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# KINASE BYPASS: A NEW STRATEGY FOR ANTI-HIV DRUG DESIGN

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**Abstract.**
The 5'-bis(trichloroethyl) phosphate derivatives of several 3'-O-acyl thymidines were prepared from the thymidine phosphate. Even though the parent nucleosides are inactive as antiviral agents, the phosphates are selective inhibitors of the proliferation of HIV. This activity is attributed to a new mechanism of action, we herein describe as "kinase by-pass".

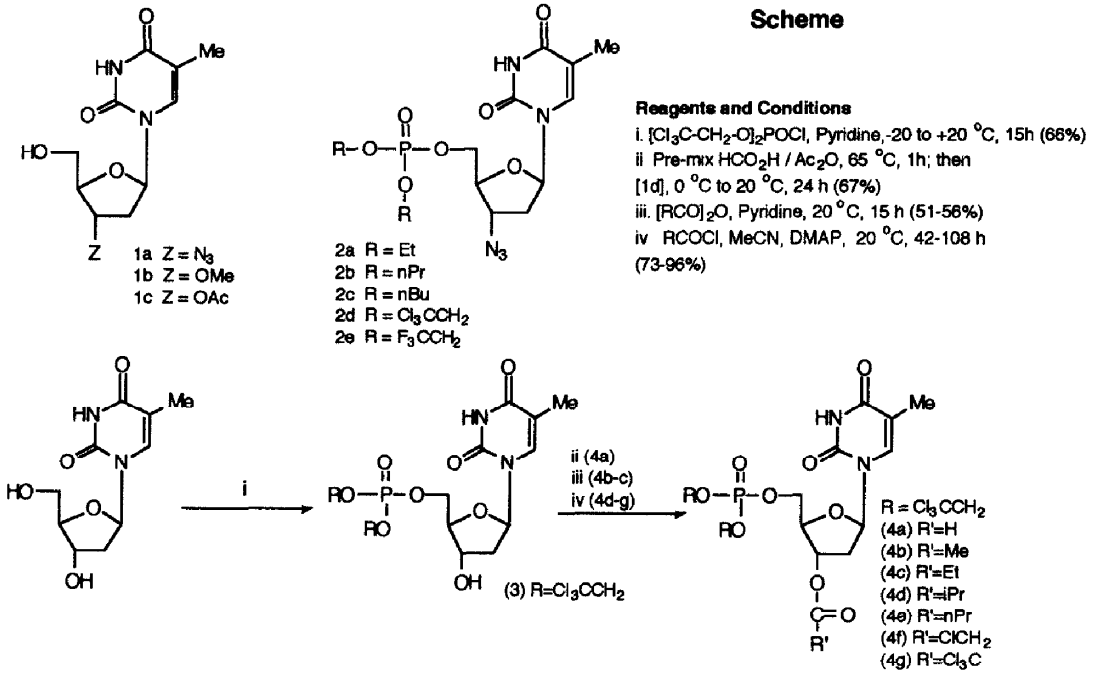
Although nucleoside analogues, such as AZT (1a) continue to dominate anti-HIV drug therapy they have a number of major limitations, such as their inherent toxicity, a dependence on kinase mediated activation to generate the bio-active (tri)phosphate forms, and the emergence of resistance.[1-2] There remains the need for potent and selective agents acting by different mechanisms and on novel targets during the process of viral replication. We herein report a recent discovery we have made which may facilitate the development of new anti-viral compounds.

We[3-6] and others[7-9] have pursued a masked phosphate approach in an attempt to improve on the therapeutic potential of the parent nucleoside analogues. In this approach, inactive phosphate derivatives of the nucleoside analogue are designed to penetrate the cell membrane and liberate the bio-active nucleotides intracellularly. Masking of the phosphate group is necessary on account of the extremely poor membrane penetration by the polar (charged) free nucleotide. As we have noted[3] such masked pro-drugs may have improved antiviral selectivity by two quite separate means. One mechanism by which masked phosphates may lead to enhanced selectivity of action arises from what we have termed "kinase bypass".[3] Thus, the complete dependence of administered (anti-HIV) nucleoside analogues on host nucleoside-kinase mediated activation places constraints upon the structures of nucleoside analogues which might be active. Nucleoside analogues which fall outside these strict constraints will be inactive, even if their 5'-triphosphates (the bio-active form) are potent and selective inhibitors of a viral target, such as reverse transcriptase (RT). Several such cases are known. For example 3'-O-methylthymidine (1b), an analogue of AZT, is inactive against HIV, whilst its triphosphate is an exceptionally potent inhibitor of HIV RT;[10] the inactivity of the nucleoside being attributed to poor phosphorylation by host kinases. If the masked phosphate strategy were able to deliver nucleotides intracellularly, the nucleoside kinase would be by-passed and the structural

