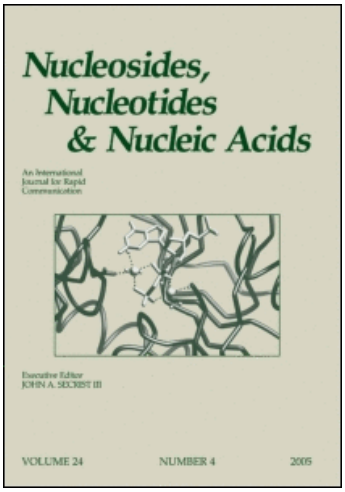

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Inhibitors Interacting with the Magnesium Binding Site of Reverse Transcriptase: Synthesis and Biological Activity Studies of 3'-(Ω-Amino-Acyl) Amino-3'-Deoxy-Thymidine

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INHIBITORS INTERACTING WITH THE MAGNESIUM BINDING SITE OF REVERSE TRANSCRIPTASE: SYNTHESIS AND BIOLOGICAL ACTIVITY STUDIES OF 3′-(Ω-AMINO-ACYL) AMINO-3′-DEOXY-THYMIDINE

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□ *Active site of reverse transcriptase contains carboxylate groups involved in the magnesium binding. We prepared some nucleoside analogs which could bind to these carboxylates preventing the binding of nucleotides. To the 3′-amino-3′-deoxy-thymidine, different N-protected ω-amino-acids were bound, the protection removed to give the 3′-(ω-amino-acyl-) amino-3′-deoxy-thymidines in good yield. Some showed moderate to low activity in HIV 1 replication test.*

Keywords 3′-Amino-3′-deoxy-thymidine; magnesium binding site; reverse transcriptase; kinases; HIV

INTRODUCTION

Magnesium ion is added in almost every test systems of phosphate transferring enzymes, even if this ion has not been shown to be required for the enzymatic activity.[1] It is not easy to demonstrate the precise role of this ion in the phosphate transfer reaction. Due to its low molecular weight, it does not show up like other heavy ions in the electronic density maps. The absence of a concrete method like an x-ray structure determination leaves the space open for mechanistic speculations. The order in which magnesium ions are added to the enzyme in the reaction pathway is not always determined. Does the phosphate ester bind to the enzyme before the

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magnesium ion to the enzyme? In a number of cases this has been shown to occur. So the phosphate ester may bind to the enzyme without magnesium ion. This has a certain implication for the rational design of inhibitors of phosphate transferring enzymes. There are even kinases where magnesium may not be involved in the binding nor in the catalysis.[1] With this fact in mind, we looked at the structure of a number of phosphate transferring enzymes and the first one was reverse transcriptase from HIV. Indeed this enzyme catalyses at two active sites, two reactions involving phosphate esters: polymerase and RNase.[2] In solution the binding of the substrate to the RNase H domain is a precondition for metal ion binding.[3] In RNase active site four acidic residues are located: Asp^{443}, Glu^{478}, Asp^{498}, and Asp^{549}. In the polymerase site three Asp^{110}, Asp^{185}, and Asp^{186} bind magnesium ions.[4–6]

The resistance of a zwitterionic RNA to the exonuclease has its origin to the binding of the ammonium of 3-amino-propyl group at C-2′ to the metal binding site, interfering with the phosphoryl transfer.[7] This induced the design of nucleoside analogs bearing a group binding to the carboxylic groups present in the active site. Their binding interferes on the subsequent binding of magnesium ion. As group binding to the carboxylate residues we chose the primary amine of a ω-amino-acyl chain. This chain is bound at C-3′ through an amide as in structure of puromycin, an antibiotic which inhibits the protein synthesis by substituting the incoming aminoacyl-tRNA.[8,9] This group of puromycin seems to be stable under physiological conditions and we inferred that the same holds for other amides at C-3′.

