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BIS-S-ACYL-2-THIOETHYL (SATE)-BEARING MONOPHOSPHATE PRODRUG OF β-L-FD4C AS POTENT ANTI-HBV AGENT

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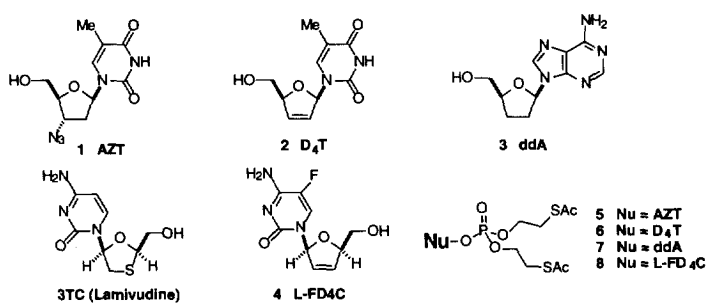
Abstract: The S-acyl-2-thioethyl (SATE)-bearing 5'-monophosphate prodrug of β-L-FD4C (**8**) was synthesized and evaluated for its activity against HBV in the 2.2.15 cell line. This pronucleotide (**8**) exhibited an excellent inhibitory effect against HBV with an EC_{50} value that is more than eight fold lower than that of the parent nucleoside (**4**) under some assay conditions. It is also important to note that pronucleotide (**8**) was capable of inhibiting HBV replication by 90%; whereas its parent, β-L-FD4C (**4**), could only inhibit virus replication no greater than 70% in the same assay. When evaluated in the standard cytotoxicity assay in CEM cell line, pronucleotide (**8**) exhibited an IC_{50} value of 52 μM, which was four times less toxic than parent β-L-FD4C (**4**) (IC_{50} = 13 μM).

Introduction

Since the approval of AZT (**1**) (3'-azido-2',3'-dideoxythymidine)[1] as a first-line therapeutic agent for the treatment of human immunodeficiency virus (HIV), considerable effort has been devoted to the design and synthesis of other nucleoside analogs that would inhibit the replication of HIV and other related viruses. Out of such efforts came the discovery of a series of 2',3'-dideoxy (dd) and 2',3'-dideoxy-2',3'-didehydro (D4) nucleoside analogs displaying potent antiviral activity such as D_4T (**2**)[2] and ddA (**3**)[3] as well as the recently discovered L-nucleosides including 3TC (Lamivudine)[4] and β-L-FD4C (**4**).[5] Since an important step in viral replication for

Figure 1: Representitive Antiviral Nucleosides and Pronucleotides



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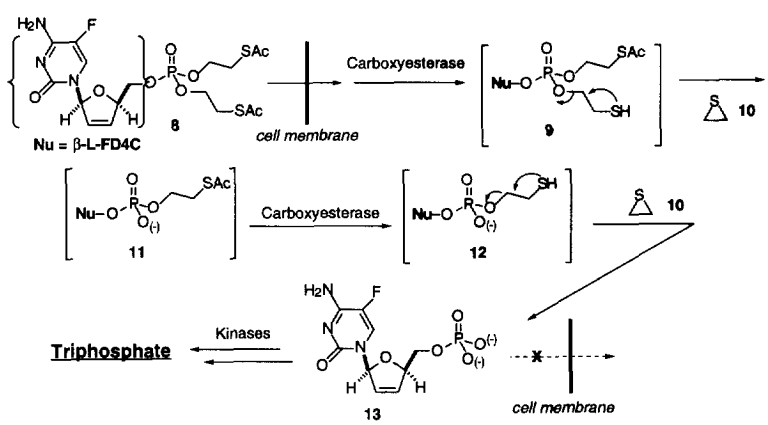
both HIV and HBV involves a virally encoded reverse transcriptase (RT), nucleoside analogs with an inhibitory effect on RT may have activity against both viruses. This assumption proved to be true for 3TC and β-L-FD4C (**4**), with the latter being 10- to 20-fold more potent against HBV than 3TC (see Figure 1 for structures).[5]

One strategy to further improve the efficacy of nucleoside analogs is to increase the intracellular availability of its 5′-monophosphate, a metabolite needed for subsequent phosphorylation to the bioactive triphosphate form.[6] It has been shown that, among the three successive phosphorylation steps, the first phosphorylation step could be rate limiting. Thus, the lack of antiviral activity of some nucleoside analogs is due to the absence of an efficient kinase activity capable of carrying out the first phosphorylation step.[7] To circumvent this problem, Gosselin and Imbach designed the bis(SATE)- bearing monophosphate prodrugs of AZT (**5**),[8] D4T (**6**)[9] and ddA (**7**)[10] that possessed impressive anti-HIV activity in a thymidine kinase-deficient (TK^-) cell line. In contrast, unmodified AZT (**1**) and D4T (**2**) were inactive in this TK^- cell line.

