Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 20, pp. 2445-2448, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00446-5

A RAPID SYNTHESIS OF SOME 5'-AMINO- NUCLEOSIDES AND NUCLEOTIDES AS POTENTIAL ANTIVIRAL COMPOUNDS.

Christopher McGuigan*, Stephen Turner, Naheed Mahmood[1] and Alan J Hay[2]

Welsh School of Pharmacy, University of Wales Cardiff, King Edward VII Avenue, Cardiff, CF1 3XF, UK.
Tel./FAX +44 1222 874537. email mcguigan@cardiff.ac.uk
[1]Medical Research Council Collaborative Centre, Mill Hill, London, NW7 1AD, UK.
[2]National Institute for Medical Research, The Ridgeway, London, NW7 1AA, UK.

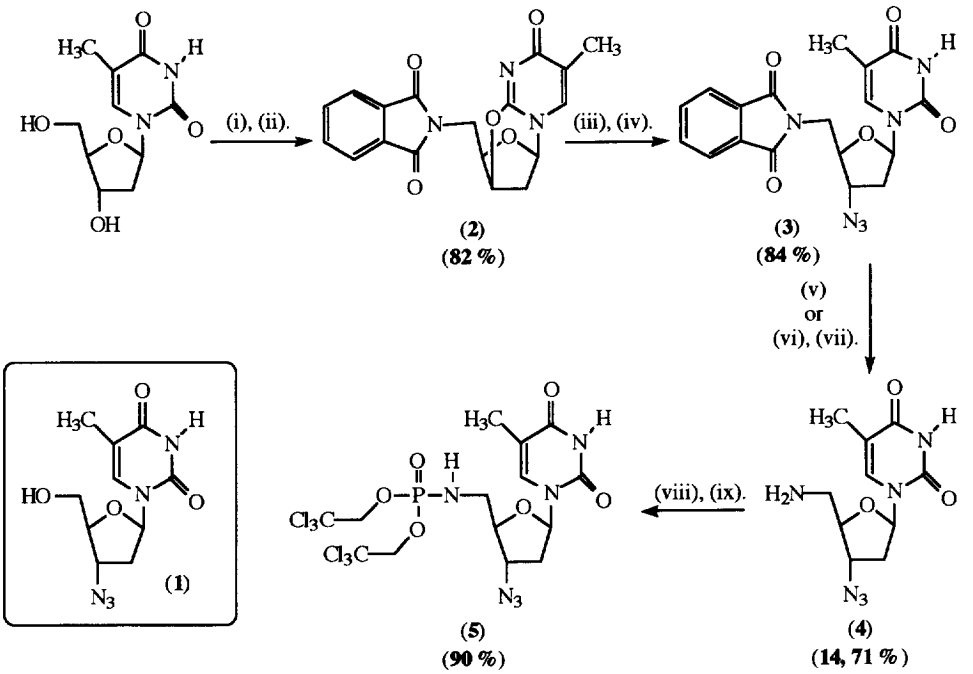
Abstract. We describe a convenient method for the synthesis of 5'-amino-3'-azido-3',5'-dideoxythymidine, and its conversion to a 5'-blocked phosphate derivative. The compounds are evaluated as inhibitors of HIV-1 in vitro, but prove to be poorly effective by comparison to their 5'-oxygen analogues.

Recently, there has been much interest in 2',3'-dideoxynucleosides as inhibitors of HIV-1, the causative agent of AIDS. The archetypal clinical agent is 3'-azido-3'-deoxythymidine (AZT, **1**), which is a potent and selective inhibitor of HIV. This, and most other nucleoside analogues suffer from an absolute dependence on (host cell) kinase-mediated activation, a dependence which may lead to poor activity and the emergence of resistance. In an effort to circumvent this dependence, we and others[1-3] have suggested the use of masked phosphate pro-drugs of the bio-active nucleotide forms of several chemotherapeutic nucleoside analogues. We have noted that simple dialkyl phosphate triesters of AZT are inactive as anti-HIV agents, whereas bis(trihaloethyl) phosphates are active.[4] We have also previously noted the anti-HIV activity of phosphoramidate derivatives of AZT,[5] and of 2',3'-dideoxy-3'-fluorothymidine (FLT)[6] and 3'-deoxy-2',3'-didehydrothymidine (d4T)[7] analogues.