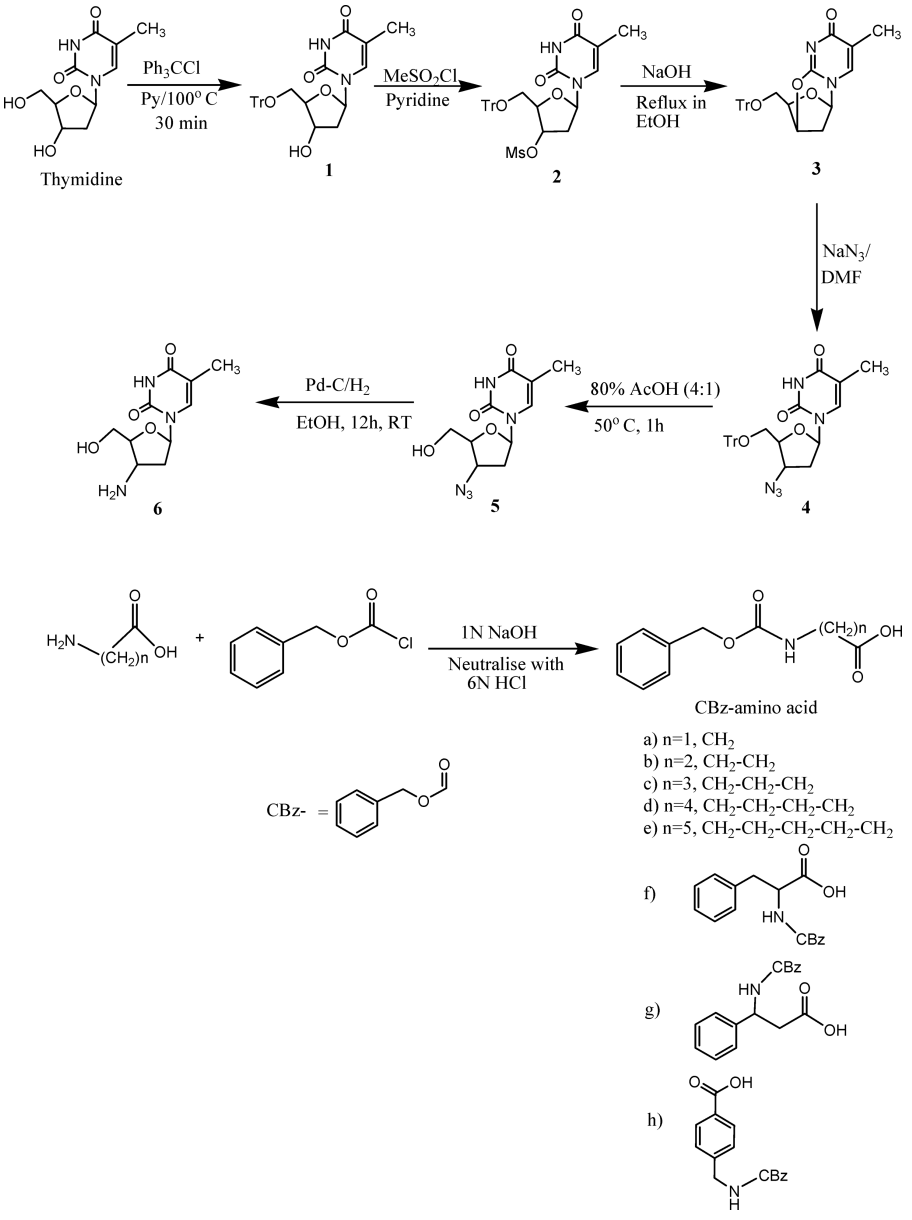
RESULTS AND DISCUSSION

Procedure

3′-Amino-3′-deoxy-thymidine (**6**) have been prepared as described previously.[10.11] The carbobenzoxyamino acids **a–h** have been condensed with the amine **6** in the presence of HBTU and diisopropylethylamine to give amides **7a–7h** in 60–70% yield (Schemes 1 and 2). The hydrogenolysis was performed with hydrogen in presence of Pd/C and the free amines **8a–8h** were obtained as solids in a yield of 60–80%. Their spectral data correspond to the proposed structure. The acids were racemates and thus the compounds **8f** and **8g** were obtained as diastereoisomers even if their NMR spectra did not show any sign of heterogeneity. We did not try to separate them.

Anticancer Activity

The anticancer properties of compounds **8a**, **8c** and **8g** were studied under the developmental program of the National Cancer Institute NCI/NIH. Results obtained indicated that at a concentration of 10^{-5} M, compounds **8c**

