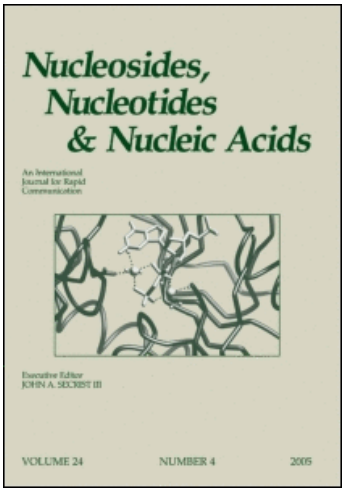

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Synthesis and RT Inhibitory Activity Evaluation of New Pyrimidine-Based *Seco*-Nucleosides

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SYNTHESIS AND RT INHIBITORY ACTIVITY EVALUATION OF NEW PYRIMIDINE-BASED *SECO*-NUCLEOSIDES

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□ *Eleven new 3′,4′-*seco *acyclic nucleosides (**4–14**) were prepared by nucleophilic substitution of protected pyrimidine bases on ethyl 3,3-diethoxypropanoate (**3**). Structures were characterized spectroscopically and a brief analysis of their conformation in solution was performed by the vicinal coupling constants* $^3J_{H2'aH3'}$ *and* $^3J_{H2'bH3'}$*. In solid state, compound **6** forms a homodimer linked by hydrogen bonding. In preliminary tests all compounds show low toxicity and gentle activity against HIV-1 RT in vitro.*

Keywords Non-nucleosides; Acyclonucleosides; *Seco*-nucleosides; RT inhibitors

INTRODUCTION

One of the most devasting diseases in the last quarter of a century is acquired immune deficiency syndrome (AIDS). This often fatal immunodeficiency disorder is caused by the retrovirus HIV-1 that targets CD4 receptors in cell-mediated immune system. During the process of viral infection, the single-stranded RNA genome of HIV is converted to double-stranded DNA by the viral encoded reverse transcriptase (RT) enzyme. Thus, a strategy to control the disease is to target the RT enzyme with drugs that bind to it, blocking their active site, thus leading to inactivation of the replicative cycle of the virus.[1]

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Address correspondence to Barbara Gordillo, Depto. de Química. CINVESTA-IPN. Av. IPN 2508 07000 México D.F. E-mail: ggordill@cinvestav.mx

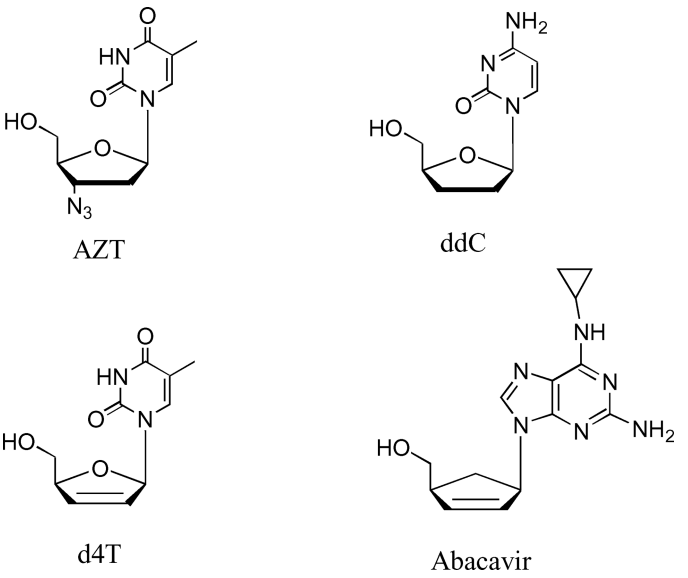


FIGURE 1 Anti-HIV ddN drugs.

The HIV-1 reverse transcriptase has a highly flexible hydrophobic binding domain with the shape of a pocket with two inhibitory binding sites named the catalytic and the allosteric site.[2] The metabolically activated 5′-triphosphates of 2′,3′-dideoxynucleoside (ddN) analogues are known to be potent antiviral drugs that interact at the catalytic site of the RT enzyme.[3,4] However, ddN analogues are toxic to normal cells because they compete with natural nucleosides for the in vivo phosphorylation step acting as DNA chain terminators in the polymerization process.[5,6] Some antiviral ddN are shown in Figure 1.

On the other hand, non-nucleosides reverse transcriptase inhibitors (NNRTIs),[7] shown in Figure 2, are less toxic since they do not interact with DNA polymerases but with the RT enzyme at the allosteric site located 10 Å apart from the catalytic site.

A striking feature of allosteric RT pocket is its considerable conformational flexibility, which is believed to be essential for its catalytic action.[8] The pocket geometries dictate the binding energies of the NNRTIs into the cavity; therefore, changes of inhibitor potency are accompanied with those changes in conformation that optimize their interaction with proteins. For example, the non-nucleoside analogues MKC-442 and TNK-651 (Figure 3) are better RT inhibitors than HEPT (i.e., $IC_{50} = 17\ \mu M$ for HEPT vs. $IC_{50} =$ 8 nM for MKC-442) because they maximize the interaction of the 6-benzyl ring with Tyr 181 residue.[9]

A common feature in all HEPT analogues is the linear substituted nature of C(1′) at the N(1) of the pyrimidine ring; however, it is interesting to note that branched C(1′) analogues (called 3′,4′-*seco* acyclic nucleosides),[10]

