Synthesis and Antiviral Activity of 4-Benzyl Pyridinone Derivatives as Potent and Selective Non-Nucleoside Human Immunodeficiency Virus Type 1 Reverse Transcriptase Inhibitors

Valérie Dollé,[†] Chi Hung Nguyen,*[,†] Michel Legraverend,[†] Anne-Marie Aubertin,[‡] André Kirn,[‡] Marie Line Andreola,[§] Michel Ventura,[§] Laura Tarrago-Litvak,[§] and Emile Bisagni[†]

UMR 176 CNRS−Institut Curie, Section de Recherche, Bitiment 110, 15 rue Georges Climenceau, 91405 Orsay, France, INSERM U74, Institut de Virologie, 3 rue Koeberlé, 67000 Strasbourg, France, and EP-REGER, CNRS−Université Victor Segalen Bordeaux 2, IFR 66 Pathologies Infectieuses, 1, rue Camille Saint Saëns, 33077 Bordeaux Cedex, France

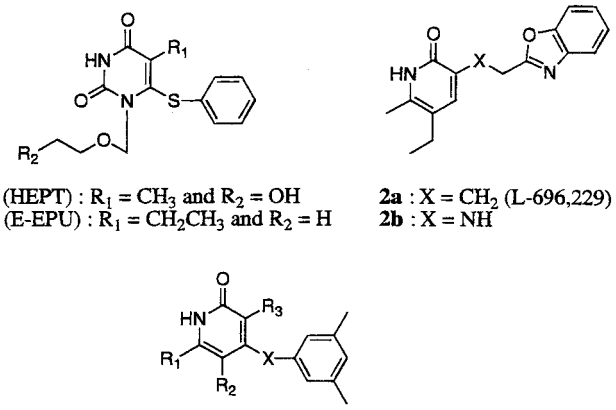
Received March 20, 2000

Several 4-benzyl analogues of 5-ethyl-6-methyl-4-(phenylthio)pyridin-2(1*H*)-ones were synthesized and evaluated for their anti-HIV-l activities. Key transformations include metalation at the 4-C-position of 5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridine (**5**) and its coupling with benzyl bromide or benzaldehyde derivatives. Biological studies revealed that some of the new 4-benzylpyridinones show potent HIV-1 specific reverse transcriptase inhibitory properties. Compounds **14**, **19**, and **27**, which inhibit the replication of HIV-1 in CEM-SS cells, with IC_{50} values ranging from 0.2 to 6 nM are the most active compounds in this series. Biochemical studies showed that compound **27** strongly inhibited the activity of a recombinant HIV-1 RT. Moreover, the infectivity of isolated HIV-1 particles was severely decreased after exposure to compound **27**. Although cross resistance is frequently observed between non-nucleoside reverse transcriptase inhibitors, compound **27** was capable of inhibiting a virus resistant to nevirapine with an IC_{50} of 40 nM.

Introduction

Our continuing interest in the search for new non-nucleoside human immunodeficiency virus type 1 (HIV-1) specific reverse transcriptase inhibitors led us recently to describe the synthesis and potent biological activities of a new series of variously substituted 4-(phenylthio)pyridin-2(1*H*)-ones **3a**,[1] which can be considered as HEPT-pyridinone[2−9] hybrid molecules. However, chemical instability of 4-(phenylthio)pyridin-2(1*H*)-ones derivatives **3a**, under basic conditions (for example, CH_3ONa/CH_3OH), leads to complex degradation mixtures and the loss of the thiophenyl group. Therefore, our concern was to obtain more chemically stable analogues in which the sulfur atom would be replaced by a methylene group (Figure 1, **3b**). Since for the 4-phenylthio series, the structure−activity relationship (SAR) studies showed that the 5-ethyl and 6-methyl substituents led to the best anti-HIV-1 activities (IC_{50} values ranging from 6 to 90 nM), 3-substituted-4-benzyl-5-ethyl-6-methylpyridinones were selected as new target molecules.

In this paper we report the synthesis and biological properties of these 4-benzylpyridinone derivatives **3b**.



1a (HEPT) : $R_1 = CH_3$ and $R_2 = OH$
1b (E-EPU) : $R_1 = CH_2CH_3$ and $R_2 = H$

2a : $X = CH_2$ (L-696,229)
2b : X = NH

3a : X = S; R_1= H, CH_3; R_2 = CH_3, CH_2CH_3; R_3 = NO_2, NH_2, CO_2Et ...
3b : X = CH_2; R_1= CH_3; R_2 = CH_2CH_3; R_3 = NH_2, $N(CH_3)_2$, NHCHO ...

Figure 1.

Chemistry

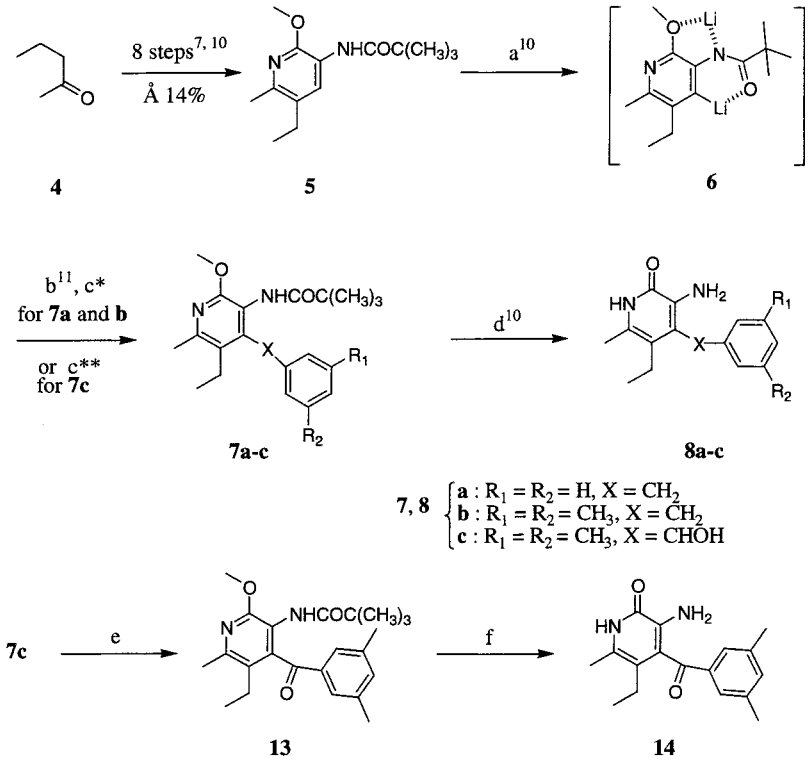
Starting from pentan-2-one (**4**), the synthesis of the intermediate substituted-pyridine **5**, bearing a pivaloylamino *ortho* directing metalation group at its 3-position, was performed with a 8-step sequence as previously described[7,10] and a 14% overall yield. Our preliminary studies showed that treatment of **5** with *n*-butyllithium at 0 °C in the presence of TMEDA (Scheme 1) led to the lithiated intermediate **6** in quantitative yield. Surprisingly it did not give efficient condensation with benzyl bromide. Then we turned to the transformation of the lithiated derivative **6** into the organocopper derivative with the $Cu^{I}I$:dimethyl sulfide[11] complex. The reaction with benzyl bromide and 3,5-dimethylbenzyl bromide[12,13] generated the expected 4-benzylpyridinones **7a,b** in 53 and 37% yields, respectively. Hydrolysis in hydrochloric acid at reflux cleaved both methoxy and pivaloyl groups, giving the pyridinones **8a**,**b** in good yields.

Fisher et al.[14] reported that organozinc species coupled efficiently with benzyl bromide in the presence of Pd-

* Corresponding author: Chi Hung Nguyen. Phone: 33 (0)1 69 86 30 89. Fax: 33 (0)1 69 07 53 81. E-mail: chi.hung@curie.u-psud.fr.
[†] UMR 176 CNRS−Institut Curie.
[‡] INSERM U74, Institut de Virologie.
[§] EP-REGER, CNRS−Université Victor Segalen Bordeaux 2.

10.1021/jm0009437 CCC: $19.00
Published on Web 10/03/2000

Scheme 1[a]



[a] Reagents: (a) *n*-BuLi (3.5 equiv)/TMEDA (3.5 equiv)/THF/−78 °C to 0 °C; (b) $Cu^{I}I$:DMS (3.5 equiv)/THF/−78 °C to 0 °C; (c*) $BrCH_2C_6H_3R_1R_2$ (4.5 equiv)/THF/−78 °C to r.t.; (c**) 3,5-dimethylbenzaldehyde (4.5 equiv)/THF/−78 °C to r.t.; (d) HCl 3 M/H_2O/Δ_{reflux} to r.t.; (e) MnO_2 (5.0 equiv)/toluene/Δ_{reflux}; (f) HCl 3 M/H_2O/Δ_{reflux} to r.t.

Table 1. Attempts To Improve the Synthesis of **7b** from **5**

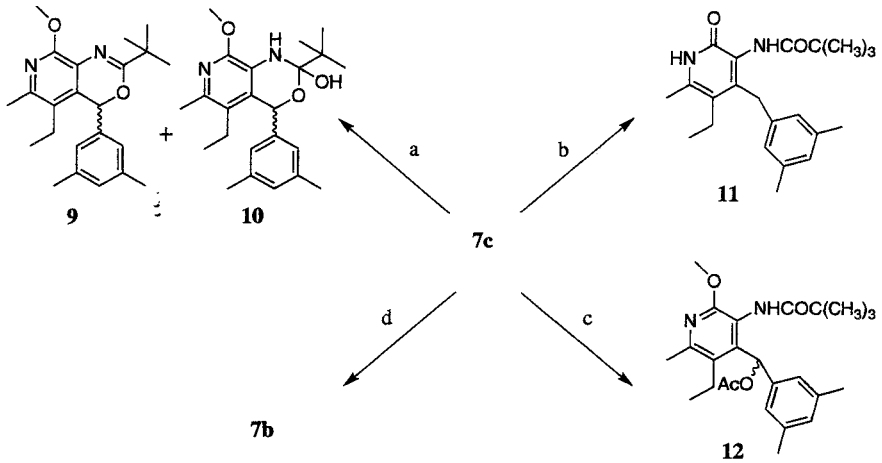
experimental conditions	% yield (expected compound)	% recovered starting material
a. *n*-BuLi (3.5 equiv)/TMEDA (3.5 equiv)/THF/−78 °C to 0 °C b. $ZnCl_2$ (0.50 equiv) c. 3,5-dimethylbenzyl bromide (4.5 equiv)/$Pd(PPh_3)_4$ (0.02 equiv)	31	46
a. *n*-BuLi (3.5 equiv)/TMEDA (3.5 equiv)/THF/−78 °C to 0 °C b. $ZnCl_2$ (1.00 equiv) c. 3,5-dimethylbenzyl bromide (4.5 equiv)/$Pd(PPh_3)_4$ (0.02 equiv)	11.5	70.5
a. *n*-BuLi (3.5 equiv)/TMEDA (3.5 equiv)/THF/−78 °C to 0 °C b. $ZnCl_2$ (0.25 equiv) c. 3,5-dimethylbenzyl bromide (4.5 equiv)/$Pd(PPh_3)_4$ (0.02 equiv)	30	67
a. *n*-BuLi (3.5 equiv)/TMEDA (3.5 equiv)/THF/−78 °C to 0 °C b. $ZnCl_2$ (0.25 equiv) c. 3,5-dimethylbenzyl bromide (4.5 equiv)/$Pd(PPh_3)_4$ (0.06 equiv)	7.5	72.5

$(PPh_3)_4$. Different experiments (summarized in Table 1 and see Experimental Section) were performed from **5** using various proportions of $ZnCl_2$ and $Pd(PPh_3)_4$, but the resulting yield did not exceed 31%. On the contrary, we observed that our lithiated intermediate **6** coupled efficiently with 3,5-dimethylbenzaldehyde[15] to give **7c** with a 71% yield, avoiding either the use of highly toxic dimethyl sulfide for the preparation of the organocopper intermediate or the use of $Pd(PPh_3)_4$ for the coupling with the organozinc compound (Scheme 1). This result gave an alternative way for obtaining 4-benzylpyridinone **7b** since it could be prepared from the benzyl alcohol **7c**. For this purpose, several attempts were performed from **7c**. First, treatment of **7c** with triethylsilane and trifluoroacetic acid[16] did not afford the expected compound **7b** but the oxazines **9** and **10**, probably resulting from an acid-catalyzed intramolecular reaction (Scheme 2). The use of zinc powder in acetic acid[17] afforded only a partial deprotection of **7c**, giving compound **11** in a very low yield (5%). Acetylation of **7c** gave acetate **12** in good yield (95%), and an attempt to replace the acetoxy group by a hydrogen atom was performed in the presence of triethylsilane in dichloromethane but totally failed (starting material **12** was recovered). Finally when **12** was subjected to hydrogenolysis[18,19] in the presence of 30% Pd/C under a 10 atm hydrogen pressure, the expected 4-benzyl derivative **7b** was obtained almost quantitatively.

To undertake SAR studies in this new series of 3-amino-4-benzylpyridin-2(1*H*)-one derivatives, the benzyl alcohol and ketone derivative **8c** and **14** have been synthesized. Thus, deprotection of **7c** in hydrochloric acid at reflux led to the pyridinone **8c** and oxidation of **7c** in the presence of manganese dioxide,[20] followed by deprotection in acidic conditions, provided compound **14** via **13**.

