44 and 7.40 (rotamers) (d, *J* = 8.3 Hz, 1H, H₇), 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, CH₂PhF₂), 5.00 and 4.98 (rotamers) (s, 2H, CH₂N), 3.04 and 2.96 (rotamers) (s, 3H, NCH₃), 2.15 and 2.11 (rotamers) (s, 3H, NAc). Anal. (C₂₄H₁₉F₄N₃O) 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 NaHCO₃ (saturated solution) and NaCl (saturated aqueous), dried (Na₂SO₄), filtered, and concentrated. The product was purified by flash chromatography eluting with 2% MeOH/CH₂Cl₂, yielding 0.10 g (0.25 mmol, 48% yield) of white crystals: mp 122–124 °C; ¹H NMR (300 MHz, CD₂C1₂) δ 7.53 (m, 1H, H_{4'}), 7.43 (dd, *J* = 7.1, 2.2 Hz, 1H, H₇), 7.34–7.28 (m, 2H, H_{5,6}), 7.24 (m, 1H, H₄), 7.08 (m, 2H, H_{3',5'}), 6.81 (m, 2H, H_{3',5'}), 5.35 (s, 2H, CH₂PhF₂), 4.90 (s, 2H, CH₂O), 3.45 (s, 3H, OCH₃). Anal. (C₂₂H₁₅F₄N₂O) 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 CH₂Cl₂, washed with H₂O, dried over Na₂SO₄, 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; ¹H NMR (300 MHz, CD₂C1₂) δ 7.55 (m, 1H, H₄), 7.50 (dd, *J* = 7.7, 1.6 Hz, 1H, H₇), 7.32 (m, 1H, H₆), 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, CH₂PhF₂), 4.2 (s, 2H, CH₂CN). Anal. (C₂₂H₁₅F₄N₃) 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 averaging²¹ 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 1.124 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

Acknowledgments

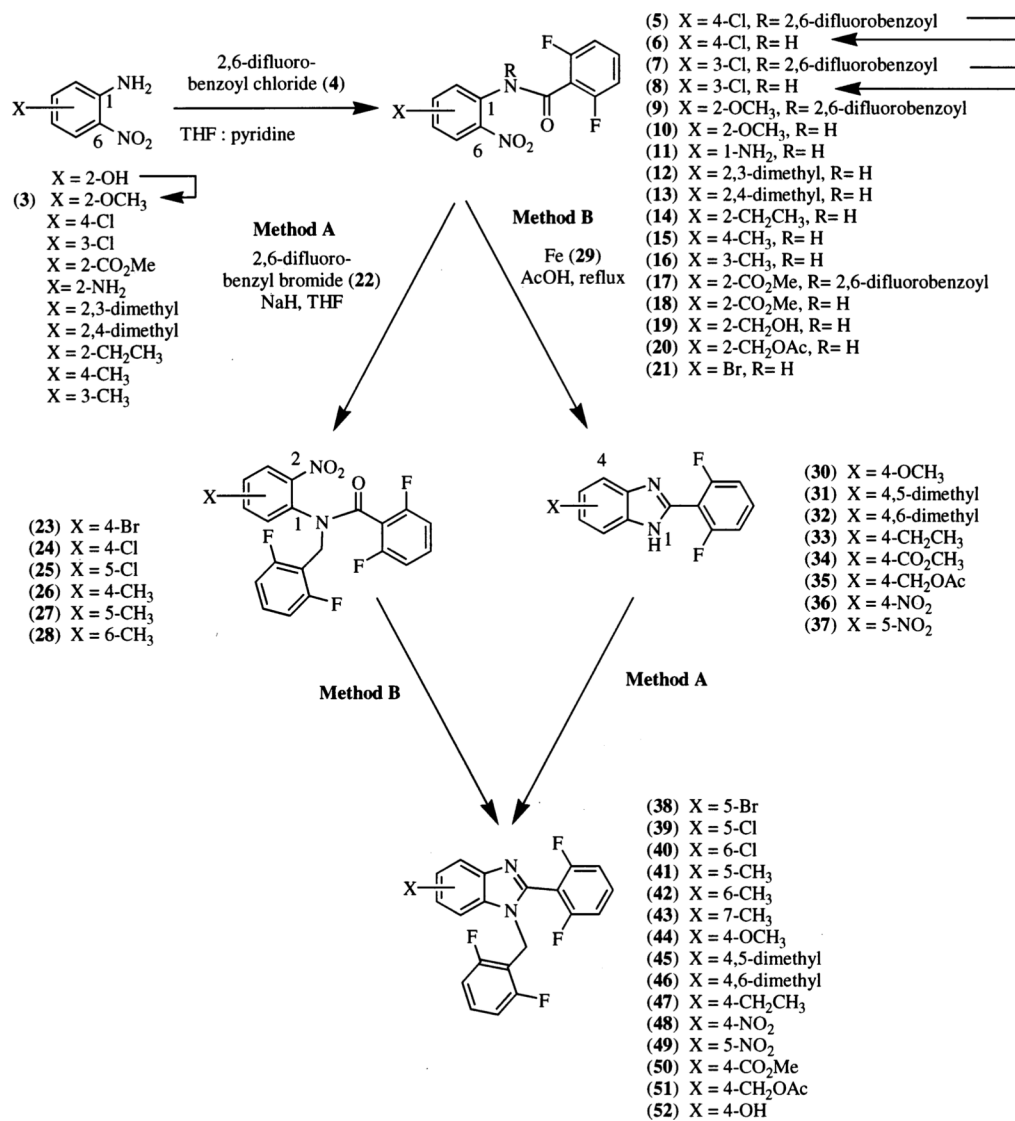
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Scheme 1.
 Synthesis of 1-(2,6-Difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazoles (BPBI)

