4-Benzyl and 4-Benzoyl-3-dimethylaminopyridin-2(1*H*)-ones: In Vitro Evaluation of New C-3-Amino-Substituted and C-5,6-Alkyl-Substituted Analogues against Clinically Important HIV Mutant Strains

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In a program to optimize the anti-HIV activity of the 4-benzyl and 4-benzoyl-3-dimethylaminopyridinones **9** and **10**, lead compounds in a new class of highly potent non-nucleoside type inhibitors of HIV-1 reverse transcriptase, modification of the alkyl substitutents at the C-5 and C-6 positions on the pyridinone ring and of the substitutents on the C-3 amino group has been studied. Of the 17 new 5/6-modified analogues prepared, compounds **31b** and **32b** substituted at C-5 by an extended nonpolar chain containing an ether function and a C-6 methyl group and compound **35** bearing a C-5 ethyl/C-6 hydroxymethyl substituent pattern were selected on the basis of their in vitro activity against wild-type HIV and the three principle mutant strains, K103N, Y181C, and Y188L. When tested further, it was shown that these molecules, and in particular compound **35**, are globally more active than **9**, **10**, and efavirenz against an additional eight single [L100I, K101E, V106A, E138K, V179E, G190A/S, and F227C] and four double HIV mutant strains [L100I + K103N, K101E + K103N, K103N + Y181C, and F227L + V106A], which are clinically relevant. Concerning modulation of the N-3 substituent, 36 new analogues were prepared. Of these, the *N*-methyl-*N*-(2-methoxyethyl)-substituted compounds **40**, **42**, and **62**, as well as the doubly modified compounds **77a** and **77b**, were selected from the initial screen and were subsequently shown to be active at sub-micromolar concentrations (IC_{50}'s) against all the other mutant strains except K103N + Y181C and F227L + V106A. Two possible, but distinct, modes of binding of these analogues in RT were suggested from molecular modeling studies. The preferred mode of binding for compound **62**, corresponding to the predicted "orientation 1", was revealed in the X-ray crystal structure of the compound **62**−RT complex.

Introduction

Nevirapine **1**, delaviridine **2**, and efavirenz **3** are at present the only non-nucleoside type reverse transcriptase inhibitors (NNRTIs) used in combination (HAART) therapy for the treatment of AIDS.[1] In view of the long-term complications that arise due to toxicity, resistance, and associated side effects (lipodystrophy, hyperlipidaemia, etc.)[2−6] using combinations of nucleoside inhibitors, protease inhibitors, and NNRTIs, it is important to find new drugs which display a high level of activity against the clinically relevant HIV single and multiple mutant strains. This criterion is of particular relevance to the development of the NNRTI class of compounds for which it has been shown that cross resistance is a major concern.[7] Promising new molecules in the NNRTI group, which are currently undergoing clinical evaluation, include DPC 083, **4**,[8] and TMC125, **5**.[9,10]

Our efforts have been directed toward the evaluation of 4-arylthiopyridinones/benzylpyridinones of general formula **8** as a new class of anti-HIV agents which target reverse transcriptase at the hydrophobic pocket common to all NNRTIs.[11,12] Preliminary SAR studies led to the identification of compounds **9** and **10** as potent inhibitors of wild-type HIV.[13] These compounds are in many respects hybrids of the HEPT analogue GCA-186, **6**,[14] and the Merck pyridinone, **7**,[15−19] in that they contain both a 3′,5′-dimethylphenyl group and a 5-ethyl-6-methyl-substituted pyridinone motif in their structure. The presence of the 3-NMe_2 substitution in **9** and **10**, as opposed to a nonfunctionalized amino group, was similarily inspired by the presence of an *i*-Pr group at

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the corresponding position in the HEPT derivatives MKC-442 and **6**.[20]

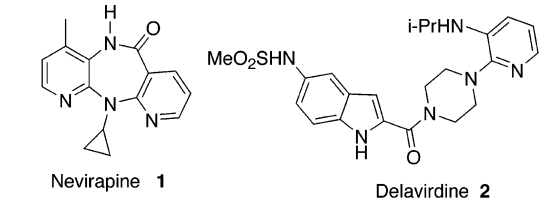
From the outset it was clear that drug resistance would be a major obstacle to the development of the arylthiopyridinones/benzylpyridinones series. Indeed, the rapid appearance of drug-resistant HIV strains bearing the Y181C, Y188L, and K103N mutations in RT ultimately led to the abandonment of the clinical development of HEPT and the Merck pyridinones. The question was, will a lack of sensitivity to these major mutant strains and others commonly encountered in AIDS patients also be an "inherent" limiation of our pyridinone family?

The increase in potency of GCA-186 over HEPT against both wild-type HIV and the Tyr-181 mutant has been correlated with an overall tighter and/or more complete fit in the hydrophobic pocket due to the presence of the 3′,5′-dimethyl groups.[14] The key to the problem was thus to find the correct combination of modifications of our initial lead molecules which would reinforce the crucial interactions in the hydrophobic region in the mutant forms of RT. Following this logic, we recently prepared and evaluated more than 100 new analogues of lead molecules **9** and **10** in which the number, position, steric hindrance, and electronic properties of the substituent(s) on the aromatic ring were varied.[13] This study permitted the identification of two new lead compounds **11** and **12a** in the 4-benzoylpyridinone series and compound **12b** in the 4-benzylpyridinone group. These molecules are potent inhibitors of wild-type HIV-1, as well as a large panel of HIV-1 mutant strains which are responsible for the onset of resistance to the NNRTIs that are currently used in HAART therapy. Globally, the activity profile for these new molecules, and in particular for **12a**, is better in vitro than that for efavirenz.

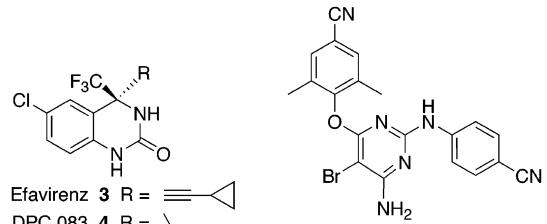
The next step of this SAR study involved looking closely at the influence of modifications at the C-5 and C-6 positions on the pyridinone ring and at the amino substitutent at C-3 on anti-HIV activity in vitro.

In earlier studies on the arylthio- and benzylpyridinones, the 5-Et/6-Me substitution pattern was generally adopted, since this combination of alkyl groups was optimized for the Merck pyridinones (cf. **7**).[18,19] To expand the SAR at these two centers, a series of 5,6-dialkyl-substituted pyridinones were thus prepared and evaluated. As can be seen from Tables 1 and 3, analogue **35**, bearing a polar hydroxymethyl group at C-6, and the analogues **31b** and **32b** with extended nonpolar chains containing an ether function at C-5 and a C-6 methyl group are highly active against the three principle mutants and against the larger panel of eight single [L100I, K101E, V106A, E138K, V179E, 190A/S, and F227C] and four double [L100I + K103N, K101E + K103N, K103N + Y181C, and F227L + V106A] HIV mutant strains.

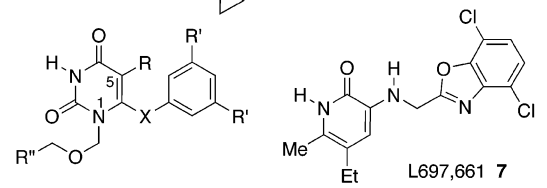
To explore the effects of modulating the 3-amino substituent, a wide range of 3-*N*,*N*-dialkylamino and cyclic amine analogues were prepared. Several of these displayed potent activity against wild-type HIV-1 and the three major mutant strains (Tables 2 and 3). In particular, the *N*-methyl-*N*-(2-methoxyethyl)-substituted compounds **40**, **42**, and **62**, as well as the doubly modified compounds **77a** and **77b** selected from the



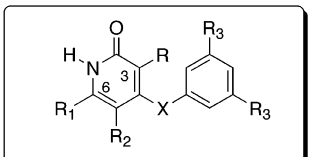
Nevirapine **1** Delavirdine **2**



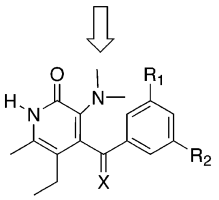
Efavirenz **3** R =
DPC 083 **4** R =
TMC125 **5**



HEPT R = Me, R' = H, R" = CH_2OH, X = S
MKC-442 R = i-Pr, R' = H, R" = Me, X = CH_2
6 GCA-186 R = i-Pr, R' = R" = Me, X = CH_2
L697,661 **7**



8 R_1/R_2 = alkyl, R_3 = H, Me
R = NH_2, X = S, CH_2, CO



9 X = H,H, $R_1 = R_2$ = Me
10 X = O, $R_1 = R_2$ = Me NB: 10=20a if R_1=H , R_2 = Me
11 X = O, R_1 = H, R_2 = CN
12a X = O, R_1 = H, R_2 =
12b X = CH_2, R_1 = H, R_2 =

initial screen, were subsequently shown to be active at sub-micromolar concentrations (IC_{50}'s) against all the mutant strains except K103N + Y181C and F227L + V106A. To gain insight as to the mode of binding of these interesting new N-3-modified analogues in the hydrophobic pocket of RT, a molecular modeling study was undertaken for compounds **9** and **62** using the HEPT−RT structure as a starting point.[20] This study revealed two possible and essentially equivalent modes of binding of these compounds (orientations 1 and 2, Figure 1), generated by rotation of the pyridinone ring about an axis defined by atoms N-1 and C-4. These orientations differ most importantly in the positioning of the extended amino side chain substituent. Note that in orientation 2 this side chain occupies the same space as the N-1 substituent in HEPT and HEPT analogues. The preferred mode of binding for compound **62**, corresponding to the predicted orientation 1, was revealed

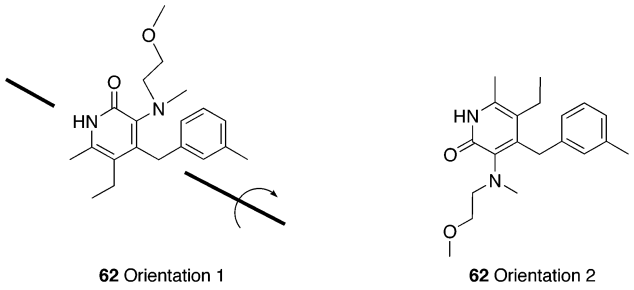
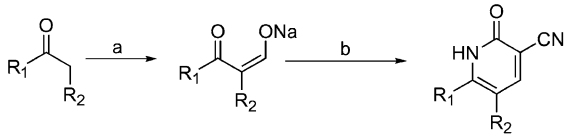


Figure 1. Orientation 1 and orientation 2 for compound **62**.

Scheme 1[a]



a : R_1 = Me, R_2 = Et
b: R_1 = R_2 = Me
c : R_1 = Et, R_2 = Me
d : R_1 = Me, R_2 = iBu
e : R_1 = iPr, R_2 = Me
f: R_1 = Me, R_2 = nPr
g : R_1 = R_2 = H
h : R_1 = CH_3, R_2 = H
i : R_1-R_2 = $(CH_2)_4$
j : R_1-R_2 = $(CH_2)_3$

[a] Conditions: (a) NaOMe, EtOH, HCO_2Et, Et_2O; (b) cyanoacetamide, piperidine, HOAc, H_2O.

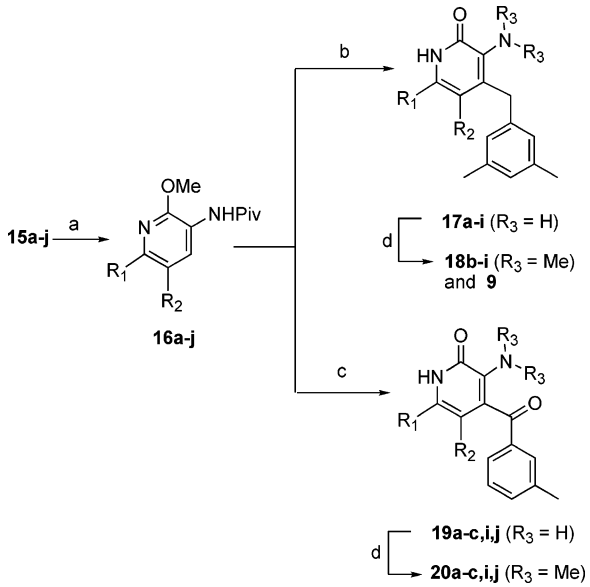
in the X-ray crystal structure of the compound **62**−RT complex.[21] The results obtained for both series of compounds broaden our view of the SAR in the 4-benzyl/4-benzyloxypyridinone family and clearly indicate directions to be taken for further analogue synthesis.

Chemistry

As for the preparation of 3-dimethylamino-4-benzylpyridinone **9**,[12] the synthesis of analogues **18b**−**i** containing different alkyl substituents at C-5 and C-6 involved four distinct stages (Schemes 1 and 2): (i) construction of the 3-cyanopyridinones **15** by condensation of the requisite ketones **13** with ethyl formate, and reaction of the derived intermediates **14** with cyanoacetamide (piperidine/HOAc),[17,22−24] (ii) conversion of intermediates **15b**−**i** to 2-methoxy-3-*N*-pivaloylaminopyridines **16b**−**i** in six steps according to a procedure established for the conversion of **15a** to **16a**,[12] (iii) reaction of the dianion of compounds **16** with 3,5-dimethylbenzyl bromide, followed by treatment of the derived condensation product with 6 N HCl to effect amide hydrolysis and liberation of the 2-pyridinone motif, and (iv) conversion of amines **17b**−**i** to the corresponding *N*,*N*-dimethylamines under reductive alkylation conditions. As described for **20a**,[13] compounds **20a**−**c**,**i**,**j** were obtained by reaction of the dianion of the requisite 2-methoxy-3-*N*-pivaloylaminopyridines **16** with 3-methylbenzaldehyde, followed by alcohol to ketone oxidation, treatment with acid, and reductive alkylation of amine intermediates **19a**−**c**,**i**,**j**.

In an initial attempt to prepare pyridinones **31a**,**b** and **32b** (Scheme 3) it was found that intermediate **23b** was acid-sensitive, precluding sucessful hydrolysis−decarboxylation of the 3-cyano group. An alternate route to the pivotal compounds **28a**,**b** was thus devised involving in the key step a Beckmann rearrangement of the oximes **25a**,**b**, obtained from the 3-acetyl-substituted pyridinones **24a**,**b**. Compounds **28** were then converted to the target molecules in a fashion similar to the preparation of compounds **20**.