A particular attraction of the masked phosphate strategy has been the apparent ability to confer antiviral action on previously inactive nucleoside analogues by judicious chemical phosphorylation, an approach we have described as "kinase bypass".[8] Using this strategy, several inactive 3'-modified nucleoside analogues were thus activated by 5'-phosphorylation.[9] In order to probe the scope of this strategy we were keen to pursue modifications in other regions of the nucleoside. Previously some of these structural modifications were avoided for fear of causing poor nucleoside kinase-mediated phosphorylation (and thus poor activity). In this manuscript we describe initial attempts at replacing the 5'-linking oxygen atom by a -NH function. It has been found that attempts to replace this oxygen with a methylene group yielded mono-nucleotide analogues which were inactive against HIV-1 in vitro.[10] However, it was hoped that the nitrogen may be more isosteric with oxygen, and may confer activity, either before or after phosphorylation. Evidence supporting this possibility is that the triphosphate of 5'-amino-5'-deoxythymidine can be incorporated with a phosphoramidate linkage into a polynucleotide by a DNA polymerase.[11] In this manuscript we report a direct synthetic strategy to the novel 5'-amino-3'-azido-3',5'-dideoxythymidine (**4**) nucleoside and its subsequent phosphorylation to a 5'-blocked phosphoramidate (**5**). The antiviral data for the modified nucleotide and all the nucleoside intermediates against HIV-1 are also presented.
Thymidine was initially converted to the 5'-protected 2,3'-O-anhydro nucleoside (**2**) in a single pot reaction. Two consecutive Mitsunobu reactions were used to couple phthalimide onto the 5'-position and form the 2,3'-O-

anhydro linkage[12] (Scheme). Compound (**2**) underwent ring opening using lithium azide generated in-situ by the reaction of sodium azide with lithium chloride[13] to give the 5'-protected AZT analogue (**3**). Phthalamide (**3**) was converted to the 5'-amino nucleoside (**4**)[14] using either hydrazine monohydrate[15] or sodium borohydride followed by hydrolysis with acetic acid.[16] Following our established methods,[4] (**4**) was converted to its 5'-bis(2,2,2-trichloroethyl) phosphoramido derivative (**5**).[17]

Scheme



Reagents and conditions: (i) Phthalimide, DIAD, PPh_3, 0 °C - RT, 1 Hr. (ii) DIAD, 0 °C - RT, 3 Hrs. (iii) NaN_3, LiCl, DMF, 100 °C, 9 Hrs. (iv) Dil. HCl. (v) $H_2NNH_2.H_2O$, CH_3OH, RT, 12Hrs. (vi) $NaBH_4$, H_2O/ iPrOH (1:6), RT, 2 Hrs. (vii) AcOH, 80 °C, 2 Hrs. (viii) $(CCl_3CH_2O)_2P(O)Cl$, C_5H_5N, 10 °C - RT, 5 Hrs. (ix) H_2O.

The novel nucleosides (**2-4**), and the phosphoramidate (**5**), were tested for their ability to inhibit the replication of HIV-1, as previously described[5] using AZT (**1**) as a reference [Table]. It is most notable that the 5'-amino nucleoside (**4**) is far (2500-fold) less active than the parent nucleoside analogue AZT (**1**). The results also show that the blocked phosphate (**5**) exhibits no significant increase in potency over the free nucleoside (**4**), and moreover, is more cytotoxic; leading to a ca. 10-fold reduction in selectivity index of (**5**) over (**4**).

Thus, the 5'-amino analogue of AZT is poorly effective as an antiviral agent; the replacement of the 5'-oxygen by a NH functionality leading to a huge decrease in activity. Moreover, this reduction in potency cannot be overcome by use of the 5'-N-bis(2,2,2-trichloroethyl) phosphoramidate, as a potential kinase bypass strategy.[8-9]
Several explanations are possible. Compound (**4**) may be a poor substrate for nucleoside kinases, leading to little intracellular 5'-N-monophosphate, and (**5**) may be a poor intracellular source of the 5'-N-monophosphate.