constraints such host enzymes impose would be obviated. In this way, wider structural variation of the nucleoside analogue would be permitted, and more specific (less toxic) inhibitors of viral function may arise. Whilst many phosphate derivatives of bio-active nucleosides have been reported, very few derivatives of inactive nucleosides have been prepared, although some activity was noted for phosphates derived from ddT and d4A (both of which are poorly active).[11-12]

A labile phosphate group is clearly a pre-requisite for the masked phosphate approach, in order to facilitate (intracellular) release of the free nucleotides. Thus, simple dialkyl phosphate derivatives (2a-c) of zidovudine are extremely resistant to phosphate hydrolysis, and they display no antiviral effect.[3] On the other hand bis(trihaloethyl) phosphate derivatives (2d-e) of AZT show significant lability towards hydrolysis, and are potent inhibitors of viral proliferation.[5] We now note that one of these phosphate masking groups, the 2,2,2-trichloroethyl moiety is successful in the kinase by-pass activation of certain inactive 3'-modified nucleosides. In particular, in this paper we report the application of this new strategy to a series of 3'-O-acyl thymidines (4a-g). A particular interest here is the possibility of esterase-induced cleavage of the acetyl function[13] providing a potential detoxification route, since it would liberate thymidine nucleotides. Much of the selectivity of the anti-cancer nucleoside analogues FU and FUDR is now attributed to a more rapid metabolic breakdown

**Scheme**



in normal cells.[14] Thus, thymidine was allowed to react with bis(2,2,2-trichloroethyl) phosphorochloridate in pyridine at low temperature to give (3) in moderate yield as we have described[5]. This was acylated at the 3'-position by treatment with the appropriate acid anhydride in pyridine, or (for more bulky acyl groups) the acid chloride in acetonitrile containing DMAP. The target compounds (4a-g) were isolated in good yield and were fully characterised by heteronuclear NMR, FAB mass spectrometry, and HPLC, all data being consistent with their structure and purity.[15]

Each of the masked phosphates (4a-g) were tested for their ability to inhibit the replication of HIV1 in C8166 T-cells, using methods we have described,[16] data being presented in the table. The parent nucleoside 3'-O-acetyl thymidine (1c), and AZT (1a) were also examined. It is apparent that whilst the nucleoside (1c) is devoid of anti-viral activity at the highest concentrations tested (200$\mu$M), the phosphate derivatives (4a-g) exert a significant anti-HIV effect at concentrations as low as 30 $\mu$m. It is notable that activity varies with the nature of the 3'-substituent; with antiviral potency peaking at the acetyl group (4b). The compounds are non-toxic at their therapeutic concentrations; with the exception of the butanoyl (4e) and chloroacetyl (4f) analogues, where no antiviral selectivity is noted.

Table.

| Compound | ED50 ($\mu$M) | TD50 ($\mu$M) |
|---|---|---|
| 1a | 0.01 | >200 |
| 1c | >200 | >200 |
| 4a | 100 | >200 |
| 4b | 30 | >200 |
| 4c | 35 | 80 |
| 4d | 40 | >200 |
| 4e | 75 | 80 |
| 4f | 50 | 50 |
| 4g | 50 | >200 |

In conclusion, we report the antiviral activity of certain masked phosphate derivatives of inactive 3'-modified nucleosides, and attribute this introduction of activity to kinase by-pass.[17] It is quite likely that the use of alternative nucleosides and / or phosphate blocking groups may yield yet more potent anti-HIV agents, and it has not escaped our attention that this discovery may have implications in other aspects of chemotherapy. Lastly, we note that the increased structural freedom which arises from the by-pass approach may have implications for dealing with the emergence of resistant strains of viruses.[18]

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