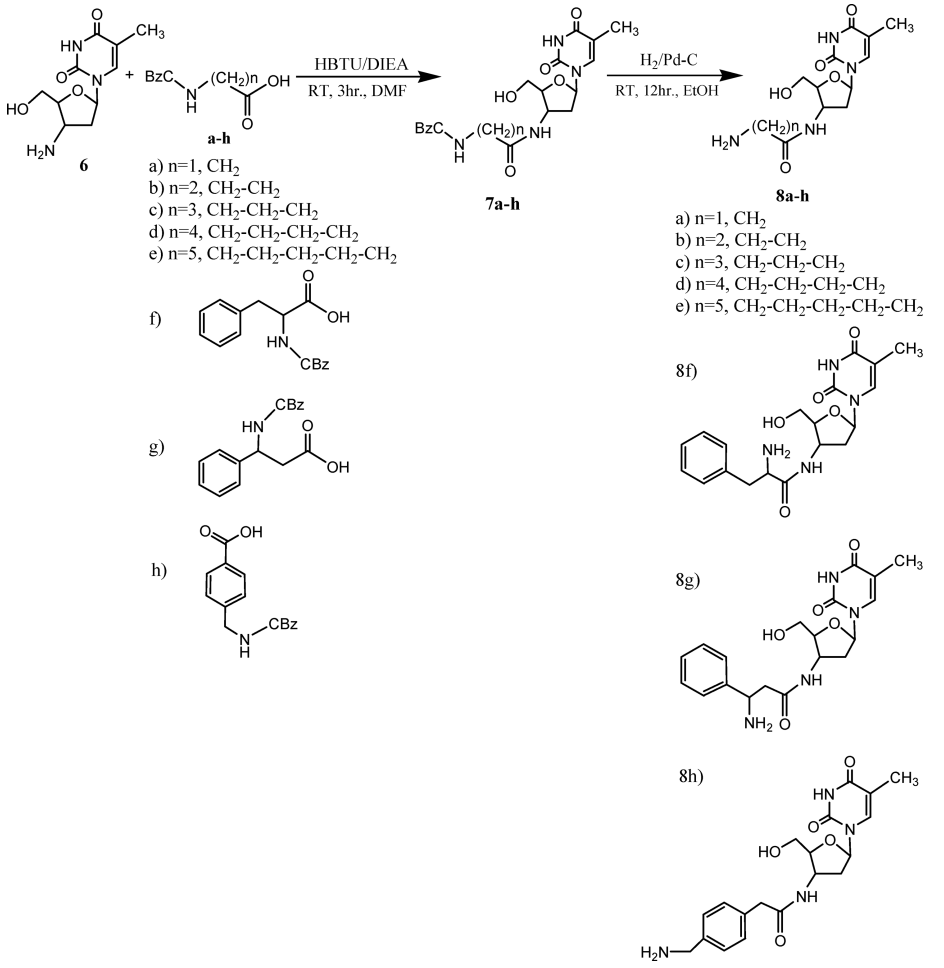


SCHEME 1 Synthesis of amino-3′-deoxy-thymidine 6 and of carbobenzoxyamino acids a–h.

and **8g** showed total inhibition of strain HCC-2998 of colon cancer, whereas **8a** did not show positive result.

Inhibition of HIV-1 Replication

Nucleoside analogs **8a–8h** were studied for anti-HIV-1 activity on CEM-SS infected by HIV-1 Lai[12] (Table 1). Compound **8a** had a modest antiviral



SCHEME 2 Synthesis of 3′-(ω-amino-acyl)-3′-deoxy-thymidine.

activity ($IC_{50} = 660\ \mu M$), while compounds **8c** and **8f** showed little activity but the IC_{50} was close to the CC_{50} cytotoxic concentrations, thus the selectivity indexes were low (2–4 CC_{50}/IC_{50}) (Table 1). Compound **8e** had a selectivity index of 1 CC_{50}/IC_{50} (Table 1). On MT4 cells, no specific antiviral activity was measurable (Table 1).

Inhibition on a Panel of Kinases

Compounds **6**, **8a**, **8b**, **8d**, **8e**, **8f,** and **8g** were tested for their inhibition on a panel of kinases. The panel of kinases included AKT1, ARK5, Aurora-A, Aurora-B, B-RAF-VE, CDK2/CycA, CDK4/CycD1, COT, FAK, EPHB4, ERBB2, EGF-R, IGF1-R, SRC, VEGF-R2, VEGF-R3, FLT3, INS-R, MET, PDGFR-beta, PLK1, SAK, TIE2, and CK2-alpha1. Data obtained showed

TABLE 1 Anti-HIV Activity on HIV-1 Lai wild type in CEM-SS

	HIV-1 LAI CEM-SS		HIV-1 IIIB MT4	
Compound	IC_{50} (μM)[a]	CC_{50} (μM)[b]	IC_{50} (μM)	CC_{50} (μM)
6	20	500	$>CC_{50}$	2.3
8a	660	$>10^3$	$>CC_{50}$	150
8b	$>10^3$	$>10^3$	$>CC_{50}$	780
8c	390	660	$>1.10^3$	$>1.10^3$
8d	$>5.10^2$	$>5.10^2$	$>5.10^2$	$>5.10^2$
8e	150	160	$>CC_{50}$	24
8f	60	220	$>CC_{50}$	22
8g	$>5.10^2$	$>5.10^2$	>500	>500
8h	240	870	$>CC_{50}$	690
AZT	$2.1\ 10^{-3}$	–	$1.9.10^{-2}$	–

[a]IC_{50} : 50% inhibitory concentration or concentration required to inhibit the replication of HIV by 50%;

[b]CC_{50} : cytotoxic concentration or concentration required to reduce the viability of uninfected cell by 50%.

that all the above mentioned compounds were found to be inactive at a concentration of 10 μM.[13]

CONCLUSION

Some of the 3′-(ω-amino-acyl) amino-3′-deoxy-thymidine derivatives were found to exhibit low activity in HIV-1 replication test. Whether this is due to the inhibition of the reverse transcriptase remains an open question. The cytotoxic effects observed with some compounds were not reflected in the results of the anticancer trials, but those trials were done at lower concentration. None of the kinases were significantly inhibited with these thymidine derivatives, which may not be too surprising since the attachment is at C-3′. Finally the question raised with these results is: Is the amide group a proper attachment of the side chain bearing the group interacting with the carboxylate groups Some analogs with chain bearing amino group linked at C-3′ by a ether had been reported as inactive against Herpes virus and HIV.[14,15] The presence of hydrophobic group in **8f** and **8h** gives an indication for the design of new analogs as well as the fact in **8c** and **8h** the distance between the amide and the amino group is a C_5 and C_6 chain. It would be interesting to determine the activity of the products with ether as link at C-3′ and the C_6 chain.

EXPERIMENTAL

General Procedure

All the reactions were carried out under an atmosphere of nitrogen. Melting points (mp) were taken on a MEL-TEMP capillary tube apparatus

and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in d_4-MeOH solution using Bruker 400 or 500 MHz.