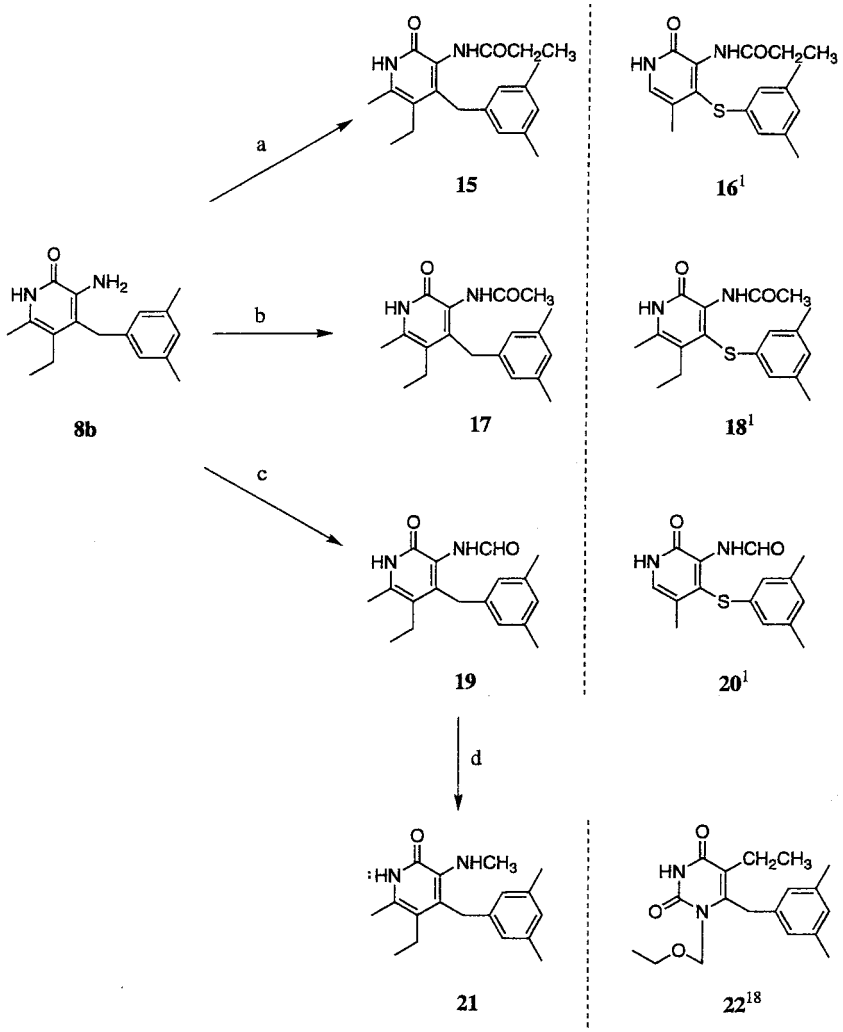
With respect to the model of 3-amino-substituted pyridinone **2b**, modifications of the amino function of

Scheme 2[a]



[a] Reagents: (a) Et_3SiH (1.2 equiv)/CF_3CO_2H/r.t.; (b) Zn (6.0 equiv)/AcOH/Δ_{reflux}; (c) 1. Ac_2O (10.0 equiv)/pyridine/r.t. to 60 °C; 2. Et_3SiH (5.0 equiv)/CH_2Cl_2/r.t. to Δ_{reflux}; (d) 1. Ac_2O (10.0 equiv)/pyridine/r.t. to 60 °C; 2. H_2/P = 10 atm/Pd/C(30%)/AcOH/H_2O/dioxane/r.t.

Scheme 3[a]

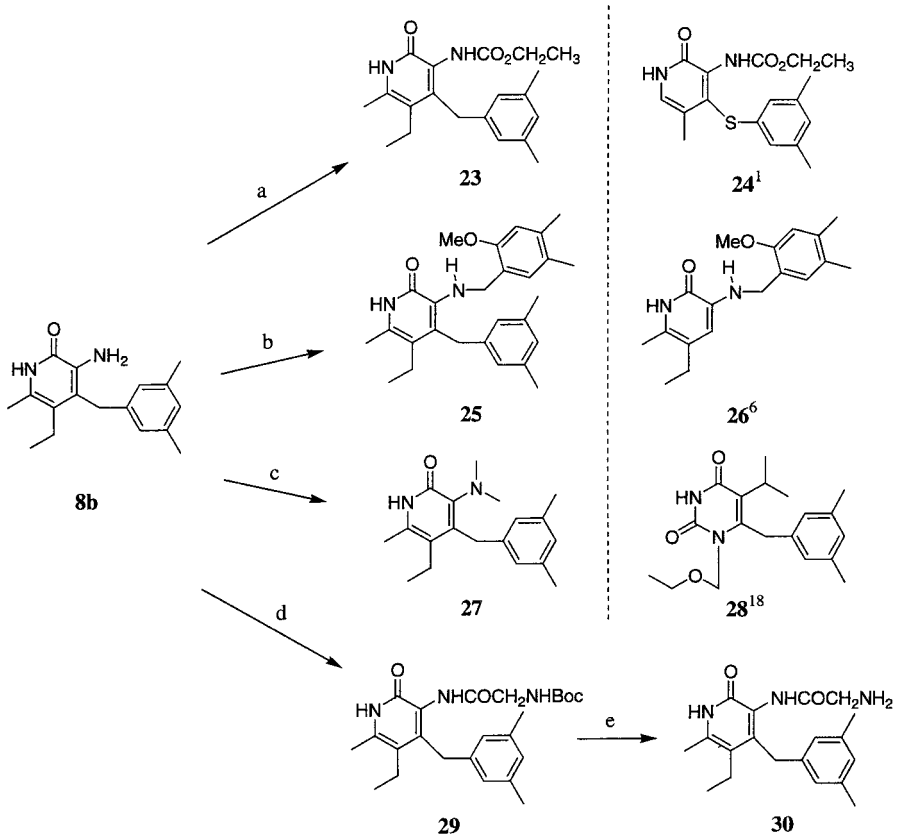


[a] Reagents: (a) $ClCOCH_2CH_3$ (1.03 equiv)/Et_3N (0.95 equiv)/CH_2Cl_2/0 °C to r.t.; (b) $(CH_3CO)_2O$ (1.4 equiv)/AcOH/Δ_{reflux}; (c) HCO_2H/HCO_2Et/Δ_{reflux}; (d) $LiAlH_4$ (5.0 equiv)/THF/Δ_{reflux}.

3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(*H*)-one (**8b**) have been performed. Thus, under the usual acylation conditions, using propionyl chloride, acetic anhydride, and ethyl formate, the amides **15**, **17**, and **19** were obtained in 44−66% yields. Reduction of the formamidopyridinone **19** in the presence of lithium aluminum hydride led to the methylaminopyridinone **21** in 57% yield (Scheme 3). The carbamate **23** was synthesized with ethyl chloroformate in 43% yield (Scheme 4).

We previously described[1] that in various conditions no condensation occurred from 3-amino-4-phenylthiopy-

Scheme 4[a]



[a] Reagents: (a) $ClCO_2CH_2CH_3$ (30.0. equiv)/Et_3N (2.5 equiv)/EtOH/Δ_{reflux}, to r.t.; (b) 1. 2-methoxy-4,5-dimethylbenzaldehyde (1.0 equiv)/AcOH/MeOH/r.t.; 2. $NaBH_4$ (2.0 equiv)/MeOH/$CHCl_3$/r.t.; (c) HCHO 37% aq (10.0 equiv)/$NaBH_3CN$ (3.0 equiv)/AcOH glacial (6.0 equiv)/CH_3CN/r.t.; (d) $BocNHCH_2CO_2H$ (7.0 equiv)/DCC (7.0 equiv)/HOBt (7.0 equiv)/NMM (7.7 equiv)/CH_2Cl_2/0 °C to r.t.; (e) HCI/EtOAc/r.t.

ridinones **3a** and 4,5-dimethyl-2-methoxybenzaldehyde. On the contrary, compound **25** was obtained under the usual conditions[6,21] in 60% yield. In the same way, the methylation of **8b** by the Eschweiler−Clarke reaction[22] using formaldehyde and sodium cyanoborohydride, which was unsuccessful with the 3-amino-4-phenylthiopyridinone derivative **3a**, afforded the dimethylamino derivative **27** in 91% yield. Condensation of *N*-Bocglycine on amine **8b** followed by deprotection gave compound **30** with a 70% overall yield (Scheme 4).

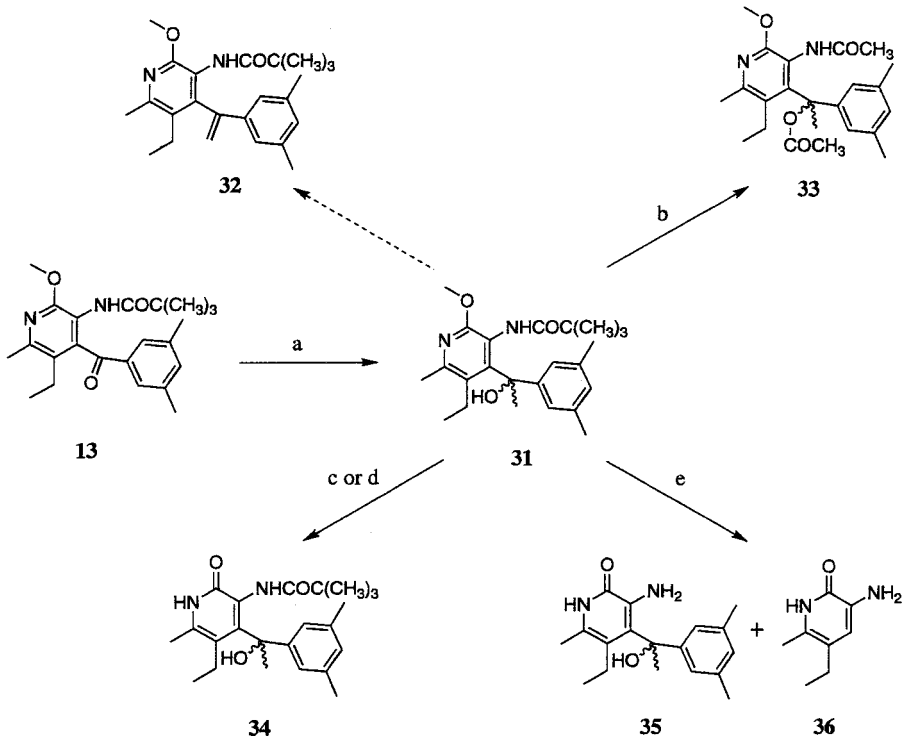
Treatment of diaryl ketone **13** with methyllithium led to compound **31** (72% yield) which could not be dehydrated under the following conditions: when acetic anhydride[23] was used at reflux only the acetamidoacetate **33** was obtained with a 9% yield beside 45% of starting material **31**; treatment of **31** with *p*-toluenesulfonic acid led to a partial deprotection to give **34** in 18−66% yields (Scheme 5); treatment of **31** in hydrochloric acid at reflux did not afford the expected product **32** but a mixture of the totally deprotected compound **35** and aminopyridinone **36**.[4] Moreover, we also tried to prepare compound **32** by using a Wittig reaction from ketone **13** under various conditions,[24−26] but no reaction occurred and **13** was totally recovered.

Biological Results

Inhibition of HIV-1 Replication. Twenty-five newly synthesized pyridinones were studied for their anti-HIV-l activity. Several molecules showed significant antiviral properties. The most active ones are compounds **27**, **19,** and **14** with IC_{50}s of 0.2, 3, and 6 nM, respectively (Table 2). Some of the best inhibitors were tested on a Nevirapine resistant strain (Table 3). Thus compound **27** exhibited a good anti-HIV activity on this resistant strain (IC_{50} = 40 nM). This last result can be compared to the activity on the same Nevirapine resistant strain found for 5-ethyl-6-methyl-3-nitro-4-[(3,5-dimethylphenyl)thio]pyridin-2(1*H*)-one,[1] which was 6-fold less active (IC_{50} = 260 nM). The evaluation of the antiviral activities was extended to different HIV-1 isolates including an AZT resistant strain, lymphotropic, and macrophage tropic viruses infecting human primary cells, peripheral blood mononuclear cells (PBMC), or monocyte-derived macrophages (Table 4). Compound **27** has, in all cases, the highest activity, inhibiting HIV-1 multiplication in primary cultures at subnanomolar concentrations. However, none of the molecules were active against HIV type 2 and, except compound **29**, were not toxic at the highest concentrations tested (100 μM to 1 μM).

Retrovirucidal Effect. It has been reported that retrotranscription can occur not only in the host cell cytoplasm but also in the extracellular virion.[27−29] This endogenous reverse transcription seems to be correlated with an increased level of infectivity. Therefore, inhibition of extracellular retrotranscription would imply that RT inhibitors enter the plasma virions. Recent work also shows that HIV-1 virions are present in the blood plasma during all stages of the AIDS disease and, more particularly, that the life span of a free virion reaches 7 to 8 h.[30] This new set of data strongly suggests that in addition to HIV-1 infected cells, free virions may

Scheme 5[a]



[a] Reagents: (a) MeLi (3.0 equiv)/Et_2O/0 °C to r.t.; (b) Ac_2O/Δ_{reflux}; (c) *p*-TsOH (2.5 equiv)/toluene/Δ_{reflux}; (d) *p*-TsOH (10.0 equiv)/xylene/Δ_{reflux}/Dean−Stark; (e) HCl 3 M/H_2O/Δ_{reflux}.