Table

COMPOUND	EC_{50} / HIV-1 / C8166 / μM	CC_{50} / C8166 / μM	SI = CC_{50}/EC_{50}
1	0.016	>1000	>62,500
2	400	>1000	>2.5
3	100	1000	10
4	40	>1000	>25
5	30	100	3.3

EC_{50} = The drug concentration (μM) required to reduce viral antigen production by 50%. CC_{50} = The concentration (μM) which reduces the viability of uninfected cells by 50%. Selectivity Ratio (S.I.) = The ratio of EC_{50} to CC_{50}.

Alternatively, it may be that the subsequent intracellular phosphorylation of the monophosphate of (**4**) to the di- and tri-phosphate is slow. Finally, it may be that the 5'-triphosphate of (**4**) is not an effective inhibitor of HIV reverse transcriptase. At the moment, it is not possible to clarify which, if any, of these explanations is more likely. It is however known that 5'-amino-5'-deoxythymidine is a good competitive inhibitor of the phosphorylation of thymidine by thymidine kinase,[18-19] and a modest inhibitor of thymidylate kinase.[20] Both these enzymes are required to metabolise AZT to its triphosphate, hence, should (**4**) have similar properties, it may be poorly phosphorylated. This poor rate of phosphorylation of 5'-amino-5'-deoxythymidine by host cell kinases is the attributing factor to the observed selectivity in antiviral activity of these compounds to HSV which has its own, less selective kinase enzymes. The replacement of the 5'-linking oxygen with a methylene group of a 3'-modified nucleotide triphosphate analogue of AZT has been found to greatly reduce the rate of RT-catalysed incorporation of the nucleoside into a DNA strand compared to thymidine triphosphate.[10] This may also be true for the incorporation of 5'-amino-3'-azido-3',5'-dideoxythymidine-5'-N-triphosphate by RT.

Regarding the poor efficacy of (**5**), it may be that alternative phosphate protection may be more efficacious; we have noted that phosphoramidates with amino acids are especially effective in the case of several 3'-modified nucleosides. It would be interesting to see if these might more significantly improve on the antiviral action of (**4**).

Acknowledgements.
We thank the AIDS Directed Programme of the MRC and the Biomedical Research Programme and Human Capital and Mobility Programme of the EC for financial support.

References

1. C. McGuigan, R.N. Pathirana, J. Balzarini and E. De Clercq, J. Med. Chem., **1993**, 36, 1048.

2. S.N. Farrow, A.S. Jones, A. Kumar, R.T. Walker, J. Balzarini and E. De Clercq, J. Med. Chem., **1990**, 33, 1400.

3. C. Perigaud, A.M. Aubertin, H. Peliano, J.L. Giradet, G. Maury, G. Gosselin, A. Kim and J.L. Imbach, Biochem. Pharmacol., **1994**, 48, 11.

4. C. McGuigan, J. O'Connor, S. R. Nicholls, C. Nickson and D. Kinchington, Antiviral Chem. Chemother.,

1990, 1, 355.

5. C. McGuigan, K.G. Devine, T.J. O'Connor and D. Kinchington, Antiviral Res., **1991**, 15, 255.

6. C. McGuigan, B.C.N.M. Jones, K.G. Devine, S.R. Nicholls, T.J. O'Connor and D. Kinchington, BioMed. Chem. Lett., **1991**, 1, 729.