$^2H_4$-MeOH instrument and chemical shifts were reported using d_4-MeOH (49.1 ppm) as $^2H_4$-MeOH internal standards. Purification by column chromatography was carried out with neutral silica gel 60 (70–230 mesh ASTM). MS spectra were determined on a Shimadzu QP-1000 spectrometer or VG 70–250S spectrometer. HRMS spectra were collected on Autospec orthogonal acceleration-time of flight mass spectrometer with a resolution of 6000 (5%). All the starting materials are purchased from Aldrich. <aq>Please provide names and locations for manufacturers used and mentioned</aq>

General Procedure for the Preparation of 3′-[(N-benzyloxycarbonyl -amino acids)-amino] -3′-deoxy-thymidine (7a–7h.

3′- Amino thymidine (**6**)[11] (100 mg, 1 equiv.), corresponding N-benzyloxycarbonyl-amino acid (1 equiv.), and HBTU (1.1 equiv.), were dissolved in dry DMF (3 ml) under nitrogen with a syringe, after few minutes diisopropyl ethyl amine (DIEA) (2 equiv.) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 hours. The solvents were evaporated under vacuum, and the crude residue was purified by flash column chromatography (gradient EtOAc—MeOH).

3′-[(N-benzyloxycarbonyl-glycyl)-amino]-3′-deoxy-thymidine **(7a).** Compound **7a** obtained as fluffy white solid. Yield (0.120 g, 67%), ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.86 (s, 3H), 2.32 (m, 2H), 3.69 (m, 1H), 3.72 (br m, 2H), 4.51 (m, 1H), 5.08 (s, 2H), 6.19 (t, 1H, J = 4.2 Hz), 7.27—7.35 (m, 5H), 7.85 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.4 (CH_3), 38.5 (CH_2), 44.8 (CH_2), 49.7 (CH), 62.3 (CH_2), 67.8 (CH_2), 85.8 (CH), 86.3 (CH), 111.4 (C), 129.0 (CH), 129.1 (CH), 129.4 (CH), 138.0 (CH), 138.0 (C), 152.0 (C), 159.0 (C), 166.3 (C), 172.3 (C). Mass spectrum (FABMS): m/z 433 $(M+H)^+$; exact mass calcd. for $C_{20}H_{25}N_4O_7$ 433.1268, found 433.1262.

3′-[(N-benzyloxycarbonyl-β−alanyl)amino]-3′-deoxy-thymidine **(7b).** Compound **7b** obtained as fluffy white solid. Yield (0.145 g, 78.3%), ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.85 (s, 3H), 2.25 (m, 2H), 2.40 (t, 1H, J = 6.8 Hz), 3.38 (t, 1H, J = 6.8 Hz), 3.71 (m, 1H), 3.82 (m, 2H), 4.52 (m, 1H), 5.0 (s, 2H), 6.18 (t, 1H, J = 4.2 Hz), 7.28 (m, 5H), 7.82 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 13.0 (CH_3), 37.6 (CH_2), 38.8 (CH_2), 39.1 (CH_2), 49.7 (CH), 62.8 (CH_2), 67.8 (CH_2), 86.2 (CH), 86.9 (CH), 111.9 (C), 129.2 (CH), 129.4 (CH), 129.8 (CH), 138.5 (CH), 138.6 (C), 152.7 (C), 159.1 (C), 166.7 (C), 174.3 (C). Mass spectrum (FABMS): m/z 447 $(M+H)^+$; exact mass calcd. for $C_{21}H_{27}N_4O_7$ 447.1801 found 447.1794.

3′-[(N-benzyloxycarbonyl-amino-γ-butyryl)amino]-3′-deoxy-thymidine **(7c).** Compound **7c** obtained as off-white solid. Yield (0.145 g, 76.3%), ^{1}H NMR

(d_4-MeOH, 500 MHz): δ 1.76 (m, 2H), 1.86 (s, 3H), 2.27 (t, 2H, J = 6.0 Hz), 3.13 (t, 2H, J = 5.2 Hz), 3.70 (m, 1H), 3.83 (m, 2H), 4.45 (m, 1H), 5.0 (s, 2H), 6.20 (t, 1H, J = 4.8 Hz), 7.29 (m, 5H), 7.84 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.4 (CH_3), 27.0 (CH_2), 34.0 (CH_2), 38.6 (CH_2), 41.2 (CH_2), 50.2 (CH), 62.4 (CH_2), 67.4 (CH_2), 85.8 (CH), 86.5 (CH), 111.5 (C), 128.7 (CH), 128.9 (CH), 129.4 (CH), 138.0 (CH), 138.3 (C), 152.2 (C), 158.8 (C), 166.3 (C), 175.6 (C). Mass spectrum (FABMS): *m/z* 461 $(M+H)^+$; exact mass calcd. for $C_{22}H_{29}N_4O_7$ 461.1958 found 461.1952.