Table 2. Anti-HIV-1 Activity of the Pyridinones on HIV-1 Lai Wild Type in CEM-SS

compd	IC_{50} (nM)	CC_{50} (nM)	SI	compd	IC_{50} (nM)	CC_{50} (nM)	SI	compd	IC_{50} (nM)	CC_{50} (nM)	SI
AZT	3	>100 000	>33 333	**13**	>10 000	>10 000	-	***24***[1]	*7 500*	*>100 000*	*>13*
5	>100 000	>100 000	-	**14**	6	>10 000	>1 666	**25**	1 000	>1 000	-
7a	>1 000	>1 000	-	**15**	4 600	>10 000	>2	***26***[20]	*8*		
7b	>100 000	>100 000	-	***16***[1]	*41 400*	*>100 000*	*>2*	**27**	0.2	>1 000	>5 000
7c	>1 000	>1 000	-	**17**	100	>10 000	>100	***28***[18]	*0.6*	*43 000*	*72 000*
8a	730	>100 000	>136	***18***[1]	*50*	*80 000*	*1 600*	**29**	6 700	86 000	12
8b	17	>10 000	>588	**19**	3	>10 000	>3 333	**30**	18	>100 000	>5 555
8c	1000	>10 000	>10	***20***[1]	*6 600*	*>100 000*	*>15*	**31**	>10 000	>10 000	-
9	>10 000	>10 000	-	**21**	30	>10 000	>333	**33**	2 500	>10 000	>4
10	4 200	>10 000	>2	***22***[18]	*1.6*	*207 000*	*130 000*	**34**	12 000	>100 000	>8
12	10 000	>10 000	>1	**23**	460	>10 000	>21	**36**	30 000	>100 000	>3

Table 3. Anti-HIV-1 Activity of the Pyridinones on HIV-1 Nevirapine Resistant Strain[1b]

compd	IC_{50} (nM)	CC_{50} (nM)	SI
TIBO R82913	>10 000	>10 000	-
8b	1 000	>10 000	>10
8c	4 500	>10 000	>2
14	1 300	>10 000	>7
17	>10 000	>10 000	-
19	2 900	>10 000	>3
21	1 700	>10 000	>5.5
27	40	>1 000	>25

be considered as a potential target for HIV-1 RT inhibitors.

Therefore, it seemed interesting to investigate whether 4-benzyl pyridinone derivatives **8b**, **14**, **19**, and **27** were active against free virions. In parallel experiments, different well-known described nonnucleoside reverse transcriptase inhibitors (HEPT, TIBO R82913, nevirapine) were also used. Viral preparations were incubated with different concentrations of each inhibitor as described in the Experimental Section. Although significant inhibition was observed with the four pyridinone derivatives, compound **27** was the most active. The infectivity of the viral suspension was severely affected, in a concentration-dependent manner, by compound **27** as shown in Figure 2. In contrast, TIBO R82913, HEPT, and nevirapine were unable to produce an antiviral effect even at concentrations as high as 10 μM. Similar results were recently described by Borkow et al.[31] and by our group[32] showing that the infectivity of isolated HIV-1 particles was inhibited by a non-nucleoside reverse transcriptase inhibitor.

Inhibition of RT. The effect of these antiviral agents on recombinant HIV-1 RT activity was analyzed. Reactions were carried out in the presence of poly(C)-oligo(dG) as template-primer. The concentration inhibiting 50% of the RT activity (IC_{50}) for each compound is given in Table 5. The best inhibitor was compound **27** with an IC_{50} value very similar to that of the best compound of the pyridin-2(1*H*)-ones **3**.[1] Compounds **8b**, **14**, and **19** were also able to inhibit HIV-1 RT, but at higher concentrations, giving IC_{50} values comparables to those of nevirapine and 1-[(benzyloxy)methyl]-6-(phenylthio)-thymine (BPT).

It has been previously shown that nonnucleoside RT inhibitors present different levels of inhibition depend-

Table 4. Anti-HIV-1 Activity of the Pyridinones on Various HIV Strains and Primary Cell Cultures

	IC_{50} (nM)/CC_{50} (nM)				
compd	HIV-1 IIIB/MT4	HIV-1 AZTres./MT4	HIV-1 IIIB/PBMC	HIV-2 D 194/PBMC	HIV-1 Bal/Mono/Macrophages
8b	500/>10 000	4 350/>10 000	310/>10 000	>10 000/>10 000	130/>10 000
8c	3 400/10 000	420/>10 000	690/>10 000	>10 000/>10 000	180/>10 000
14	85/>10 000	25/>10 000	20/>10 000	>10 000/>10 000	17/>10 000
17	900/>10 000	440/>10 000	67/>10 000	>10 000/>10 000	200/>10 000
19	26/>10 000	71/>10 000	1.8/>10 000	>10 000/>10 000	21/>10 000
21	310/>10 000	76/>10 000	16/>10 000	>10 000/>10 000	720/>10 000
27	2.4/>1 000	0.2/>1 000	0.58/>1 000	>1 000/>1 000	0.004/>1 000

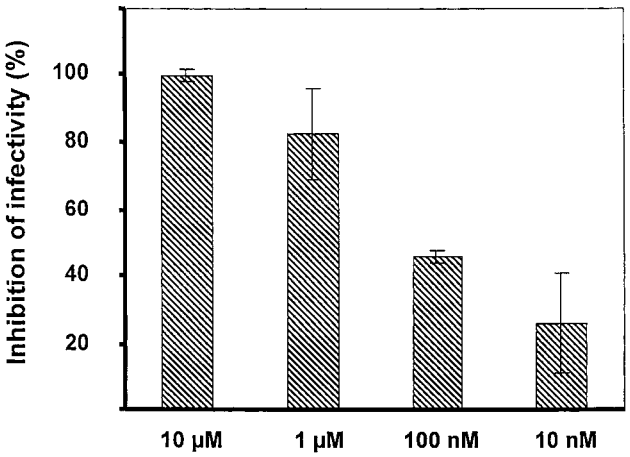


Figure 2. Effect of compound **27** on free virions. The infectivity of HIV-1 in the presence of the inhibitor was determined as described in the Experimental Section. 0% represents the infectivity of virions in the absence of inhibitor.

Table 5. Inhibition of HIV-1 Reverse Transcriptase (RT)[a]

compd	IC_{50} (nM)
nevirapine	200
5-ethyl-6-methyl-3-nitro-4-[(3,5-dimethylphenyl)thio]pyridin-2(1*H*)-one[1]	30
BPT	300
8b	100
14	100
19	200
27	20

[a] RT activity was measured in the presence of poly(C)-oligo(dG), as described in the Experimental Section.

ing on the template-primer used to measure the RT activity. We determined the inhibition produced by these compounds using different template-primers. The best inhibition was obtained in the presence of poly(C)-oligo(dG) as compared to that obtained with poly(A)-oligo(dT).

Besides utilizing synthetic template-primers to measure the inhibitory effect of compound **27**, a system closer to the native one was used, where the template was a fragment of HIV-1 RNA containing the primer binding site (PBS) sequence, and the primer was either $tRNA^{Lys3}$, the natural primer of HIV-1, or an oligodeoxynucleotide complementary to the PBS (anti PBS). In both cases, the addition of compound **27** produced a strong inhibitory effect in a concentration-dependent manner (Figure 3) with IC_{50} values of 6−8 nM.

As all derivatives of the present work were targeted at HIV-1 RT, inhibition studies, carried out with **27**, (Figure 4), show that this optimized compound is a linear, noncompetitive inhibitor against dGTP in reactions with poly (C)-oligo(dG) as template-primer. The K_i for **27**, determined from the replot of the slopes (Figure 4, inset) was 35 nM.

All nonnucleoside inhibitors thus far identified on the basis of their activities against HIV-1 do not inhibit RT from HIV-2. Nevertheless all the compounds were tested with both RTs. At concentrations where HIV-1 RT was well inhibited, the activity of HIV-2 RT was not affected (results not shown). This discriminatory behavior toward HIV-1 vs HIV-2 RT makes these compounds share another common property of non-nucleoside inhibitors.

To determine a possible synergism in the inhibition of RT, we investigated the effect of combining 4-benzyl pyridinone derivatives and nucleoside analogues. Synergistic inhibition by combination of compound **27** and AZT-triphosphate was analyzed as described by Caroll et al.,[33] using the method of fractional inhibitory concentrations.

Preliminary results showed synergistic inhibition when both inhibitors were used at low concentrations (Figure 5): an important percentage of the data points fall below and to the left of the line of additivity.

Discussion

In this work, biological studies were carried out on various 3,4-disubstituted 5-ethyl-6-methylpyridin-2(1*H*)-one derivatives of general formula **3b**. Results are reported in Table 2.

From these first SAR studies limited to 25 new 4-C-aralkylpyridinone derivatives, some general comments can be made. (a) As previously described[1] by us and as in the HEPT series,[18] the methyl groups at the 3- and 5-positions of the 4-benzyl substituent play an important role for biological activity. Thus, the nonmethylated 4-benzyl compound **8a** displayed a moderate activity (IC_{50} = 730 nM), whereas the 3,5-dimethylbenzyl derivative **8b** exhibited a pronounced inhibitory effect (IC_{50} = 17 nM). (b) When an hydroxy group is present on the methylene linking group, a striking decrease of biological activity was observed: thus, compound **8c** is 60-fold less active than its analogue **8b**. On the contrary, when a ketone function is introduced at this position as it is the case for ketone **14**, the IC_{50} is improved and reached 6 nM. (c) In all cases, when pyridinones are protected (2-OMe and 3-NHPiv), no activity was observed (**7a**, **7b**, **7c**, **12**, **13**, **31**, **33**, **34**). (d) Concerning the replacement of the sulfur atom by a methylene group, when similar compounds, bearing the same substituent at their 3-position, are compared (compound **8b** vs 3-amino-5-ethyl-6- methyl-4-[(3,5-dimethylphenyl)thio]pyridin-2(1*H*)-one,[1] **15** vs **16**, **19** vs **20**, and **23** vs **24**), the biological activity is generally maintained or improved. In the case of the formamido derivative **19**, IC_{50} reached 3 nM compared to 6600 nM for its corresponding 4-thiophenyl analogue **20**. This replacement of the

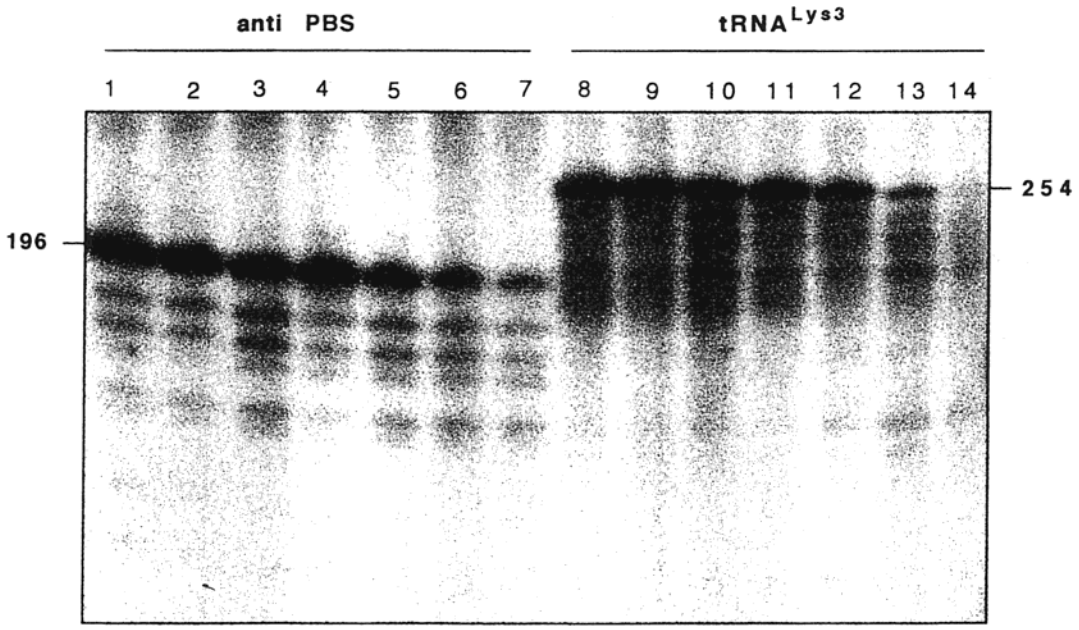


Figure 3. Inhibition of cDNA synthesis by HIV-1 RT in the presence of compound **27.** Reverse transcription was performed as described in the Experimental Section. Reactions primed with tRNA^{Lys3} yielded a cDNA product of 254 nucleotides; those primed with anti PBS, a cDNA of 196 nucleotides. Products were analyzed by denaturing polyacryl amide gels. Lanes 1 and 2: complete system primed with anti PBS, in the absence of compound **27**. Lanes 3 to 7: 2, 4, 6, 8, and 10 nM, respectively, of compound **27**. Lanes 8 and 9: complete system primed with tRNA^{Lys3}. Lanes 10 to 14: 2, 4, 6, 8, and 10 nM, respectively, of compound **27**.