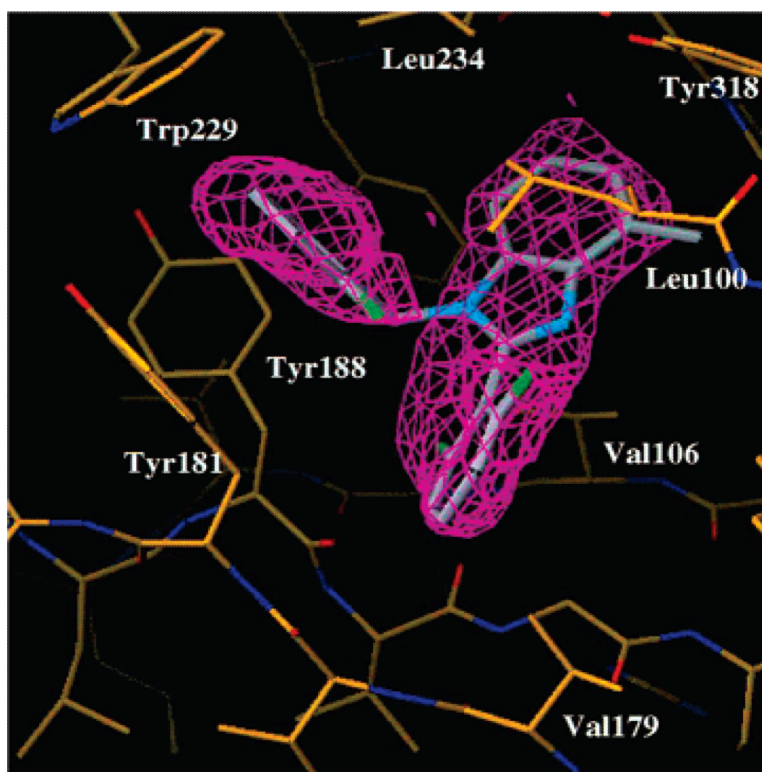


Figure 1. Electron density of 4-methyl BPBI (**1**) in the binding pocket of HIV-1 RT. The piece of electron density covering the inhibitor is from a $|F_o| - |F_c|$ map calculated prior to inclusion of the inhibitor in structure refinement.