3′-[(N-benzyloxycarbonyl-amino-δ−valeryl)amino]-3′-deoxy-thymidine **(7d).** Compound **7d** obtained as fluffy white solid. Yield (0.180 g, 91.8%), ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.48 (m, 2H), 1.62 (m, 2H), 1.86 (s, 3H), 2.23 (t, 2H, J = 7.2 Hz), 2.32 (m, 2H), 3.11 (t, 2H, J = 6.8 Hz), 3.72 (m, 1H), 3.84 (m, 2H), 4.47 (m, 1H), 5.0 (s, 2H), 6.20 (t, 1H, J = 6.0 Hz), 7.28 (m, 5H), 7.84 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.5(CH_3), 24.0 (CH_2), 30.3 (CH_2), 37.0 (CH_2), 38.7 (CH_2), 41.4 (CH_2), 50.2 (CH), 62.5 (CH_2), 67.3 (CH_2), 85.9 (CH), 86.5 (CH), 111.5 (C), 128.7 (CH), 128.9 (CH), 129.5 (CH), 138.2 (CH), 138.4 (C), 152.3 (C), 158.8 (C), 166.4 (C), 176.0 (C). Mass spectrum (FABMS): *m/z* 475 $(M+H)^+$; exact mass calcd. for $C_{23}H_{31}N_4O_7$ 475.2114 found 475.2109.

3′- [(N-benzyloxycarbonyl-amino-ε-hexanoyl)amino]-3′-deoxy-thymidine **(7e).** Compound **7e** obtained as fluffy white solid. Yield (0.123 g, 81.4%), ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.32 (m, 2H), 1.50 (m, 2H), 1.62 (m, 2H), 1.86 (s, 3H), 2.19 (m, 2H), 2.26—2.32 (m, 2H), 3.0 (m, 2H), 3.73 (m, 1H), 3.85 (m, 2H), 4.48 (m, 1H), 5.0 (s, 2H), 6.20 (t, 1H, J = 6.0 Hz), 7.26 (m, 5H), 7.82 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.5 (CH_3), 26.5 (CH_2), 27.3 (CH_2), 30.5 (CH_2), 36.8 (CH_2), 38.7 (CH_2), 41.6 (CH_2), 50.3 (CH), 62.6 (CH_2), 67.3 (CH_2), 85.9 (CH), 86.6 (CH), 111.5 (C), 128.7 (CH), 128.9 (CH), 129.4 (CH), 138.0 (CH), 138.4 (C), 152.3 (C), 158.8 (C), 166.3 (C), 176.2 (C). Mass spectrum (FABMS): *m/z* 489 $(M+H)^+$; exact mass calcd. for $C_{24}H_{33}N_4O_7$ 489.2305 found 489.2298.

3′-(N-benzyloxycarbonyl-dl-phenylalanyl)amino-3′-deoxy-thymidine **(7f).** Compound **7f** obtained as off-white solid. Yield (0.142 g, 65.7%), ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.85 (s, 3H), 2.20 (m, 2H), 2.27 (m, 1H), 2.95 (m, 1H), 3.03 (m, 2H), 3.29 (m, 1H) 3.55—3.69 (m, 2H), 4.30 (m, 1H), 4.33 (m, 1H), 5.0 (s, 2H), 6.11 (t, 1H, J = 6.0 Hz), 7.18—7.29 (m, 10 H), 7.78 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.4 (CH_3), 38.4 (CH_2), 39.4 (CH_2), 50.0 (CH), 58.0 (CH), 62.3 (CH_2), 67.6 (CH_2), 85.9 (CH), 86.1 (CH), 111.5 (C), 127.9 (CH), 128.9 (CH), 129.5 (CH), 129.54 (CH), 130.4 (CH), 138.1 (CH), 138.4 (CH), 152.2 (C), 158.4 (C), 166.3 (C), 174.0 (C), 174.1 (C). Mass spectrum (FABMS): *m/z* 523 $(M+H)^+$; exact mass calcd. for $C_{27}H_{31}N_4O_7$ 523.2114 found 523.2110.

3′-(N-benzyloxycarbonyl-β-phenyl-β-dl-alanyl)amino-3′-deoxy-thymidine **(7g).** Compound **7g** obtained as off-white solid. Yield (0.130 g, 60.1%), ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.86 (s, 3H), 2.20—2.28 (m, 2H), 2.49 (m, 1H),

2.62 (m, 1H), 2.89 (m, 1H), 3.61 (m, 1H), 3.77 (m, 2H) 4.33 (m, 1H), 4.41 (m, 1H), 5.01 (s, 2H), 6.13 (s, 1H), 7.18—7.31 (m, 10 H), 7.81 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.4 (CH_3), 38.7 (CH_2), 38.8 (CH_2), 45.6 (CH_2), 50.1 (CH), 54.4 (CH), 62.3 (CH_2), 85.9 (CH), 86.2 (CH), 111.5 (C), 127.6 (CH), 128.2 (CH), 129.4 (CH), 129.5 (CH), 129.7 (CH), 138.1 (CH), 142.0 (CH), 144.7 (CH), 152.3 (C), 164.9 (C), 173.5 (C), 175.3 (C). Mass spectrum (FABMS): *m/z* 523 $(M+H)^+$; exact mass calcd. for $C_{27}H_{31}N_4O_7$ 523.2114 found 523.2107.

