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TRANS-ESTERIFICATION REACTIONS YIELD NOVEL MASKED PHOSPHATE DERIVATIVES OF THE ANTI-CANCER AGENT araC

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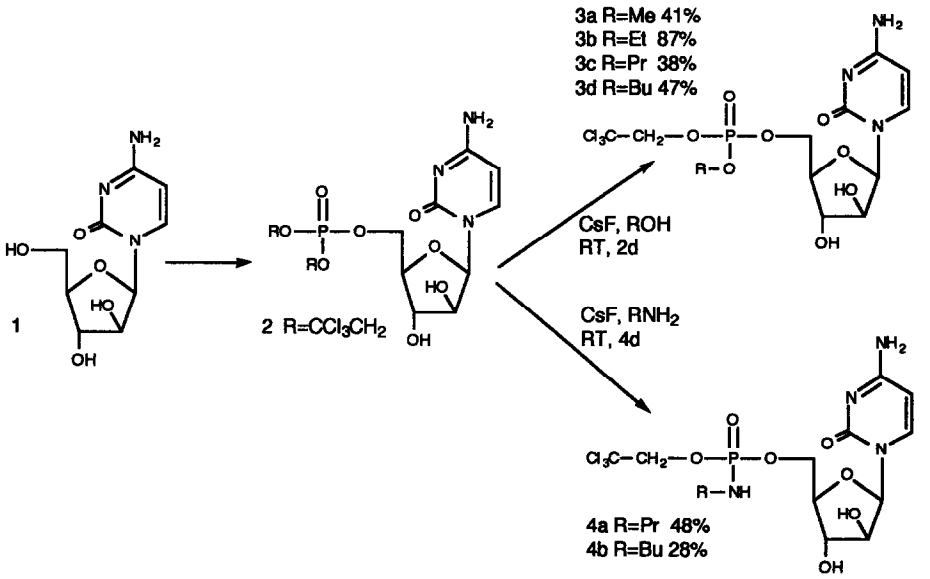
Abstract. Phosphate and phosphoramidate derivatives of the anti-leukemic agent araC have been prepared by a fluoride-ion mediated trans-esterification reaction. The resulting compounds, which are chiral at the phosphate centre, were tested for their ability to inhibit the incorporation of thymidine into the DNA of mammalian cells in tissue culture, and clear structure activity relationships emerged.

Although widely used in the chemotherapy of cancer and of viral infections, nucleoside analogues have a number of limitations. These arise, in part, from the absolute necessity for kinase-mediated activation of the nucleoside drugs. The potential advantages of circumventing the requirement for nucleoside kinase activation by using masked phosphate pro-drugs have been described[1-4]. In the case of the anti-leukemic agent araC (cytarabine; 1) we have found[5] that simple 5'-dialkyl phosphate derivatives are resistant to chemical and enzymic degradation and yet exert an effect on DNA synthesis by mammalian cells. Moreover, a clear relationship exists between increasing alkyl chain length and biological activity. We attribute this to increasing lipophilicity, and hence improved membrane penetration, with increasing chain length. We recently described[6] the preparation and potent biological properties of bis(haloethyl) phosphate analogues, in which the P-O-alkyl groups were labilised. In this communication we note that one of these, the bis(trichloroethyl) compound can be converted to mixed phosphate esters and to phosphoramidates, by a trans-esterification reaction.

Thus, bis(2,2,2-trichloroethyl) phosphorochloridate was allowed to react with araC in pyridine, to yield (2) as we have described[6-7]. Using the method developed by Ogilvie[8-9] this was allowed to react with CsF in methanol, to give the mixed compound (3a). This gave a single resonance in the ^{31}P NMR (δ 1.67), which suggested either the preferential formation of one diastereoisomer, by selective reaction of one specific haloethyl chain, or the coincidence of the ^{31}P signals for the two diastereomers. Further spectroscopic data[10] strongly support the former suggestion, with no evidence of the presence of two isomers in the product. This suggests a high degree of diastereoselectivity in the displacement reaction, although the yield is sufficiently low that selective isolation of one isomer cannot be excluded. A similar reaction of (2) with CsF in ethanol, 1-propanol,

and 1-butanol gave the homologues (3b-d) respectively. The reactions to produce (3c,d) proceeded as noted above. However, the reaction in ethanol was anomalous. In particular, the product was clearly seen as a pair of diastereoisomers, in the ^{31}P NMR (δ -2.62, -2.66), and in the ^{13}C and ^{1}H spectra[11]. Analytical HPLC also uniquely revealed two closely spaced signals for (3b); preparative HPLC being used to separate the isomers. The reasons for this unusual behavior specifically in the case of the ethanol reaction remain unclear, although it is of interest to note that the yield was double that of all of the other reactions. Thus, it would appear that both isomers can be isolated in the case of (3b), but that for (3a) and (3c-d) only one isomer is isolated It seems likely that the failure to generate the other isomer in the latter cases arises at the second stage of the reaction, the displacement of fluoride from the intermediate phosphorofluoridate by the n-alcohol[8-9], as the starting material (2) is entirely consumed in every case (as assessed by TLC). A very polar side-product is also noted on TLC in each of the reactions, it is likely that this is a phosphate diester, although it was not isolated nor characterised.