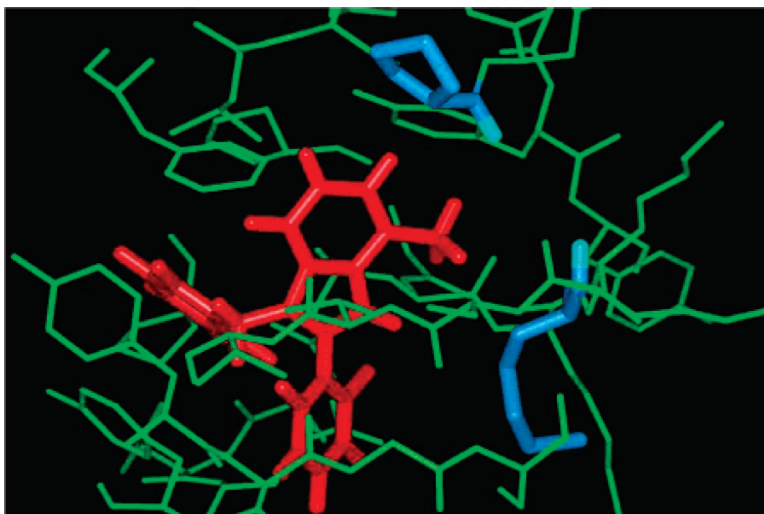


Figure 2. Orientation of 4-methyl BPBI in the binding pocket of HIV-1 RT allows for expansion of substituents in the 4 position of the benzimidazole ring.

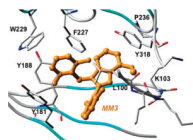
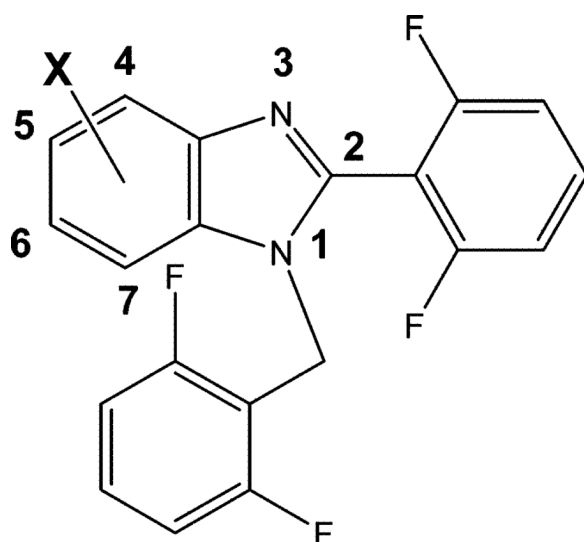


Figure 3.
Orientation of 4-methoxy BPBI (**44**) in the binding pocket of HIV-1 RT.

Table 1

Enzyme Inhibition Data for Substituted Benzimidazoles^a

compd	X	% inhibition ^b (1 μ M)
(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

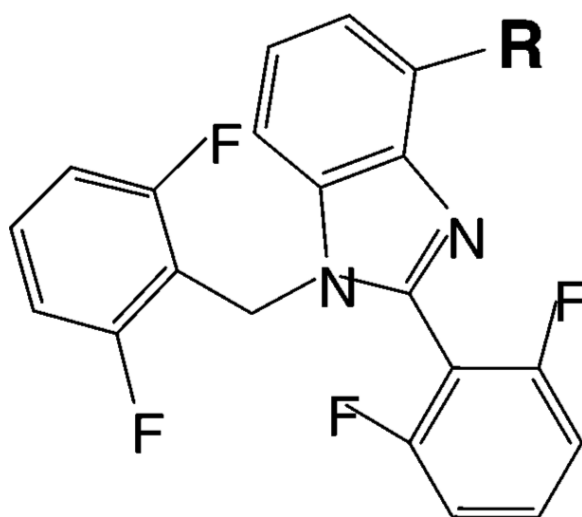
^aEnzyme assays done with WT RT.^b% inhibition is the average of two experiments done in triplicate.