***3′-(N-benzyloxycarbonyl-4-aminomethylbenzoyl) amino-3′-deoxy-thymidine* (7h).** Compound **7h** obtained as fluffy white solid. Yield (0.050 g, 68.5%), mp 180–185 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.88 (s, 3H), 2.42—2.44 (m, 2H), 3.78 (dd, 1H, J = 3.5 Hz), 3.86 (dd, 1H, J = 3.5 Hz), 4.0 (m, 1H), 4.33 (s, 2H), 4.68 (m, 1H), 6.26 (t, 1H, J = 5.2 Hz), 7.32—7.36 (m, 7H), 7.77 (d, 2H, J = 8.5 Hz), 7.88 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.5 (CH_3), 38.9 (CH_2), 44.5 (CH_2), 50.7 (CH), 62.5 (CH_2), 67.7 (CH_2), 86.1 (CH), 86.4 (CH), 111.6 (C), 128.3 (CH), 128.7 (CH), 128.8 (CH), 129.0 (CH), 129.5 (CH), 134.0 (C), 138.3 (CH), 145.0 (C), 152.4 (C), 159.1 (C), 166.5 (C), 170.2 (C). Mass spectrum (FABMS): *m/z* 509 $(M+H)^+$; exact mass calcd. for $C_{26}H_{29}N_4O_7$ 509.1958 found 509.1951.

General Procedure for the Preparation of 3′-[(Amino acids)-amino] -3′-deoxy-thymidine (8a–8h)

Substrates (**7a–7h**) (100 mg) were dissolved in EtOH (10 ml), followed by 10% palladium on charcoal (70 mg) was exposed to a positive pressure of hydrogen gas at room temperature for overnight. The reaction mixture was filtered through celite pad that was successively washed with DCM and ethanol. Solvents were removed under vacuum from the combined filtrates to afford compounds **8a–8h** as white solids.

***3′-glycylamino-3′-deoxy-thymidine* (8a).** Compound **8a** obtained as white solid. Yield (0.078 g, 63.4%), mp 140–145 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.84 (s, 3H), 2.30 (m, 2H), 3.70 (m, 1H), 3.82 (m, 2H), 4.48 (m, 1H), 6.19 (s, 1H), 7.84 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.5 (CH_3), 38.8(CH_2), 44.7 (CH_2), 48.7 (CH), 62.4 (CH_2), 85.1 (CH), 85.5 (CH), 111.5 (C), 138.2 (CH), 152.4 (C), 166.5 (C), 174.6 (C). Mass spectrum (FABMS): *m/z* 299 $(M+H)^+$; exact mass calcd. for $C_{12}H_{19}N_4O_5$ 299.1268, found 299.1262.

***3′-β-alanylamino-3′-deoxy-thymidine* (8b).** Compound **8b** obtained as white solid. Yield (0.089 g, 88.1%), mp 135–140 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.83 (s, 3H), 2.30 (m, 2H), 2.37 (t, 2H, J = 6.4 Hz), 2.89 (t, 2H, J = 6.4 Hz), 3.69 (m, 1H), 3.81 (m, 2H), 4.45 (m, 1H), 6.17 (t, 1H, J = 4.2 Hz), 7.82 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 13.0 (CH_3), 39.1 (CH_2), 39.15 (CH_2), 39.2 (CH_2), 50.7 (CH), 62.9 (CH_2), 86.3 (CH), 86.9 (CH), 111.9 (C), 138.5 (CH), 153.0 (C), 167.1 (C), 174.7 (C). Mass

spectrum (FABMS): *m/z* 313 $(M+H)^+$; exact mass calcd.for $C_{13}H_{21}N_4O_5$ 313.1434 found 313.1426.

***3′-(γ-amino-butyryl)amino-3′-deoxy-thymidine* (8c).** Compound **8c** obtained as white solid. Yield (0.098 g, 72.5%), mp 110–115 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.77 (m, 2H), 1.87 (s, 3H), 2.25—2.34 (m, 4H), 2.67 (m, 2H), 3.70 (m, 1H), 3.85 (m, 2H), 4.47 (m, 1H), 6.21 (t, 1H, $J = 4.2$ Hz), 7.84 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.5 (CH_3), 28.9 (CH_2), 34.2 (CH_2), 38.6 (CH_2), 41.6 (CH_2), 50.2 (CH), 62.4 (CH_2), 84.5 (CH), 85.0 (CH), 111.5 (C), 138.0 (CH), 152.8 (C), 167.0 (C), 175.7 (C). Mass spectrum (FABMS): *m/z* 327 $(M+H)^+$; exact mass calcd. for $C_{14}H_{23}N_4O_5$ 327.1668 found 327.1673.

***3′-(δ-aminovaleryl)amino-3′-deoxy-thymidine* (8d).** Compound **8d** obtained as white solid. Yield (0.100 g, 76.9%), mp 170–175 °C, ^{1}H NMR (D_2O, 500 MHz): δ 1.68 (m, 4H), 1.92 (s, 3H), 2.36—2.44 (m, 4H), 2.94 (m, 2H), 3.77 (m, 1H), 3.88 (m, 1H), 4.0 (br s, 1H), 4.50 (m, 1H), 6.30 (s, 1H), 7.63 (s, 1H). ^{13}C NMR (D_2O 125 MHz): δ 12.3(CH_3), 22.2 (CH_2), 27.3 (CH_2), 34.9 (CH_2), 36.4 (CH_2), 39.3 (CH_2), 48.8 (CH), 61.0 (CH_2), 83.8 (CH), 84.6 (CH), 111.6 (C), 136.6 (CH), 156.5 (C), 173.0 (C), 176.2 (C). Mass spectrum (FABMS): *m/z* 341 $(M+H)^+$; exact mass calcd. for $C_{15}H_{25}N_4O_5$ 341.1825 found 341.1830.