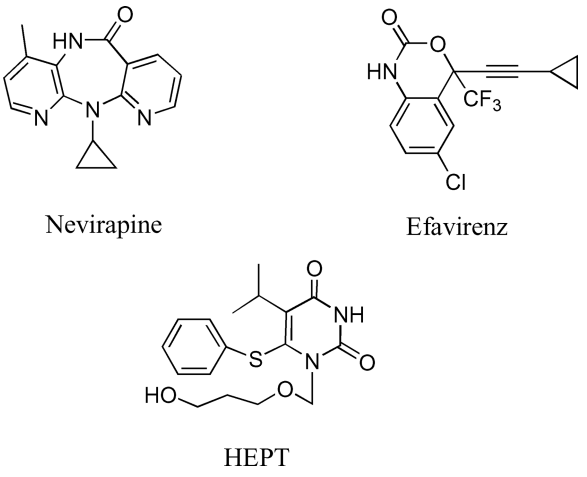


FIGURE 2 Anti-HIV NNRTIs drugs.

which resembles more closely to natural nucleosides (Figure 4), are in fact less active RT inhibitors,[11] as well as some others *seco* nucleosides.[12,13] This behavior may attend to the fact that nucleoside analogues target the catalytic site of the enzyme and non-nucleoside analogues the allosteric, as mentioned above.

Taking into consideration that in the allosteric RT pocket, non-covalent interactions as hydrogen bonding or hydrophobic interactions, among others, are of importance to form the RT-NNRTIs complex, we synthesized several 3′,4′-*seco* acyclic nucleosides combining an ether function as observed in MKC-442 and an ester function, as hydrogen bonding acceptor, in the C(1′) branch (compounds **4–14** in Figure 5), to test their activity in vitro against the enzyme RT of HIV-1. The conformation of the *seco* nucleosides in solution is reported as well as the X-ray diffraction of compound **6**.

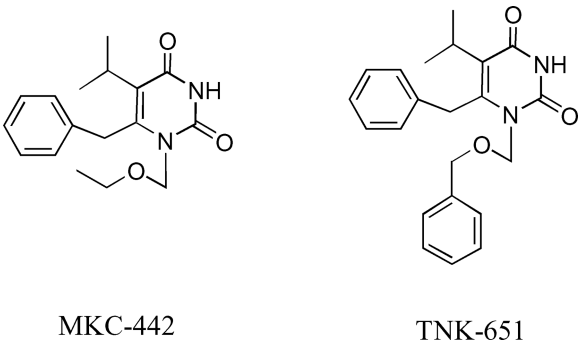


FIGURE 3 NNRTIs, analogues of HEPT.

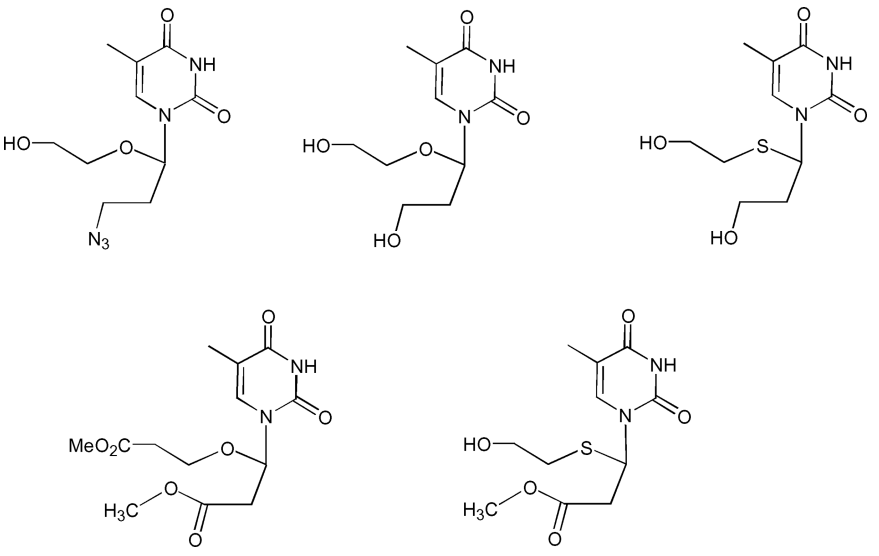
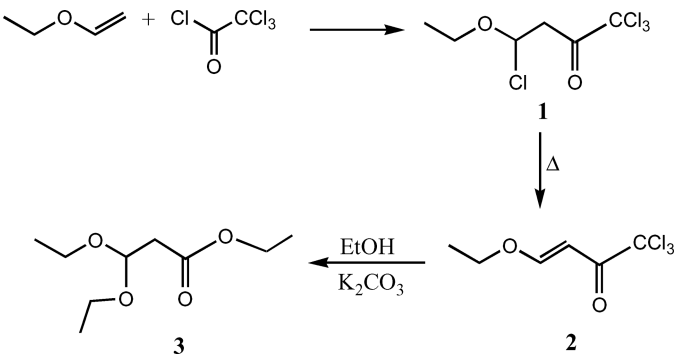


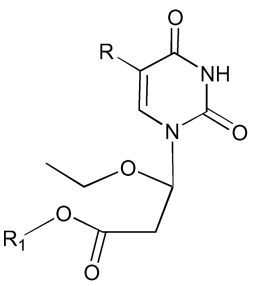
FIGURE 4 3′,4′-*Seco* acyclic nucleosides.