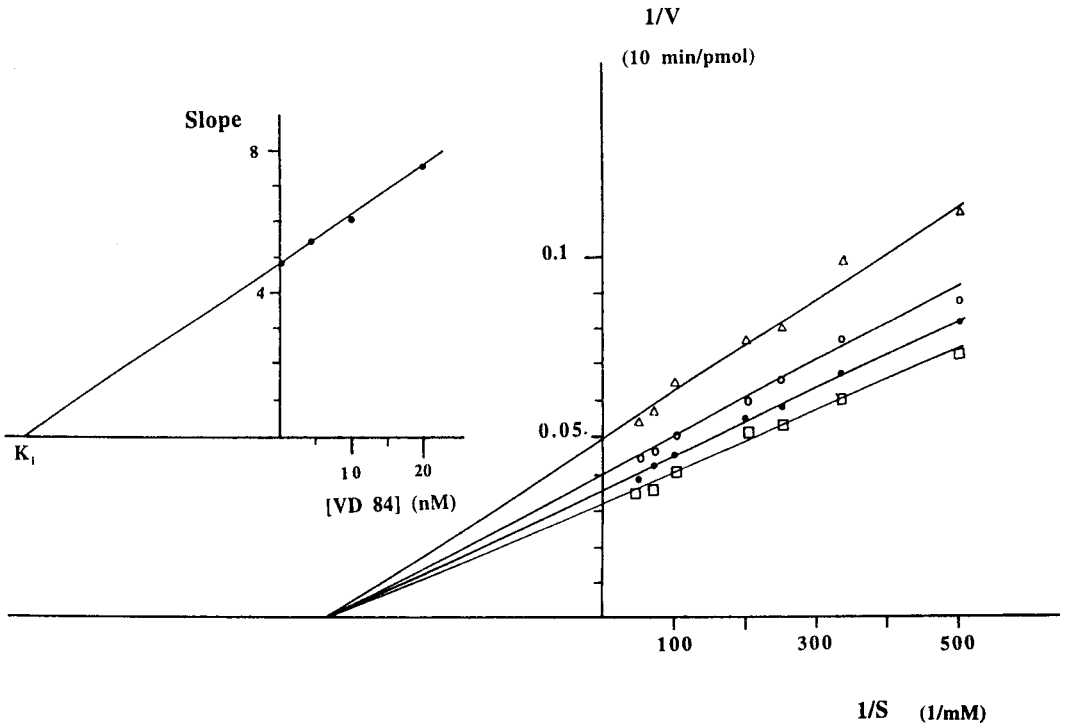


Figure 4. Inhibition by **27** of reverse transcriptase-catalyzed synthesis. The activity was measured with poly(C)-oligo(dG) as template-primer and dGTP as substrate. RT was incubated, as described in the Experimental Section, in the absence (□), or in the presence of 4 nM (●), 10 nM (○), or 20 nM (△) of **27.** Data were fit as a double-reciprocal plot according to Lineweaver−Burk. Inset: the apparent K_i was determined from the secondary plot of slopes versus inhibitor concentration.

arylthio group by an arylmethyl substituent, leading to more stable pyridinones and to the more reactive 3-amino function in these 4-benzyl analogues, allowed us to obtain the 3-dimethylaminopyridinone **27** which is the best compound in this series. (e) When an amino acid is present on the 3-amino function in the case of compound **30**, the activity is maintained (IC_{50} = 18 nM). On the contrary, when this last compound is still protected by a *tert*-butoxycarbonyl group (compound **29**), the biological activity is completely abolished (IC_{50} reached a value 400-fold higher than that of compound **30**). (f) Since these new pyridinones are considered as **[1a**,**b** (HEPT derivatives)-**2a**,**b** (pyridinones)] hybrid molecules, we tried to compare activities of some of these pyridinones with related HEPT or Merck-pyridinone compounds. Activities of the monomethylamino and dimethylamino pyridinones **21** and **27** were thus compared to those of the HEPT derivatives **22** and **28**. As

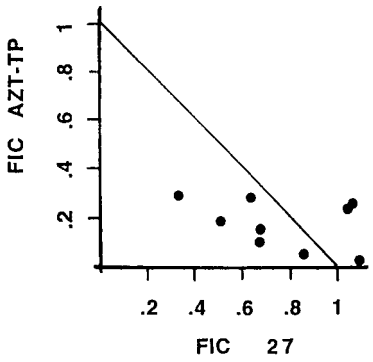


Figure 5. Effect of combination of compound **27** and AZT-triphosphate on HIV-1 RT inhibition. Inhibition of RT activity by the combination of compound **27** and AZT-TP was determined by the method of fractional inhibitory concentrations (FIC). The FIC of each inhibitor was calculated as the concentration of the inhibitor present in the reaction divided by the concentration required to give the same degree of inhibition when the inhibitor was used alone. The right line indicates additivity of inhibition.

it can be seen for these compounds, IC_{50} values are of the same order of magnitude. On the other hand, compound **25** is 125-fold less active than the pyridinone **26**. The bulkiness of the two groups at 3- and 4-positions probably prevents compound **25** to fit in the allosteric site of the RT and accounts for this result.

Due to a limited solubility in the culture medium and as the highest concentrations tested were not toxic, the selectivity indexes could not be determined.

Conclusion

This work led us to elaborate two different ways to obtain 4-benzyl-3,5,6-trisubstituted pyridin-2(1*H*)-one derivatives. These compounds constitute a new series of potent non-nucleoside HIV-1 RT inhibitors related both to HEPT and Merck-pyridinone series and chemically more stable than their 4-phenylthio analogues.

Biological studies revealed that some of new 4-benzylpyridinones show potent HIV-1 specific reverse transcriptase inhibitory properties. Compounds **14**, **19**, and **27**, which inhibit the replication of HIV-1 in CEM-SS cells, with IC_{50} values ranging from 0.2 to 6 nM are the most active compounds in this series. They are very efficient in blocking the replication of lymphotropic and macrophage tropic isolates in primary lymphocytes or monocyte-derived macrophages. Biochemical studies showed that compound **27** strongly inhibited the activity of a recombinant HIV-1 RT, with IC_{50} values of 6−8 nM. The new 4-benzyl derivatives showed different levels of inhibition depending on the synthetic template-primer used. Whereas better levels of inhibition were obtained with poly(C)-oligo(dG) as template-primer, as compared to poly(A)-oligo(dT), enzyme kinetic analysis of RT inhibition by these compounds indicated that they were noncompetitive with respect to the substrate dGTP, and the compounds were shown to be specifically active against HIV-1 RT, but not against HIV-2 RT. These 4-benzylpyridinones are therefore HIV-1 specific noncompetitive RT inhibitors which share similar properties with other non-nucleoside inhibitors of this enzyme.

Moreover, besides inhibiting HIV-1 replication in cell culture, compound **27** is able to strongly decrease the infectivity of isolated HIV-1 particles. This compound, which was capable of inhibiting a virus resistant to nevirapine with an IC_{50} of 40 nM, can be considered as a potent HIV-1 inhibitor which has the unusual capability to reach the reverse transcription complex inside the extracellular virions.

Experimental Section

Chemistry. TLC were carried out on precoated plates of silica gel 60F254 (Merck). To reveal the compounds, TLC plates were exposed to UV-light. Purifications were performed on silica gel (40−60 μm, SDS) columns by medium pressure chromatography. In all experiments involving lithium derivatives or amino acid coupling, the glassware was dried in the oven for a 24 h period before use. Tetrahydrofuran (THF) was systematically freshly distilled from sodium/benzophenone. All melting points were measured on an Electrothermal 9200 apparatus and were uncorrected. ^{1}H NMR spectra were recorded in the given solvents at 294 K (except when mentioning) with a Bruker AC 200 or a Bruker AMX 300 apparatus using the hydrogenated residue of deuterated solvents ($CHCl_3$, $\delta = 7.25$ ppm and DMSO, $\delta = 2.54$ ppm) as internal standards (*, # = interchangeable assignments). Chemical shifts (δ) were reported in ppm units, downfield from TMS (s, d, t, q, m, br for singlet, doublet, triplet, quadruplet, multiplet, and broad, respectively), and coupling constants (J) were given in hertz (Hz). Elemental analyses, performed by the "Service Central de Microanalyses du CNRS", 91190 Gif-sur-Yvette, France, were within 0.3% of the theoretical values calculated for C, H, N, O, and Cl. Mass spectra (MS) were obtained on a NERMAG R10-10-C by direct introduction for the chemical ionization (IC).

CAUTION: Since dimethyl sulfide is very toxic and very irritating for eyes, this reagent must be used very cautiously with an appropriate respirator.

4-(3,5-Dimethylbenzyl)-5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridine (7b). (i) By Lithiation of 5: The starting material **5** and the 3,5-dimethylbenzyl bromide were dried in the presence of phosphorus pentoxide under vacuum at room temperature during 24 h. Copper iodide (Cu^II) was dried in the presence of phosphorus pentoxide under vacuum at 50 °C for 24 h. 5-Ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridine[10] (**5**) (1.06 g, 4.23 mmol) and freshly distilled (on calcium hydride) TMEDA (2.24 mL, 14.82 mmol) were dissolved in dry THF (26 mL), and the mixture was cooled at −78 °C under a nitrogen atmosphere. *n*-Butyllithium (1.6 M in hexane, 9.26 mL, 14.82 mmol) was added dropwise. The mixture was stirred for 1 h at 0 °C. An orange-yellow precipitate was observed. On another hand, the Cu^II:dimethyl sulfide complex was prepared at −78 °C under a nitrogen atmosphere by addition of dimethyl sulfide (14.00 mL, 190.54 mmol) to a suspension of copper iodide (2.82 g, 14.82 mmol) in dry THF (52 mL). The Cu^II:dimethyl sulfide complex was then added dropwise to the mixture at −78 °C. The black mixture was stirred at 0 °C for a 30 min period and cooled again at −78 °C to allow the addition of 3,5-dimethylbenzyl bromide[4,5] (3.81 g, 19.05 mmol) dissolved in THF (4 mL). The resulting mixture was stirred at 0 °C for 3 h and at room temperature for 12 h. Water (16 mL) and 28% aqueous ammonium hydroxide solution (20 mL) were added. The blue aqueous layer was extracted with 3 × 80 mL of ether. The combined organic layers were washed with 40 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography using cyclohexane−ethyl acetate (1:0 to 8:2) as eluent, giving **7b** (577 mg, 37%) as a white solid and 544 mg (51%) of starting material **5**. **7b**: mp 138−139 °C; ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t, J = 7.4 Hz, CH_3CH_2), 1.19 (9H, s, $COC(CH_3)_3$), 2.21 (6H, s, CH_3-3′ and 5′), 2.46 (3H, s, CH_3-6), 2.57 (2H, q, J = 7.6 Hz, CH_3CH_2), 3.89 (5H, s, CH_3O and $CH_2C_6H_5$), 6.56 (2H, s, H-2′ and 6′), 6.78 (s, 1H, H-4′). Anal. ($C_{23}H_{32}N_2O_2 \cdot 0.25H_2O$) C, H, N.

(**ii) By Hydrogenolysis of 12:** A mixture of (+,−)-1-(5-ethyl-2-methoxy-6-methyl-3- pivaloylaminopyridin-4-yl)-1-(3,5-

dimethylphenyl)methyl acetate (**12**) (850 mg, 1.99 mmol) and Pd−C (30%, 850 mg) in acetic acid−water-dioxane (42.5 mL, 2:1:2, v/v/v) was stirred at room temperature for 24 h under 10 atm of hydrogen. The catalyst was removed by filtration and washed with ethanol. The combined filtrates were concentrated to dryness under reduced pressure, giving **7b** (726 mg, 99%) as a white solid which was identical to that described above.

(+,−)-1-(3,5-Dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)methanol (7c). The starting materials were dried in the presence of calcium chloride under vacuum at room temperature during 12 h. 5-Ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridine[10] (**5**) (620 mg, 2.47 mmol) and freshly distilled (on calcium hydride) TMEDA (1.31 mL, 8.67 mmol) were dissolved in dry THF (15 mL), and the mixture was cooled at −78 °C under a nitrogen atmosphere. *n*-Butyllithium (1.6 M in hexane, 5.42 mL, 8.67 mmol) was added dropwise. The mixture was stirred for 1 h at 0 °C. The formation of an orange precipitate was observed. The mixture was cooled again at −78 °C to allow the addition of 3,5-dimethylbenzaldehyde[15] (1.49 g, 11.14 mmol), dissolved in dry THF (30 mL). The resulting mixture was stirred at 0 °C for 2 h and at room temperature for 12 h. The organic layer was washed with 30 mL of water. The aqueous layer was extracted with 3 × 60 mL of ether. The combined organic layers were washed with 30 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was washed with 10 mL of cyclohexane to give the product **7c** (570 mg) after filtration. The filtrate was evaporated, and the residue was purified by column chromatography using cyclohexanes−ethyl acetate (1:0 to 8:2) as eluent giving the product **7c** (104 mg, total yield = 71%) as a white solid and 72 mg (12%) of starting material **5**. **7c**: mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, t, J = 7.4 Hz, CH_3CH_2), 1.09 (9H, s, $COC(CH_3)_3$), 2.24 (6H, s, CH_3-3′ and 5′), 2.47 (3H, s, CH_3-6), 2.68 (2H, m, CH_3CH_2), 3.90 (3H, s, CH_3O), 4.94 (1H, d, J = 7.7 Hz, OH), 5.94 (1H, d, J = 7.4 Hz, CH), 6.81 (4H, br s, H-2′, 4′, 6′ and NH). Anal. ($C_{23}H_{32}N_2O_3$) C, H, N, O.