As one can see in Figure 2, pronucleotide (**8**) is a protected neutral 5′-monophosphate triester, thus it can enter cells much more readily than its corresponding free 5′-phosphoric acid. After entering the cell, pronucleotide (**8**) should decompose via a sequence of enzymatic thioester hydrolyses followed by subsequent fragmentation with the concomitant release of episulfide (**10**), to its corresponding 5′-monophosphate (**13**), a pathway reported previously by Gosselin and Imbach for pronucleotides (**5-7**).[6,8,9,10] As also shown in Figure 2, the monophosphate dianion (**13**) generated inside the cell can not escape because it is negatively charged. One of the remaining options for **13** is to undergo further phosphorylation to its corresponding bioactive triphosphate.[6]

Figure 2: Proposed Decomposition Pathway of Pronucleotide (8)



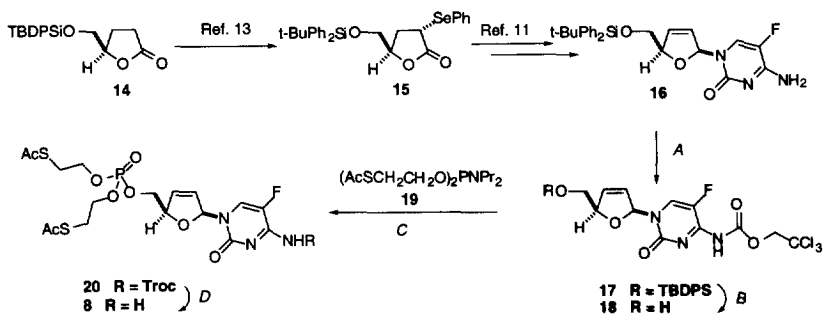
To take advantage of these findings, we utilized a similar strategy to prepare the pronucleotide of β-L-FD4C (**8**) with the aim of further improving its potency against HIV and

HBV replication. In this communication, we wish to report the synthesis and preliminary biological evaluation of the bis(SATE)-bearing prodrug of β-L-FD4C (**8**).

Chemistry

The synthetic route employed for the synthesis of pronucleotides (**8**) is outlined in Scheme 1. The starting material for our prodrug synthesis (**16**) was prepared from 2α-phenylselenolactone (**15**)[11] in four-steps with an overall yield of 75%. The key intermediate (**15**) was prepared in turn from the known γ-lactone (**14**)[12] in a highly stereoselective manner, according to the protocol newly developed in our laboratory.[13] Protection of the N-4 nitrogen and removal of the 5′-TBDPS silyl group in (**16**) afforded N_4-Troc protected intermediates (**18**) in an overall yield of 57%. Subsequent 5′-phosphorylation of (**18**) using the appropriate phosphoroamidite (**19**)[14] (in the presence of 1-H tetrazole) provided, after in situ MCPBA oxidation of the resulting intermediate, the desired 5′-phosphates (**20**) in an almost quantitative yield. Final removal of the N_4-Troc protecting group was accomplished by gently heating of a slurry of (**20**) with zinc in methanol, which afforded the desired pronucleotide (**8**) (71%).[15]

Scheme 1: Synthesis of Pronucleotide 8



Reagents & Conditions: (A) TrocCl/Pyr./DMAP/CH_2Cl_2, **17** (75-91%); (B) Et_3N-$(HF)_3$/THF, **18** (76%); (C) 1-H Tetrazole/**19**/THF, then MCPBA/CH_2Cl_2, **20** (95%); (D) Zn/MeOH, **8** (71%).

Biological Evaluation

* *HBV assay*

The human hepatoblastoma cell line HepG2 2.2.15 (2.2.15 cells)[16] was used for evaluation of the compounds for inhibition of hepatitis B virus in vitro. Antiviral activity of various antiviral agents was determined by the method described by Korba and Milman;[17] cells were treated with increasing concentrations of the agent and the extracellular HBV DNA in the supernatant was measured by dot blot hybridization technology.

Direct comparison of anti-HBV activity of the parent drug (**4**) and its corresponding bis-(SATE) pronucleotide (**8**) was performed twice in 2.2.15 cell lines. In the first experiment, both agents, (**4**) and (**8**), were dosed every 2 days for 4 total doses. Medium was collected on Day 9 and

was assyed for the presence of HBV DNA according to the method mentioned above. The result obtained from this experiment showed that β-L-FD4C (**4**) inhibited viral DNA synthesis by 50% at a concentration of 7 nM. The β-L-Fd4C prodrug (**8**) inhibited HBV DNA synthesis 50% at 4 nM. More notably, the parent drug (**4**) did not reach 90% inhibition with the highest doses tested (40 nM); however, the prodrug (**8**) reached 90% inhibition at a concentration of 19 nM (see Figure 3).