RESULTS AND DISCUSSION

The synthesis of compounds **4–14** was performed by nucleophilic substitution of protected pyrimidine bases on ethyl 3,3-diethoxypropanoate (**3**). Compound (**3**) was prepared according to the procedure reported by Tietze (Scheme 1).[14,15] Ethyl vinyl ether was reacted with trichloroacetyl chloride at 0–4°C for 5 h and then stirred to room temperature for 12 h to produce the 1,1,1-trichloro-4-chloro-4-ethoxy-butan-2-one (**1**). The dehydrohalogenation of **1** was performed by heating and distilling off the product 1,1,1-trichloro-4-ethoxy-buten-2-one (**2**), obtained in 94% yield. Compound (**2**) was treated with an excess of K_2CO_3 in ethanol to obtain the propanoate (**3**) in 98% yield.[16]



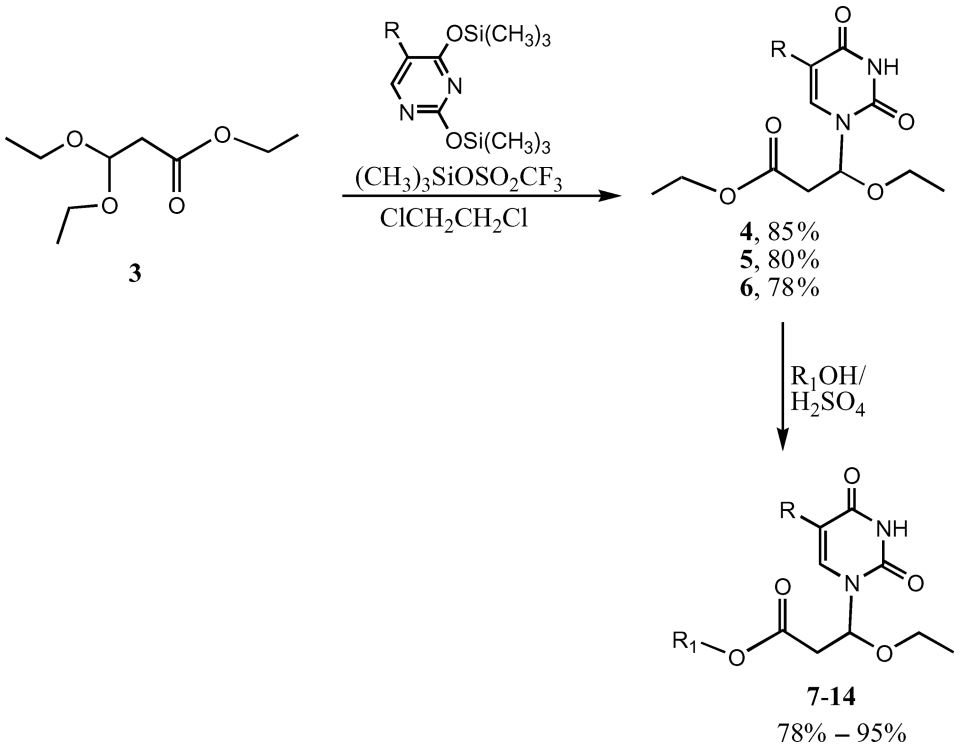
SCHEME 1



4, R= H; R_1= CH_2CH_3
5, R= CH_3; R_1= CH_2CH_3
6, R= F; R_1 = CH_2CH_3
7, R= H; R_1= CH_3
8, R= CH_3; R_1= CH_3
9, R= H; R_1= $CH_2CH_2CH_3$
10, R= CH_3; R_1= $CH_2CH_2CH_3$
11, R= H; R_1= $CH_2CH_2CH_2CH_3$
12, R= CH_3; R_1 = $CH_2CH_2CH_2CH_3$
13, R= H; R_1= $CH_2CH(CH_3)_2$
14, R= CH_3; R_1= $CH_2CH(CH_3)_2$

FIGURE 5 Pyrimidine based *seco* acyclic nucleosides (**4–14**).

In order to obtain the acyclic nucleosides **4–6**, compound **3** was treated with the corresponding persilylated pyrimidine nucleobases under Vorbrüggen conditions (Scheme 2).[17,18] We found that the products were obtained in better yields if the reaction mixture was stirred at room temperature for long periods of time (10–12 days) rather than be heated (Scheme 2). Compounds **7–14** were obtained by transesterification of **4** or **5** with heating at 70°C under acidic conditions (Scheme 2). The reaction took place more efficiently with methanol than with other alcohols as it was carried out at



