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ATTEMPTS TO INTRODUCE CHEMOTHERAPEUTIC NUCLEOTIDES INTO CELLS: STUDIES ON THE ANTI-HIV AGENT FDT

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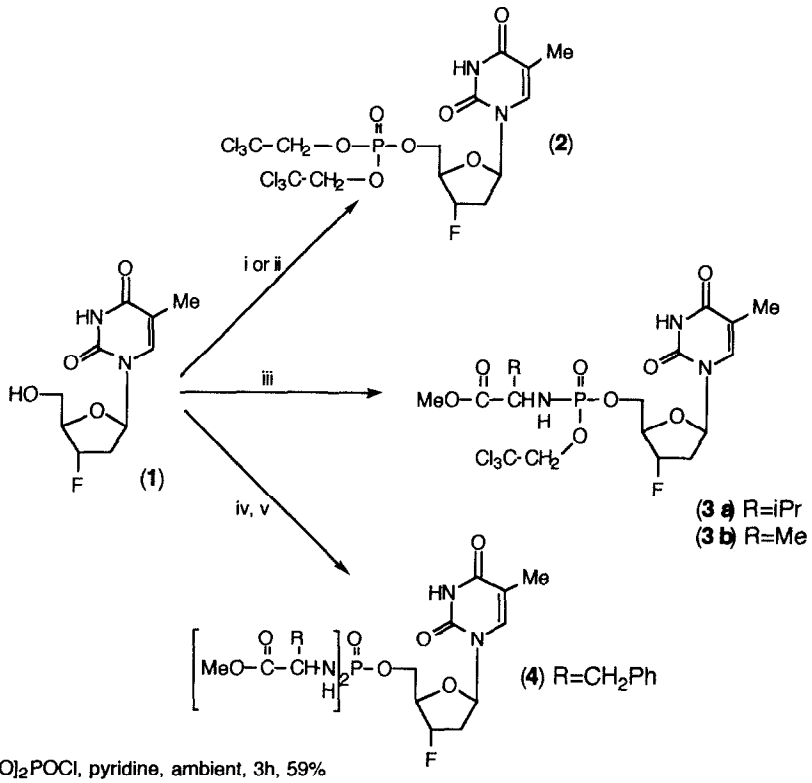
Abstract. Phosphate, phosphoramidate, and phosphorodiamidate derivatives of the anti-HIV nucleoside analogue FdT were prepared as potential pro-drugs of the bio-active free nucleotide. Two synthetic routes were adopted. The anti-viral activity of the derivatives varies greatly with the phosphate structure, but several are active below 1μM.

Recently, there has been much interest in 2',3'-dideoxynucleosides as inhibitors of HIV-1, the causative agent of AIDS.[1-3] The 3'-fluoro analogue FdT (**1**) has been noted to be a very potent inhibitor of HIV,[2] indeed rather more potent than the established anti-retroviral agent AZT.[3] These, and other nucleoside analogues suffer from an absolute dependence on (host cell) kinase-mediated activation, a dependence which can lead to poor activity, the emergence of resistance, and clinical toxicity.[4] In an effort to circumvent this dependence, we,[5] and others[6] have suggested the use of masked phosphate pro-drugs of the bio-active nucleotide forms of several chemotherapeutic nucleoside analogues. We now report the preparation and biological evaluation of various phosphate derivatives of FdT, designed to act as intracellular sources of the free 5'-monophosphate FdTMP.

FdT was prepared essentially by the method described by Herdewijn[2] using DAST fluorination of the 5'-trityl protected threo thymidine. As noted[2] considerable decomposition ensued in the detritylation, despite using a reduced temperature (80 ^{o}C).

We have recently noted that simple dialkyl phosphate triesters of AZT are inactive as anti-viral agents,[7] whereas bis(trihaloethyl) phosphates are active.[8] Therefore, FdT was allowed to react with bis(2,2,2-trichloroethyl)phosphorochloridate to give the target compound (**2**), the reaction being quicker, and the yield rather higher in THF/N-methylimidazole[9] than in pyridine (Scheme).
As anticipated[8], this material displayed one signal in the ^{31}P NMR (δ_P -5) , and much (^{31}P, ^{19}F) coupling in the H-decoupled ^{13}C spectrum.[10]