3-Amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (8b). A 3 M aqueous hydrochloric acid solution (150 mL) was added to a suspension of 4-(3,5-dimethylbenzyl)-5-ethyl- 2-methoxy-6-methyl-3-pivaloylaminopyridine (**7b**) (2.36 g, 6.41 mmol) in water (300 mL). The mixture was refluxed for 3.5 h and then stirred at room temperature for 12 h. The solution was basified by addition of concentrated ammonium hydroxyde and extracted with 3 × 800 mL of ethyl acetate. The combined organic layers were washed with 110 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure giving **8b** (1.79 g, ≈100%) as a light orange solid: mp 204−205 °C; ^{1}HNMR ($CDCl_3$) δ 1.00 (3H, t, J = 7.5 Hz, CH_3CH_2), 2.25 (6H, s, CH_3-3′ and 5′), 2.27 (3H, s, CH_3-6), 2.39 (2H, q, J = 7.5 Hz, CH_3CH_2), 3.81 (4H, br s, $CH_2C_6H_5$ and NH_2), 6.72 (2H, s, H-2′ and 6′), 6.83 (1H, s, H-4′), 10.65 (1H, br s, NH-1). Anal. ($C_{17}H_{22}N_2O\cdot0.2H_2O$) C, H,N,O.

(+,−)-3-Amino-4-[1-(3,5-dimethylphenyl)-1-hydroxy]-methyl-5-ethyl-6-methylpyridin-2(1H)- one (8c). Starting from (+,−)-1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3- pivaloylaminopyridin-4-yl)-methanol (**7c**) (150 mg, 0.39 mmol) and 3 M aqueous hydrochloric acid (9 mL), the reaction was performed as above (for **8b**). The residue was purified by column chromatography using dichloromethane−ethanol (100:0 to 95:5) as eluent giving the starting material **7c** (70 mg, 47%) and **8c** (19 mg, 17%) as white crystals (after recrystallization from dichloromethane): mp 238−239 °C; ^{1}H NMR (DMSO-d_6) δ 0.94 (3H, t, J = 7.0 Hz, CH_3CH_2), 2.13 (3H, s, CH_3-6), 2.25 (6H, s, CH_3-3′ and 5′), 2.32 (2H, m, CH_3CH_2), 4.84 (2H, br s, NH_2), 5.80 (1H, s, CH), 6.08 (1H, s, OH), 6.87 (1H, s, H-4′), 6.96 (2H, s, H-2′ and 6′), 11.26 (1H, br s, NH-1). Anal. ($C_{17}H_{22}N_2O_2\cdot0.25H_2O$) C, H, N, O.

***(+,−)*-2-*tert*-Butyl-4-(3,5-dimethylphenyl)-5-ethyl-8-methoxy-6-methyl-4*H*-pyrido[3,4-*d*]-*m*-oxazine (9) and (+,−)-2-*tert*-Butyl-4-(3,5-dimethylphenyl)-5-ethyl-8-methoxy-6-methyl-2,4-dihydro-1*H*-pyrido[4,3-*d*]-*m*-oxazin-2-ol (10).** A mixture of (+,−)-1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)-methanol (**7c**) (200 mg, 0.52 mmol), triethylsilane (0.10 mL, 0.62 mmol), and trifluoroacetic acid (1 mL) was stirred at room temperature for 27 h (the flask was fitted with a calcium chloride drying tube) and evaporated to dryness under reduced pressure. To the residue were added 10 mL of water. The aqueous layer was basified by addition of concentrated ammonium hydroxyde and extracted with 3 × 20 mL of dichloromethane. The combined organic layers were washed with 10 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane as eluent to give 53 mg (26.5%) of **10** as a yellow oil and 125 mg (65%) of **9** as a yellow solid. **9**: mp 111−1 12 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, J = 7.5 Hz, CH_3-CH_2), 1.03 (9H, s, $C(CH_3)_3$), 2.18−2.30 (8H, m, CH_3CH_2, CH_3-3′ and CH_3-5′), 2.40 (3H, s, CH_3-6), 4.03 (3H, s, OCH_3), 6.14 (1H, s, H-4), 6.78 (2H, s, H-2′ and 6′), 6.91 (1H, s, H-4′); MS (IC): 384 $[M + NH_4]^+$, 367 $[M + H]^+$; 309. Anal. ($C_{23}H_{30}N_2O_2\cdot0.25H_2O$) C, H, N, 0. **10**: ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, t, J = 7.2 Hz, CH_3CH_2), 1.29 (9H, s, $C(CH_3)_3$), 2.27 (6H, s, CH_3-3′ and 5′), 2.42 (3H, s, CH_3-6), 2.57−2.80 (2H, m, CH_3CH_2), 3.96 (4H, s, OCH_3 and OH), 6.79 (2H, s, H-2′ and 6′), 6.92 (1H, s, H− 4′), 7.18 (1H, s, H-4); MS (IC): 385 $[M + H]^+$; 367; 283. Anal. ($C_{23}H_{32}N_2O_3$) C, H, N.

4-(3,5-Dimethylbenzyl)-5-ethyl-6-methyl-3-pivaloylaminopyridin-2(1*H*)-one (11). Zinc powder (127 mg, 1.95 mmol) was added to a solution of (+,-)-l-(3,5-dimethylphenyl)-1-(5-ethyl-2- methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)-methanol (**7c**) (300 mg, 0.78 mmol) in acetic acid (2.50 mL). The heterogeneous mixture was refluxed for 4.5 h. An additional 127 mg of zinc powder (1.95 mmol) was still added, and the mixture was again refluxed for 22 h, then filtered. The solvent was evaporated under reduced pressure and the residue was taken up in 20 mL of ethyl acetate. The organic layer was washed with 10 mL of an aqueous saturated sodium bicarbonate solution, 10 mL of water, and 10 mL of brine, dried over magnesium sulfate, and concentrated under pressure. The residue was purified by column chromatography using dichloromethane−ethanol (100:0 to 95:5) to give **11** (40 mg, 5%) as a yellow solid: mp 237−238C; ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, J = 7.4 Hz, CH_3CH_2), 1.23 (9H, s, $COC(CH_3)_3$), 2.23 (6H, s, CH_3-3′ and 5′), 2.33 (3H, s, CH_3-6), 2.41 (2H, q, J = 7.6 Hz, CH_3CH_2), 3.90 (2H, s, CH_2), 6.60 (2H, s, H-2′ and 6′), 6.80 (1H, s, H-4′), 7.17 (1H, br s, NH-1). Anal. ($C_{22}H_{30}N_2O_2\cdot0.9H_2O\cdot0.9$ EtOH) C, N, O.

(+,−)-1-(5-Ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)-1-(3,5- dimethylphenyl)methyl acetate (12). A total of 8.34 g (21.70 mmol) of (+,−)-1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)-methanol (**7c**) was dissolved in pyridine (200 mL) and added to acetic anhydride (10.24 mL, 108.51 mmol), and the solution was stirred for 1.5 h at room temperature and for 60 h at 60 °C. An additional 10.24 mL of acetic anhydride (108.51 mmol) was added, and heating was continued at 60 °C for 24 h. The pyridine was evaporated under reduced pressure, and the residue was taken up in 500 mL of ethyl acetate. The organic layer was washed with 170 mL of an aqueous saturated sodium bicarbonate solution, 170 mL of water, and 170 mL of brine, dried over magnesium sulfate, and concentrated to dryness. The residue was purified by column chromatography using dichloromethane−ethanol (100:0 to 95:5) to give **12** (8.78 g, 95%) as a white mass: mp 70−71 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 7.4 Hz, CH_3CH_2), 1.11 (9H, s, $COC(CH_3)_3$), 2.17 (3H, s, $OCOCH_3$), 2.24 (6H, s, CH_3-3′ and 5′), 2.45 (3H, s, CH_3-6), 2.52−2.68 (2H, m, CH_3CH_2), 3.92 (3H, s, OCH_3), 6.75 (2H, s, H-2′ and 6′), 6.87 (1H, s, H-4′), 7.15 (1H, br s, NH), 7.18 (1H, s, CH). Anal. ($C_{25}H_{34}N_2O_4$) C, H, N.

1-(3,5-Dimethylphenyl)-l-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)methanone (13). A solution of (+,−)-1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)-methanol (**7c**) (1.66 g, 4.32 mmol) in 80 mL of toluene was heated at reflux. Manganese dioxide (1.88 g, 21.58 mmol) in suspension in 40 mL of toluene was added, and the mixture was then stirred at reflux for 6 h.

The catalyst was filtered off, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography using dichloromethane−ethyl acetate (100:0 to 95:5) as eluent giving **13** (1.48 g, 89.5%) as a white solid: mp 143−144 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, s, $COC(CH_3)_3$), 0.97 (3H, t, J = 7.3 Hz, CH_3CH_2), 2.27 (6H, s, CH_3-3′ and 5′), 2.28−2.45 (2H, m, CH_3CH_2), 2.46 (3H, s, CH_3-6), 3.92 (3H, s, CH_3O), 6.78 (1H, s, NH), 7.14 (1H, s, H-4′), 7.38 (2H, s, H-2′ and 6′). Anal. ($C_{23}H_{30}N_2O_3$·0.25H_2O) C, H, N.

3-Amino-4-(3,5-dimethylbenzoyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (14). Starting from 1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)-methanone (**13**) (400 mg, 1.05 mmol) and 3 M aqueous hydrochloric acid (24 mL), the reaction was performed as above (for **8b**). The residue was purified by column chromatography using dichloromethane−ethanol (100:0 to 95:5) as eluent giving 75 mg (19%) of starting material **13** and 191 mg (64%) of **14** as a yellow solid: mp 212−213 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, J = 7.2 Hz, CH_3CH_2), 2.19 (2H, q, J = 7.5 Hz, CH_3CH_2), 2.30 (3H, s, CH_3-6), 2.34 (6H, s, CH_3-3′ and 5′), 4.11 (2H, br s, NH_2), 7.24 (1H, s, H-4′), 7.50 (2H, s, H-2′ and 6′), 12.85 (1H, br s, NH-1). Anal. ($C_{17}H_{20}N_2O_2$·0.45H_2O) C, H, N.

4-(3,5-Dimethylbenzyl)-5-ethyl-6-methyl-3-propionamidopyridin-2(1*H*)-one (15). To a solution of 3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (**8b**) (200 mg, 0.74 mmol) and triethylamine (97.9 μL, 0.70 mmol) in dichloromethane (9 mL) was added freshly distilled propionyl chloride (65.9 μL, 0.76 mmol) dropwise at 0 °C (the flask was fitted with a calcium chloride drying tube). The mixture was stirred at room temperature during 3.5 h. The solvent was evaporated under reduced pressure, 10 mL of water was added, and the solid was filtered off. After washings with 10 mL of cyclohexane and drying in the presence of calcium chloride under vacuum, 133 mg (55%) of **15** was obtained as a light beige solid: mp 246−247 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, J = 7.4 Hz, CH_3CH_2), 1.16 (3H, t, J = 7.1 Hz, CH_3CH_2CO), 2.23−2.38 (13H, m, CH_3CH_2, CH_3CH_2CO, CH_3-3′, 5′ and 6), 3.91 (2H, s, CH_2), 6.59 (2H, s, H-2′ and 6′), 6.80 (1H, s, H-4′), 6.89 (1H, br s, N*H*$COCH_2CH_3$), 11.60 (1H, br s, NH-1). Anal. ($C_{20}H_{26}N_2O_2$·0.25H_2O) C, H, N, O.

3-Acetylamino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (17). A solution of the 3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (**8b**) (200 mg, 0.74 mmol) and acetic anhydride (106 mg, 1.03 mmol) in acetic acid (40 mL) was heated at reflux for 2 h. After evaporation of the volatile materials under reduced pressure, 20 mL of ice water was added, and the mixture was neutralized at 0 °C with diluted ammonium hydroxyde. After filtration, the residue was washed with 10 mL of cyclohexane giving **17** (101 mg, 44%) as a beige solid: mp 234−235 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.2 Hz, CH_3CH_2), 2.08 (3H, s, CH_3-CO), 2.23 (9H, s, CH_3-6, 3′ and 5′), 2.31 (2H, q, J = 7.3 Hz, CH_3CH_2), 3.90 (2H, s, $CH_2C_6H_5$), 6.60 (2H, s, H-2′ and 6′), 6.81 (1H, s, H-4′), 6.88 (1H, br s, N*H*$COCH_3$), 10.90 (1H, br s, NH-1). Anal. ($C_{19}H_{24}N_2O_2$·0.25H_2O) C, H,O.