SCHEME 2

TABLE 1 *Seco*-Nucleosides **4–14** Synthesized in This Work

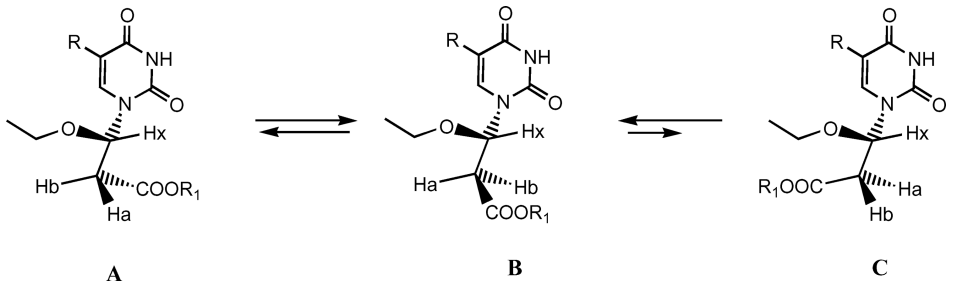
Compound	Isolated yield (%)	Melting point (°C)	MS (m/z)	Analysis (%)[a]		
				C	H	N
4	85	106–107	256 (M^+)	51.74 (51.56)	6.32 (6.25)	11.05 (10.93)
5	80	149–151	270 (M^+)	53.46 (53.33)	6.74 (6.66)	10.68 (10.37)
6	78	115–116	274 (M^+)	48.24 (48.17)	5.58 (5.47)	[b]
7	95	108–109	242 (M^+)	49.83 (49.58)	5.83 (5.78)	11.63 (11.57)
8	93	119–120	256 (M^+)	52.03 (51.56)	6.42 (6.25)	11.06 (10.93)
9	83	90–91	270 (M^+)	53.56 (53.33)	6.78 (6.66)	10.21 (10.37)
10	81	85–86	284 (M^+)	55.00 (54.92)	7.15 (7.04)	9.97 (9.85)
11	81	45–47	284 (M^+)	55.34 (54.92)	7.56 (7.04)	9.27 (9.85)
12	80	67–68	298 (M^+)	56.47 (56.37)	7.49 (7.38)	9.39 (9.39)
13	84	87–88	284 (M^+)	55.00 (54.92)	7.15 (7.04)	9.92 (9.85)
14	76	78–79	298 (M^+)	56.59 (56.37)	7.48 (7.38)	9.77 (9.39)

[a]Calculated values are given in parentheses.
[b]Undetermined.

reflux temperature. However, for all other alcohols, the transesterification reaction was performed at lower than reflux temperature as the starting material (**3**) decomposes at temperatures greater than 70°C. The physical properties and chemical analysis of compounds **4–14** are summarized in Table 1.

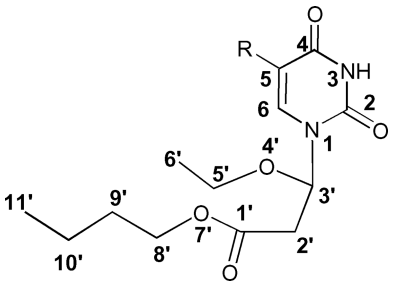
The spectra were obtained at 27°C using $CDCl_3$ as solvent. The complete assignments of proton and carbon-13 signals are shown in Tables 2 and 3, respectively.

In all acyclic nucleosides, the methylene and methine protons at C2′ and C3′ respectively, comprise ABX systems centered at around δ 2.72, 2.80 and 6.07 ppm (Table 1). The methylene C2′ signal is for almost all the compounds a doublet of doublets with coupling constants $^3J_{H2'aH3'}$ and $^3J_{H2'bH3'}$, which differ in 1.5–2.6 Hz. On the basis of the calculated $^3J_{HH}$(anti) = 11.0 Hz and $^3J_{HH}$(gauche) = 3.28 Hz, it can be proposed that the molecule is in conformational equilibrium between two staggered conformations (A and B of Scheme 3) where conformation C is not likely.[19]



SCHEME 3

TABLE 2 ^{1}H NMR Chemical Shifts (in ppm) for Compounds **4–14** in $CDCl_3$

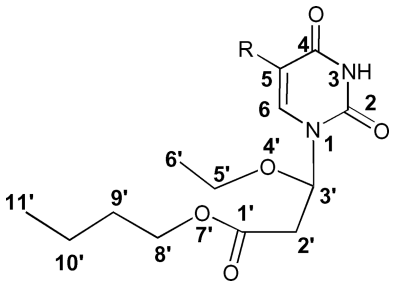


Chemical shifts (δ)	Compound										
	4	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
H2′a	2.73	2.73	2.72	2.73	2.72	2.72	2.72	2.72	2.72	2.75	2.73
H2′b	2.80	2.80	2.78	2.81	2.81	2.77	2.80	2.78	2.81	2.81	2.81
H3′	6.07	6.09	6.03	6.06	6.08	6.05	6.09	6.05	6.09	6.07	6.09
H5′	3.58	3.56	3.59	3.58	3.56	3.55	3.55	3.55	3.57	3.59	3.56
Me (H6′)	1.28	1.27	1.26	1.21	1.21	1.18	1.20	1.19	1.22	1.22	1.21
H8′a	4.18	4.18	4.14	3.72	3.72	4.05	4.07	4.10	4.13	3.90	3.88
H8′b	4.18	4.18	4.14	3.72	3.72	4.05	4.07	4.10	4.13	3.93	3.92
H9′	1.22	1.21	1.22	—	—	1.62	1.65	1.59	1.62	1.95	1.93
H10′	—	—	—	—	—	0.91	0.94	1.35	1.38	0.94	0.92
H11′	—	—	—	—	—	—	—	0.91	0.94	—	—
Me (9′)	—	—	—	—	—	—	—	—	—	0.94	0.92
H3	9.17	9.25	9.83	9.38	8.19	9.89	9.50	9.95	9.23	8.94	8.94
H5	5.80	—	—	5.82	—	5.80	—	5.82	—	5.82	—
H6	7.41	7.20	7.46	7.40	7.19	7.39	7.20	7.40	7.20	7.41	7.20
Me(5)	—	1.96	—	—	1.96	—	1.95	—	1.97	—	1.96

A splitting of the H_5 signal (δ∼5.8 ppm) of around 1.4–2.0 Hz that corresponds to a cross-ring N–H coupling in the uracils substituted acyclic nucleosides (**4**, **7**, **9**, and **13**) was observed. In ^{13}C NMR spectra, the chemical shifts of C2′, C3′ or C5′, C6′ are similar for all the compounds (Table 3).