Phosphoramidate derivatives of therapeutic nucleoside analogues have long been of interest, on account of the potential enzymatic or chemical lability of the P-N bond.[11] Recently, we have noted that phosphoramidate derivatives of AZT are inhibitors of HIV, particularly when the amine moiety is a carboxyl-protected amino acid.[12] It was of interest to determine whether the same applied to FdT, and whether the same, rather precise structure activity relationships would hold. Thus, methyl valine hydrochloride was allowed to react with 2,2,2-trichloroethyl phosphorodichloridate in the presence of triethylamine to give the required phosphorochloridate,[13] which reacted with FdT to yield the target compound (**3a**) in moderate yield.



i. $[Cl_3CCH_2O]_2POCl$, pyridine, ambient, 3h, 59%
ii. $[Cl_3CCH_2O]_2POCl$, THF, N-methylimidazole, ambient, 20min, 88%
iii. $[Cl_3CCH_2O][MeOC(O)CHRNH]POCl$, THF, N-methylimidazole, ambient, 48h, 55-60%
iv. $POCl_3$, $(EtO)_3PO$, 0 °C, 17h; v. $MeOC(O)CHRNH_2.HCl$, Et_3N, 0 °C, 4d, 33%

Compound (**3a**) gave two closely spaced signals in the ^{31}P NMR, corresponding to the mixed stereochemistry at the phosphate centre, the isomers being almost 1:1. The ^{31}P chemical shift was α 10 ppm downfield of (**2**), as expected on introduction of the phosphoramidate group.[14] We have noted recently an unusually high activity in the AZT series from the trichloroethyl methoxy alanine compound,[15] and it was of interest to see whether this was unique to AZT or applied to FdT also. Therefore, the alanine analogue (**3b**) was prepared by identical methodology, and displayed very

similar spectra to the earlier homologue.

Phosphorodiamidates of AZT have been noted also to have anti-HIV activity, again when the amines are carboxyl-protected amino acids,[16] and we now prepared an analogous FdT phosphorodiamidate. Here, the deactivating effect[17] of the attached amines necessitates the use of an alternative synthetic strategy. Thus, FdT was allowed to react with phosphoryl chloride in triethyl phosphate, conditions reported to enhance the 5'-selectivity of this reagent.[18] The phosphorodichloridate intermediate was then allowed to react with an excess of methoxy phenylalanine hydrochloride in the presence of triethylamine to give the target compound (**4**) in moderate yield. Unlike (3a-b) this only gives one signal in the ^{31}P NMR (δ_P 11), as expected an account of the symmetric phosphate.

The parent nucleoside (**1**), the phosphate (**2**), the phosphoramidates (**3a-b**) and the phosphorodiamidate (**4**) were tested for their ability to inhibit the replication of HIV-1 in a human T-cell line, by methods we have described,[19] the results being displayed in the table.[20]

Table.

Compound	ED50 (μM)	TD50 (μM)
1	0.005	100
2	0.01	100
3a	0.3	>100
3b	0.3	>100
4	5	>100

Thus, it is notable that the activity of the derivatives varies over 3-orders of magnitude with variation in the phosphate structure. The diamidate (**4**) is rather poorly active. This contrasts to the situation with AZT[16] where the analogous derivative is 100-times more active than this; indeed the phenylalanine compound was chosen in this study on the basis of its especially efficacious properties in the AZT series. The amidates (**3a-b**) are approximately 20-times more active than (**4**). However, the major distinction between these two amino acids when used as AZT phosphate blocking groups does not apply in the case of FdT. The most active of the derivatives is the first one prepared, the haloethyl compound (**2**). This displays rather similar activity to the parent nucleoside (**1**) and is almost 100-times more active than the corresponding AZT analogue. Although these data are obtained from pre-clinical *in vitro* assay, and as such may be a poor measure of the *in vivo* or clinical properties of such drugs, the similar activities of (**1**) and (**2**) suggest that the pro-drug warrants further biological study.

In conclusion, the anti-HIV activity of phosphate derivatives of FdT varies greatly with the nature of the phosphate blocking group. Rather different structure activity correlations apply to those previously noted for AZT, with the bis(trichloroethyl) phosphate being particularly active in the case of FdT. Unfortunately, it may be that conclusions drawn from studies on phosphate derivatives of one

nucleoside analogue may not readily be extended to another analogue, and that each case may require careful optimisation.

Acknowledgements

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20. ED50 is the drug concentration needed to reduce viral antigen production by 50%; TD50 is the concentration of drug which inhibits protein synthesis in uninfected cells by 50%.