4-(3,5-Dimethylbenzyl)-5-ethyl-3-formamido-6-methylpyridin-2(1*H*)-one (19). To a solution of 3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1H)-one (**8b**) (400 mg, 1.47 mmol) in ethyl formate (previously distilled over calcium hydride) (30 mL) was added formic acid (7 mL). The mixture was heated under reflux for 3 h. After evaporation of the volatile materials, the residue was washed with ethanol and dried in the presence of calcium chloride during 12 h under vacuum at room temperature to give 255 mg of the expected product **19**. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by column chromatography using dichloromethane−ethyl acetate−ethanol (100:0:0 to 47.5:47.5:5) as eluent to give 35 mg (total yield = 66%) of **19** as white crystals (after recrystallization from ethanol): mp 246−247 °C; ^{1}H NMR (DMSO-d_6; 294 K) δ 0.83−0.85 (3H, m, CH_3CH_2), 2.20−2.29 (11H, m, CH_3CH_2, CH_3-3′, 5′ and 6), 3.78 (0.61H, s, CH_2), 3.89 (0.39H, s, CH_2), 6.71−6.74 (2H, m, H-2′ and 6′), 6.83 (1H, s, H-4′), 8.08 (0.39H, d, J = 12.0 Hz, NH*CHO* strans), 8.17 (0.61H, s, NH*CHO* scis), 8.94 (0.39H, d, J = 12.0 Hz, *NH*CHO strans), 9.23 (0.61H, s, *NH*CHO scis), 11.71−11.81 (1H, m, NH-1); ^{1}H NMR (DMSO-d_6; 360 K) δ 0.89 (3H, t, J = 6.0 Hz, CH_3CH_2), 2.25 (9H, s, CH_3-3′, 5′ and 6), 2.35 (2H, q, J = 6.0 Hz, CH_3CH_2), 3.88 (1H, s, CH_2), 6.74 (2H, s, H-2′ and 6′), 6.84 (1H, s, H-4′), 8.16 (1H, s, NH*CHO*), 8.67 (1H, br s, *NH*CHO), 11.38 (1H, br s, NH-1). Anal. ($C_{18}H_{22}N_2O_2$·0.25H_2O) C, H, N.

4-(3,5-Dimethylbenzy)-5-ethyl-3-methylamino-6-methylpyridin-2(1*H*)-one (21). Lithium aluminum hydride (25 mg, 0.67 mmol) was cautiously added to a suspension of 4-(3,5-dimethylbenzyl)-5-ethyl-3-formamido-6-methylpyridin-2(1*H*)-one (**19**) (100 mg, 0.33 mmol) in dry THF (6 mL). The mixture was heated at reflux for 2 h. An additional 38 mg (1.00 mmol) of lithium aluminum hydride was added, and stirring was continued for 2 h (the flask was fitted with a calcium chloride drying tube). A total of 10 mL of water was slowly added. The two layers were separated and the aqueous layer was acidified by addition of a 3 M aqueous hydrochloric acid solution and then extracted with 3 × 10 mL of ether. The combined organic layers were washed with 5 mL brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane−ethanol (1:0 to 95:5) as eluant, giving **21** (54 mg, 57%) as a light brown solid (after washing with cyclohexane and drying): mp 153−154 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t, J = 7.4 Hz, CH_3CH_2), 2.25−2.30 (11H, m, CH_3CH_2, CH_3-3′, 5′ and 6), 2.66 (3H, s, NHCH_3), 3.98 (2H, s, CH_2), 6.60 (1H, br s, *NH*CH_3), 6.72 (2H, s, H-2′ and 6′), 6.81 (1H, s, H-4′), 12.84 (1H, br s, NH-1). Anal. ($C_{18}H_{24}N_2O$·0.2H_2O) C, H, N.

4-(3,5-Dimethylbenzyl)-3-ethoxycarbonylamino-5-ethyl-6-methylpyridin-2(1*H*)-one (23). Triethylamine (0.26 mL, 1.85 mmol) was added to a solution of 3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (**8b**) (200 mg, 0.74 mmol) in ethanol (7 mL). To this mixture, cooled in ice water, was added dropwise freshly distilled ethyl chloroformate (2.12 mL, 22.19 mmol), and the mixture was stirred at room temperature for 3 h, at reflux for 4 h, and then at room temperature for 96 h. After evaporation of the solvent under reduced pressure, 10 mL of water was added. After filtration of the precipitate and washings with 10 mL of cyclohexane, the solid was purified by column chromatography using dichloromethane−ethanol (100:0 to 95:5) as eluent to give the recovered amine **8b** (10 mg, 5%) and **23** (108 mg, 43%) as a yellow light solid: mp 192−193 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, t, J = 7.3 Hz, CH_3CH_2), 1.24 (3H, t, J = 7.0 Hz, CH_3CH_2-CO_2), 2.23−2.36 (11H, m, CH_3CH_2, CH_3-3′, 5′ and 6), 3.95 (2H, s, CH_2), 4.14 (2H, d, J = 7.4 Hz, $CH_3CH_2CO_2$), 6.15 (1H, br s, *NH*$CO_2CH_2CH_3$), 6.62 (2H, s, H-2′ and 6′), 6.80 (1H, s, H− 4′), 12.25 (1H, br s, NH-1). Anal. ($C_{20}H_{26}N_2O_3$·0.1H_2O) C, H, N.

4-(3,5-Dimethylbenzyl)-3-[N-(4,5-dimethyl-2-methoxybenzyl)amino]-5-ethyl-6-methylpyridin-2(1*H*)-one (25). To a solution of 3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (**8b**) (200 mg, 0.74 mmol) and of 4,5-dimethyl-2-methoxybenzaldehyde[6,21] (121.5 mg, 0.74 mmol) in 10 mL of methanol was added a drop of glacial acetic acid. The mixture was stirred at room temperature for 2.4 h. The yellow precipitate was filtered off and dissolved in 10 mL of a mixture of methanol−chloroform (1:1, v/v), and 56 mg of sodium borohydride (1.48 mmol) was added. A discoloration of the solution was observed. The solvents were evaporated under reduced pressure, and the resulting residue was taken up in 50 mL of dichloromethane. The organic layer was washed with 2 × 10 mL of water and 10 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure to give **25** (185 mg, 60%) as a yellow light solid: mp 179−180 °C; ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.1 Hz, CH_3CH_2), 2.10−2.22 (17H, m, CH_3CH_2, CH_3-3′, 4", 5′, 5" and 6), 3.68 (3H, s, CH_3O), 3.87 (2H, s, CH_2), 3.96 (2H, d, J = 6.9 Hz, NHCH_2), 4.53 (1H, t, J = 7.0 Hz, *NH*CH_2), 6.70 (2H, s, H-2′ and 6′), 6.74 (1H, s, H-6″), 6.84 (2H, s, H-3″ and 4′), 11.30 (1H, br s, NH-1). Anal. ($C_{27}H_{32}N_2O_2$·1.75H_2O) C, H, N, O.

3-Dimethylamino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (27). To a stirred solution of 3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-

one (**8b**) (200 mg, 0.74 mmol) and 37% aqueous formaldehyde (0.60 mL, 7.39 mmol) in 5 mL of acetonitrile was added 139 mg (2.22 mmol) of sodium cyanoborohydride. Glacial acetic acid (0.07 mL, 2.22 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 2 h. An additional 0.07 mL (2.22 mmol) of glacial acetic acid was added, and stirring was continued for 30 min. The solvent was evaporated, and 15 mL of ether was added to the resulting residue. The organic layer was washed with 3 × 30 mL of 1 N aqueous potassium hydroxide and 3 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure to give **27** (200 mg, 91%) as a yellow solid: mp 229−230 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, J = 7.3 Hz, CH_3CH_2), 2.24−2.29 (11H, m, CH_3CH_2, CH_3-3′, 5′ and 6), 2.73 (6H, s, $N(CH_3)_2$), 4.10 (2H, s, CH_2), 6.66 (2H, s, H-2′ and 6′), 6.78 (1H, s, H-4′), 11.42 (1H, br s, NH-1). Anal. ($C_{19}H_{26}N_2O\cdot0.3H_2O$) C, H, N.

3-[*N*-(*tert*-Butoxycarbonyl)glycyl]amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (29). The starting materials were dried in the presence of calcium chloride under vacuum at room temperature, and the 1-hydroxybenzotriazole hydrate was dehydrated in the presence of calcium chloride at 70 °C under vacuum for 12 h. The 1,3-dicyclohexylcarbodiimide (4.81 g, 23.30 mmol) was added to the mixture, cooled at 0 °C, of dehydrated 1-hydroxybenzotriazole (3.15 g, 23.30 mmol, commercial) and *N*-(*tert*-butoxycarbonyl)glycine (4.08 g, 23.30 mmol) in 25 mL of anhydrous dichloromethane. A slight exothermic reaction occurred, and the formation of a white precipitate was observed. The mixture was stirred at 0 °C under a nitrogen atmosphere for 30 min. Then the 3-amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (**8b**) (0.90 g, 3.33 mmol), dissolved in 25 mL of dichloromethane, was added dropwise. After 5 min, freshly distilled (barium oxide), *N*-methylmorpholine (2.82 mL, 25.63 mmol) was added. The resulting mixture was stirred at 0 °C for 1.5 h and at room temperature for 3.5 h. The 1,3-dicyclohexylurea was filtered off, and the solvent was evaporated under reduced pressure. The residue was taken up in 250 mL of ethyl acetate. This organic layer was washed successively with a saturated aqueous sodium bicarbonate solution (2 × 50 mL), a saturated aqueous citric acid solution (50 mL), water (50 mL), and brine (50 mL). It was then dried over magnesium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography using dichloromethane−ethanol (98:2 to 95:5) as eluent giving **29** (1.00 g, 70.5%) as a yellow light solid: mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, t, J = 7.0 Hz, CH_3CH_2), 1.38 (9H, s, $COC(CH_3)_3$), 2.22 (6H, s, CH_3-3′ and 5′), 2.31 (3H, s, CH_3-6), 2.35 (2H, q, J = 7.0 Hz, CH_3CH_2), 3.88 (4H, s, 2 CH_2), 5.29 (1H, br s, NH-3), 6.59 (2H, s, H-2′ and 6′), 6.79 (1H, s, H-4′), 7.52 (1H, br s, NH−Boc), 12.58 (1H, br s, NH-1). Anal. ($C_{24}H_{33}N_3O_4\cdot0.25H_2O$) C, H, N.

4-(3,5-Dimethylbenzyl)-5-ethyl-3-(*N*-glycyl)amino-6-methylpyridin-2(1*H*)-one (30). To a suspension of 3-[*N*-(*tert*-butoxycarbonyl)glycyl]amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(1*H*)-one (**29**) (500 mg, 1.17 mmol) in 40 mL of ethyl acetate was added concentrated aqueous hydrochloric acid (2.70 mL). The solution obtained was stirred at room temperature for 10 min and basified with concentrated ammonium hydroxyde. Water (60 mL) was added, and the aqueous layer was extracted with ethyl acetate (3 × 120 mL). The combined organic layers were washed with 40 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure to give 380 mg (99%) of the deprotected product **30** as a white solid: mp 148−149 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (3H, t, J = 6.9 Hz, CH_3CH_2), 2.22 (11H, br s, CH_3-3′, 5′, 6 and CH_3CH_2), 3.23 (2H, s, CH_2-4), 3.38 (2H, br s, NH_2), 3.77 (2H, s, CH_2NH_2), 6.73 (2H, s, H-2′ and 6′), 6.81 (1H, s, H-4′), 6.86 (1H, br s, NH-3). Anal. ($C_{19}H_{25}N_3O_2\cdot0.7H_2O$) C, H.

(+,−)-1-(3,5-Dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)ethan-1-ol (31). The starting material **13** was dried in the presence of calcium chloride at room temperature under vacuum for 12 h. To a solution of 1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy- 6-methyl-3-pivaloylaminopyridin-4-yl)methanol (**13**) (400 mg, 1.05 mmol) in 20 mL of anhydrous ether was added methyllithium (1.6 M in ether, 1.96 mL, 3.14 mmol) dropwise under a nitrogen atmosphere at 0 °C. The mixture was stirred at this temperature during 2 h and then at room temperature for 12 h. The reaction was quenched by addition of 20 mL of water. The two layers were separated, and the aqueous layer was extracted with 3 × 40 mL of ether. The combined organic layers were washed with 20 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane−ethanol (99:1 to 95:5) as eluent giving **31** (302 mg, 72.5%) as a white solid: mp 159−160 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (9H, s, $COC(CH_3)_3$), 1.02 (3H, t, J = 7.4 Hz, CH_3CH_2), 2.17 (3H, s, CH_3-3′ or 5′*), 2.28 (3H, s, CH_3-5′ or 3′*), 2.39−2.48 (8H, m, CH_3-6, CH_3CH_2 and $CH_3C(OH)$), 3.93 (3H, s, OCH_3), 6.77 (1H, s, H-4′), 6.97 (1H, s, H-2′ or 6′#), 7.08 (1H, s, H-6′ or 2′#). Anal. ($C_{24}H_{34}N_2O_3$) C, H, N, O.

(+,−)-1-(3-Acetylamino-5-ethyl-2-methoxy-6-methylpyridin-4-yl)-1-(3,5-dimethylphenyl)ethyl acetate (33). A solution of (+,−)-1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)ethan-l-ol (**31**) (100 mg, 0.25 mmol) in 5 mL of acetic anhydride was heated at reflux for 72 h. The solvent was evaporated under reduced pressure, and the resulting residue was taken up in 10 mL of water and 10 mL of dichloromethane. The aqueous layer was basified by addition of 1 N aqueous potassium hydroxide and extracted twice with 10 mL of dichloromethane. The combined organic layers were washed with 5 mL of brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane−ethyl acetate (99:1 to 95:5) as eluent to give **33** (9 mg, 9%) as a yellow light oil and 45 mg (45%) of the recovered pyridine **31**: ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, t, J = 7.5 Hz, CH_3CH_2), 2.07 (6H, s, CH_3-3′ and 5′), 2.19 (3H, s, CH_3-6), 2.30 (3H, s, CH_3COO), 2.34−2.48 (2H, m, CH_3CH_2), 2.43 (3H, s, $COCH_3$), 2.54 (3H, s, CH_3), 3.93 (3H, s, OCH_3), 7.01 (1H, s, H-2′ or 6′*), 7.11 (1H, s, H-6′ or 2′*), 7.37 (1H, s, H-4′); MS (IC): 399 $[M + H]^+$, 355 $[M\text{-}COCH_3]^+$. Anal. ($C_{23}H_{30}N_2O_4\cdot0.5H_2O$) C, H.