Table 2

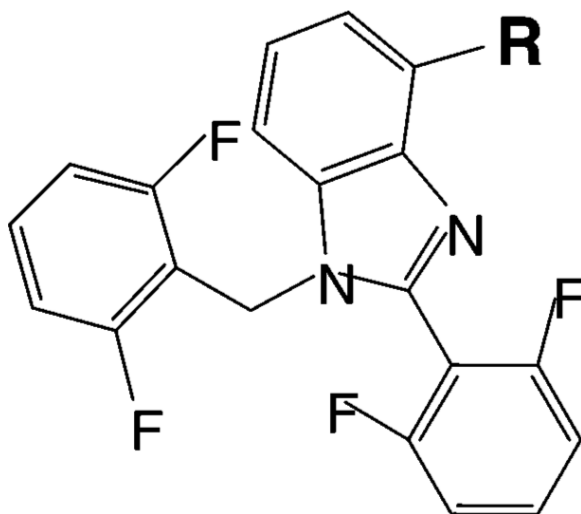
X-ray Crystallographic Data and Refinement Statistics

X-ray Diffraction Data		
PDB code	2B6A	
X-ray source	CHES F1	CHES F1
data collection temp (K)	268	104
no. of crystals	10	1
wavelength (Å)	0.918	0.918
space group	C2	C2
cell constants		
<i>a</i> (Å)	227.8	223.9
<i>b</i> (Å)	70.2	68.8
<i>c</i> (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	

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 ₂ CH ₃	17.5	0.82	26.1
48	NO ₂	1.88	2.4	30.1
50	CO ₂ Me	157.8	NRVC ^c	57.7
51	CH ₂ OAc	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 ₂ H	ND ^b	NRVC ^c	23.5
65	isopropyl	ND ^b	6.34	27.0
66	isoprenyl	ND ^b	1.44	28.3
67	CH ₂ OH	ND ^b	0.023	58.1



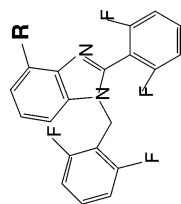
compd	R	IC ₅₀ (μ M)	EC ₅₀ (μ M)	TC ₅₀ (μ M)
68	CHO	ND ^b	0.12	0.82
69	CH ₂ Cl	ND ^b	0.18	13.4
70	CH ₂ N ₃	ND ^b	0.045	>100
71	CH ₂ NH ₂	ND ^b	0.037	49.3
72	CH ₂ NHAc	ND ^b	NRVC ^c	20.8
73	CH ₂ NCH ₃ Ac	ND ^b	0.58	20.6
74	CH ₂ OCH ₃	ND ^b	0.06	ND ^b
75	CH ₂ CN	ND ^b	0.06	ND ^b
	nevirapine	ND ^b	0.035	ND ^b

^aIC₅₀ is the quantity of drug required to reduce WT RT enzyme activity by 50%. EC₅₀ is the quantity of drug required to reduce cell killing of HIV-1 infected CEM cells by 50%. TC₅₀ is the quantity of drug required to reduce cell viability by 50%.

^bND: not determined.

^cNRVC: no reduction in viral cytopathic effect.

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



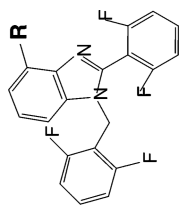
isolate	R group															
	CH ₃ 1	OCH ₃ 44	Cl 55	Br 54	NH ₂ 53	Et 47	NHAc 56	NAcCH ₃ 57	CH ₂ N ₃ 70	CH ₂ OAc 51	CH ₂ OH 67	CH ₂ NH ₂ 71	CH ₂ Cl 69	CH ₂ CN 75	NVP ^b	
NL4-3 (WT)	0.5	0.06	0.5	0.5	0.4	0.9	1.97	0.04	0.045	0.062	0.048	1.36	0.16	0.06	0.1	
L100I	0.3	0.16	0.3	0.5	1.2	2.48	3.25	1.66	0.08	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% (EC₅₀). All values reported in μ M.

^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 ₂ OAc	ΔG_{expt}^b CH ₂ OAc	ΔG_{calc}^c CH ₂ OAc	EC ₅₀ CH ₂ CN	ΔG_{expt}^b CH ₂ CN	ΔG_{calc}^c CH ₂ CN	EC ₅₀ CH ₂ N ₃	ΔG_{expt}^b CH ₂ N ₃	ΔG_{calc}^c CH ₂ N ₃
NLA-3 (WT)	0.062	-10.24	-10.29	0.06	-10.24	-9.48	0.045	-10.43	-10.6
L1001	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 $\Delta G_{\text{expt}} = -RT \ln \text{EC}_{50}$.

^c See ref 19.

^d ND: not determined.