***3′-(δ-aminohexanoyl)amino-3′-deoxy-thymidine* (8e).** Compound **8e** obtained as white solid. Yield (0.078 g,), mp 115–120 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.35 (m, 2H), 1.52 (m, 2H), 1.62 (m, 2H), 1.87 (s, 3H), 2.22 (m, 2H), 2.27—2.31 (m, 2H), 3.72 (m, 1H), 3.86 (m, 2H), 4.48 (m, 1H), 6.21 (t, 1H, $J = 6.0$ Hz), 7.84 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.6 (CH_3), 26.6 (CH_2), 27.4 (CH_2), 32.0 (CH_2), 38.8 (CH_2), 41.9 (CH_2), 50.3 (CH), 62.5 (CH_2), 85.9 (CH), 86.5 (CH), 111.6 (C), 138.1 (CH), 128.9 (CH), 152.9 (C), 167.2 (C), 176.2 (C). Mass spectrum (FABMS): *m/z* 355 $(M+H)^+$; exact mass calcd. for $C_{16}H_{27}N_4O_5$ 355.1981 found 355.1983.

***3′- dl-phenylalanylamino-3′-deoxy-thymidine* (8f).** Compound **8f** obtained as white solid. Yield (0.085 g, 64.8%), mp 90–95 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.86 (s, 3H), 2.30 (m, 2H), 2.92 (m, 2H), 3.54 (m, 1H), 3.65 (m, 1H), 3.78 (m, 1H), 4.41 (m, 1H), 4.62 (m, 1H), 6.10 (s, 1H), 7.25 (m, 5H), 7.78 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.4 (CH_3), 38.6 (CH_2), 42.8 (CH_2), 50.0 (CH), 57.8 (CH), 62.4 (CH_2), 85.9 (CH), 86.1 (CH), 111.5 (C), 127.8 (CH), 129.6 (CH), 130.5 (CH), 138.2 (CH), 138.8 (C), 152.4 (C), 166.5 (C), 176.8 (C). Mass spectrum (FABMS): *m/z* 389 $(M+H)^+$; exact mass calcd. for $C_{19}H_{25}N_4O_5$ 389.1825 found 389.1824.

***3′-(β-amino-β-phenyl-dl-alanyl) amino-3′-deoxy-thymidine* (8g).** Compound **8g** obtained as white solid. Yield (0.075 g, 57.1%), mp 95–100 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.87 (s, 3H), 2.19—2.27 (m, 2H), 2.58 (m, 1H), 2.62 (m, 1H), 2.90 (m, 1H), 3.60 (m, 1H), 3.77 (m, 2H) 4.33 (br s, 1H), 4.41 (m, 1H), 6.12 (t, 1H, $J = 6.0$ Hz), 7.14—7.31 (m, 5H), 7.81 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.5 (CH_3), 38.7 (CH_2), 45.6 (CH_2),

50.1 (CH), 54.5 (CH), 62.3 (CH_2), 86.0 (CH), 86.5 (CH), 111.5 (C), 127.6 (CH), 129.5 (CH), 129.7 (CH), 138.1 (CH), 142.0 (C), 152.3 (C), 164.9 (C), 173.5 (C). Mass spectrum (FABMS): *m/z* 389 $(M+H)^+$; exact mass calcd. for $C_{19}H_{25}N_4O_5$ 389.1829 found 389.1825.

***3′-(4-aminomethyl-benzoyl) amino-3′-deoxy-thymidine* (8h).** Compound **8h** obtained as white solid. Yield (0.030 g, 83.0%), mp 85–90 °C, ^{1}H NMR (d_4-MeOH, 500 MHz): δ 1.89 (s, 3H), 2.42 (s, 2H), 2.46 (m, 2H), 3.78 (dd, 1H, J = 3.5 Hz), 3.88 (dd, 1H, J = 3.5 Hz), 4.0 (m, 1H), 4.72 (m, 1H), 6.27 (t, 1H, J = 6.0 Hz), 7.26 (d, 2H, J = 8.0 Hz), 7.72 (d, 2H, J = 8.0 Hz), 7.89 (s, 1H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 12.5 (CH_3), 38.7 (CH_2), 43.0 (CH_2), 50.6 (CH), 62.5 (CH_2), 86.1 (CH), 86.4 (CH), 111.6 (C), 128.5 (CH), 130.2 (CH), 132.4 (C), 138.3 (CH), 143.7 (C), 152.4 (C), 166.5 (C), 170.5 (C). Mass spectrum (FABMS): *m/z* 375 $(M+H)^+$; exact mass calcd. for $C_{18}H_{22}N_4O_5$ 375.1590 found 375.1587.