Scheme 2[a]



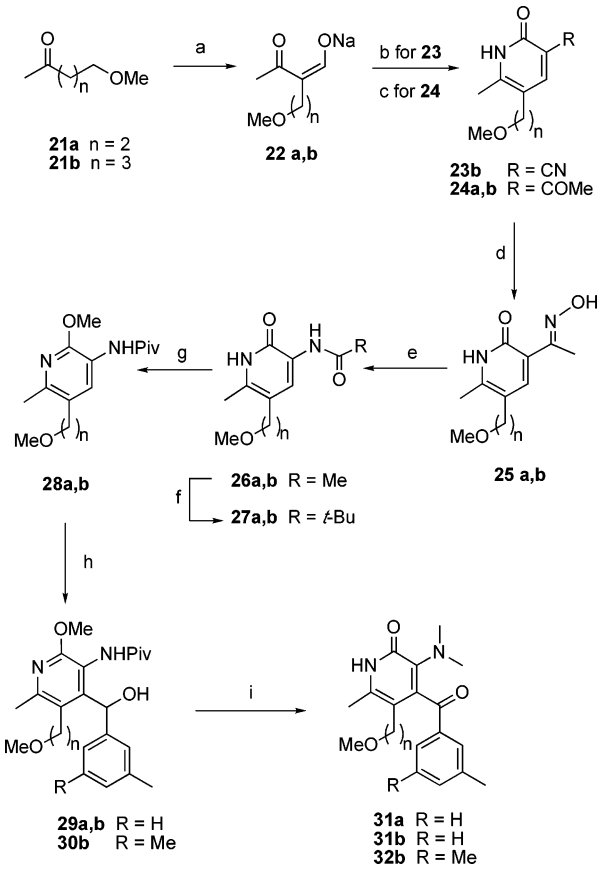
[a] Conditions: (a) (i) 6 N HCl, reflux; (ii) HN_3/H_2SO_4); (iii) $POCl_3$, $BnEt_3NCl$, CH_3CN, reflux; (iv) NaOMe, MeOH; (v) H_2, Raney Ni; (vi) *t*-BuCOCl, CH_2Cl_2, Et_3N; (b) (i) *n*-BuLi, TMEDA, 3,5-dimethyl-1-bromomethylbenzene; (ii) 6 N HCl; (c) (i) *n*-BuLi, TMEDA, 3-methylbenzaldehyde; (ii) MnO_2, toluene, reflux; (iii) 3 N HCl; (d) HCHO, $NaBH_3CN$, HOAc.

Ready access to the interesting 6-hydroxymethyl derivative **35** of lead molecule **10** was achieved by formation of the *N*-oxide of **33**, its reaction with Ac_2O under Boekelheide conditions, acid hydrolysis to give **34**, and reductive amination (Scheme 4).

The "mixed" *N*,*N*-dialkylpyridinone analogues **38** and **39** and in particular compounds **40**−**43** in which a sulfur or oxygen atom is present in the longer of the two alkyl chains were obtained by N-formylation of **17a**, reduction of the *N*-formyl group in **36**,[12] and reaction of the derived monomethylated intermediate **37**[12] with the requisite aldehyde and cyanoborohydride (Scheme 5). The mono-*N*-Et (**47**), *N*-Pr (**48**), and *N*-Bu (**49**) derivatives were similarily prepared by LAH reduction of amides **44**−**46**. Reaction of **17a** with a large excess of the aldehyde component and cyanoborohydride produced the symmetrical dialkyl compounds **50** and **51**, whereas with a smaller amount of benzaldehyde the mono- and di-*N*-benzylated compounds **52** and **53** were obtained as a separable mixture. Reductive amination conditions were also employed to prepare the cyclic morpholine and piperidine based analogues **54** and **55**. By the simple reaction of **17a** with 2,5-dimethoxytetrahydrofuran, the pyrrole analogue **56** was produced (Scheme 6).

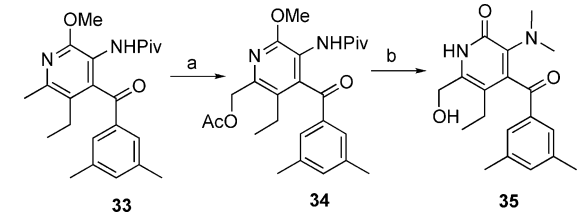
Following the successive reductive alkylation strategy, we prepared the mixed dialkylpyridinones **59**−**63**, related to 3′-methyl-3-dimethylaminopyridinone **20a**, from intermediate **57**[13] (Scheme 7). However, for the preparation of **64**−**66**, alkylation of the initially formed mono-*N*-methyl compound **58** using the requisite chloroalkylnitrile reagent was preferred. Through reaction of **57** with ethyl and phenyl isothiocyanate, respectively, the thiourea analogues **67** and **68** were formed. Alternative reaction of **57** with NH_4SCN and benzoyl chloride

Scheme 3[a]



[a] Conditions: (a) HCO_2Et, NaOMe, Et_2O; (b) cyanoacetamide, piperidine, H_2O; (c) acetoacetamide, piperidine, H_2O; (d) EtOH, $H_2NOH{\cdot}HCl$, pyr; (e) HCO_2H, heat; (f) (i) 3 N HCl; (ii) Piv-Cl, Et_3N, CH_2Cl_2; (g) MeI, Ag_2CO_3, CH_2Cl_2; (h) *n*-BuLi, TMEDA, 3-methylbenzaldehyde or 3,5-dimethylbenzaldehyde; (i) (i) Jones reagent; (ii) 3 N HCl; (iii) HCHO, $NaBH_3CN$, HOAc.

Scheme 4[a]



[a] Conditions: (a) (i) *m*-CPBA, CH_2Cl_2; (ii) Ac_2O, reflux; (b) (i) 12 N HCl; (ii) HCHO, $NaBH_3CN$, HOAc.

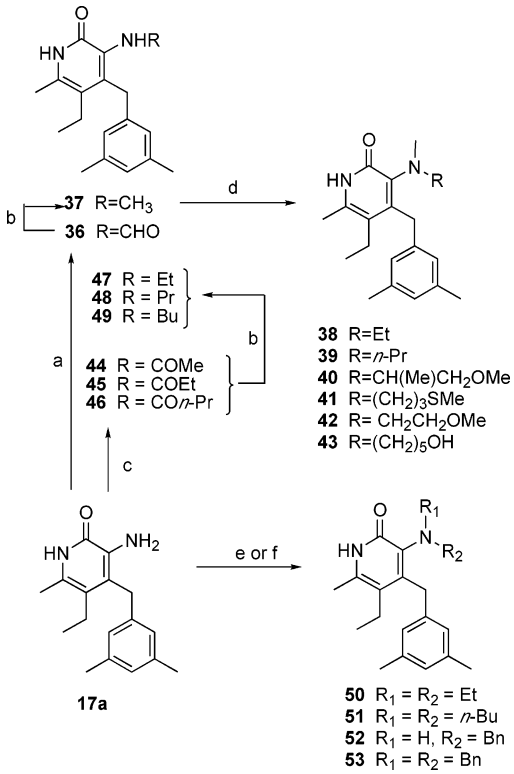
gave compound **69**, and by treatment of this product with 3 N NaOH, thiourea **70** was obtained.

In view of the interesting biological activities observed for compounds **42** and **62**, the 4-benzoylpyridinone analogue **72** possessing the 2-methoxyethylamine side chain was also prepared via **19a** and **71** (Scheme 8). Further, given the very potent anti-HIV properties of compound **12b** bearing a 3′-acrylonitrile function on the phenyl ring, it was similarily pertinent to construct compounds **77a** and **77b** where this modification and the 2-methoxyethylamine motif are combined in the same molecule (Scheme 9).

Modeling

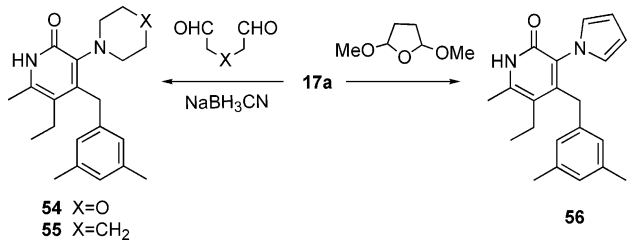
Manual Docking. Compounds **9** and **62** were constructed and superimposed onto the heterocycle component in the HEPT ligand of the HEPT−RT X-ray crystal structure (PDB code: 1RTI) using the SYBYL modeling package.[25] For each molecule, two orientations of the pyridinone ring were considered (Figures 1 and 2). Then, with the exception of the N-3 side chain in orientation 2 which was modeled to match the corresponding HEPT side chain conformation, the substituents at the 3-, 4-, and 5-positions of the pyridinone ring were oriented toward the hydrophobic key residues (Y181, Y188, W229, and P95). The four resulting models were subsequently minimized using the Tripos force field. The protein structure was then modified to reflect the conformational changes occurring upon binding of potent HEPT analogues bearing bulky substituents at C-5.[20] In particular, Y181 was set in the "switched" conformation, thus, enabling the N-side chain to extend deeper into the hydrophobic pocket and to interact directly with the key hydrophobic residues. A local minimization was performed in the Y181 region to account for the conformational adjustments of the surrounding residues triggered by the tyrosine switch. Finally, the four ligand models were separately merged into the modified protein structure, and the four complexes were minimized using the Tripos force field along

Scheme 5[a]

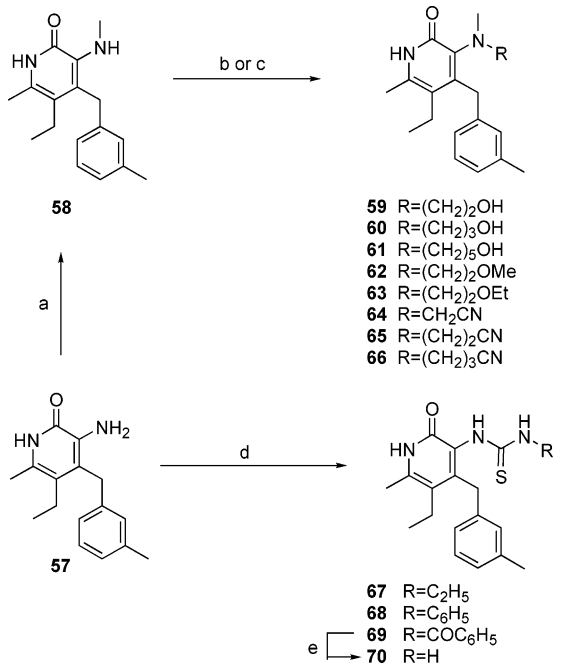


[a] Conditions: (a) HCO_2Et, HCO_2H; (b) $LiAlH_4$, THF; (c) RCOCl, Et_3N; (d) R′CHO, $NaBH_3CN$; (e) R′CHO (excess), $NaBH_3CN$; (f) PhCHO (1.5 equiv), $NaBH_3CH$.

Scheme 6

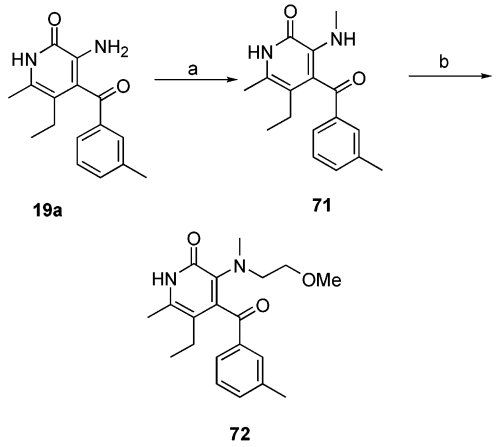


Scheme 7[a]



[a] Conditions: (a) (i) HCO_2Et, HOAc; (ii) $LiAlH_4$; (b) R′CHO, $NaBH_3CN$ (for **59**−**63**); (c) RCl, Et_3N (for **64**−**66**); (d) RNCS (for **67** and **68**) or C_6H_5COCl/NH_4SCN for (for **69**); (e) 3 N NaOH.

Scheme 8[a]

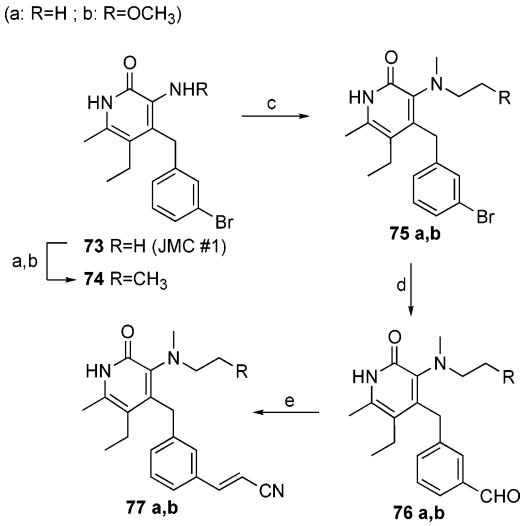


[a] Conditions: (a) (i) HCO_2Et, HOAc; (ii) $LiAlH_4$; (b) $MeOCH_2CHO$, $NaBH_3CN$.

with Gasteiger−Hückel charges for the ligands and Kollman-all-atoms charges for the protein. During the minimization, all residues included in a 10 Å sphere around the ligand were allowed to move, while the remaining part of the protein structure was kept rigid. It is to be noted that the minimization process takes into account all atoms in the complex when calculating its internal energy.

De Novo Design. The "optimize" mode of the de novo design module Leapfrog[25] was used for this analysis. In this mode, the software was first provided with the RT protein structure taken from the HEPT−RT X-ray crystal structure (PDB code: 1RTI) from which the ligand has been removed. The protein structure was modified in the same way as described above. In a second step, compound **10** was constructed and manually docked within the RT protein. On the basis of the protein alone, the process generated an interaction map

Scheme 9[a]



[a] Conditions: (a) HCO_2Et, HCO_2H; (b) $LiAlH_4$; (c) RCH_2CHO, $NaBH_3CN$; (d) *n*-BuLi, DMF; (e) $(EtO)_2POCH_2CN$, *t*-BuOK.

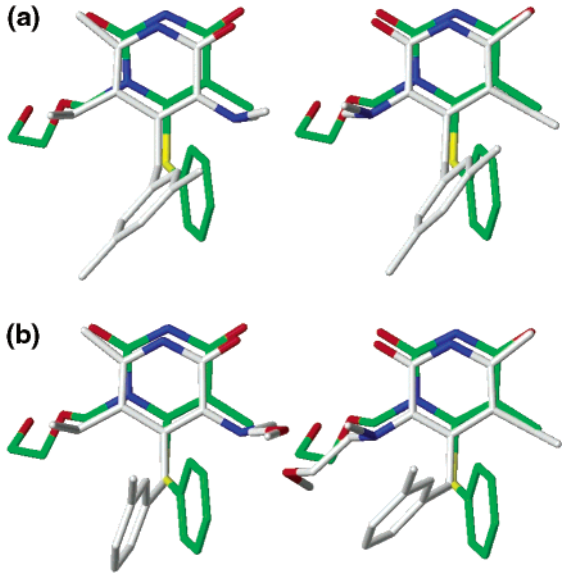


Figure 2. (a) Compound **9** superimposed onto the HEPT crystal structure. Only the six-member ring was used for the alignment. For the sake of clarity, compound **9** was shifted to show matching atoms: left, orientation 1; right, orientation 2. (b) Compound **62** superimposed onto the HEPT crystal structure. Only the six-member ring was used for the alignment. For the sake of clarity, compound **62** was shifted to show matching atoms: left, orientation 1; right, orientation 2.