We have noted potent biological properties for phosphoramidate derivatives of anti-HIV nucleosides such as 3'-azidothymidine[12-13], and wondered if the same would apply to araC, and whether the reaction employed above with alcohols would also function with amines, to yield corresponding amidates. Thus, compound (2) was allowed to react with CsF in propylamine, and in butylamine to give phosphoramidates (4a,b) respectively. These compounds were very readily distinguishable from the earlier series by the ^{31}P NMR chemical shift (δ ca. 12). Again, spectroscopic data clearly showed the presence of only one isomer in each case, and the yields were rather low, as noted for all but the ethanol case above



A similar reaction between (2) and CsF in a secondary amine (Et_2NH) failed to yield any of the desired amidate product, indicating that the amine reaction is only feasible for primary amines.

Compounds (1), (2), (3a), (3c), (4a, b) and the separated isomers of (3b) were tested for their ability to inhibit the incorporation of tritiated thymidine into mammalian DNA in tissue culture, by methods we have previously described[1,5]. The results are displayed in table (1).

Compound	% I (SEM)
1	83 (5)
2	91 (3)
3a	22 (6)
3b (fast isomer)[14]	31 (2)
3b (slow isomer)	30 (4)
3c	40 (4)
4a	45 (3)
4b	48 (7)

Table 1 Inhibition by nucleosides and nucleotides of tritiated thymidine incorporation by mammalian epithelial cells. The mean percentage inhibition produced by 30μM drug (final concentration) is shown. The figures in parenthesis are the Standard Errors of the Means. Assays were repeated 3 times in each case.

Each of the compounds tested significantly inhibits DNA synthesis in this assay. The bis(trichloroethyl) compound (2) is more active than the parent nucleoside (1), as we have recently noted[6]. All of the mixed compounds are less active than (1) or (2) in this assay. Interestingly, the two diastereoisomers of (3b) are identical in their activity, suggesting the relative un-importance of the stereochemistry at the phosphate centre. This contrasts to our recent observations relating to the anti-viral effect of mixed phosphoramidate derivatives of AZT, where the isomers differ 10-fold in their activities.

It is notable that the activity increases consistently along the series (3a-c). This is exactly as we noted for symmetrical dialkyl phosphates of araC[5], and is attributed to the likely enhanced lipophilicity as the alkyl chains lengthened. Each homologue is more active than the corresponding simple, symmetrical dialkyl phosphates[5], indicating the enhancement in activity caused by the presence of one haloethyl chain; which is further enhanced by the presence of two such chains (as in [2]).

The amidates (4a, b) do not differ significantly in their activities, nor do they differ greatly from the corresponding alkyl compound (3c).

In conclusion, we have demonstrated that mixed phosphate triesters can be synthesised from the bis(trichloroethyl) phosphate of the anti-cancer agent araC, by treatment with CsF in the appropriate alcohol. The analogous reaction in primary amines yields the phosphoramidates. With the sole exception of the ethanol reaction, a single isomer is isolated. The stereochemistry at the phosphate does not affect the biological activity of the resulting mixed phosphate esters, although it appears that chain length / lipophilicity is a major determinant of activity. In each case the presence of a haloethyl chain contributes positively to the biological effect, and the presence of two trihaloethyl chains on the phosphorus leads to a more pronounced biological activity.

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10. Selected data: $\delta_P(CDCl_3)$ 1.67; $\delta_C(CDCl_3)$ (doublets due to phosphorus coupling) 167.6(C4), 158.2(C2), 144.6(C6), 95.9(d, CCl_3, J=11.3Hz), 94.9(C5), 88.8(C1'), 84.6(d, C4', J=6.8Hz), 78.5(d, CH_2OP, J=4 4Hz), 77.9(C2'), 76.2(C3'), 70 1(d, C5', J=5.8Hz), 56.9(d, OMe, J=8 6Hz); m/e (FAB) 494(MNa^+, $2x^{37}Cl$, <1%), 492(MNa^+,^{37}Cl, 3), 490(MNa^+, 5), 471(M^+, $2x^{37}Cl$, 3), 469(M^+,^{37}Cl, 6), 467(M^+, 8), 112(100), Found C 29.14%, H 4.39, N 8.43, $C_{12}H_{17}Cl_3N_3O_8P$ $[H_2O]_{1.5}$ requires C 29.08, H 4 07, N 8.48.
11 Selected data: $\delta_C(CDCl_3)$ 167.7(C4), 158.2(C2), 144.56/144.53(C6), 95.9(d, CCl_3, J=11.3Hz), 94.92/94 88(C5), 88 84/88.72(C1'), 84.5(d, C4', J=6 6Hz), 78.5(d, $CCl_3\underline{C}H_2OP$, J=4.4Hz), 77.89/77.38(C2'), 76 16/76.08(C3'), 70 6(d, C5', J=6.0Hz), 64 8(d, $CH_3\underline{C}H_2$, J=5.1Hz), 16.0(d, Me, J=6 2Hz), m/e (FAB) 506(MNa^+,^{37}Cl, 4%), 504(MNa^+, 6), 486(MH^+, $2x^{37}Cl$, 4), 484(MH^+,^{37}Cl, 10), 482(MH^+, 11), 112(100); Found C 30.73%, H 4.66, N 8.10, $C_{13}H_{19}Cl_3N_3O_8P.[H_2O]_{1.5}$ requires C 30.64, H 4 35, N 8 24; HPLC (Spherisorb CN 5μM column; eluant of water (A), acetonitrile (B), with 85% A for 0-12 min, then a linear gradient to 20% A at t=30 min) retention times 11.56, 11.84 min.
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14. "Fast" and "slow" refer to the relative chromatographic mobility of the diastereoisomers of (3b) on reverse phase HPLC