Biological Evaluation of the Antiviral Activity of the Compounds. The effects of the compounds on the replication of HIV-1 were evaluated as previously described.[12]

REFERENCES

1. Miallau, L.; Alphey, M.S.; Kemp, L.E.; Leonard, G.A.; Mc Sweeney, S.M.; Hecht, S.; Bacher, A.; Eisenreich, W.; Rohdich, F.; Hunter, W. N. Biosynthesis of isoprenoids: crystal structure of 4-diphosphocytidyl-2C-methyl-D-erythritol kinase. *Proc. Natl. Acad. Sci. USA* **2003**, 100, 9173–9178.
2. Didierjean, J.; Isel, C.; Querre, F.; Mouscadet, J.-F.; Aubertin, A-M.; Valnot, J.-Y.; Piettre, S.R.; Marquet, R. Inhibition of human immunodeficiency virus type 1 reverse transcriptase, RNase H, and integrase activities by hydroxytropolones. *Antimicrob. Agents Chemother.* **2005**, 49, 4884–4894.
3. Pari, K.; Mueller, G.A.; DeRose, E.F.; Kirby, T.W.; London, R.E. Solution structure of the RNase H domain of the HIV-1 reverse transcriptase in the presence of magnesium. *Biochemistry* **2003**, 42, 639–650.
4. Davies, J.F. II, ; Hostomska, Z.; Hostomsky, Z.; Jordan, S.R.; Matthews, D.A. Crystal-Structure of the Ribonuclease-H Domain of HIV-1 Reverse Transcriptase. *Science* **1991**, 252, 88–95.
5. Huang, H.; Chopra, R.; Verdine, G.L.; Harrison, S.C. Structure of a covalently trapped catalytic complex of HIV-I reverse transcriptase: Implications for drug resistance. *Science* **1998**, 282, 1669–1675.
6. Klumpp, K.; Hang, J.Q.; Rajendran, S.; Yang, Y.; Derosier, A.; In P, W.K.; Overton, H.; Parkes, K.E.B.; Cammack, N.; Martin, J. Two-metal ion mechanism of RNA cleavage by HIV RNase H and mechanism-based design of selective HIV RNase H inhibitors. *Nucleic Acids Res.* **2003**, 31, 6852–6859.
7. Teplova, M.; Wallace, S.T.; Tereshko, V.; Minasov, G.; Symons, A.M.; Cook, P.D.; Manoharan, M.; Egli, M. Structural origins of the exonuclease resistance of a zwitterionic RNA. *Proc. Natl. Acad. Sci. USA* **1999**, 96, 14240–14245.
8. Lee, H.; Fong, K.-L.; Vince R. Puromycin Analogs–Effect of Aryl-substituted Puromycin analogs on the Ribosomal peptidyltransferase Reaction. *J. Med. Chem.* 1981, 24, 304–308.
9. Nguyen-Trung, N.Q.; Botta, O.; Terenzi, S.; Strazewski, P. A practical route to 3 '-amino-3 '-deoxyadenosine derivatives and puromycin analogues. *J. Org. Chem.* 2003, 68, 2038–2041.
10. Sochacka, E.; Nawrot, B.; Pankiewicz, K.W.; Watanabe, K.W. Synthesis of 1-methyl 5-(3-azido-2,3-dideoxy-.beta.-D-erythro-pentofuranosyl)uracil and 1-methyl-5-(3-azido-2,3-dideoxy-2-fluoro-.beta.-D-arabinofuranosyl)uracil. The C-nucleoside isostere of 3′-azido-3′-deoxythymidine and its 2′-"up"-fluoro analog. *J. Med. Chem.* **1990**, 33, 1995–1999.
11. Lin, T.-S.; Prusoff, W.H. Novel synthesis and biological Activity of several 5-halo-5′-amino analogs of deoxyribopyrimidine nucleosides. *J. Med. Chem.* **1978**, 21, 109–112.

12. Dolle, V.; Nguyen, C.H.; Legraverend, M.; Aubertin, A.-M.; Kirn, A.; Andreola, M.L.; Ventura, M.; Tarrago-Litvak, L.; Bisagni, E. Synthesis and antiviral activity of 4-benzyl pyridinone derivatives as potent and selective non-nucleoside human immunodeficiency virus type 1 reverse transcriptase inhibitors. *J. Med. Chem.* **2000**, 43, 3949–3962.
13. Moulin, E.; Barluenga, S.; Totzke, F.; Winssinger, N. Diversity-oriented synthesis of pochonins and biological evaluation against a panel of kinases. *Chem. Eur. J.* **2006**, 12, 8819–8834.
14. El-Kousy, S.; Pedersen, E.B.; Nielsen, C. Synthesis and investigation of antiviral activity of 3′-O-(aminoalkyl)-thymidines and their quaternary ammonium salts. *Monatsh. Chem.* **1994**, 125, 713–721.
15. Abdel, A.; Abdel, A.H.; Larsen, E.; Pedersen, E.B.; Nielsen, C. Synthesis of 3′-O-(2-aminoethyl)-2′-deoxyuriidnes. *Acta Chem. Scand.* **1995**, 49, 609–614.