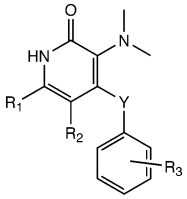
by calculating the nonbounded interactions (steric, electrostatic, and hydrophobic) between a C^+ probe and each atom of the binding site. Leapfrog then built new substituents that matched the required interactions and resulted in a decrease of the estimated binding energy.

Results and Discussion

The pyridinone analogues described in Schemes 1−9 were evaluated in vitro against wild-type HIV-1 (HVTL IIIB, LAI cell line) and against the three principle mutant strains, K103N, Y181C, and Y188L, which confer resistance to the NNRTIs currently used in the clinic.[26] The results are presented in Tables 1 and 2.

From the data in Table 1, one sees that the 5,6-dimethyl compound **18b**, the 6-ethyl/5-methyl compound

Table 1. Activity (IC_{50}, μM) versus HIV-1



					IC_{50} (μM)				
compd	R_1	R_2	R_3	Y	LAI	SI[a]	K103N	Y181C	Y188L
9	CH_3	C_2H_5	3,5-diCH_3	CH_2	0.008	12 589	0.032	0.100	0.251
10	CH_3	C_2H_5	3,5-diCH_3	CO	0.004	2 512	0.010	0.063	0.158
18b	CH_3	CH_3	3,5-diCH_3	CH_2	0.005	1 995	0.398	0.398	3.162
18c	C_2H_5	CH_3	3,5-diCH_3	CH_2	0.010	10 000	0.100	0.251	5.012
18d	CH_3	*i*-C_4H_9	3,5-diCH_3	CH_2	0.050	1 995	0.063	0.158	0.398
18e	*i*-C_3H_7	CH_3	3,5-diCH_3	CH_2	0.794	126	nd[b]	nd[b]	nd[b]
18f	CH_3	*n*-C_3H_7	3,5-diCH_3	CH_2	0.050	1 995	0.079	0.316	0.631
18g	H	H	3,5-diCH_3	CH_2	7.943	10	39.811	100	nd[b]
18h	CH_3	H	3,5-diCH_3	CH_2	0.398	251	31.623	79.43	100
18i	$-(CH_2)_4-$	$-(CH_2)_4-$	3,5-diCH_3	CH_2	0.010	158	0.316	0.501	31.620
20a	CH_3	C_2H_5	3-CH_3	CO	0.0025	39 811	nd[b]	nd[b]	1.995
20b	CH_3	CH_3	3-CH_3	CO	0.016	6 310	0.398	2	50.119
20c	C_2H_5	CH_3	3-CH_3	CO	0.100	1 000	nd[b]	nd[b]	nd[b]
20i	$-(CH_2)_4-$	$-(CH_2)_4-$	3-CH_3	CO	0.063	158	nd[b]	nd[b]	nd[b]
20j	$-(CH_2)_3-$	$-(CH_2)_3-$	3-CH_3	CO	0.016	3 162	nd[b]	nd[b]	nd[b]
31a	CH_3	$(CH_2)_2OCH_3$	3-CH_3	CO	0.004	7 943	0.158	1.26	10
31b	CH_3	$(CH_2)_3OCH_3$	3-CH_3	CO	0.008	1 259	0.126	0.160	0.158
32b	CH_3	$(CH_2)_3OCH_3$	3,5-diCH_3	CO	0.002	12 589	0.01	0.05	0.2
35	CH_2OH	C_2H_5	3,5-diCH_3	CO	0.001	12 589	0.003	0.072	0.631

[a] Selectivity index or ratio of CC_{50} to IC_{50} relative to LAI (fold). [b] nd, not determined.

18c, and the bicyclic compound **18i** are essentially equipotent to lead molecule **9** against wild-type HIV. However, these same molecules display a very low level of activity toward the crucial Y188L mutant. A similar loss in sensitivity toward the Y188L mutant was observed for compound **20b** relative to the reference compound **20a**. These findings suggested that even minor changes in the nature of the 5- and 6-alkyl substituents will strongly influence the positioning of the molecule in the hydrophobic pocket of the Y188L RT mutant. Consistent with this idea, the mono-(C-6)-methyl-substituted analogue **18h** fails against the mutant strains, and the unsubstituted compound **18g** is totally inactive. However, the results for analogues **18d** and **18f** show that modification at C-5 is possible without substantial loss of activity against the Y188L and the two other HIV mutant strains. It is probable that the presence of the 6-methyl substituent in these molecules is important for their activity. Consistent with these observations and using HEPT as a model,[20] the 4-benzoyl-type-pyridinone analogues **31a**, **31b**, and **32b** were all shown to be active at nanomolar concentrations against wild-type HIV. Compound **31a** fails against Y181C and Y188L, but the closely related compound **31b** remains active against all three mutants, and the profile for **32b** is, in fact, better than that for lead molecule **9**.

Contrary to what might be deduced from the data for the C-6-ethyl-substituted compound **18c**, incorporation of a hydroxyl group into the C-6 methyl side chain, as in **35**, did not lead to loss of activity. In fact, this analogue is a potent inhibitor of the three mutant strains and in particular the crucial K103N mutant. Conception of this molecule issues from the following molecular modeling considerations. In HEPT, the N-1 substituent and the C-2 carbonyl are oriented toward a

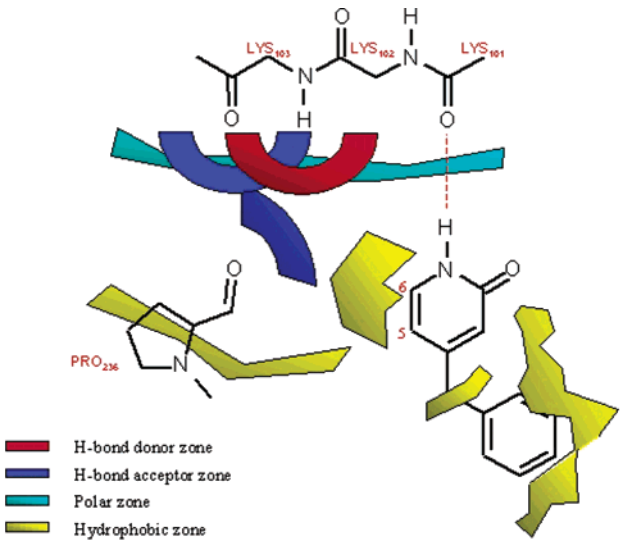
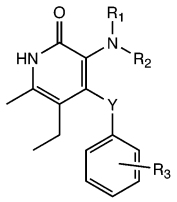


Figure 3. Schematic drawing of the interaction map generated by Leapfrog (white background).

cavity lined by the three lysines K101, K102, and K103 on one hand and by the "P236 hairpin" on the other hand. Examination of the interaction map generated by Leapfrog clearly shows that the "lysine wall" is responsible for a polar interaction surface due to a connection of accessible NH−C=O amide bonds, while the "P236 hairpin" originates a hydrophobic interaction surface due to the presence of hydrophobic side chains (Figure 3). Noteworthy are the electrostatic interaction fields generated by the carbonyl and NH groups of K103 and the carbonyl groups of K101 and P236. In light of this information, the modeling predicted that new pyridinone derivatives bearing a C-6 hydroxymethyl group may be active, since the OH group would point toward

Table 2. Activity (IC_{50}, μM) versus HIV-1



					IC_{50} (μM)				
compd	R_1	R_2	R_3	Y	LAI	SI[a]	K103N	Y181C	Y188L
36	H	CHO	3,5-diCH_3	CH_2	0.079	1 259	0.794	7.943	0.316
37	H	CH_3	3,5-diCH_3	CH_2	0.01	5 012	0.079	1	1.995
38	CH_3	C_2H_5	3,5-diCH_3	CH_2	0.008	3 981	0.079	0.316	1.259
39	CH_3	C_3H_7	3,5-diCH_3	CH_2	0.016	6 310	0.1	0.398	0.398
40	CH_3	$CH(CH_3)CH_2OCH_3$	3,5-diCH_3	CH_2	0.006	7 943	0.079	0.398	0.251
41	CH_3	$(CH_2)_3SCH_3$	3,5-diCH_3	CH_2	0.025	398	1	0.501	nd[b]
42	CH_3	$CH_2CH_2OCH_3$	3,5-diCH_3	CH_2	0.002	19 953	0.063	0.316	0.1
43	CH_3	$(CH_2)_5OH$	3,5-diCH_3	CH_2	0.004	12 589	0.1	0.126	2.512
44	H	$COCH_3$	3,5-diCH_3	CH_2	0.398	251	19.95	25.12	100
45	H	COC_2H_5	3,5-diCH_3	CH_2	3.981	25	100	100	nd[b]
46	H	COC_3H_7	3,5-diCH_3	CH_2	100	1	100	100	nd[b]
47	H	C_2H_5	3,5-diCH_3	CH_2	0.016	126	0.316	100	100
48	H	C_3H_7	3,5-diCH_3	CH_2	0.02	398	1.585	15.85	100
49	H	C_4H_9	3,5-diCH_3	CH_2	0.126	398	1.995	5.012	nd[b]
50	C_2H_5	C_2H_5	3,5-diCH_3	CH_2	0.016	6 310	0.398	1.259	2.512
51	C_4H_9	C_4H_9	3,5-diCH_3	CH_2	50.119	2	100	100	nd[b]
52	H	$CH_2C_6H_5$	3,5-diCH_3	CH_2	0.251	251	3.162	10	nd[b]
53	$CH_2C_6H_5$	$CH_2C_6H_5$	3,5-diCH_3	CH_2	100	1	50.12	79.43	nd[b]
54	$(CH_2CH_2)_2O$	$(CH_2CH_2)_2O$	3,5-diCH_3	CH_2	0.158	251	100	10	nd[b]
55	$(CH_2)_5$	$(CH_2)_5$	3,5-diCH_3	CH_2	0.631	158	63.096	39.81	nd[b]
56	−CH=CH−CH=CH−	−CH=CH−CH=CH−	3,5-diCH_3	CH_2	0.0126	1 259	0.398	1.259	1.995
59	CH_3	$(CH_2)_2OH$	3-CH_3	CH_2	0.005	7 943	0.794	1.259	12.59
60	CH_3	$(CH_2)_3OH$	3-CH_3	CH_2	0.003	31 623	0.316	1.585	5.012
61	CH_3	$(CH_2)_5OH$	3-CH_3	CH_2	0.01	6 310	0.501	1.585	5.012
62	CH_3	$(CH_2)_2OCH_3$	3-CH_3	CH_2	0.001	10 000	0.079	0.794	0.398
63	CH_3	$(CH_2)_2OC_2H_5$	3-CH_3	CH_2	0.013	7 943	nd[b]	nd[b]	nd[b]
64	CH_3	CH_2CN	3-CH_3	CH_2	0.004	25 119	0.316	1.585	12.59
65	CH_3	$(CH_2)_2CN$	3-CH_3	CH_2	0.016	6 310	nd[b]	nd[b]	nd[b]
66	CH_3	$(CH_2)_3CN$	3-CH_3	CH_2	0.005	12 589	0.316	1.995	3.162
67	H	NH−CS−NHC_2H_5	3-CH_3	CH_2	25.119	4	nd[b]	nd[b]	nd[b]
68	H	NH−CS−NHC_6H_5	3-CH_3	CH_2	3.162	25	nd[b]	nd[b]	nd[b]
69	H	NH−CS−$NHCOC_6H_5$	3-CH_3	CH_2	6.31	16	100	100	100
70	H	NH−CS−NH_2	3-CH_3	CH_2	0.316	316	nd[b]	nd[b]	nd[b]
72	CH_3	$(CH_2)_2OCH_3$	3-CH_3	CO	0.001	79 433	0.020	0.631	1.259
77a	CH_3	C_2H_5	3-CH=CHCN	CH_2	0.001	10 000	0.006	0.032	0.251
77b	CH_3	$(CH_2)_2OCH_3$	3-CH=CHCN	CH_2	0.001	10 000	0.02	0.04	0.316

[a] Selectivity index or ratio of CC_{50} to IC_{50} relative to LAI (fold). [b] nd, not determined.

the polar surface and engage in a hydrogen-bonding interaction with one of the accessible backbone amide functions of the lysines or proline. The model was built by complexing the RT crystal structure with the Leapfrog orientation of compound **35**. The complex was minimized using the procedure described above for manual docking. The minimized structure revealed that the hydroxyl group of compound **35** engages in a direct hydrogen bond with the backbone carbonyl of P236 (Figure 4). This became possible through a substantial conformational change of the "P236 hairpin" which is known to be able to enlarge or contract the binding site in order to optimize contacts with the different RT substrates.[20] This interaction along with the crucial pyridinone N-1−H hydrogen-bond with K101 and the hydrophobic stacking interactions made by the dimethylphenyl substituent with the key hydrophobic residues Y181 and W229 provides a good explanation for the strong binding and thus the good activity of **35** against the different HIV strains.

Next, we turn to the analysis of the data in Table 2. It was shown earlier that, whereas the formamide analogue **36**[12] displayed a good level of activity against wild-type HIV (IC_{50} = 0.08 μM), the corresponding *N*-acetyl compound **44** is less active and the *N*-propionyl compound **45** is only marginally active (IC_{50} = 3.98 μM). It was therefore not suprising that the *N*-butyryl analogue **46** was inactive. Further evaluation confirmed that none of these molecules displayed activity against the three mutant strains. The thioureas **67**−**69** were also uninteresting. These results created uncertainty as to whether it would be possible to modify the C-3 amino position such that more bulky aliphatic type substitutents could be present. This doubt was reinforced by the data for the corresponding monosubstituted amines **47**−**49** and compound **52** against the three mutant HIV strains. Similarily, the morpholino/piperidine compounds **54** and **55** and the pyrrole analogue **56** were poorly active against the mutant strains, and the bulky dialkyl-substituted analogues **51** and **53** were devoid of activity even against wild-type HIV.

