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Selective Action of 4'-Benzoylated Thymidine on DNA Polymerases and Reverse Transcriptases[a])

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Abstract. 4'-Acylated thymidine triphosphates **1** have been synthesized and tested as substrates for DNA polymerases and reverse transcriptases. The 4'-benzoylated thymidine **1d** was selectively acting as a chain terminator for the DNA synthesis catalyzed by retroviral RT. No incorporation of **1d** was observed when DNA polymerases were used. The selective targeting of the action performed by retroviral enzymes is a requirement for an effective antiviral agent with a minimum of toxicity.

The chemotherapeutic action of the antibiotics bleomycin and neocarzinostatin is initiated by abstraction of H-atoms from the 4'- and/or 5'- position of the deoxyribose of DNA [1]. In order to study the chemistry of these radicals, we synthesized 4'-acylated thymidines and incorporated them into oligonucleotides by the phosphoramidite approach [2]. Photolysis of the 4'-acylated oligonucleotides led to the formation of 4'-DNA radicals and subsequent site-selective cleavage of the DNA strand [3]. Recently, we have shown that some of the 4'-acylated thymidine triphosphates **1** (*Fig. 1*) are substrates for DNA polymerases and reverse transcriptases (RT) [4].

To study the mechanism of action of the 4'-acylated thymidine triphosphates **1**, we designed an assay where a 40mer DNA template demands the incorporation of a thymidine analogue opposite to an adenosine residue in position 30 (*Fig. 2, A*). When the *Klenow* fragment of *E.coli* DNA polymerase I or modified T7 DNA polymerase were used to promote DNA strand elongation in the presence of dATP, dGTP, dCTP, and the methylketone **1a** or ethylketone **1b**, a reaction product was formed which comigrated with a 30mer line marker on a PAGE gel (for modified T7 DNA polymerase see *Fig. 2, B*, lanes 2–5) [4]. The formation of a 30mer reaction product showed that **1a** and **1b** were incorporated by the enzyme into the synthesized DNA strand, but no further DNA strand elongation was observed after incorporation of **1a** or **1b**. In contrast, the bulkier *tert*-butylketone **1c** and phenylketone **1d** were not incorporated into the growing DNA strand as shown by the formation of a 29mer, the same reaction product which is formed in the absence of any thymidine triphosphate analogue (for modified T7 DNA polymerase see *Fig. 2, B*, lanes 1, 6–9).

In the cases where DNA strand synthesis was performed in the presence of **1a** and **1d** the use of HIV-1 RT led to different results. The incorporation of **1a** did not lead to DNA chain termination anymore (*Fig. 2, C*, lanes 2, 3) [4]. In contrast to the results obtained when the *Klenow* fragment of *E.coli* DNA polymerase I or modified T7 DNA polymerase were used to promote DNA strand elongation, the 4'-benzoylated thymidine **1d** was a substrate for the HIV-1 RT and its incorporation led to termination of DNA synthesis (*Fig. 2, C*, lanes 8, 9) [5].

Thus, **1d** acts as a selective chain terminator for the DNA synthesis catalyzed by retroviral RT because it is not a substrate for DNA polymerases. The selec-

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[a]) These results were presented as an oral contribution at the autumn meeting of the New Swiss Chemical Society (NSCS) in Basel, Switzerland, on November 21, 1996.