The conformation of compound **6** was analyzed in solid state, the X-ray structure indicates that the molecule is in a preferred staggered conformation B (Figure 6), with a gauche geometry (60°) between H2b′ and H3′ and an anti geometry (179°) between H2a′ and H3′ (Table 4). The ability of compound **6** to form hydrogen bonding through the geometry of the homodimer in Figure 7. The arrangement [N–H···O] is symmetrical with bond angle equal to 169.0° and bond lengths for the [H···O] bonding of 1.96′ and for the non-bonded distance [N···O] of 2.84′. It is remarkable that these two molecules are even closer in distance than the same in the heterodimer *n*-octylthymine/*n*-octyladenine with bond lengths for the [H···O] bonding of 2.09′ and for the non-bonded distance [N···O] of 2.97′.[20] Noncovalent interactions are important to support the activity of NNRTIs in the hydrophobic cavity of the allosteric site of the RT enzyme.[21,22]

TABLE 3 ^{13}C NMR Signal Assignments in Compounds **4–14**[a]



	Chemical shifts (δ)													
Compound	C-1′	C-2′	C-3′	C-5′	C-6′	C-8′	C-9′	C-10′	C-11′	C-2′	C-4′	C-5′	C-6	Me-C5
4	168.4	40.4	82.0	65.6	14.3	61.3	14.8	—	—	163.0	150.0	103.4	139.0	—
5	168.4	40.5	81.6	65.4	14.3	61.3	14.8	—	—	164.0	151.0	112.0	134.7	12.9
6	168.2	40.2	82.5	65.8	14.3	61.4	14.8	—	—	149.0	142.8	122.9	139.6	—
7	168.9	40.2	82.0	65.6	14.8	52.3	—	—	—	163.6	150.9	103.4	139.0	—
8	169.2	40.4	81.7	65.6	15.0	52.5	—	—	—	164.4	151.3	112.2	134.7	13.0
9	168.5	40.4	82.0	65.5	14.8	66.9	22.5	10.4	—	163.8	151	103.3	139.0	—
10	168.7	40.6	81.7	65.5	15.5	67.0	22.5	10.8	—	164.6	151.4	112.1	134.8	13.1
11	168.5	40.5	82.1	65.6	14.9	65.3	30.7	19.2	14.9	163.2	150.7	103.3	139.0	—
12	168.5	40.5	81.7	65.1	14.6	64.9	30.5	18.9	13.8	163.9	150.7	111.9	134.7	12.6
13[b]	168.5	40.4	82.1	65.6	14.9	71.4	27.8	19.2	—	163.0	150.0	103.4	139.0	—
14[b]	168.8	40.7	81.5	65.6	14.8	71.6	27.8	19.4	—	164.4	151.2	112.2	134.8	12.8

[a]Chemical shifts (d) in ppm from TMS in $CDCl_3$.
[b]Me-C9′ ($\delta = 19.2$).
[c]Me-C9′ ($\delta = 19.4$).

Biological Assays

The ability of *seco* nucleosides **4–8**, **10**, **12–14** to inhibit the HIV-1 reverse transcriptase was tested in vitro and compared to Nevirapine. This

TABLE 4 Selected Bond Lengths (′), Bond Angles (θ) in Deg, and Torsion Angles (ω) in deg for Compound **6**

Bond lengths		Bond and torsion angles	
N1-C2	1.385 (3)	N1-C2-N3	113.04 (19)
C2-N3	1.435 (3)	N3-C4-C5	111.67 (19)
N3-C4	1.431 (3)	C6-N1-C3′	121.85 (16)
C4-C5	1.439 (3)	N1-C3′-C2′	108.80 (17)
C5-C6	1.378 (3)	N1-C3′-O4′	111.89 (17)
C6-N1	1.439 (3)	C4-N3-C2-N1	2.64 (0.37)
C2-O2	1.258 (3)	C4-C5-C6-N1	1.04 (0.35)
N3-H3	0.89 (3)	C6-N1-C3′-C2′	−56.73 (0.26)
C4-O4	1.271 (3)	C2-N1-C3′-C2′	129.09 (0.21)
C5-F8	1.399 (3)	N1-C3′-C2′-C1′	179.20 (0.18)
N1-C3′	1.548 (3)	H3′-C3′-C2′-H2′a	179.13 (1.52)
C3′-C2′	1.479 (3)	H3′-C3′-C2′-H2′b	60.14 (1.46)
C3′-O4′	1.379 (3)	O4′-C3′-C2′-C1′	58.56 (0.24)