However, a change in potency began to make itself noticed for the dialkyl analogues **38** and **39** wherein one of the N-3 methyl groups in lead compound **9** was

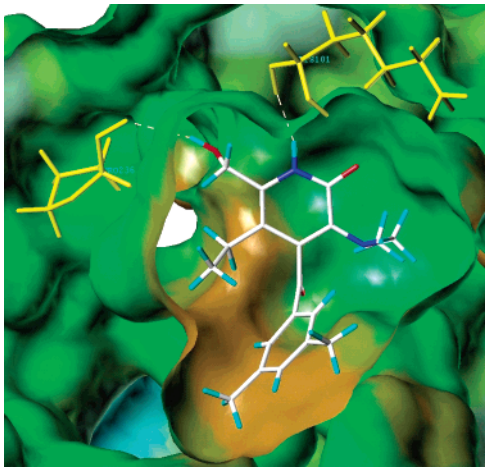


Figure 4. Binding mode of compound **35**. The ligand has been manually docked in the RT according to Leapfrog's orientation, and the complex was fully minimized. The Connolly surface of the NNRTI binding pocket has been clipped off to reveal the binding mode of compound **35**. Especially interesting are the H-bonds made with Lys101 and Pro236 (dashed yellow lines). The molecular lipophilic potential has been calculated and mapped onto the Connolly surface (color coding: from brown (hydrophobic region) to blue (polar region)).

elongated by one and two carbons, respectively. With the exception of a weak activity for **38** against the Y188L mutant, these two compounds displayed submicromolar activities against the three mutant strains. Exploring further, we found that compounds **40** and **42** with a terminal methoxy group were also active, displaying significantly better activity against the Y188L mutant. In a subsequent effort to "adjust" the substituents on the aryl group to accommodate the presence of the large substituents on N-3, compounds **59**−**66** and the 4-benzoyl analogue **72** which all have a single methyl substituent on the aromatic ring were tested. However, the data showed that the compounds **62**, **63**, and **72** containing the ether motif offered no advantage over **40** and **42** against the three mutant strains. Interestingly, analogues **64**−**66** with a terminal cyano substituent failed against the Y181C and Y188L mutations. Similarily, although compounds **59**−**61** with a polar hydroxyl substituent at the end of the alkyl chain were highly active against wild-type HIV, they failed against the mutants.

In view of the positive contribution to the activity provided by the introduction of an ethyl and in particular a 2-methoxyethyl substituent to the N-3 nitrogen, it was of interest to incorporate these modifications into the acrylonitrile lead compound **12b**. As seen, this combination resulted in a very significant increase in potency for compound **77a** relative to **38** against all three mutant strains and in a further 10-fold increase in the potency of **77b** relative to **42** against the Y181C mutant.

The finding that compounds **40**, **42**, and **59**−**66** are active against wild-type HIV at nanomolar concentrations, and further that analogues **40**, **42**, **62**, **72**, and **77b** with the elongated methoxy terminated N-3 side chains display activity against the two crucial Y181C and Y188L mutations present in the hydrophobic pocket of RT, led us to ask whether the activities observed for these analogues and the lead compounds **9** and **10** were not the consequence of two entirely different modes of binding (Figure 1). Indeed, although backbone hydrogen bonding is potentially possible, it was unexpected that compounds **59**−**61** with a polar hydroxyl substituent would bind effectively in the hydrophobic pocket. The idea was born that whereas compounds **9** and **10** most probably bind to RT in a manner very similar to GCA-186 [i.e., where the *N*-dimethyl substituent replaces the GCA-186 5-*i*-Pr group (orientation 1)], the other molecules, bearing longer N-side chains, might bind in a mode that places this N-substituent in the same orientation as the N-1 side chain in HEPT (orientation 2). In this conformation, obtained by a simple 180° rotation about an axis through N-1 and C-4 of the pyridinone ring, the crucial N-1−NH···K101 carbonyl H-bond is retained, while the carbonyl group flips to a new position where it now functions like the C-2 carbonyl in HEPT. These two options were studied for compounds **9** and **62** by molecular modeling using results from HEPT as the starting template.

Visual inspection of the four minimized complexes for **9** and **62** for the two orientations showed no unrealistic structural distortion (Figure 2). Comparison with the starting protein structure revealed that the major movements that occurred upon binding impact residues Y181, Y188, and W229 as well as the position of the "P236 hairpin". These changes are consistent with previous observations highlighting two key features for inhibitory activity, namely, a discrete conformational switch of Y181 and more continuous variations in the position of the "P236 hairpin".[20] During the minimization, a slight shift of the ligands occurred with respect to the HEPT starting position. This was needed to allow the bulkier mono- or dimethylbenzyl moiety versus the more compact thiophenyl substituent of HEPT to fit optimally in the hydrophobic pocket. For both compounds, the protein−ligand interaction pattern in the hydrophobic key residues area in either conformation was found to be compatible with the observed activities. Thus, it was not possible to choose one orientation over the other.

A significant parameter that may help to distinguish between ligand orientations 1 and 2 is the internal energy of the inhibitor−RT complexes. For both compounds, the lowest absolute energy, which translates to enhanced stability, was obtained when the ligand adopted the orientation 1 in the binding site. However, the relative energy difference within each orientation couple was 5.0 and 2.1 kcal/mol for compounds **9** and **62**, respectively. A recent study on the energetics of the conformational changes druglike molecules experience upon binding revealed that approximately 60% of the ligands were calculated to bind with strain energies lower than 5 kcal/mol.[27] Strain energies over 9 kcal/mol were calculated in at least 10% of the cases. A clear correlation was found between acceptable strain energy and ligand flexibility, while there was no correlation between strain energy and binding affinity. This indicates that expensive conformational rearrangements can be tolerated in some cases without penalizing the tightness of the binding. The low-energy differences that we calculated in our case do not permit us to favor one orientation over the other. Moreover, close examination of the X-ray crystal structure of the GCA-186−RT complex (PDB code: 1C1B) shows that the two carbonyl

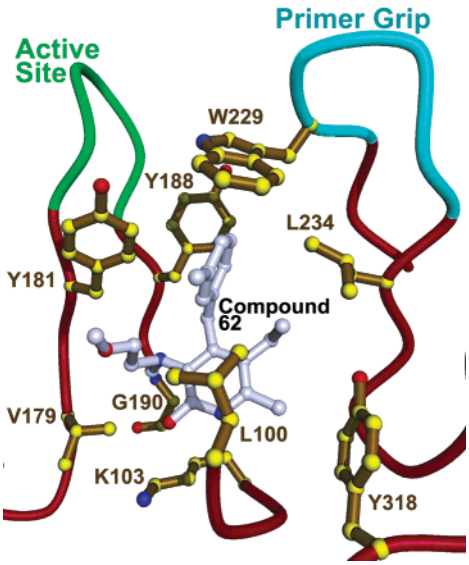


Figure 5. Crystal structure of **62**−RT. Shown is the X-ray crystal structure of compound **62** bound in the NNRTI binding pocket of RT, near the polymerase catalytic site. The ligand conformation, which corresponds to "orientation 1" (see text), allows both the 3-(2-methoxyethyl)methylamino and 4-benzyl substitutents to interact extensively with the hydrophobic core. The (2-methoxyethyl)methylamino group interacts primarily with Y181, while the 4-benzyl ring has extensive contacts with Y188 and W229. Both the Y181 and Y188 side chains point toward the active site to accommodate the ligand. The structure was determined at a resolution of 2.95 Å from a 95.5% complete data set. The R and R_{free} factors, respectively, were 24.6% and 30.7%.[21]

groups of GCA-186 form water-mediated H-bonds with K101 and the K103 backbone amino groups.[14] Although no solvent molecule has been taken into account in our calculations, it can be anticipated that when the ligands adopt the orientation 2 the pyridinone carbonyl group would be ideally positioned to form a similar water-mediated H-bond with K103 lowering further, by approximately 2 kcal/mol, the energy difference between the two orientation patterns.

On the basis of these arguments, neither the structural nor the energetic elements were significant enough to allow us to discriminate between the two ligand orientations. For this reason, the X-ray crystal structure analysis of the **62**−RT complex was obtained for compound **62** complexed with HIV-1 RT (Figure 5), determined to a resolution of 2.95 Å.[21] This structure is consistent with the first orientation hypothesis.

Contacts with Y181 and Y188. Especially striking is the effect of the bulky (2-methoxyethyl)methylamino substituent in the 3-position of the pyridinone ring of compound **62** on the conformation of the Y181 side chain. Compared to its position in the unliganded RT, the side chain of this tyrosine rotates ~100° about the side chain dihedral angle $\chi 1$.[28,29] In the crystal structure of the **62**−RT complex, the (2-methoxyethyl)methylamino substituent at position 3 is aligned with the aromatic 4-benzyl ring and points toward the hydrophobic core of the NNRTI inhibitor binding pocket formed by the amino acid residues Y181, Y188, W229, P95, L100, and L234 (Figure 5).

In this orientation, this long chain nitrogen substituent makes extensive contacts with Y181 that are stabilized by hydrophobic contacts which the terminal methoxy group makes with the p51 residue E138, as well as hydrophobic contacts which the terminal *N*-methyl makes with V179 and G190. From the structure, it is evident that the Y181C mutation would have a significant effect on an RT-bound **62**. Since this part of **62** is interacting primarily with Y181, the Y181C mutation would cause the loss of this inhibitor−protein interaction, resulting in a significant problem of resistance. Accordingly, although compounds **38**, **40**, **42**, **59**−**62**, **64**, and **66** tend to be potent against wild-type RT (probably because of extensive hydrophobic interactions with Y181), all of them are significantly vulnerable to resistance from the Y181C mutation.

The 3′-methylbenzyl group at C-4 of the pyridinone ring has substantial interactions with Y188, W229, and L234. The interactions with W229 receive additional stabilization from a contact which the 3′-methyl group makes with P95. Consistent with this observation, a Y188L mutation offers considerable resistance to compound **62** as well as most other compounds in this series. It is possible, however, to introduce other interactions that compensate for the loss of contacts with Y188.

Compensatory Interactions. Structural studies[30a−e] suggest that contacts with the highly conserved residue W229 and the ability to form hydrophobic stacking interactions with p66 amino acid residues Y181 and/or Y188 are important for NNRTI binding. The substituent pattern on a ligand should permit positioning in the binding pocket so as to optimize these interactions, while it is desirable that the backbone of the compound be flexible enough to permit conformational and positional adaptation in response to mutations of other residues in the pocket.[10] Other compensatory interactions are desirable as well, so that inhibitor activity is not lost when either Y181 or Y188 mutates. At the 5-position of the pyridinone ring, a substituent containing an ethyl or *n*-Pr chain can interact with L234, as seen in the **62**−RT structure. A longer substituent (e.g., the 3-methoxypropyl in compounds **31b** and **32b)** can interact with V106, F227, and P236, similar to what is seen in RT complexes with HEPT-like compound TNK-651,[31] and flexibility can help redefine its orientiation in response to mutations at these positions (106 and 236 are positions for common drug resistance mutations). Compound **31a**, whose 2-methoxyethyl substituent is not long enough to interact extensively with residues V106, F227, and P236, has 10-fold poorer activity against Y181C and Y188L than compound **31b**.

The **62**−RT structure indicates that substituents on the C-6 atom of the pyridinone ring will be positioned to interact with Y318. If either this or the interactions described above for substituents on the 5-position are lost, then ligand binding relies more heavily on contacts of the benzyl ring with Y188 and contacts with Y181, so that mutations at these residues are likely to introduce significant resistance to the inhibitor. Bulky substituents at the 6-position may be unable to interact with Y318 or find other compensatory contacts. Compounds such as **18i**, **20i**, and **20j** that conjoin C-5 and C-6 with a bulky aliphatic ring reduce the backbone flexibility of the ligand. Consequently, these compounds are limited in their ability to adapt to changes in the shape of the pocket such as in a Y188L mutation.

Table 3. Activity (IC_{50}, μM) versus HIV-1

compd	LAI	SI[a]	K103N	Y181C	Y188L	L100I	K101E	V106A	E138K	V179E	G190A	G190S	F227C	L100I + K103N	K101E + K103N	K103N + Y181C	F227L + V106A
31b	0.008	1259	0.126	0.160	0.158	0.020	0.032	0.040	0.020	0.008	0.016	0.032	0.631	0.063	0.158	0.200	0.040
32b	0.002	12 589	0.01	0.05	0.2	0.008	nd[bb]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]
35	0.001	12 589	0.003	0.072	0.631	0.001	0.005	nd[b]	0.004	0.001	0.003	0.002	0.234	0.020	0.007	0.149	0.794
38	0.008	3981	0.079	0.316	1.259	0.063	0.079	0.316	0.063	0.032	0.063	0.316	1.119	0.598	0.139	3.122	nd[b]
40	0.006	7943	0.079	0.398	0.251	0.079	0.058	nd[b]	0.017	0.009	0.078	0.062	0.869	1.000	0.362	8.680	10
42	0.002	19 953	0.063	0.316	0.1	0.003	0.010	nd[b]	0.010	0.003	0.016	0.042	0.339	0.121	0.069	3.344	2.512
62	0.001	10 000	0.079	0.794	0.398	0.006	0.016	0.063	0.008	0.004	0.1	0.039	0.79	0.794	0.1	10	3.981
72	0.001	79 433	0.020	0.631	1.259	0.008	0.003	nd[b]	0.003	0.001	0.003	0.003	0.224	0.017	0.015	2.811	0.251
77a	0.001	10 000	0.006	0.032	0.251	0.010	0.006	0.008	0.006	0.002	0.010	0.125	0.794	0.2	0.158	0.199	3.162
77b	0.001	10 000	0.02	0.04	0.316	0.008	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	0.05	nd[b]	0.398	1
9	0.008	12 589	0.032	0.1	0.251	0.05	0.016	0.04	nd[b]	nd[b]	0.063	nd[b]	nd[b]	nd[b]	nd	0.794	nd[b]
10	0.004	2512	0.01	0.063	0.158	0.006	0.008	0.006	0.005	0.002	0.013	0.004	0.631	0.04	0.013	0.158	0.398
NVP[c]	0.032	5012	6.310	10	100	0.316	0.316	5.012	0.050	0.195	7.943	0.044	0.135	1.452	0.509	100	0.163
EFV[c]	0.001	10 000	0.04	0.002	0.158	0.04	0.006	0.04	0.002	0.005	0.01	0.251	0.158	10	0.158	0.04	0.025

[a] Selectivity index or ratio of CC_{50} to IC_{50} relative to LAI (fold). [b] nd, not determined. [c] NVP, nevirapine; EFV, efavirenz.

Compound **35** is 4-fold more potent than **9** against wild-type RT (Table 1). Compounds **9** and **35** differ only in that **9** has a methyl at the 6-position, whereas **35** has a hydroxymethyl there. The **62**−RT structure suggests that a hydroxymethyl at the 6-position can form a hydrogen bond with the main chain carbonyl of K101. A hydrogen bond between the hydroxyl and K101 is consistent with this improved potency. However, this interaction is not sufficient to improve inhibitory activity against Y181C or Y188L mutants (Table 1).

Having determined that the C-5-modified analogues **31b** and **32b**, the C-6 hydroxymethyl-substituted analogue **35**, the *N*-3 ethyl/methyl analogues **38**, and **77a** and the series of N-modified compounds **40**, **42**, **62**, **72** and **77b** all displayed significant activities against the three key HIV mutant strains, these compounds were further evaluated against the larger panel of HIV mutants. The results are presented in Table 3 along with the values for **9**, **10**, and the two clinically used NNRTI type anti-HIV agents nevirapine and efavirenz.

From the data, one sees that nevirapine has a poor activity profile in vitro against the three crucial mutants K103N, Y181C, and Y188L, whereas the lead molecule **10** and efavirenz are equipotent inhibitors of nearly all the single and double mutant strains present in the panel. Indeed, the only major differences between these two molecules is that the pyridinone **10** is approximately 30 times less potent than efavirenz against the Y181C mutant and 60- and 250-fold more potent than this clinically used NNRTI type anti-HIV agent against the G190S and the L100I + K103N double mutant, respectively. Discussion of the new pyridinone analogues will thus focus mainly on their activity against these three mutant strains as well as against F227C and the three other double mutant strains (right side of Table 3).