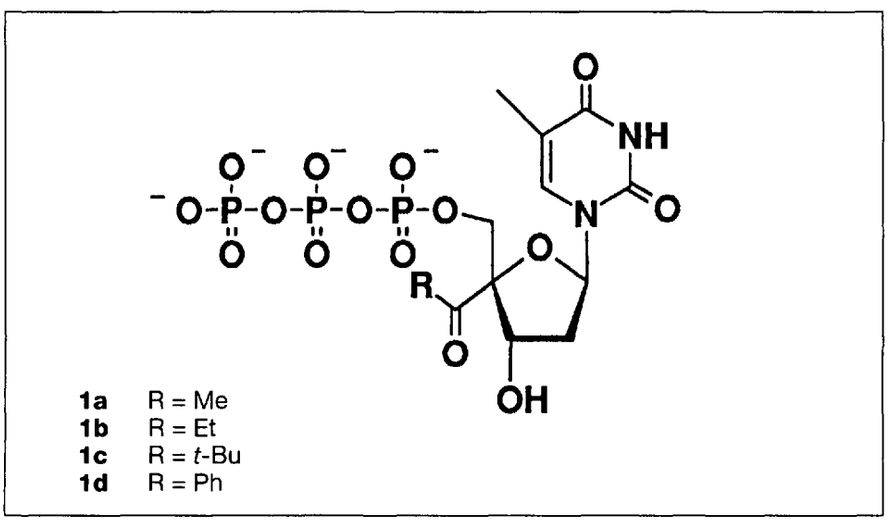

Fig. 1. *4'-Acylated thymidine triphosphates* **1a–d**

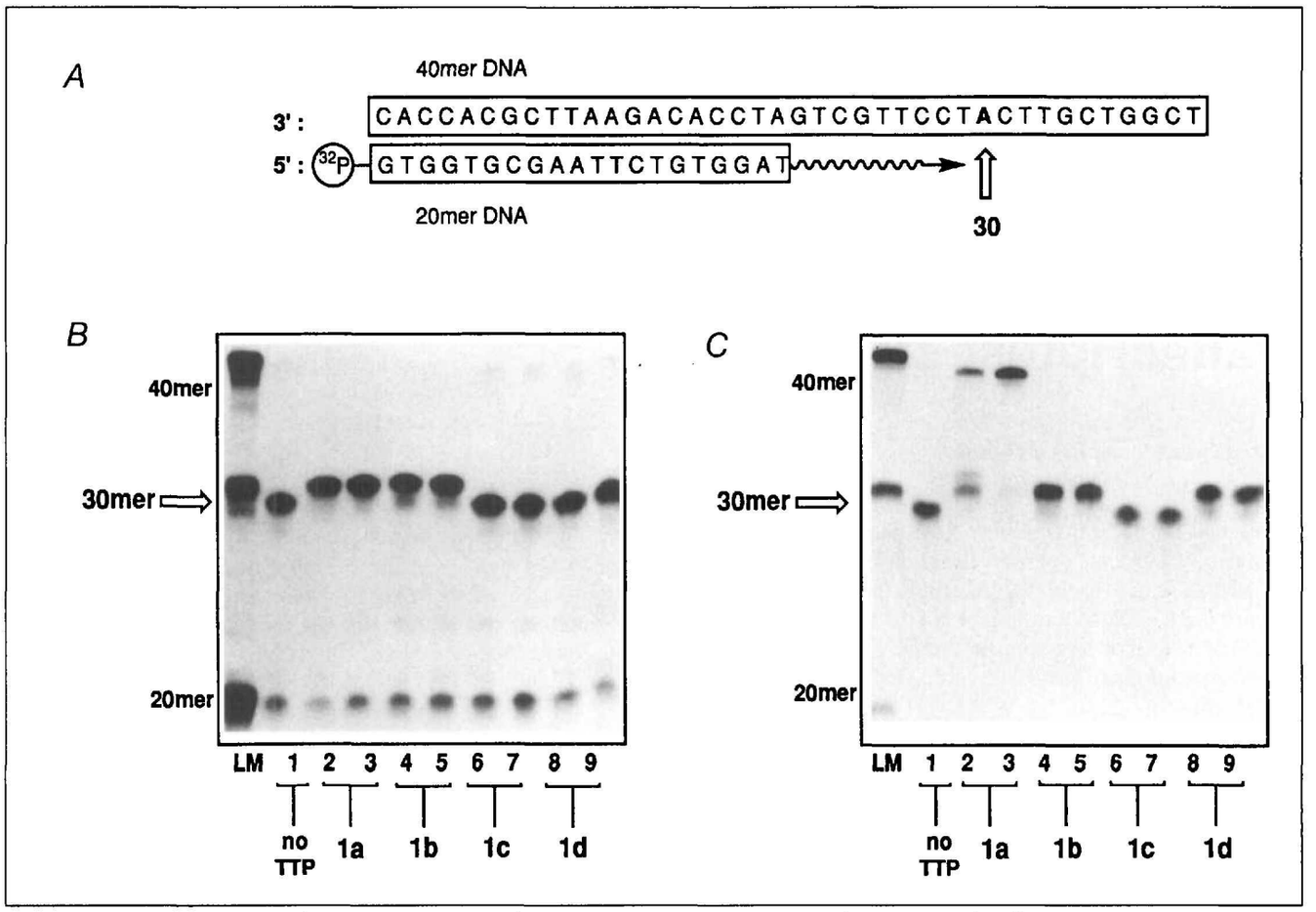

Fig. 2. A: *The assay we used.* B: *Effect of 4'-acylated thymidine triphosphates* **1a–d** *on DNA strand synthesis catalyzed by modified T7 DNA polymerase.* Shown is an autoradiogram of a 19% denaturing PAGE gel. LM: line marker. Lane 1: ^{32}P-5'-end-labeled primer (0.05 pmol), template (0.20 pmol), dATP, dGTP, dCTP (2.5 μM), incubated at 37° for 5 h in a buffer containing 40 μM Tris-HCl (pH 7.5), 20 μM $MgCl_2$, 50 μM NaCl, and 0.13 units modified T7 DNA polymerase. Lanes 2, 3: as in lane 1, but with **1a** (100 μM), incubated for 2 h and for 5 h. Lanes 4, 5: as in lane 1, but with **1b** (100 μM), incubated for 2 h and for 5 h. Lanes 6, 7: as in lane 1, but with **1c** (100 μM), incubated for 2 h and 5 h. Lanes 8, 9: as in lane 1, but with **1d** (100 μM), incubated for 2 h and 5 h. C: *Same as in* B, *but with HIV-1 RT.* Incubated in a buffer containing 20 μM Tris-HCl (pH 7.5), 6 μM $MgCl_2$, 40 μM KCl, 0.5 μM DTT, dATP, dGTP, dCTP (1.0 μM), and 0.15 units HIV-1 RT.

tive targeting of the action performed by retroviral enzymes is a requirement for an effective antiviral agent with a minimum of toxicity. The finding that selectivity towards retroviral enzymes can be achieved by changing the nature of the 4'-substituent makes 4'-acylated nucleotides very interesting tools in the search for new antiviral drugs.

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[5] **1d** was a substrate for AMV RT or M-MuLV RT and was acting as chain terminator as well.