In the second experiment, cells were treated with drugs (**4** and **8**) every 3 days for 9 days. Using this modified dosing protocol yielded a greater disparity between the parent drug and the prodrug. The parent β-L-Fd4C (**4**) reached EC_{50} at a concentration of 17 nM. Even at 40 nM, inhibition did not go much below 50% in these experiments. On the other hand, the β-L-FD4C prodrug (**8**) did not lose potency using this dosing regime. The EC_{50} value for (**8**) was found to be 2 nM. More importantly, greater than 85% inhibition of extracellular HBV DNA was achieved with the β-L-FD4C prodrug (**8**) at the concentration of 10 nM (see Figure 4).

* *Cytotoxicity assay*

The measurement of cytotoxicity of the nucleoside (**4**) and its monophosphate nucleotide (**8**) was performed using the MTT assay[18] in five cell lines. The results obtained from this study are listed in Table 1. The cytotoxicities of (**4**) and (**8**) in human T-cell lymphoblastic leukemia cell line (CEM) were found to be 13 μM and 52 μM, respectively, indicating prodrug (**8**) was four times less cytotoxic than its parent drug (**4**). Both (**4**) and (**8**) were not cytotoxic in the human hepatoma cells (2.2.15), murine colon cancer cells (C26), murine melanoma cell line (B16) and murine lung carcinoma cell line (M109) at the highest concentration tested (70 μM).

Table 1: In Vitro Cytotoxicity Evaluation of Analogs **7** & **9**:

Compounds	IC_{50} (μM)**				
	CEM	**2.2.15**	**C26**	**B16**	**M109**
β-L-FD4C (7)	13	>70	>70	>70	>70
Prodrug (9)	52	>70	>70	>70	>70

CEM: Human T-cell lymphatic leukemia cell. 2.2.15: Human hepatoma cell.
C26: Murine colon cancer. B16: Murine melanoma. M109: Murine lung carcinoma.

In conclusion, we have completed the synthesis and preliminary in vitro evaluation of the S-acetyl-2-thioethyl (SATE)-bearing monophosphate prodrugs of β-L-FD4C (**8**). When compared with β-L-FD4C (**4**), the prodrug (**8**) exhibited improved activity against hepatitis B virus (HBV) yet reduced cytotoxicity. Furthermore, the prodrug (**8**) was capable of inhibiting HBV proliferation to a greater extent than that achieved with the parent β-L-FD4C. These results clearly demonstrate the advantage of pronucleotide approach.[19]

Acknowledgment

We would like to thank Professor Y-C Cheng for his helpful suggestions and discussions.

Figure 3

Comparason of ß-L-FD4C with ß-L-FD4C prodrug (4 doses, 2 days apart)

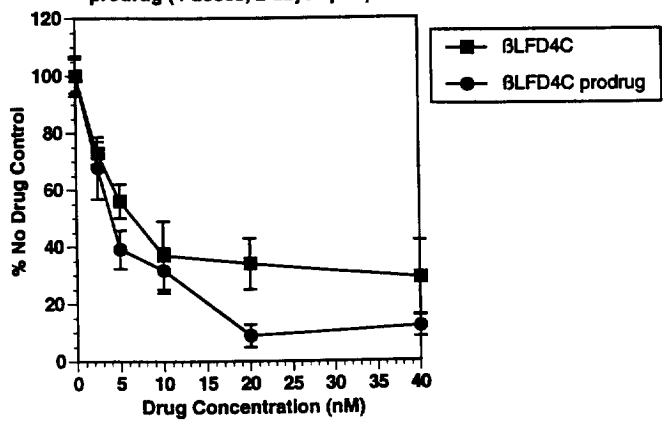
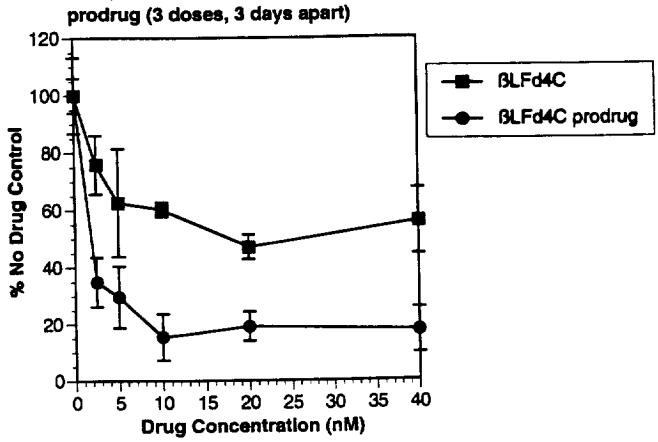


Figure 4

Comparason of ß-L-FD4C with ß-L-FD4C prodrug (3 doses, 3 days apart)



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