Looking first at the IC_{50} values against Y181C, we see that all the new N-3- and C-5/C-6-modified compounds are sub-micromolar inhibitors of this mutant, but only **32b**, **35**, and the doubly modified analogues **77a** and **77b** are interesting relative to efavirenz. Against the important Y188L mutant, the N-3-modified compounds **38** and **72** are weakly active, whereas the other pyridinone analogues are not significantly less potent than efavirenz. Moving further across the table, we find that all the new molecules are equipotent or more active than efavirenz against the G190S mutant. Against the F227C mutant, compound **38** fails, and compounds **40**, **62**, and **77a** are only moderately active inhibitors. In contrast, a small but significant gain in sensitivity toward this mutant was observed for compound **35**, relative to the lead molecule **10**.

Concerning the double mutants, all the new compounds were much more active than efavirenz against the L100I + K103N double mutant, but when compared to **10**, an important loss in sensitivity was observed for the more bulky N-3-modified analogues **38**, **40**, and **62**. Globally, all the N-3 analogues were "comparable" to efavirenz and **10** in their activity toward the K101E + K103N mutant. However, they displayed little or no activity against one or both of the two remaining double mutant strains. In contrast, the C-5-modified analogue **31b** remains active toward these double mutants, suggesting that there is still considerable opportunity to do SAR at this position. Very interesting also was the very pronounced gain in activity for compound **35** relative to both efavirenz and lead compound **10** against the K101E + K103N mutant strain. This observation is consistent with the prediction that the hydroxyl substitutent in **35** interacts with polar residues in the vicinity of amino acids 101−103 in RT.

In summary, the N-3-modified analogues **42**, **77a**, **77b**, and, to a lesser degree, **62** display in vitro activity profiles which are comparable to those for **10** and efavirenz. However, the data for the K103N + 181C and F227L + V106A mutants show that increasing the steric bulk at the N-3 motif has a negative influence on activity. Indeed, these results clearly reveal the "Achilles' heal" to the development of new pyridinone analogues wherein the alkyl substituents on the C-3 nitrogen are modified. More encouraging, however, is that modification of the C-5 substituent in **9** and **10** to give **31b** did not reduce the potency toward these two double mutants. Compound **35** is less active against the F227L + V106A mutant, but like **31b**, this molecule offers considerable promise for future development. This avenue of investigation is being persued.

Experimental Section

Chemistry. General Remarks. All solvents were reagent grade. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone under argon. Acetonitrile and dichloromethane (CH_2Cl_2) were distilled from calcium hydride. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide. *N*,*N*-Dimethylformamide (DMF) was purchased from Aldrich and used without purification unless otherwise noted. All reactions were monitored by thin-layer chromatography (TLC) using E. Merk $60F_{254}$ procoated

silica gel plates. Flash column chromatography was performed with the indicated solvents and using E. Merk silica gel 60 (particle size of 0.035−0.070 mm unless otherwise stated). Melting points were taken on a Kofler melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AC-300 (300 MHz) spectrometer at ambient temperature using an internal deuterium lock. Chemical shifts (δ) were reported in ppm units (s, d, t, q, m, and br for singlet, doublet, triplet, quadruplet, multiplet, and broad, respectively). Elemental analyses, performed by the "Service Central de Microanalyse du CNRS" Gif-sur-Yvette (France), were within 0.4% of the theoretical values calculated for C, H, and N.

Preparation of 2-Oxo-1,2-dihydropyridin-3-ylcarbonitriles 15a−j. 2-Oxo-1,2-dihydropyridin-3-ylcarbonitriles **15a**,[23] **15b**,[22] **15c**,[22] **15f**,[17] **15i**,[24] and **15j**[24] have been described previously. Compounds **15g** and **15h** are commercially available.

6-Methyl-2-oxo-5-isobutyl-1,2-dihydropyridin-3-ylcarbonitrile 15d (via 14d). According to the protocol for **15e** described below, compound **15d** (mp 214 °C, Et_2O) was prepared in 16% overall yield from 5-methylhexan-2-one **13d**.

5-Methyl-2-oxo-6-isopropyl-1,2-dihydropyridin-3-ylcarbonitrile 15e (via 14e). A solution of 2-methylpentan-3-one **13e** (100 g, 1 mol) and HCO_2Et (81.4 g, 1.1 mol) was added slowly at 5 °C to a 30% solution of MeONa in MeOH (210 mL, 1.1 mol) in Et_2O (1000 mL). The mixture was stirred at room temperature for 8 h. The mixture was then concentrated under reduced pressure providing **14e** as a yellow oil (45 g, 30%). This intermediate, in its crude form (0.3 mol), and 2-cyanoacetamide (84 g, 1 mol) were added to a solution of piperidine (63.7 g, 0.75 mol) and HOAc (43 mL, 0.75 mol) in water (1000 mL), and the resultant mixture was refluxed for 8 h. Further HOAc (165 mL) was subsequently added at 0 °C, and the precipitate formed was collected, washed with water, and dried in vacuo. Compound **15e** crystallized from Et_2O as a white solid (44 g, 83%): mp 241 °C.

Preparation of *N*-(5,6-Disubstituted-2-methoxypyridin-3-yl)-2,2-dimethylpropionamides 16a−j. Compounds **16a**,[12] **16g**,[32] and **16h**[33] have been previously described.

***N*-(2-Methoxy-5-methyl-6-isopropylpyridin-3-yl)-2,2-dimethylpropionamide 16e: Example of the General Method. Step 1: Hydrolysis/Decarboxylation.** A solution of compound **15e** (42 g, 0.24 mol) in 6 N HCl (400 mL) was refluxed for 6 days, then poured into ice water and basified with NH_4OH. The precipitate was collected, washed with water, and dried in vacuo to give 5-methyl-6-isopropylpyridin-2(1*H*)-one (33 g, 91%) as a white solid.

Step 2: Nitration. Nitric acid (d 1.52, 16.6 mL, 0.4 mol) was added slowly to a solution of 5-methyl-6-isopropylpyridin-2(1*H*)-one (30 g, 0.2 mol) in 98% H_2SO_4 (300 mL), making sure that the temperature did not exceed 15 °C. The mixture was stirred at 5 °C for 1 h and poured into ice water. The precipitate was collected, washed with water, and dried in vacuo to give 5-methyl-3-nitro-6-isopropylpyridin-2(1*H*)-one (35 g, 90%) as a white powder.

Step 3: Chlorination. $POCl_3$ (47 mL, 0.5 mol) was added slowly at room temperature to a solution of 5-methyl-3-nitro-6-isopropylpyridin-2(1*H*)-one (33 g, 0.168 mol) and benzyltriethylammonium chloride (19.2 g, 0.084 mol) in CH_3CN (350 mL). The mixture was stirred at 80 °C for 8 h, then concentrated to dryness. The residue was taken up in ice water, basified with NH_4OH, and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to give 2-chloro-5-methyl-3-nitro-6-isopropylpyridine as an oil (37 g, 100%).

Step 4: Methoxylation. A solution of 30% NaOMe in MeOH (98 mL, 0.517 mol) was added slowly at room temperature to a solution of 2-chloro-5-methyl-3-nitro-6-isopropylpyridine (37 g, 0.172 mol) in MeOH (350 mL). The mixture was stirred at room temperature for 8 h, then poured into ice water and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated to give 6-methoxy-3-methyl-5-nitro-2-isopropylpyridine (32 g, 88%) as an oil.

Step 5: NO_2 Reduction. A mixture of 6-methoxy-3-methyl-5-nitro-2-isopropylpyridine (20 g, 0.095 mol) in 1/1 MeOH/THF (200 mL) was hydrogenated under 2 atm pressure at room temperature for 1 h using Raney Ni (10 g) as catalyst. 2-Methoxy-5-methyl-6-isopropylpyridin-3-ylamine was obtained as a yellow oil (16.4 g, 96%).

Step 6: N-Pivaloylation. 2,2-Dimethylpropionyl chloride (12 g, 0.1 mol) in CH_2Cl_2 (100 mL) was added at 5 °C to a mixture of 2-methoxy-5-methyl-6-isopropylpyridin-3-ylamine (16.2 g, 0.09 mol) and triethylamine (13.9 mL) in CH_2Cl_2 (100 mL). The mixture was stirred at 5 °C for 1 h. Water (150 mL) was then added, and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to give the compound **16e** as a white solid (24.2 g, 100%): mp 45 °C (Et_2O); ^1H NMR (DMSO-d_6) δ 1.16 (6 H, d, $J = 6.7$ Hz), 1.20 (9 H, s), 2.20 (3 H, s), 3.11 (1 H, m, $J = 6.7$ Hz), 3.88 (3 H, s), 7.79 (1 H, s), 8.38 (1 H, s). Anal. ($C_{15}H_{24}N_2O_2$) C, H, N.

Preparation of 5,6-Disubstituted-3-dimethylamino-4-(3,5-dimethylbenzyl)pyridin-2(1*H*)-ones 18b−i and Compound 9 (via 17a−i). Compound **9** was obtained as previously described.[12]

3-Dimethylamino-4-(3,5-dimethylbenzyl)-5-methyl-6-isopropylpyridin-2(1*H*)-one 18e: Example of the General Method. Step 1: Lithiation/Reaction with $ArCH_2Br$. *n*-BuLi (1.6 M, 83 mL, 132 mmol) was added dropwise at −78 °C under 1 atm of nitrogen to a solution of *N*-(2-methoxy-5-methyl-6-isopropylpyridin-3-yl)-2,2-dimethylpropionamide **16e** (10 g, 38 mmol) and TMEDA (20 mL, 132 mmol) in THF (50 mL). The mixture was stirred at 0 °C for 1 h, then recooled to −78 °C. A solution of bromomethyl-3,5-dimethylbenzene (26.3 g, 132 mmol) in THF (50 mL) was added dropwise. The mixture was stirred at 5 °C for 3 h, then poured into water (100 mL) and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated, and the residue was silica gel column chromatographed (CH_2Cl_2/EtOAc 9/1). *N*-[4-(3,5-Dimethylbenzyl)-6-isopropyl-2-methoxy-5-methyl-pyridin-3-yl]-2,2-dimethylpropionamide (3.7 g, 25%) was obtained as a yellow oil.

Step 2: *N*-Pivaloyl/*O*-Methyl Imidate Cleavage. A solution of the above alkylation product (3.0 g, 8.0 mmol) in 6 N HCl (85 mL) was refluxed overnight, then poured into ice water, basified with solid K_2CO_3, and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was silica gel column chromatographed (CH_2Cl_2/MeOH/NH_4OH 96/4/0.5). 3-Amino-4-(3,5-dimethylbenzyl)-5-methyl-6-isopropyl-pyridin-2(1*H*)-one **18e** was obtained as a white solid (0.45 g, 20%).

Step 3: N-Dimethylation. $NaBH_3CN$ (1.0 g, 15 mmol) was added portionwise at room temperature to a solution of **17e** (1.42 g, 5 mmol) and HCHO (37% in H_2O, 36 mmol) in CH_3CN (35 mL). The mixture was stirred at room temperature for 15 min before slow addition of HOAc (1 mL). The reaction was stirred at room temperature for 8 h, then basified with 10% aqueous K_2CO_3 and extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from CH_3CN provided compound **18e** as a white solid (1.2 g, 77%): mp 182 °C (CH_3CN); ^1H NMR ($CDCl_3$) δ 1.35 (6 H, d, $J = 7.0$ Hz), 1.90 (3 H, s), 2.28 (6 H, s), 2.80 (6 H, s), 3.15 (1 H, m), 4.16 (2 H, s), 6.71 (2 H, s), 6.83 (1 H, s), 11.35 (1 H, br s). Anal. ($C_{20}H_{28}N_2O \cdot 0.33H_2O$) C, H, N.

Preparation of the Compounds 5,6-Disubstituted-3-dimethylamino-4-(3-methylbenzoyl)pyridin-2(1*H*)-ones 20a−c,i,j and 10 (via 19a−c,i,j). Compounds **10** and **20a** were obtained as described previously.[13]

3-Dimethylamino-4-(3-methylbenzoyl)-5,6-dimethylpyridin-2(1*H*)-one 20b: Example of the General Method. Step 1: Lithiation/Condensation with ArCHO. *n*-BuLi (1.6 M, 33 mL, 53 mmol) was added dropwise at −78 °C under 1 atm of nitrogen to a mixture of *N*-(2-methoxy-5,6-dimethylpyridin-3-yl)-2,2-dimethylpropionamide **16b** (3.54 g, 15 mmol) and TMEDA (8 mL, 53 mmol) in THF (50 mL). The mixture was stirred at 0 °C for 1 h and cooled again to −78 °C. A

solution of 3-methylbenzaldehyde (5.5 g, 46 mmol) in THF (50 mL) was added dropwise. The mixture was stirred at 0 °C for 3 h, then poured into water (50 mL) and extracted with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was crystallized from cyclohexane to give *N*-[4-((3-methylphenyl)-hydroxymethyl)-2-methoxy-5,6-dimethylpyridin-3-yl]-2,2-dimethylpropionamide as a white solid (2.9 g, 40%).

Step 2: Oxidation. MnO_2 (10 g) in toluene (54 mL) was added portionwise at reflux to a solution of the above alcohol (8.5 g, 24 mmol) in toluene (100 mL), and the mixture was refluxed for 2 h. The solids were removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. The residue was crystallized from Et_2O providing *N*-[2-methoxy-5,6-dimethyl-4-(3-methylbenzoyl)pyridin-3-yl]-2,2-dimethylpropionamide as a white powder (8.2 g, 95%).

Step 3: *N*-Pivaloyl/*O*-Methyl Imidate Cleavage. A mixture of the derived 4-benzoylpyridinone (7.1 g, 20 mmol) in 6 N HCl (72 mL) was refluxed for 3 h, then poured into ice water and basified with concentrated NH_4OH. The precipitate that formed was collected and taken up in CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was crystallized from Et_2O providing 3-amino-5,6-dimethyl-4-(3-methylbenzoyl)pyridin-2(1*H*)-one **19b** as a white solid (5 g, 96%).

Step 4: N-Methylation. Intermediate **19b** (6.7 g, 26.1 mmol) was dimethylated following the procedure for **18e**. Compound **20b** was obtained as a white solid (2.2 g, 30%): mp 250 °C (Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.82 (3 H, s), 2.35 (3 H, s), 2.44 (3 H, s), 2.64 (6 H, s), 7.33−7.47 (2 H, m), 7.62 (1 H, d, J = 7.0 Hz), 7.73 (1 H, s), 13.20 (1 H, br s). Anal. ($C_{17}H_{20}N_2O_2 \cdot 0.20H_2O$) C, H, N.