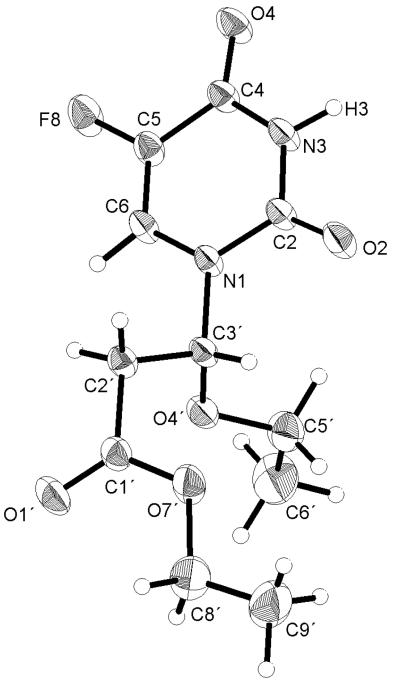


FIGURE 6 ORTEP projection of the molecular structure of ethyl 3′-ethoxy-3′-(5-fluoro-2,4-dioxo-3,4-dihydro-1(2*H*)-pyrimidinyl)propanoate (**6**). (H3 atom was located from a difference Fourier map and refined. All others H atoms were placed in calculated positions and refined using a riding model.)

compound was used as a reference because it is already in clinical use for AIDS treatment.[23,24] The RT inhibitor activity for each compound was obtained as indicated in the experimental part. The range of concentrations analyzed for Nevirapine was 0.01–1000 μM. The IC_{50} activity of Nevirapine was 0.62 ± 0.26 μM.[25] The IC_{50} reported in some studies[9,26,27] for Nevirapine ranged from 1.35 ± 0.43 μM to 0.088 ± 0.032 μM, depending upon the method used for the determination. The dose-effect curves for

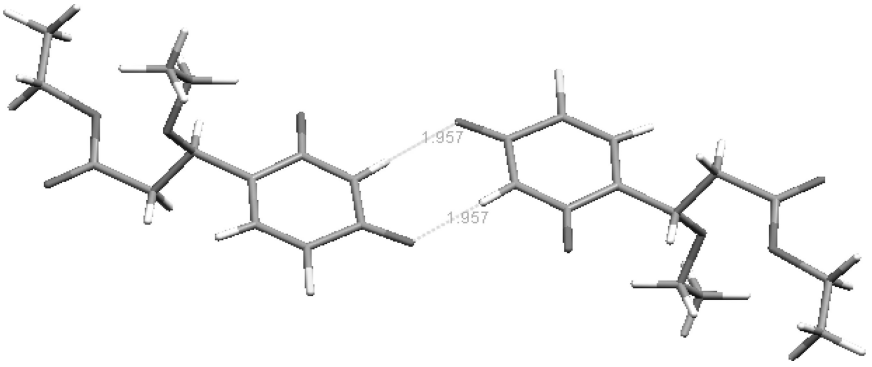


FIGURE 7 View of the crystal packing in the unit cell of compound **6**.

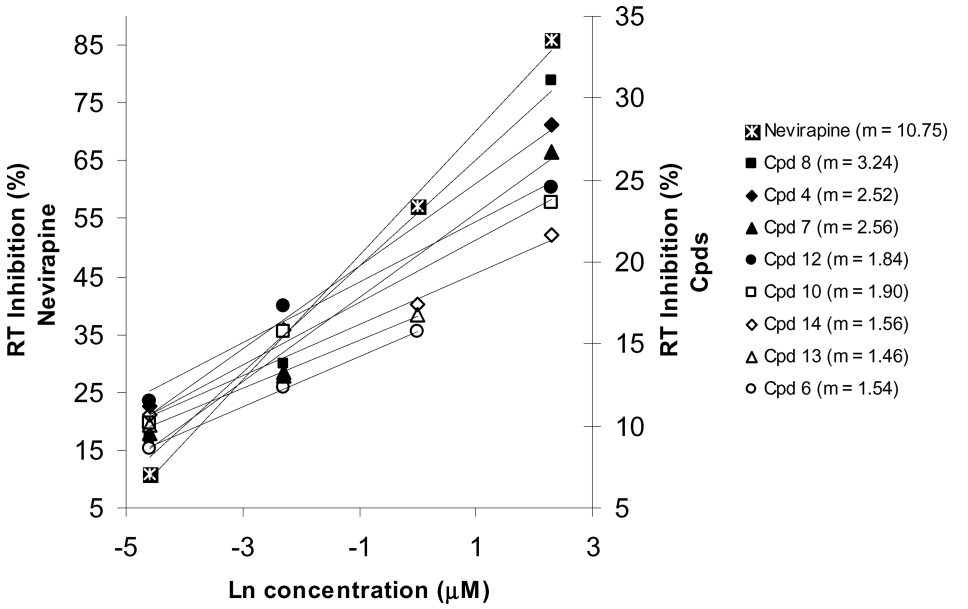


FIGURE 8 Relation between RT inhibition percentage and Ln of concentration for *seco* acyclic nucleosides.

acyclic nucleosides and Nevirapine in the range 0.01–10 μM were practically indistinguishable from a straight line (Figure 8). In basis of the slope values, it is observed that Nevirapine is at least three times more active than the acyclic nucleoside **8**. In this incipient series of moderately active RT inhibitors, the maximum potency was obtained with derivatives belonging to the thymine series (**8** and **14**), whereas derivatives belonging to the uracil series (**7** and **13**) or fluorouracil (**6**) were less potent (m = 3.24 and 2.56 for **8** and **7**, respectively; m = 1.56 and 1.46 for **14** and **13**, respectively). The antiviral activity is related inversely with the presence of bulky substituents at R_1 of the ester group. Thefore, the slope of the lines decreased in the order: methyl > propyl > butyl > isobutyl for the thymine series (compounds **8**, **10**, **12**, and **14** correspondingly) and in the order: methyl > ethyl > isobutyl for the uracil series (compounds **7**, **4**, and **13**). The acyclic nucleoside **8** was the most active RT inhibitor, followed by compounds **4** and **7** (Figure 8).