(+,−)-4-[1-(3,5-Dimethylphenyl)-1-hydroxy]ethyl-5-ethyl-6-methyl-3-pivaloylaminopyridin-2(1*H*)-one (34). To a solution of (+,−)-1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)ethan-l-ol (**31**) (100 mg, 0.25 mmol) in 10 mL of toluene was added *p*- toluenesulfonic acid (119 mg, 0.63 mmol). The mixture was stirred at reflux for 21 h. The toluene was evaporated under reduced pressure, and 10 mL of water was added to the resulting residue. The aqueous layer was extracted with 3 × 15 mL of dichloromethane. The combined organic layers were washed with 10 mL of a saturated aqueous sodium bicarbonate solution, dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane−ethanol (1:0 to 95:5) as eluent giving **34** (64 mg, 66%) as a white solid: mp 257−258 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (9H, s, $COC(CH_3)_3$), 1.03 (3H, t, J = 7.1 Hz, CH_3CH_2), 2.20 (3H, s, CH_3-3′ or 5′*), 2.27 (3H, s, CH_3-5′ or 3′*), 2.31−2.41 (5H, m, CH_3-6 and CH_3CH_2), 2.48 (3H, s, $CH_3C(OH)$), 6.98 (1H, s, H-2′ or 6′#), 7.08 (1H, s, H-6′ or 2′#), 7.40 (1H, s, H-4′), 12.36 (1H, br s, NH-1). Anal. ($C_{23}H_{32}N_2O_3\cdot0.5H_2O$) C, H, N.

(+,−)-3-Amino-4-[1-(3,5-dimethylphenyl)-1-hydroxy]-ethyl-5-ethyl-6-methylpyridin-2(1*H*)-one (35) and 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one[4] (36). Starting from (+,−)-1-(3,5-dimethylphenyl)-1-(5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl)ethan-1-ol (**31**) (100 mg, 0.25 mmol) and 3 M aqueous hydrochloric acid (6 mL), the reaction was performed as above (for **8b**). The residue was purified by column chromatography using dichloromethane−ethanol (100:0 to 95:5) as eluent giving 9 mg (9%) of the recovered pyridine **31**, 45 mg (60%) of **35** as a yellow-brown solid and 7 mg (18%) of **36** as a violet solid. **35**: mp 144−145 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, t, J = 7.4 Hz, CH_3CH_2), 2.13 (2H, q, J = 7.5 Hz, CH_3CH_2), 2.23−2.34 (9H, m, CH_3-3′, 5′ and 6), 2.41 (3H, s, $CH_3C(OH)$), 4.71 (2H, br s, NH_2), 7.05 (2H, s, H-2′ and 6′), 7.12 (1H, s, H-4′), 12.66 (1H, br s, NH-1). Anal. ($C_{18}H_{24}N_2O_2\cdot$

0.4EtOH) C, N, O. **36**: mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (3H, t, J = 7.5 Hz, CH_3CH_2), 2.21 (3H, s, CH_3-6), 2.32 (2H, q, J = 7.5 Hz, CH_3CH_2), 3.93 (2H, br s, NH_2), 6.54 (1H, s, H-4), 11.81 (1H, br s, NH-1); MS (IC): 153 $[M + H]^+$. Anal. ($C_8H_{12}N_2O \cdot 0.01H_2O$) C, H, O.

Biology. Evaluation of Antiviral Activity of the Compounds. The effects of the compounds on the replication of HIV-1 were evaluated (Table 2), as previously described, in CEM-SS cells (a cell line of the lymphocytic lineage) acutely infected with HIV-1 LAI.[34] CEM-SS cells were obtained from Peter Nara, the nevirapine resistant HIV-1 (N119) strain bearing a point mutation at RT codon 181 and AZT resistant HIV-1 (A018 G910-6) = AZT res. from D. Richman, HIV-1 Bal is a macrophage tropic virus and received from S. Gartner, M. Popovic, and R. Gallo through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

The compounds were solubilized in DMSO at an initial concentration of 10−2 M. The solubility in aqueous phase was tested with a dilution 1:100 in culture medium. When nesessary, in case of insolubility, a 10−3 M solution in DMSO, or lower concentration, was diluted at 1% in culture medium. This solution, as well as serial dilutions in culture medium, were used in the antiviral assays.

The production of virus was measured by quantification of the RT activity associated with the virus particles released in the culture supernatant. Briefly, cells were infected with 100 $TCID_{50}$ for 30 min; after virus adsorption, unbound particles were eliminated by two washes, and cells were cultured in the presence of different concentrations of test compounds for 5 days before virus production determination. The 50% inhibitory concentration of virus multiplication (IC_{50}) was derived from the computer-generated median effect plot of the dose−effect data.[35] In parallel experiments, cytotoxicity of the molecules for uninfected cells was measured after an incubation of 5 days in their presence using a colorimetric assay (MTT test) based on the capacity of mitochondrial dehydrogenases of living cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into formazan.[35] The 50% cytotoxic concentration (CC_{50}) is the concentration at which OD_{540} was reduced by one-half and was calculated using the program mentioned above. The assay procedures for measuring the anti-HIV activity of the compounds in the different cells infected with other virus isolates were also based on a quantitative detection of reverse transcriptase activity associated with the virus particles liberated in the culture supernatant, except for HIV-1 IIIB tested in MT4 cells for which the assay is based on the virus-induced cytopathogenicity (test MTT). The experimental protocoles have been detailed previously, note that monocyte-derived macrophages were maintained, in absence of serum, in medium AIM V (Gibco) supplemented with 100 U/mL GM-CSF (Genzyme, TEBU, France).[1b,34]

Retrovirucidal Effect. HIV-1 viral suspensions were obtained by coculture of MT4 cells and H9 cells chronically infected by HIV-1_{Lai} isolate. A cell supernatant (200 μL) containing viral particles (HIV-1_{Lai}: 100 $TCID_{50}$) was incubated at room temperature with various concentrations of different inhibitors. After 3 h, virions were washed through 0.02 μm Anopore membrane in 1.5 mL Vectaspin tube (Whatman) for 10 min at 5 000*g*. Each of the three subsequent washes was performed in the same conditions after the viral concentrate was refilled with 500 μL of RPMI medium. Then, the viral concentrate was readjusted to the initial volume with RPMI plus 10% fetal calf serum (FCS). The residual infectivity was assayed on P4 cells as described by Charneau et al.[36] Briefly, P4 cells were plated using 100 μL of DMEM medium plus 10% FCS in 96 plate multi-wells at 20 × 10^5 cells per milliliter. After overnight incubation at 37 °C, the supernatant was discarded, and the viral preparation (200 μL) was added. One day later the wells were washed three times in PBS. Each well was refilled with 200 μL of a reaction buffer containing 50 mM Tris-HC1 pH 8.5, 100 mM 2-mercaptoethanol, 0.05% Triton X-100, and 5 mM 4-methylumbelliferyl β-D-galactopyranoside (MUG). After 3 h at 37 °C, the level of the reaction was measured in a fluorescence microplate reader.

Expression and Purification of the Recombinant HIV-1 RT Enzyme. Yeast cells, transformed with the vector pAB 24/RT-4, were used to purify the recombinant HIV-1 RT enzyme as described previously.[37]

The system for the expression of recombinant HIV-2 RT in *Escherichia coli* was a kind gift of Dr. R. Goody. HIV-2 RT was purified as HIV-1 RT.[38]

Reverse Transcriptase Assays. Incubation was carried out at 37 °C for 10 min in the presence of different template-primers. (a) Poly(C)-oligo(dG): the reaction mixture contained in a final volume of 0.05 mL, 50 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 4 mM dithiothreitol, 0.48 A_{260} units/mL of poly(C)-oligo (dG) (5:1), 1.0 μCi [^{3}H]dGTP (28 Ci/mmol), 2 μM dGTP, 80 mM KC1, 1 μg bovine serum albumin and 20−50 nM RT. (b) Poly-(A)-oligo(dT): same conditions as in (a), except that 0.48 A_{260} units/mL of poly(A)-oligo(dT) (5:1), 0.5 μCi of [^{3}H]dTTP (46 Ci/mmol), 20 μM dTTP were used.

Reactions were stopped by the addition of 1 mL of cold 10% trichloroacetic acid plus 0.1 M sodium pyrophosphate. The precipitates were filtered through nitrocellulose membranes, washed with 2% trichloroacetic acid, dried, and counted in a PPO/POPOP/toluene scintillation mixture.

Reverse Transcription. The plasmid pAV4 containing the 50−997 HIV-1 nucleotide fragment (MAL strain) in pSP64, under the control of the bacteriophage T7 promoter, was a kind gift from Dr. J.-L. Darlix (INSERM-Lyon, France). *E. coli* HB 101 recA$^-$ was used for plasmid amplification. After digestion of this clone with PstI and in vitro transcription using T7 RNA polymerase, a HIV-1 genomic RNA fragment starting at position +50 of the MAL sequence was obtained. In vitro transcription using T7 RNA polymerase was performed as follows. Three micrograms of linearized plasmid DNA were transcribed in 100 μL of 40 mM Tris-HCl pH 8.0, 8 mM $MgCl_2$, 10 mM spermidine, 25 mM NaCl, 10 mM dithiothreitol, 0.5 mM of each ribonucleoside triphosphate, with 100 units of T7 RNA polymerase and in the presence of 20 units of human placenta ribonuclease inhibitor, for 2 h at 37 °C. After treatment with 12 units of RNase-free DNase I (for 10 min at 37 °C), the RNA transcripts were extracted with 1 volume of phenol/chloroform/isoamyl alcohol (24:24:1) and with chloroform and precipitated in 2.5 volumes of ethanol and 0.3 M ammonium acetate (pH 5.5).

Reverse transcription was performed in a total volume of 50 μL containing 50 mM Tris-HC1 pH 8.0, 6 mM $MgCl_2$, 2 mM dithiothreitol, 12 mM NaCl, 150 nM HIV-1 RNA, and either 200 nM of a synthetic oligodeoxynucleotide primer (18-mer ODN) complementary to the PBS of HIV-1 RNA, or 200 nM $tRNA^{Lys3}$. When the 18-mer ODN was used as primer, incubation was carried out at 37 °C with the template and 300 nM RT. After 30 min, 10 μCi[(α-^{32}P]dGTP (3000 Ci/mmol) and 0.1 mM of each dNTP were added, and the incubation proceeded for 30 min at 37 °C. With $tRNA^{Lys3}$ as primer, the same conditions were used except that tRNA and RNA were prehybridized by heating for 2 min at 90 °C and then slowly cooled. Samples were extracted with phenol−chloroform and collected by ethanol precipitation. Reaction products were analyzed on 8% polyacrylamide−TBE (90 mM Tris pH 8.3, 90 mM borate, 2 mM EDTA)−7 M urea gels.

Inhibition Experiments. All compounds were dissolved in dimethylsulfoxide. Controls were made in the presence of the same final concentration of DMSO.

Acknowledgment. The authors thank Drs. Claude Monneret and David Grierson for many fruitful discussions and advice. We thank the French Agency for Research Against AIDS (ANRS) for financial support including fellowships to V.D. We thank Dr. Daniel Dauzonne and the Drug Synthesis and Chemistry Branch, Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, for supplying us with 2-methoxy-4,5-

dimethylbenzaldehyde and nevirapine, respectively. This work was also supported by the Conseil Regional d'Aquitaine. The expert technical assistances by Ms. Christina Calmels (Supported by SIDACTION), Sylvie Schmidt, and Géraldine Albrecht are acknowledged.

Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Bisagni, E.; Dolle, V.; Nguyen, C.-H.; Legraverend, M.; Aubertin, A.-M.; Kirn, A.; Andreola, M. L.; Tarrago-Litvak, L. 4-Aryl-thio-pyridin-2(1*H*)-ones, medecines containing them and their uses in the treatment of illnesses linked to HIV-1 and 2. Fr. Demande Appl. 9509323, 30 Jul 1995. (b) Dolle, V.; Fan, E.; Nguyen, C. H.; Aubertin, A.-M.; Kirn, A.; Andreola, M. L.; Jamieson, G.; Tarrago-Litvak, L.; Bisagni, E. A New Series of Pyridinone Derivatives as Potent Non-Nucleoside Human Immunodeficiency Virus Type 1 Specific Reverse Transcriptase Inhibitors. *J. Med. Chem.* **1995**, *38*, 4679−4686.

(2) Goldman, M. E.; O'Brien, J. A.; Ruffing, T. L.; Schleif, W. A.; Sardana, V. V.; Byrnes, V. W.; Condra, J. H.; Hoffman, J. M.; Emini, A. E. A Nonnucleoside Reverse Transcriptase Inhibitor Active on Human Immunodeficiency Virus Type 1 Isolates Resistant to Related Inhibitors. *Antimicrob. Agents Chemother.* **1993**, *37*, 947−949.