5-(3-Methoxypropyl)-6-methyl-2-oxo-1,2-dihydropyridin-3-ylcarbonitrile 23b (via 22b). As described for **14e**, 6-methoxyhexan-2-one **21b**[34] (16 g, 123 mmol) was converted to sodium 3-oxo-2-(3-methoxypropyl)but-1-en-1-olate **22b** (white solid; 6.6 g, 30%). This product was contaminated with small amounts of sodium 3-oxo-7-methoxyhept-1-en-1-olate. The crude mixture of **22b** (13.3 g, 80 mmol) and 2-cyanoacetamide (7.4 g, 88 mmol) in water (150 mL) was added to a solution of piperidine (5.1 g, 60 mmol) and HOAc (3.4 mL, 60 mmol) in water (150 mL), and the resultant mixture was refluxed for 48 h. Further HOAc (10 mL) was then added at 0 °C, and the precipitate formed was collected, washed with H_2O, and dried in vacuo. It was dissolved in CH_2Cl_2, washed with 10% aqueous K_2CO_3, dried over $MgSO_4$, and concentrated. Compound **23b** crystallized from Et_2O as a white solid (5.5 g, 33% yield): mp 172 °C; ^{1}H NMR ($CDCl_3$) δ 1.73−1.86 (2 H, m), 2.47 (3 H, s), 2.54 (3 H, t, J = 7.3 Hz), 3.61 (3 H, s), 3.37 (2 H, t, J = 5.8 Hz), 7.73 (1 H, s), 13.6 (1 H, s).

3-Acetyl-5-(2-methoxyethyl)-6-methylpyridin-2(1*H*)-one 24a (via 22a). As described for **14e**, 5-methoxypentan-2-one **21a**[35] (16 g, 138 mmol) was converted to sodium 3-oxo-2-(2-methoxyethyl)but-1-en-1-olate **22a** (white solid; 6.9 g, 30%). This product was contaminated with small amounts of sodium 3-oxo-6-methoxyhex-1-en-1-olate. The crude mixture of **22a** (21 g, 126 mmol) and 2-acetoacetamide (14 g, 139 mmol) in water (100 mL) was added to a solution of piperidine (8.1 g, 95 mmol) and HOAc (5.5 mL, 95 mmol) in water (100 mL), and the resultant mixture was refluxed for 48 h. Further HOAc (10 mL) was then added at 0 °C, and after 30 min, the mixture was extracted with CH_2Cl_2, dried over $MgSO_4$, and concentrated to afford a residue that was silica gel column chromatographed (CH_2Cl_2/MeOH/NH_4OH 97/3/0.1). Crystallization from 2-propanone afforded **24a** as a white solid (1.5 g, 5%): mp 172 °C; ^{1}H NMR ($CDCl_3$) δ 2.46 (3 H, s), 2.71 (2 H, t, J = 6.6 Hz), 2.72 (3 H, s), 3.34 (3 H, s), 3.52 (2 H, t, J = 6.5 Hz), 8.14 (1 H, s), 13.55 (1 H, s). Anal. ($C_{11}H_{14}N_2O_2$) C, H, N.

3-Acetyl-5-(3-methoxypropyl)-6-methylpyridin-2(1*H*)-one 24b (via 22b). Following the procedure for **24a**, we reacted sodium 3-oxo-2-(3-methoxypropyl)but-1-en-1-olate **22b** (see protocol for **23b**) with 2-acetoacetamide. Compound **24b** was obtained as a white solid (5% yield) after silica gel column chromatography (CH_2Cl_2/MeOH 95/5) and crystallization from acetone: mp 142 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (2 H, m), 2.44 (3 H, s), 2.56 (2 H, t, J = 7.8 Hz), 2.73 (3 H, s), 3.35 (3 H, s), 3.38 (2 H, t, J = 6.0 Hz), 8.13 (1 H, s), 13.45 (1 H, s).

***N*-[2-Methoxy-5-(2-methoxyethyl)-6-methylpyridin-3-yl]-2,2-dimethylpropionamide 28a. Step 1: Oximation.** A solution of **24a** (8.4 g, 40 mmol) and hydroxyamine (2 g, 60 mmol) in ethanol (100 mL) was stirred at room temperature for 2 h. A 10% solution of potassium carbonate (50 mL) was then added, and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from 2-propanone/Et_2O. 3-(1-Hydroxyiminoethyl)-5-(2-methoxyethyl)-6-methylpyridin-2(1*H*)-one **25a** was obtained as a white solid (9 g, 100%): mp 172 °C.

Step 2: Beckmann Rearrangement. A solution of oxime **25a** (8.5 g, 38 mmol) in formic acid (100 mL) was stirred at 100 °C for 1 h. The mixture was then concentrated to dryness, redissolved in CH_2Cl_2, and washed with 10% aqueous K_2CO_3. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from 2-propanone/Et_2O. *N*-[5-(2-Methoxyethyl)-6-methyl-2-oxo-1,2-dihydropyridin-3-yl]-acetamide **26a** was obtained as a white solid (7.1 g, 83%): mp 206 °C.

Step 3: Hydrolysis/Amine Protection. A solution of **26a** (6.7 g, 30 mmol) in 3 N HCl (50 mL) was refluxed for 2 h. The mixture was then concentrated to dryness, and the residue was recrystallized from 2-propanone/Et_2O to give 3-amino-5-(2-methoxyethyl)-6-methylpyridin-2(1*H*)-one (6 g, 52%) as a white solid (mp 160 °C). To this 3-aminopyridinone intermediate (8.9 g, 49 mmol) in CH_2Cl_2 (100 mL) and Et_3N (20.8 mL) was added slowly at 5 °C a solution of 2,2-dimethylpropionyl chloride (5.9 g, 49 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred at room temperature for 1 h. The resulting mixture was washed with 10% K_2CO_3 solution, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from 2-propanone/Et_2O. Compound **27a** was obtained as a white solid (2.5 g, 19%): mp 175 °C; ^{1}H NMR (DMSO-d_6) δ 1.21 (9 H, s), 2.15 (3 H, s), 2.74 (2 H, t, J = 6.5 Hz), 3.24 (3 H, s), 3.40 (2 H, t, J = 6.5 Hz), 8.10 (1 H, s), 8.47 (1 H, s), 11.90 (1 H, s).

Step 4: O-Methylation. A mixture of **27a** (10 g, 38 mmol), MeI (10.6 g, 75 mmol), and Ag_2CO_3 (20.6 g, 75 mmol) in CH_2Cl_2 (100 mL) was stirred at room temperature for 4 days. The mixture was then filtered through Celite, washed with CH_2Cl_2, and concentrated. The residue was silica gel column chromatographed (cyclohexane/EtOAc 90/10). Compound **28a** crystallized from petroleum ether as a white solid (5.1 g, 48%): mp 85 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (9 H, s), 2.36 (3 H, s), 2.74 (2 H, t, J = 6.5 Hz), 3.24 (3 H, s), 3.47 (2 H, t, J = 6.5 Hz), 3.87 (3 H, s), 7.88 (1 H, s), 8.37 (1 H, s).

***N*-[2-Methoxy-5-(3-methoxypropyl)-6-methylpyridin-3-yl]-2,2-dimethylpropionamide 28b.** Compound **28b** (a white solid) was prepared from 3-acetyl-5-(3-methoxypropyl)-6-methylpyridin-2(1*H*)-one **24b** by the four step procedure used to obtain **28a**. The yields for the individual steps were comparable: mp 140 °C (Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.21 (9 H, s), 1.70 (2 H, m), 2.34 (3 H, s), 2.51 (2 H, t, J = 6.5 Hz), 3.25 (3 H, s), 3.31 (2 H, m), 3.87 (3 H, s), 7.85 (1 H, s), 8.35 (1 H, s).

5-(2-Methoxyethyl)-6-methyl-3-dimethylamino-4-(3-methylbenzoyl)pyridin-2(1*H*)-one 31a (via 29a). As for compound **20b**, the 5-(2-methoxyethyl)-substituted pyridin-2(1*H*)-one **31a** was prepared in four steps from **28a**.

Step 1: Lithiation/Condensation with 3-Methylbenzaldehyde. Intermediate **29a** was obtained as a white solid in 60% yield (15 mmol scale).

Step 2: Oxidation. Jones' reagent (20 mmol) was added slowly at 5 °C to a solution of **29a** (4 g, 10 mmol) in 2-propanone (60 mL). The mixture was stirred at 5 °C for 1 h, then poured into ice water, basified using solid K_2CO_3, and filtered through Celite (washing with CH_2Cl_2), and the filtrate was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Crystallization of the residue from Et_2O provided *N*-[2-methoxy-5-(2-methoxyethyl)-6-methyl-4-(3-methylbenzoyl)pyridin-3-yl]-2,2-dimethylpropionamide as a white solid (1.9 g, 47%).

Step 3: *N*-Pivaloyl/*O*-Methyl Imidate Cleavage. 3-Amino-5-(2-methoxyethyl)-6-methyl-4-(3-methylbenzoyl)pyridin-2(1*H*)-one was obtained as a white solid after crystallization from CH_3CN (46%, 8 mmol scale).

Step 4: N-Methylation. Compound **31a** was obtained as a white solid (33%, 8 mmol scale): mp 182 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 1.65 (2 H, s), 2.41 (3 H, s), 2.44 (3 H, s), 2.62 (6 H, s), 3.19 (3 H, s), 3.30 (2 H, s), 7.38−7.42 (2 H, m), 7.65 (1 H, d, J = 7.1 Hz), 7.72 (1 H, s), 12.75 (1 H, br s). Anal. ($C_{19}H_{24}N_2O_3 \cdot 0.50H_2O$) C, H, N.

5-(3-Methoxypropyl)-6-methyl-3-dimethylamino-4-(3-methylbenzoyl)pyridin-2(1*H*)-one 31b (via 29b). Following the protocol for **31a**, we prepared compound **31b** in four steps (43, 95, 100, and 50% yields) from *N*-[2-methoxy-5-(3-methoxypropyl)-6-methylpyridin-3-yl]-2,2-dimethylpropionamide **28b**: mp 172 °C (Et_2O); ^1H NMR (DMSO-d_6) δ 1.29−1.32 (2 H, m), 1.94−2.11 (2 H, m), 2.19 (3 H, s), 2.38 (3 H, s), 2.44 (6 H, s), 3.08 (3 H, s), 3.10−3.15 (2 H, m), 7.38−7.50 (2 H, m), 7.55 (1H, d, J = 8.8 Hz), 7.62 (1 H, s), 11.75 (1 H, br s). Anal. ($C_{20}H_{26}N_2O_3$) C, H, N.

5-(3-Methoxypropyl)-6-methyl-3-dimethylamino-4-(3,5-dimethylbenzoyl)pyridin-2(1*H*)-one 32b (via 30b). Following the protocol for **31a**, we prepared compound **32b** in four steps (46, 100, 60, and 22% yields) from *N*-[2-methoxy-5-(3-methoxypropyl)-6-methylpyridin-3-yl]-2,2-dimethylpropionamide **28b**: mp 210 °C (*i*-Pr_2O); ^1H NMR (DMSO-d_6) δ 1.29−1.57 (2 H, m), 1.81−2.17 (2 H, m), 2.18 (3 H, s), 2.33 (6 H, s), 2.44 (6 H, s), 3.02−3.20 (5 H, m), 7.29 (1 H, s), 7.38 (2 H, s), 11.70 (1 H, br s). Anal. ($C_{21}H_{28}N_2O_3 \cdot 0.20H_2O$) C, H, N.

4-(3,5-Dimethylbenzoyl)-5-(2,2-dimethylpropionylamino)-3-ethyl-6-methoxypyridin-2-ylmethyl Acetate 34. To *N*-[5-ethyl-2-methoxy-6-methyl-4-(3,5-dimethylbenzoyl)pyridin-3-yl]-2,2-dimethylpropionamide **33**[12] (12.3 g, 32 mmol) in CH_2Cl_2 (120 mL) was added *m*-chloroperoxybenzoic acid (11.1 g, 64 mmol) at 5 °C. The mixture was stirred at 5 °C for 1 h, then at room temperature for 3 days. The mixture was poured into water, extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography over silica gel with $CH_2Cl_2/CH_3OH/NH_4OH$ 96/4/0.1 as eluent to afford the intermediate *N*-[4-(3,5-dimethylbenzoyl)-5-ethyl-2-methoxy-6-methyl-1-oxide-pyridin-3-yl]-2,2-dimethylpropionamide (1.2 g, 9%) that was used without further purification in the next step.

The pyridine *N*-oxide obtained above (3.0 g, 7.5 mmol) in acetic anhydride (30 mL) was refluxed for 2 h, then poured out on ice, basified with NH_4OH and extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography over silica gel with cyclohexane/EtOAc 75/25 as eluent to give a product, which was crystallized from CH_3CN to afford the titled compound **34** (1.1 g, 33%) that was used without further purification or characterization in the following steps.

3-Dimethylamino-4-(3,5-dimethylbenzoyl)-5-ethyl-6-hydroxymethylpyridin-2(1*H*)-one 35. 4-(3,5-Dimethylbenzoyl)-5-(2,2-dimethylpropionylamino)-3-ethyl-6-methoxypyridin-2-ylmethyl acetate (**34**, 800 mg, 1.8 mmol) in concentrated HCl (25 mL) was refluxed for 4 h, poured out on ice, basified with solid K_2CO_3 and extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography over silica gel with CH_2Cl_2/CH_3OH 98/2 as eluent to afford 3-amino-4-(3,5-dimethylbenzoyl)-5-ethyl-6-hydroxymethylpyridin-2(1*H*)-one (54 mg, 10%) that was submitted to N-methylation as described for **18e** giving the titled compound **35** (20 mg, 34%) after chromatography over silica gel using CH_2Cl_2/CH_3OH 98/2 as eluent: mp > 200 °C (diethyl ether); ^1H NMR (DMSO-d_6) δ 0.82 (3 H, t, J = 7.6 Hz), 2.28 (6 H, s), 2.50 (2 H, q, J = 7.6 Hz), 3.32 (6 H, s), 4.62 (2 H, br s), 4.88 (1 H, br s), 7.22 (1 H, s), 7.41 (2 H, s), 13.14 (1 H, s). Anal. ($C_{19}H_{24}N_2O_3$) C, H, N.

Preparation of 3-Methylamino-Substituted Pyridinones 38−43. 4-(3,5-Dimethylbenzyl)-5-ethyl-3-(ethylmethylamino)-6-methylpyridin-2(1*H*)-one 38: Example of the General Method. Sodium cyanoborohydride (2.0 g, 32 mmol) was added portionwise at room temperature to a solution of **37**[12] (3.0 g, 10.5 mmol) and acetaldehyde (2.90 mL, 52 mmol) in CH_3CN (70 mL). The mixture was stirred at room temperature for 15 min before slow addition of HOAc (0.1 mL). The reaction was stirred at room temperature for 12 h, then basified with 10% aqueous K_2CO_3 and extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried over $MgSO_4$, and concentrated over reduced pressure. The residue was silica gel column chromatographed (CH_2Cl_2/MeOH 98.5/1.5). The pure fractions were collected, and the solvent was evaporated. Crystallization of the residue from *i*-Pr_2O provided compound **38** as a white solid: mp 170 °C (2.30 g, 70% yield); ^1H NMR ($CDCl_3$) δ 0.93 (6 H, m), 2.27 (11 H, m), 2.71 (3 H, s), 3.09 (2 H, m), 4.15 (2 H, m), 6.69 (2 H, s), 6.81 (1 H, s), 12.80 (1H, br s). Anal. ($C_{20}H_{28}N_2O \cdot 0.20H_2O$) C, H, N.