The cytotoxicity of acyclic nucleosides **6**, **7**, and **14** at 60 μM in Vero cells[28] was similar to that of Nevirapine (Table 5). In this study was included AZT, another drug used in AIDS infected people,[24,29–31] which was more toxic than compounds **6**, **7**, **14**, and Nevirapine. AZT is more potent anti HIV-1 compound than Nevirapine; however, it is also more toxic [EC_{50}(AZT) = 0.01 μM; EC_{50} (Nevirapine) = 0.3 μM].[32] According to the results summarized in Table 5, the acyclic nucleosides **6**, **7**, and **14** are less toxic than AZT and comparable in toxicity to Nevirapine at 60-μM doses.

TABLE 5 Cytotoxicity in vero Cells of Some Acyclic Nucleosides[a]

Compound	% of Survival
AZT	35.3 ± 1.7
Nevirapine	72.7 ± 2.3
6	72.3 ± 3.1
7	77.7 ± 2.5
14	77.0 ± 5.0

[a]Data represent $\bar{X} \pm$ SD (n = 3).

In summary, the *seco* nucleosides reported here have RT inhibitor activity and similar cytotoxicity than Nevirapine at 60-μM doses. The introduction of bulky substituents at the ester functionality led to a decrease in potency. This preliminary studies of activity and cytotoxicity are encouraging for further studies on structure-activity relationships to optimize their inhibition against HIV-1 multiplication.

EXPERIMENTAL

Chemistry

Melting points are uncorrected. Nuclear magnetic resonance spectra were recorded on Bruker Avance 300 spectrometer in $CDCl_3$ (δ 7.26, ^{1}H; δ 77.0, ^{13}C), ^{1}H at 300.1 MHz, and ^{13}C at 75.5 MHz. The abbreviations used for the ^{1}H NMR spectra are: s = singlet, d = doublet, dd = double of doublets, t = triplet, q = quartet, m = multiplet, bs = broad signal. Mass spectra (El) were measured on a Hewlett Packard 5989A spectrometer using electron impact (El) at 70 eV. All chemical and solvents used here were of reagent grade; solvent 1,2-dichloroethane was dried and distilled immediately before use from calcium hydride. Products were purified by succesive recrystallization.[33] Yields are given for isolated products. Microanalyses of **4**, **5**, and **7–14** were recorded in a Thermo Finnigan Flash 1112 analyzer. Galbraith Laboratories, Inc., Knoxville TN performed microanalyses of **6**.

1,1,1-Trichloro-4-ethoxy-buten-2-one (2).[14] In a round-bottomed 250-mL flask fitted with dropping funnel and a stirbar were placed 86.5 g (0.48 mol) of trichloroacetyl chloride. The liquid was stirred at 0°C and 68.5 g (0.95 mol) of ethyl vinyl ether were added dropwise through the dropping funnel. The mixture was kept on stirring at 0°C for 5 h and then at room temperature for 12 h. The dropping funnel was replaced by distiller apparatus and the mixture was heated under vacuum. The product was obtained as a transparent liquid of bp 84–86°C/1 mm Hg in 94% yield (78 g). ^{1}H NMR

δ 1.39 (t, J = 7.0 Hz, 3 H), 4.09 (q, J = 7.0 Hz, 2 H), 6.14 (d, J = 12.4 Hz, 1 H), 7.84 (d, J = 12.4 Hz, 1 H).

Ethyl 3,3-Diethoxypropanoate (3).[14] In a round-bottomed 250-mL flask fitted with a stirbar were placed 50 g (0.23 mol) of **2** and 50 mL (0.86 mol) of ethyl alcohol. The solution was stirred and 3 g of potassium carbonate were added in portions. The mixture was left under stirring for 15 h at room temperature. The residual potassium carbonate was filtered off and the product purified by destillation under vacuum 58–60°C/1 mmHg, to give 38.2 g (87%). ^{1}H NMR δ 1.15 (t, J = 7.0 Hz, 6 H), 1.21 (t, J = 7.0 Hz, 3 H), 2.60 (d, J = 6.0 Hz, 2 H), 3.40 (q, J = 7.0 Hz, 2 H), 3.70 (q, J = 7.0 Hz, 2 H), 4.10 (q, J = 7.0 Hz, 2 H), 4.90 (t, J = 6.0 Hz, 1 H).