(3) Saari, W.8.; Hoffman, J. M.; Wai, J. S.; Fisher, T. E.; Rooney, C. S.; Smith, A. M.; Thomas, C. M.; Goldman, M. E.; O'Brien, J. A.; Nunberg, J. H.; Quintero, J. C.; Schleif, W. A.; Emini, A. E.; Stern, A. M.; Anderson, P. S. 2-Pyridinone Derivatives: A New Class of Nonnucleoside, HIV-1-Specific Reverse Transcriptase Inhibitors. *J. Med Chem.* **1991**, *34*, 2922−2925.

(4) Hoffman, J. M.; Wai, J. S.; Thomas, C. M.; Levin, R. B.; O'Brien, J. A.; Goldman, M. E. Synthesis and Evaluation of 2-Pyridinone Derivatives as HIV-1 Specific Reverse Transcriptase Inhibitors. 1. Phthalimido alkyl and -alkylamino Analogues. *J. Med. Chem.* **1992**, *35*, 3784−3791.

(5) Saari, W. S.; Wai, J. S.; Fisher, T. E.; Thomas, C. M.; Hoffman, J. M.; Rooney, C. S.; Smith, A. M.; Jones, J. H.; Bamberger, D. L.; Goldman, M. E.; O'Brien, J. A.; Nunberg, J. H.; Quintero, J. C.; Schleif, W. A.; Emini, A. E.; Anderson, P. S. Synthesis and Evaluation of 2-Pyridinone Derivatives as HIV-1-Specific Reverse Transcriptase Inhibitors. 2. Analogues of 3-Aminopyridin-2(1*H*)-one. *J. Med. Chem.* **1992**, *35*, 3792−3802.

(6) Wai, J. S.; Williams, T. M.; Bamberger, D. L.; Fisher, T. E.; Hoffman, J. M.; Hudcosky, R. J.; MacTough, S. C.; Rooney, C. S.; Saari, W. S.; Thomas, C. M.; Goldman, M. E.; O'Brien, J. A.; Emini, E. A.; Nunberg, J. H.; Quintero, J. C.; Schleif, W. A.; Anderson, P. S. Synthesis and Evaluation of 2-Pyridinone Derivatives as Specific HIV-1 Reverse Transcriptase Inhibitors. 3. Pyridyl and Phenyl Analogues of 3-Aminopyridin-2(1*H*)-one. *J. Med. Chem.* **1993**, *36*, 249−255.

(7) Hoffman, J. M.; Smith, A. M.; Rooney, C. S.; Fisher, T. E.; Wai, J. S.; Thomas, C. M.; Bamberger, D. L.; Barnes, J. L.; Williams, T. M.; Jones, J. H.; Olson, B. D.; O'Brien, J. A.; Goldman, M. E.; Nunberg, J. H.; Quintero, J. C.; Schleif, W. A.; Emini, A. E.; Anderson, P. S. Synthesis and Evaluation of 2-Pyridinone Derivatives as HIV-1-Specific Reverse Transcriptase Inhibitors. 4. 3-[2-(Benzoxazol-2-yl)ethyl]-5-ethyl-6-methylpyridin-2(1*H*)-one and Analogues. *J. Med. Chem.* **1993**, *36*, 953−966.

(8) Saag, M. S.; Emini, A. E.; Laskin, O. L.; Douglas, J.; Lapidus, W. I.; Schleif, W. A.; Whitley, R. J.; Hildebrand, C.; Byrnes, V. W.; Kappes, J. C.; Anderson, K. W.; Massari, F. E.; Shaw, G. M. The L-697,661, Working Group. A Short-Term Clinical Evaluation of L-697, 661, A Non-Nucleoside Inhibitor of HIV-1 Reverse Transcriptase. *N. Engl. J. Med.* **1993**, *329*, 1065−1072.

(9) Houpis, I. N.; Molina, A.; Lynch, J.; Reamer, R. A.; Volante, R. P.; Reider, P. J. Condensation of 2-Methylbenzoxazole with Aromatic Aldehydes Bearing Acidic Protons. A Convenient Coupling in the Synthesis of the HIV−Reverse Transcriptase Inhibitor L-696,229. *J. Org. Chem.* **1993**, *58*, 3176−3178.

(10) Doll6, V.; Nguyen, C. H.; Bisagni, E. Studies towards 4-C-Alkylation of Pyridin-2(1H)-one Derivatives. *Tetrahedron* **1997**, *53*, 12505−12524.

(11) Casas, R.; Cavé, C.; d'Angelo, J. *Ortho*-metalated Aromatic Tertiary Amides: New Synthetic Applications. *Tetrahedron Lett.* **1995**, *36* (7), 1039−1042.

(12) Kelley, J. L.; Linn, J. A.; Selway, J. W. T. Synthesis and Antirhinovirus Activity of 6-(dimethylamino)-2-(trifluoromethyl)-9-(substitutedbenzyl)-9H-purines. *J. Med. Chem.* **1989**, *32*, 1757−1763.

(13) Olah, G. A.; Balaram Gupta, B. G.; Malhotra, R.; Narang, S. C. Chlorotrimethylsilane/Lithium Bromide and Hexamethyl disilane/Pyridinium Bromide Perbromide: Effective and Selective Reagents for the Conversion of Alkyl (Cycloalkyl and Aralkyl) Alcohols into Bromides. *J. Org. Chem.* **1980**, *45*, 1638−1639.

(14) Fisher, L. E.; Labadie, S. S.; Reuter, D. C.; Clark, R. D. Palladium-Mediated Benzylation of Dimetalated 1-Butylindole-3-carboxylic Acid. *J. Org. Chem.* **1995**, *60*, 6224−6225.

(15) Schaefer, T.; Takeuchi, C. S. A Positive ^{6}J (*H, CHO*) in some *meta* Derivatives of Benzaldehyde. A Simple Model. *Can. J. Chem.* **1989**, *67*, 827−830.

(16) Bisagni, E.; Rautureau, M.; Huel, C. 1-Functionalized 5,6-dimethyl-6H-pyrido[4,3-b]carbazoles (Ellipticines) and Analogues: A New Rapid Synthesis. *Heterocycles* **1988**, *27* (7), 1671−1678.

(17) Komatsu, Y.; Minami, N. Synthesis of a Novel Dual Inhibitor of Thromboxane A_2 Synthetase and 5-Lipoxygenase (E3040) via the Direct Coupling Reaction of Hydroquinone with 3-Pyridine carboxaldehyde. *Chem. Pharm. Bull.* **1995**, *43* (10), 1614−1616.

(18) Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Inouye, N.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. Synthesis and Antiviral Activity of 6-Benzyl Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) as Potent and Selective Anti-HIV-1 Agents. *J. Med. Chem.* **1995**, *38*, 2860−2865.

(19) Suzuki, K.; Tatsuoka, T.; Murakami, T.; Ishihara, T.; Aisaka, K.; Inoue, T.; Ogino, R.; Kuroki, M.; Miyazaki, T.; Satoh, F.; Miyano, S.; Sumoto, K. 2-Arylmethyl-1,4-benzoquinones. I. Novel Inhibitors of Platelet Aggregation: Synthesis and Pharmacological Evaluation. *Chem. Pharm. Bull.* **1996**, *44*, (1), 139−144.

(20) (a) Highet, R. J.; Wildman, W. C. Solid Manganese Dioxide as an Oxidizing Agent. *J. Am Chem. Soc.* **1955**, *77*, 4399−4401. (b) Gritter, R. J.; Wallace, T. J. The Manganese Dioxide Oxidation of Allylic Alcohols. *J. Org. Chem.* **1959**, *24*, 1051−1056. (c) Harfenist, M.; Bavley, A.; Lazier, W. A. The Oxidation of Allyl and Benzyl Alcohols to the Aldehydes. *J. Org. Chem.* **1954**, *19*, 1608−1616.

(21) The 2-methoxy-4,5-dimethylbenzaldehyde was provided by Dr. Dauzonne, Institut Curie, Section de Recherche, Paris.

(22) Borch, R. F.; Hassid, A. I. A New Method for the Methylation of Amines. *J. Org. Chem.* **1972**, *37*, 1673−1674.

(23) Nguyen, C. H.; Bisagni, E.; Lhoste, J.-M. Synthesis of N-5 substituted 5*H*-pyrido[4,3-*b*]benzo[*f*]indole derivatives, isomers of 6*H*-pyrido[4,3-*b*]carbazoles (ellipticines). *Can. J. Chem.* **1986**, *64*, (3), 545−551.

(24) Nicolaou, K. C.; Barnette, W. E.; Ma, P. A Remarkably Simple, Highly Efficient, and Stereoselective Synthesis of Steroids and Other Polycyclic Systems. Total Synthesis of Estra-1,3,5(10)-trien-17-one *via* Intramolecular Capture of *o*-Quinodimethanes Generated by Cheletropic Elimination of SO_2. *J. Org. Chem.* **1980**, *45*, 1463−1470.

(25) Dollé, F.; Hetru, C.; Rousseau, B.; Sobrio, F., Blais, C.; Lafont, R.; Descamp, M.; Luu, B. Synthesis of a Tritiated 3-Dehydroecdysteroid Putative Precursor of Ecdysteroid Biosynthesis. *Tetrahedron* **1993**, *49*, 2485−2498.

(26) Wittig, G.; SchoellKopf, U. Methylenecyclohexane. *Org. Synth.* **1973**, *V*, 751−754.

(27) Trono, D. Partial Reverse Transcripts in Virions from Human Immunodeficiency and Murine Leukemia Viruses. *J. Virol.* **1992**, *66*, 4893−4900.

(28) Lori, F.; Veronese, F. M.; DeVico A. L.; Lusso, P.; Reitz, M. S.; Gallo, R. C. Viral DNA Carried by Human Immunodeficiency Virus Type 1 Virions. *J. Virol.* **1992**, *66*, 5067−5074.

(29) Zhang, H.; Zhang Y.; Spicer, T. P.; Abbott, L. Z. Abbott, M.; Poiesz, B. J. Reverse Transcription Takes Place within Extracellular HIV-1 Virions: Potential Biological Significance. *AIDS Res. Hum. Retroviruses* **1993**, *9*, 1287−1296.

(30) Ho, D. D.; Neumann, A. U.; Perelson, A. S.; Chen, W.; Leonard, J. M.; Markowitz, M. Rapid Turnover of Plasma Virions and CD4 Lymphocytes in HIV-1 Infection. *Nature* **1995**, *373*, 123−126.

(31) Borkow, G.; Barnard, J.; Nguyen, T. M.; Belmonte, A.; Wainberg, M. A.; Parniak, M. A. Chemical Barriers to Human Immunodeficiency Virus Type 1 (HIV-1) Infection: Retrovirucidal Activity of UC781, a Thiocarboxanilide Nonnucleoside Inhibitor of HIV-1 Reverse Transcriptase. *J. Virol.* **1997**, *71*, 3023−3030.

(32) Ventura, M.; Tarrago-Litvak, L.; Dollé, V.; Nguyen, C. H.; Legraverend, M.; Fleury, H. J. A.; Litvak, S. Effect of nucleoside analogues and nonnucleoside inhibitors of HIV-1 reverse transcriptase on cell-free *virions. Arch. Virol.* **1999**, *144*, 513−523.

(33) Carroll, S. S.; Stahlhut, M.; Geib, J.; Olsen, D. B. Inhibition of HIV-1 Reverse Transcriptase by a Quinazolinone and Comparison with Inhibition by Pyridinones. Differences in the Rates of Inhibitor Binding and in Synergistic Inhibition with Nucleoside Analogues. *J. Biol. Chem.* **1994**, *269*, 32351−32357.

(34) Moog, C.; Wick, A.; Le Ber, P.; Kirn, A.; Aubertin, A. M. Bicyclic Imidazo Derivatives, a New Class of Highly Selective Inhibitors for the Human Immunodeficiency Virus Type 1. *Antiviral Res.* **1994**, *24*, 275−288.

(35) Chou, J.; Chou, T.-C. Dose−effect Analysis with Microcomputers: Quantitation of ED_{50}, LD_{50}, Synergism, Antagonism, Low-dose Risk, Receptor Binding and Enzyme Kinetics. *Computer Software for Apple II Series and IBM-PC and Instruction Manual*; Elsevier-Biosoft, Elsevier Science Publishers: Cambridge, U.K., 1985; pp 19−28.
(36) Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(37) Charneau, P.; Mirambeau, G.; Roux, P.; Paulous, S.; Buc, H.; Clavel, F. HIV-1 Reverse Transcription. A Termination Step at the Center of the Genome. *J. Mol. Biol.* **1994**, *241*, 651−662.
(38) Sallafranque-Andreola, M. L.; Robert, D.; Barr, P. J.; Fournier, M.; Litvak, S.; Sarih-Cottin, L.; Tarrago-Litvak, L. Human Immunodeficiency Virus Reverse Transcriptase Expressed in Transformed Yeast Cells. *Eur. J. Biochem*. **1989**, *184*, 367−374.
(39) Müller, B.; Restle, T.; Kühnel, H.; Goody, R. S. Expression of the Heterodimeric Form of Human Immunodeficiency Virus Type 2 Reverse Transcriptase in *E. coli* and Characterization of the Enzyme. *J. Biol. Chem*. **1991**, *266*, 14709−14713.

JM0009437