***N*-[4-(3,5-Dimethylbenzyl)-5-ethyl-6-methyl-2-oxo-1,2-dihydropyridin-3-yl]butyramide 46.** Butyryl chloride (0.95 g, 8.9 mmol) was added dropwise at 10 °C to a suspension of **17a** (2.0 g, 7.4 mmol) and Et_3N (1.55 mL, 11.1 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred for 1 h and poured out into a H_2O and 10% K_2CO_3 solution. The precipitate was filtered off, washed with CH_2Cl_2, and dried. Compound **46** was obtained as a white powder (1.94 g, 77%): mp 250 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 1.01 (6 H, m), 1.76 (2 H, m), 2.36 (13 H, m), 3.96 (2 H, s), 6.65 (2 H, s), 6.85 (1 H, s), 7.02 (1 H, br s), 12.85 (1 H, br s). Anal. ($C_{21}H_{28}N_2O_2$) C, H, N.

3-Butylamino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one 49. A mixture of **46** (1.12 g, 3.3 mmol) in THF (10 mL) was stirred at room temperature under N_2 flow, and $LiAlH_4$ (0.50 g, 13.2 mmol) was added. The mixture was stirred for 15 h and cooled to 5 °C. Water (2 mL) was added dropwise, then CH_2Cl_2 (30 mL) was added. The precipitate was filtered off and washed with CH_2Cl_2. The filtrate was dried ($MgSO_4$) and filtered, and the solvent was evaporated into dryness. The residue was silica gel column chromatographed ($CH_2Cl_2/CH_3OH/NH_4OH$ 98.5/1.5/0.1). Crystallization of the residue from Et_2O provided compound **49** as a white solid (0.50 g, 46%): mp 106 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 0.83 (3 H, t, J = 7.3 Hz), 0.96 (3 H, t, J = 7.3 Hz), 1.24 (2 H, sextuplet, J = 7.3 Hz), 1.45 (2 H, quintuplet, J = 7.3 Hz), 2.28 (11 H, m), 2.91 (2 H, t, J = 7.3 Hz), 3.99 (2 H, s), 4.20 (1 H, br s), 6.75 (2 H, s), 6.84 (1 H, s), 12.80 (1 H, br s). Anal. ($C_{21}H_{30}N_2O$) C, H, N.

In an identical fashion, compounds **44**[12] and **45**[12] were converted to **47** and **48**, respectively.

Compound 47. Yield 45%; mp 137 °C (Et_2O); ^1H NMR (DMSO-d_6) δ 0.95 (3 H, t, J = 7.5 Hz), 1.07 (3 H, t, J = 7.1 Hz), 2.31 (11 H, m), 2.96 (2 H, q, J = 7.5 Hz), 3.99 (3 H, m), 6.74 (2 H, s), 6.84 (1H, s), 12.40 (1 H, br s). Anal. ($C_{19}H_{26}N_2O$) C, H, N.

Compound 48. Yield 42%; mp 108 °C (Et_2O); ^1H NMR (DMSO-d_6) δ 0.73 (3 H, t, J = 7.4 Hz), 0.84 (3 H, t, J = 7.2 Hz), 1.32 (2 H, sextuplet, J = 7.2 Hz), 2.15 (11 H, m), 2.76 (2 H, q, J = 7.2 Hz), 3.86 (2 H, s), 4.20 (1 H, t, J = 7.2 Hz), 6.70 (2 H, s), 6.80 (1 H, s), 11.40 (1 H, br s). Anal. ($C_{20}H_{28}N_2O$) C, H, N.

3-Diethylamino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one 50. As for **51**, compound **50** was prepared from **17a** (55% yield): mp 188 °C (*i*-Pr_2O); ^1H NMR (DMSO-d_6) δ 0.79 (9 H, m), 2.17 (11 H, m), 2.97 (4 H, m), 4.12 (2 H, s), 6.64 (2 H, s), 6.78 (1 H, s), 11.30 (1 H, br s). Anal. ($C_{21}H_{30}N_2O$) C, H, N.

3-Dibutylamino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one 51. Sodium cyanoborohydride (1.4 g, 22.2 mmol) was added portionwise at room temperature to a solution of **17a** (2.0 g, 7.4 mmol) and butyraldehyde (5.30 g, 74 mmol) in MeOH (60 mL). The mixture was stirred at room temperature for 15 min before slow addition of HOAc (0.1 mL). The reaction was stirred at room temperature for 12 h, then basified with 10% aqueous K_2CO_3 and extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was silica gel column chromatographed (CH_2Cl_2/MeOH 97/3). The pure fractions were collected, and the solvent was evaporated. Crystallization of the residue from a small amount

of CH_3OH provided compound **51** as a white solid (2.1 g, 74%): mp 109 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 0.75 (9 H, m), 1.15 (8 H, m), 2.13 (5 H, m), 2.19 (6 H, s), 2.90 (4 H, m), 4.10 (2 H, s), 6.60 (2 H, s), 6.80 (1 H, s), 11.25 (1 H, br s). Anal. ($C_{25}H_{38}N_2O$) C, H, N.

3-Benzylamino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylmethylpyridin-2(1*H*)-one 52 and 3-Dibenzylamino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one 53. Sodium cyanoborohydride (1.5 g, 24 mmol) was added portionwise at room temperature to a solution of **17a** (2.2 g, 8 mmol) and benzaldehyde (1.27 g, 12 mmol) in CH_3CN (50 mL). The mixture was stirred at room temeperature for 15 min before slow addition of HOAc (0.1 mL). The reaction was stirred at room temperature for 12 h, then basified with 10% K_2CO_3 solution. The precipitate was filtered off, washed with H_2O, and dried. The residue was silica gel column chromatographed (CH_2Cl_2/MeOH/NH_4OH 98/2/0.1). The pure fractions were collected, and the solvent was evaporated. Crystallization of the residue from *i*-Pr_2O provided compound **52** as a white solid (1.3 g, 44%): mp 137 °C (*i*-Pr_2O); ^{1}H NMR (DMSO-d_6) δ 0.82 (3 H, t, J = 7.2 Hz), 2.15 (11 H, m), 3.86 (2 H, s), 4.07 (2 H, d, J = 7.2 Hz), 4.70 (1 H, t, J = 7.2 Hz), 6.66 (2 H, s), 6.80 (1 H, s), 7.20 (5 H, m), 11.40 (1 H, br, s). Anal. ($C_{24}H_{28}N_2O\cdot 0.25H_2O$) C, H, N.

The mother layer was extracted with CH_2Cl_2, the organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/MeOH/NH_4OH 98/2/0.1). Crystallization of the residue from *i*-Pr_2O provided compound **53** as a white solid (0.55 g, 15%): mp 188 °C (*i*-Pr_2O); ^{1}H NMR (DMSO-d_6) δ 0.59 (3 H, t, J = 7.1 Hz), 2.00 (2 H, q, J = 7.1 Hz), 2.10 (9 H, s), 3.70 (2 H, s), 4.13 (4 H, s), 6.44 (2 H, s), 6.70 (1 H, s), 7.15 (10 H, m), 11.45 (1 H, br s). HRMS: calcd for $C_{31}H_{34}N_2O$ $(MH)^+$ *m/z* 451.1275; found, 451.1275.

4-(3,5-Dimethylbenzyl)-5-ethyl-6-methyl-3-(morpholin-4-yl)pyridin-2(1*H*)-one 54. Sodium cyanoborohydride (2.1 g, 33 mmol) was added slowly to a solution of **17a** (3.0 g, 11 mmol) and 2,2′-oxybis(acetaldehyde), prepared in situ from 1,4-anhydroerythritol[36] (1.12 g, 11 mmol; 0.4 M in H_2O/CH_3CN). After addition, HOAc (0.8 mL) was added. The mixture was stirred at room temperature for 2 h, poured out into H_2O, and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/MeOH/NH_4OH 97/3/0.1). The pure fractions were collected, and the solvent was evaporated. Crystallization of the residue from Et_2O/petroleum ether provided compound **54** as a white solid (1.32 g, 35%): mp 163 °C (Et_2O/petroleum ether); ^{1}H NMR (DMSO-d_6) δ 0.81 (3 H, t, J = 7.2 Hz), 2.18 (11 H, m), 2.25 (2 H, m), 3.60 (6 H, m), 4.00 (2 H, s), 6.65 (2 H, s), 6.76 (1 H, s), 11.40 (1 H, br s). Anal. ($C_{21}H_{28}N_2O_2\cdot 0.50H_2O$) C, H, N.

4-(3,5-Dimethylbenzyl)-5-ethyl-6-methyl-3-(piperidin-1-yl)pyridin-2(1*H*)-one 55. Sodium cyanoborohydride (465 mg, 7.4 mmol) was added slowly to a solution of **17a** (2.0 g, 7.4 mmol) and glutaraldehyde (2.22 g, 22.2 mmol) in CH_3CN (50 mL). After addition, HOAc (0.1 mL) was added. The mixture was stirred at room temperature for 2 h, poured out into H_2O, and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/MeOH/NH4OH 97/3/0.1). The pure fractions were collected, and the solvent was evaporated. Crystallization of the residue from CH_3OH provided compound **55** as a white solid (0.88 g, 33%): mp 208 °C (CH_3OH); ^{1}H NMR (DMSO-d_6) δ 0.81 (3 H, t, J = 7.1 Hz), 1.37 (6 H, m), 2.12 (3 H, s), 2.19 (6 H, s), 2.20 (2 H, m), 2.51 (2 H, m), 3.39 (2 H, m), 3.99 (2 H, s), 6.68 (2 H, s), 6.80 (1 H, s), 11.30 (1 H, br s). Anal. ($C_{22}H_{30}N_2O$) C, H, N.

4-(3,5-Dimethylbenzyl)-5-ethyl-6-methyl-3-(pyrrol-1-yl)pyridin-2(1*H*)-one 56. 2,5-Dimethoxytetrahydrofuran (1.36 g, 10.3 mmol) was added dropwise at 0 °C to a stirred suspension of **17a** (2.33 g, 8.6 mmol) in HOAc (40 mL). The mixture was refluxed for 2 h, cooled, and poured out into H_2O, NH_4OH, and ice. The precipitate was filtered off and taken up in CH_2Cl_2 while stirring. The mixture was filtered and washed with CH_2Cl_2. The filtrate was dried ($MgSO_4$) and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/CH_3OH/NH_4OH 97.5/2.5/0.2). The desired fractions were collected, and the solvent was evaporated. Crystallization from 2-propanone and *i*-Pr_2O provided compound **56** as an oil (0.87 g, 32%): ^{1}H NMR (DMSO-d_6) δ 0.81 (3 H, t, J = 7.3 Hz), 2.19 (11 H, m), 3.53 (2 H, s), 6.07 (2 H, s), 6.59 (4 H, m), 6.79 (1 H, s), 11.90 (1 H, br s). Anal. ($C_{21}H_{24}N_2O$) C, H, N.

5-Ethyl-6-methyl-3-(methylamino)-4-(3-methylbenzyl)-pyridin-2(1*H*)-one 58. A mixture of **57**[13] (20 g, 78 mmol) in ethyl formate (460 mL) was stirred at room temperature for 15 min, then HOAc (250 mL) was added, and the mixture was refluxed for 4 h. The solvent was evaporated till dryness, and the residue was taken up in H_2O, basified with a concentrated NH_4OH solution, and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/CH_3OH/NH_4OH 92/8/0.1). Crystallization of the residue from Et_2O provided the formamide intermediate as a white powder (20.4 g, 92%) which was treated by addition portionwise at room temperature of $LiAlH_4$ (7.7 g, 200 mmol) in THF. The mixture was stirred at room temperature for 3 h, and H_2O (8 mL) was added dropwise at 0 °C. The organic layer was separated, washed with CH_2Cl_2, and dried. Crystallization from Et_2O provided (9.0 g, 46%) of compound **58** as a white solid: mp 204 °C (Et_2O); ^{1}H NMR (DMSO-d_6) δ 0.85 (3 H, t, J = 7.4 Hz), 2.12 (5 H, m), 2.26 (3 H, s), 2.67 (3 H, s), 4.15 (2 H, s), 4.31 (1 H, s), 7.41 (4 H, m), 12.75 (1 H, br s).

Preparation of 3-Methylamino-Substituted Pyridinones 59−63. 4-(3-Methylbenzyl)-5-ethyl-3-[*N*-(3-hydroxypropyl)-*N*-methyl]amino-6-methylpyridin-2(1*H*)-one 60: **Example of the General Method.** Sodium cyanoborohydride (1.38 g, 22 mmol) was added portionwise at room temperature under N_2 flow to a mixture of **58** (2.0 g, 7.4 mmol) and 3-hydroxypropionaldehyde[37] (1.1 g, 18.5 mmol) in CH_3CN (50 mL). The mixture was stirred for 5 h, then H_2O was added, and the mixture was extracted with AcOEt. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/CH_3OH 94/6). Crystallization of the residue from Et_2O/*i*-Pr_2O provided compound **60** as a white powder (500 mg, 20%): mp 110 °C (Et_2O/*i*-Pr_2O); ^{1}H NMR (DMSO-d_6) δ 0.79 (3 H, m), 1.43 (2 H, s), 2.16 (8 H, m), 2.54 (3 H, s), 2.98 (2 H, m), 3.32 (2 H, s), 4.14 (2 H, m), 4.26 (1 H, s), 6.82 (1 H, m), 6.88 (1 H, m), 6.95 (1 H, m), 7.14 (1 H, m), 11.30 (1 H, br s). Anal. ($C_{20}H_{28}N_2O_2\cdot 0.20H_2O$) C, H, N.

***N*-{[4-(3-Methylbenzyl)-5-ethyl-6-methyl-2-oxo-1,2-dihydropyridin-3-yl]}-4-(methylamino)butyronitrile 66.** A mixture of **58** (3.0 g, 11 mmol), 4-chlorobutyronitrile (1.35 g, 13 mmol), and Et_3N (1.8 mL, 13 mmol) in DMF (30 mL) was stirred at 100 °C for 48 h, poured out into H_2O, and extracted with AcOEt. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/ 2-propanol 95/5). Crystallization of the residue from Et_2O/*i*-Pr_2O provided compound **66** as a white powder (100 mg, 3%): mp 130 °C (Et_2O/*i*-Pr_2O); ^{1}H NMR ($CDCl_3$) δ 0.94 (3 H, t, J = 7.4 Hz), 1.70 (2 H, m), 2.12 (2 H, m), 2.33 (8 H, m), 2.65 (3 H, s), 3.20 (2 H, m), 4.17 (2 H, s), 6.87 (2 H, m), 7.03 (1 H, d, J = 7.5 Hz), 7.14 (1 H, m), 12.65 (1 H, br s). Anal. ($C_{21}H_{27}N_3O$) C, H, N.