General Procedure for the Preparation of Ethyl 3′-Ethoxy-3′-(substituted Pyrimidinyl)propanoates 4–6. Thymine, uracil, or 5-fluorouracil nucleobases were persilylated by the procedure proposed by Vorbrüggen:[17] In a round-bottomed 50-mL flask fitted with reflux condenser and rubber septa were placed 7.9 mmol of the nucleobase, 23.69 mmol of 1,1,1,3,3,3-hexamethyldisilazane, and 2.76 mmol of trimethylchlorosilane. The resulting suspension was stirred and heated under reflux until the solution was transparent (between 5 and 12 h). The excess of HMDS was removed under vacuum (1 mmHg) at 40°C. The procedure continued as follow:

To the flask containing the persylilated nucleobase were added 10 mL of dry 1,2-dichloroethane. A solution that contained 7.9 mmol of ethyl 3,3-diethoxypropanoate **3** in 5 mL of dry 1,2-dichloroethane was then added via syringe, followed by the addition via syringe of 4.0 mmol of trimehylsilyltrifluoromethane sulphonate in 5 mL of dry 1,2-dichloroethane. The mixture was stirred at room temperature (warning: heating of the reaction at this point led to several by-products) until no starting material **3** was observed by thin layer chromatography (10 days for compounds **4** and **5** and 12 days for compound **6**). The product was washed with 200.0 mL of water, extracted with methylene chloride (3 × 50 mL), and the organic layer was dried over sodium sulfate. The solvent was removed with a rotary evaporator to yield an oily product in all cases. Purification of the crude products was accomplished by recrystallization in CH_2Cl_2/hexane (1:4).

General Procedure for the Preparation of Alkyl-3′-Ethoxy-3-(substituted Pyrimidinyl)propanoate 7–14. In a round-bottomed 25-mL flask, fitted with reflux condenser and rubber septa, were placed 2.0 mmol of the propanoate **4** or **5** and 10 mL of the corresponding alcohol. The solution was stirred and four drops of 95–98% sulfuric acid were added via syringe. The mixture was heated at 70°C and the transesterification reaction was folowed by thin-layer chromatography of aliquots taken at different times.

The reaction was maintained under stirring and heating until completed (8 h for **7** and **8** and 8–12 days for **9–14**). After cooling, the suspension was concentrated under vacuum and the residue washed with 25.0 mL of an aqueous solution of 10% sodium bicarbonate. The product was extracted with methylene chloride (3×20 mL) and the organic layer was dried over sodium sulfate. The solvent was removed with a rotary evaporator. Purification of the crude product was accomplished by recrystallization in CH_2Cl_2/hexane (1:4).

In Vitro RT Inhibition Assays

The activity of acyclic nucleosides **4–8**, **10**, **12–14**, and Nevirapine to inhibit the HIV-1 reverse transcriptase was tested in vitro by using the kit Lenti-RT™ (Cavidi Tech, Sweden), which contains poly (rA) as the enzyme template, an oligo (dT) as the primer and BrdUTP as the non-radioactive triphosphate substrate. Quantification of the BrdUMP incorporated in DNA by the RT enzyme was performed after reaction with alkaline phosphatase enzyme-conjugated anti-BrdU monoclonal antibody in the presence of *p*-nitrophenylphosphate (pNPP).[34–36] The RT inhibitor activity was obtained through the absorbance measurements at $\lambda = 405$ nm for each compound after 1 h incubation. *Seco* nucleosides and Nevirapine were dissolved in DMSO and all experiments were carried out in triplicate.

Cytotoxicity Assays

Compounds **6**, **7**, and **14**, as well as AZT and Nevirapine, were evaluated for cytotoxicity in Vero[37,38] (African green monkey kidney) cells. Cells were cultured in Dulbecco's modified Eagle's basal medium (Sigma Chemical Co., St. Louis, MO), supplemented with 10% calf serum (Gibco BRL, Grand Island, NY) in 95% air, 5% CO_2 incubator at 37°C. Cultures in confluence were washed twice with PBS and removed using Trypsin-EDTA. Cells (1×10^5 cells/cm^2) were cultured in 24-well cell culture plates newly. Cultures at subconfluence were washed twice with PBS to remove bond-attached cells and exposed to 60 μM of compounds dissolved in DMSO. At 40 h of exposure cytotoxicity assays were carried out in cultures using Trypan blue dye.[39,40] Control cultures contained only DMSO and assays were carried out in triplicate.

X-Ray Crystallography

Crystallographic work was performed in an Enraf-Nonius Kappa CCD diffractometer. Data collection: COLLECTION software.[41] Cell refinement and data reduction: Denzo/Scalepack software[42] and WinGX version 1.7 software.[43] The structure of compound **6** was solved and refined by SHELXS 97.[44] Molecular graphics: CAMERON software.[45] The crystal structure has

been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCD 292628.

CONCLUSIONS

A simple method for the synthesis of 3′,4′-*seco* acyclic nucleosides, analogues of the antiviral compound MKC-442, is reported. The coupling constants $^{3}J_{H2'aH3'}$ and $^{3}J_{H2'bH3'}$ suggest that most compounds adopt the same conformation in solution regardless of the substituent (R_1) at the ester function. The hydrogen-bonding interactions in the homodimer of the solid structure of compound **6** are of the type [N–H···O]; they are symmetrical with bond angle equal to 169.0° and bond lengths for the [H···O] bonding of 1.96′ and for the non-bonded distance [N···O] of 2.84′. All the compounds are, in vitro, less active than Nevirapine against the enzyme RT of HIV-1. Compounds substituted with thymine nucleobase and with small R_1 substituents in the ester group are more active than those substituted with uracil nucleobase and with bulky R_1 groups. At doses of 60 μM, the *seco* nucleosides **6**, **7**, and **14** showed similar cytotoxicity than Nevirapine and lower cytotoxicity than AZT.

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