Compounds **64** and **65** were similarly prepared from **58** through reaction with chloroacetonitrile and 3-chloropropionitrile, respectively.

Compound 64. Yield 28%; ^{1}H NMR (DMSO-d_6) δ 0.78 (3 H, t, J = 7.2 Hz), 2.16 (3 H, s), 2.24 (5 H, s), 2.61 (3 H, s), 4.04 (2 H, s), 4.11 (2 H, s), 6.84 (1 H, m), 6.99 (2 H, m), 7.13 (1 H, m), 11.60 (1 H, br s). Anal. ($C_{19}H_{23}N_3O$) C, H, N.

Compound 65. Yield 5%; mp 166 °C (Et_2O/petroleum ether); ^{1}H NMR (DMSO-d_6) δ 0.97 (3 H, t, J = 7.4 Hz), 2.29 (10 H, m), 2.67 (3 H, s), 3.38 (2 H, m), 4.20 (2 H, m), 6.89 (2 H, m), 7.02 (1 H, m), 7.16 (1 H, m), 13.07 (1 H, br s). Anal. ($C_{20}H_{25}N_3O$) C, H, N.

1-Ethyl-3-[4-(3-methylbenzyl)-5-ethyl-6-methyl-2-oxo-1,2-dihydropyridin]-3-ylthiourea 67. According to the protocol for **68**, compound **67** was obtained by reaction of **57** with ethylisothiocyanate: yield 34%; mp 112 °C (Et_2O); ^1H NMR (DMSO-d_6) δ 0.74 (3 H, t, J = 7.3 Hz), 1.02 (3 H, t, J = 7.1 Hz), 2.19 (8 H, m), 3.42 (2 H, m), 3.80 (2 H, s), 6.98 (3 H, m), 7.09 (1 H, m), 7.45 (1 H, br s), 8.45 (1 H, br s), 11.65 (1 H, br s). Anal. ($C_{19}H_{25}N_3OS$) C, H, N.

1-[4-(3-Methylbenzyl)-5-ethyl-6-methyl-2-oxo-1,2-dihydropyridin]-3-yl-3-phenylthiourea 68. A mixture of **57** (1.9 g, 7.4 mmol) and phenylisothiocyanate (1.2 g, 8.9 mmol) in THF was stirred and refluxed for 20 h, cooled to room temperature, poured out into H_2O, and extracted with EtOAc. The precipitate was filtered off and dried. Crystallization of the residue from H_2O/EtOAc provided compound **68** as a white solid (1.2 g, 41%): mp 176 °C (H_2O/AcOEt); ^1H NMR (DMSO-d_6) δ 0.78 (3 H, t, J = 7.2 Hz), 2.25 (8 H, m), 3.90 (2 H, s), 7.02 (3 H, m), 7.25 (6 H, m), 8.80 (1 H, br s), 9.65 (1 H, br s), 11.70 (1 H, br s). Anal. ($C_{23}H_{25}N_3OS$) C, H, N.

1-Benzoyl-3-[4-(3-methylbenzyl)-5-ethyl-6-methyl-2-oxo-1,2-dihydropyridin]-3-ylthiourea 69. Benzoyl chloride (9.8 g, 70 mmol) was added on stirring at room temperature to a solution of ammonium thiocyanate (5.32 g, 70 mmol) in 2-propanone (200 mL), and the mixture was refluxed for 30 min. Aminopyridinone **57** (15.4 g, 60 mmol) was added slowly portionwise, and the mixture was refluxed for 3 h, then basified with 10% K_2CO_3. The precipitate was filtered off, washed with H_2O, and dried. Crystallization of the residue from 2-propanone provided compound **69** as a white solid (17.9 g, 71%): mp 237 °C (2-propanone); ^1H NMR (DMSO-d_6) δ 0.82 (3 H, t, J = 7.3 Hz), 2.22 (8 H, m), 3.86 (2 H, s), 6.93 (3 H, m),7.10 (1 H, m), 7.52 (2 H, m), 7.62 (1 H, m), 7.95 (2 H, m), 11.62 (1 H, s), 11.66 (1 H, s), 11.79 (1 H, br s). Anal. ($C_{24}H_{25}N_3O_2S$) C, H, N.

[4-(3-Methylbenzyl)-5-ethyl-6-methyl-2-oxo-1,2-dihydropyridin]-3-ylthiourea 70. A mixture of **69** (2.05 g, 4.9 mmol) in 3 N NaOH (30 mL) was stirred and refluxed for 8 h, poured out on ice, basified with NH_4OH, and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. Crystallization of the residue from pentane provided compound **70** as a white solid (210 mg, 14%): mp 122 °C (pentane); ^1H NMR (DMSO-d_6) δ 0.74 (3 H, t, J = 7.2 Hz), 2.21 (8 H, m), 3.80 (2 H, m), 6.99 (4 H, m), 7.10 (2 H, m), 8.65 (1 H, br s), 11.65 (1 H, br s). Anal. ($C_{17}H_{21}N_3Os$) C, H, N.

5-Ethyl-6-methyl-3-methylamino-4-(3-methylbenzoyl)-pyridin-2(1*H*)-one 71. A mixture of **19a**[13] (20.3 g, 75 mmol) in ethyl formate (460 mL) was stirred at room temperature for 15 min. Acetic acid (248 mL) was added, then the mixture was refluxed for 3 h. The solvent was evaporated till dryness, and the residue was taken up in H_2O. The mixture was basified with concentrated NH_4OH solution and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (eluent: CH_2Cl_2/CH_3OH/NH_4OH 99/1/0.1 and 80/20/0.1). The pure fractions were collected, and the solvent was evaporated. The residue was crystallized from 2-propanone/diethyl ether giving the *N*-formyl intermediate. $LiAlH_4$ (8.2 g, 210 mmol) was added portionwise at room temperature to this intermediate in THF, and the mixture was stirred at room temperature for 3 h, cooled, poured out into H_2O (24 mL) and 15% NaOH (8 mL), then filtered over Celite, washed with CH_2Cl_2, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was silica gel column chromatographed (CH_2Cl_2/CH_3OH/NH_4OH 94/5/0.5). The pure fractions were collected to provide compound **71** as a white powder (1.0 g, 5%) which was directly transformed into **72** without any further purification.

5-Ethyl-3-[(2-methoxyethyl)methylamino]-6-methyl-4-(3-methylbenzoyl)pyridin-2(1*H*)-one 72. Sodium cyanoborohydride (630 mg, 10 mmol) was added portionwise at room temperature to a solution of **71** (1.0 g, 3.5 mmol) and methoxyacetaldehyde (4.27 M in H_2O, 4.12 mL, 17.6 mmol) in CH_3CN (10 mL). The mixture was stirred at room temperature for 8 h, basified with 10% K_2CO_3, and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (CH_2Cl_2/CH_3OH/NH_4OH 95/5/0.5). The pure fractions were collected, and the solvent was evaporated. The residue was purified again by column chromatography over C 18 (eluent, CH_3OH/H_2O 65/35; column, KROMASIL C 18 3.5 μm). The pure fractions were collected, and the solvent was evaporated. The residue was crystallized from petroleum ether. The precipitate was filtered off and dried to provide compound **72** as a white solid (210 mg, 18%): mp 126 °C (petroleum ether); ^1H NMR ($CDCl_3$) δ 0.97 (3 H, t, J = 7.5 Hz), 2.37 (5 H, m), 2.43 (3 H, s), 2.61 (3 H, s), 3.15 (7 H, m), 7.38 (2 H, m), 7.60 (1 H, d, J = 9.0 Hz), 7.70 (1 H, s), 13.20 (1H, br s). Anal. ($C_{20}H_{26}N_2O_3$) C, H, N.

3-{3-[5-Ethyl-3-(ethylmethylamino)-6-methyl-2-oxo-1,2-dihydropyridin-4-ylmethyl]-phenyl}acrylonitrile 77a.
Step 1: N-Formylation. A solution of the brominated 3-aminopyridinone **73**[13] (20.0 g, 62 mmol) in ethyl formate (500 mL) and formic acid (100 mL) was refluxed for 8 h, then poured into ice water, and basified using concentrated NH_4OH. The precipitate was filtered off, providing the formamide intermediate (21.5 g, 98%) as a pale yellow solid.

Step 2: Reduction with $LiAlH_4$. $LiAlH_4$ (7.0 g, 185 mmol) was added portionwise at 5 °C to a solution of the above formamide intermediate (21.5 g, 62 mmol) in THF (300 mL). The mixture was stirred at 5 °C for 2 h, then hydrolyzed with water in the presence of EtOAc. The precipitate was filtered off, and the filtrate was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under vacuum. 4-(3-Bromobenzyl)-5-ethyl-6-methyl-3-methylaminopyridin-2(1*H*)-one **74** (11.0 g, 53%) was obtained as a white solid.

Step 3: Reductive Amination. Sodium cyanoborohydride (1.7 g, 27 mmol) was added at room temperature to a solution of the 3-aminopyridinone **74** (3.0 g, 9 mmol) and acetaldehyde (2.5 mL, 45 mmol) in acetonitrile (65 mL). Acetic acid (1.0 mL) was added, and the reaction was stirred at room temperature for 8 h. The mixture was poured into H_2O, basified with 10% aqueous K_2CO_3, and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was silica gel column chromatographed (CH_2Cl_2/CH_3OH 95/5) providing 4-(3-bromobenzyl)-5-ethyl-3-(ethylmethylamino)-6-methylpyridin-2(1*H*)-one **75a** (2.6 g, 80%) as a white solid.

Step 4: Formylation via Metalation. *n*-Butyllithium (18 mmol) was added dropwise at −70 °C to a solution of **75a** (2.6 g, 7.2 mmol) in THF (50 mL) under nitrogen. The mixture was stirred at −10 °C for 30 min; DMF (5.5 mL, 72 mmol) was added slowly. The mixture was stirred at −10 °C for 1 h. This was followed by addition at room temperature of H_2O and extraction with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was silica gel column chromatographed (CH_2Cl_2/CH_3OH/NH_4OH 99/1/0.1 to 85/15/2) providing 3-[5-ethyl-3-(ethylmethylamino)-6-methyl-2-oxo-1,2-dihydro-pyridin-4-ylmethyl]-benzaldehyde **76a** (1.7 g, 79%) as a pale yellow solid.

Step 5: Wittig−Horner Reaction. Potassium terbutoxide (0.8 g, 6.8 mmol) was added at 5 °C under nitrogen to a solution of diethyl diethylcyanophosphonate (1.1 mL, 6.8 mmol) in THF (20 mL), and the mixture was stirred at 5 °C for 30 min. A solution of the benzaldehyde derivative **76a** (1.7 g, 5.7 mmol) in THF (20 mL) was added dropwise, and the reaction was stirred at room temperature for 8 h before addition of H_2O and extraction with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was silica gel column chromatographed (cyclohexane/*i*-PrOH 87/13), and the concentrated product fractions were crystallized from petroleum ether, providing the titled compound **77a** (0.26 g, 13%) as a pale yellow solid: mp 196 °C; ^1H NMR (DMSO-d_6) δ 0.70−0.89 (6 H, m), 2.14 (3 H, s), 2.21 (2 H, t, J = 7.1 Hz), 2.55 (3 H, s), 2.95 (2 H, m), 4.14 (2 H, s), 6.40 (1 H, d, J = 16.7 Hz), 7.09 (1 H, d, J = 7.5 Hz), 7.28−7.40

(2 H, m), 7.49 (1 H, d, $J = 7.8$ Hz), 7.63 (1 H, d, $J = 16.7$ Hz), 11.4 (1 H, br s). Anal. ($C_{21}H_{25}N_3O$) C, H, N.

3-(3-{5-Ethyl-3-[(2-methoxyethyl)methylamino]-6-methyl-2-oxo-1,2-dihydropyridin-4-ylmethyl}phenyl)-acrylonitrile 77b. This compound was obtained in three steps (36, 41, and 10% yields) from methylaminopyridinone **74** in the same manner to that described above for the parent compound **77a**: mp 169 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, t, $J = 7.3$ Hz), 2.28−2.40 (5 H, m), 2.65 (3 H, s), 3.22−3.37 (7 H, m), 3.93−4.56 (2 H, m), 5.86 (1 H, d, $J = 16.7$ Hz), 7.16 (1 H, d, $J = 6.6$ Hz), 7.20 (1 H, s), 7.24−7.32 (2 H, m), 7.38 (1 H, d, $J = 16.7$ Hz), 12.6 (1 H, br s). Anal. ($C_{22}H_{27}N_3O_2$) C, H, N.

Biology. Evaluation of Antiviral Activity of the Compounds. Cells and Viruses. MT4 cells are human T-lymphoblastoid cells that are highly sensitive to HIV infection, producing a rapid and pronounced cytopathic effect. All cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics in a humidified incubator with a 5% CO_2 atmosphere at 37 °C.

Site-Directed Mutants. Mutant RT coding sequences were generated from a pGEM vector containing the HIV-1 LAI (clone HXB2) protease (PR) and the RT coding sequence, using the QuikChange site-directed mutagenesis kit (Stratagene) and HPLC-purified primers (Genset Oligos). Plasmids were checked by sequencing to confirm that they contained the desired mutations. Mutant viruses were created by recombination of the mutant PR−RT sequence with a PR−RT deleted HIV-1 HXB2 proviral clone.[26]

Drug Sensitivity Assays. The antiviral activity of compounds against laboratory adapted strains, site-directed mutants, and clinical sample derived recombinant viruses was tested using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay as previously described.[26,38] Briefly, various concentrations of the test compounds were added to wells of a flat-bottom microtiter plate. Subsequently, virus and MT4 cells were added to a final concentration of 200 $CCID_{50}$/well and 30 000 cells/well, respectively. To determine the toxicity of the test compound, mock-infected cell cultures containing an identical compound concentration range were incubated in parallel with the virus-infected cell cultures. After 5 days of incubation (37 °C, 5% CO_2), the viability of the cells was determined using MTT. The results of drug susceptibility assays were expressed as an EC_{50} defined as the concentration of drug at which there was 50% infection compared with the drug-free control. In some cases a fold change in susceptibility was calculated by dividing the EC_{50} for the tested virus by the EC_{50} for the wild-type virus (HIV-1 LAI) tested in parallel. Toxicity results are expressed as CC_{50}, defined as the concentration of drug at which the cell viability was reduced by 50% compared to the drug-free control.

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Supporting Information Available: Synthetic procedure and intermediate and final product characterzation. This material is available free of charge via the Internet at http://pubs.acs.org.

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