7. C. McGuigan, D. Cahard, H. M. Sheeka, E. De Clercq and J. Balzarini, BioMed. Chem. Lett., **1996**, 6, 1183.

8. C. McGuigan, D. Kinchington, S. R. Nicholls, C. Nickson and T. J. O'Connor, BioMed. Chem. Lett., **1993**, 3, 1207.

9. C. McGuigan, P. Bellevergue, H. Sheeka, N. Mahmood and A.J. Hay, FEBS Lett., **1994**, 351, 114.

10. G. A. Freeman, J. L. Rideout, W. H. Miller and J. E. Reardon, J. Med. Chem., **1992**, 35, 3192.

11. R. L. Letsinger, J. S. Wilkes and L. B. Dumas, J. Am. Chem. Soc., **1972**, 94, 292.

12. S. Czernecki and J.-M. Valéry, Synthesis, **1991**, 239.

13. W. G. Finnegan, R. A. Henry and R. Lofquist, J. Am. Chem. Soc., **1958**, 80, 3908.

14. Selected data for [**4**]: δ_H (CD_3OD) 7.51 (1 H, s, H6); 6.24 (1 H, *pseudo* t ($J_{1',2'\alpha}$ = 5.66 Hz, $J_{1',2'\beta}$ = 3.14 Hz), H6); 4.61-4.69 (2 H, m, $N\underline{H}_2$); 4.25-4.32 (1 H, m, H3'); 3.88-3.92 (1 H, m, H4'); 3.20-3.38 (2 H, m, H5'); 2.19-2.33 (2 H, m, H2'); 1.82 (3 H, s, 5-$C\underline{H}_3$). δ_C ($CDCl_3$/ CH_3OD) 164.29 (C2); 154.53 (C4); 136.32 (C6); 110.98 (C5); 85.36 (C1'); 82.70 (C4'); 60.21 (C3'); 41.75 (C5'); 36.31 (C2'); 11.96 (5-$\underline{C}H_3$). IR (Thin Film, KBr Disc) 3365.6, 3172.7, 2928.2, 2105.3, 1696.0, 1471.4, 1368.6, 1272.5, 1072.2, 958.3, 766.0. FAB MS (Argon, NBA Matrix) 267 (MH^+, Base Peak); 141 (MH^+-Thymine, 32); 127 (ThymineH$^+$, 23). Analytical HPLC Retention Time 25.37 min. (ACS Quarternary system, using Techsphere Si 5 μM (250 x 4.6 mm) column, and an isocratic elution of methanol in DCM (6:94), Flow rate 1.00 cm^3/min).

15. T. Sasaki, K. Minamoto and H. Itoh, J. Org. Chem., **1978**, 43, 2320.

16. J. O. Osby, M. G. Martin and B. Ganem, Tetrahedron Lett., **1984**, 25, 2093.

17. Selected data for [**5**]: δ_P ($CDCl_3$) 7.425 (s). δ_H ($CDCl_3$) 9.21 (1 H, Br s, $N^3\underline{H}$)*; 7.07 (1 H, d ($J_{6,5CH3}$ = 1.16 Hz), H6); 5.85 (1 H, *pseudo* t ($J_{1',2'\beta}$ = 6.76 Hz, $J_{1',2'\alpha}$ = 6.56 Hz), H1'); 4.62, 4.63 (2 H, d ($J_{\underline{H}COP}$ = 5.99 Hz), $C\underline{H}_2OP$); 4.43 (1 H, dt ($J_{3',4'}$ = 8.12 Hz, $J_{3',2'}$ = 5.40 Hz), H3'); 4.29 - 4.37 (1 H, dt ($J_{\underline{H}NP}$ = 8.30 Hz, $J_{NH,5'}$ = 7.15 Hz), 5'-N$\underline{H}$)*; 3.90 - 3.95 (1 H, m, H4'); 3.30 - 3.49 (2 H, m, H5'); 2.72 (1 H, ddd ($J_{2'\beta,2'\alpha}$ = 14.10 Hz, $J_{2'\beta,1'}$ = 6.73 Hz, $J_{2'\beta,3'}$ = 1.74 Hz), H5'β); 2.39 (1 H, ddd ($J_{2'\alpha,2'\beta}$ = 14.10 Hz, $J_{2'\alpha,1'}$ = 6.54 Hz, $J_{2'\alpha,3'}$ = 5.41 Hz), H2'α); 1.93 (3 H, d ($J_{5CH3,6}$ = 1.16 Hz), 5-$C\underline{H}_3$) * Exchanges with a D_2O shake. δ_C ($CDCl_3$) 164.24 (C2); 150.63 (C4); 137.31 (C6); 111.69 (C5); 95.18 (d ($J_{\underline{C}COP}$ = 11.31 Hz), $\underline{C}Cl_3$); 87.41 (C1'); 83.31 (d ($J_{\underline{C}CNP}$ = 5.65 Hz), C4'); 76.59 (d ($J_{\underline{C}OP}$ = 2.25 Hz), $\underline{C}H_2OP$); 60.65 (C3'); 42.91 (C5'); 36.68 (C2'); 12.63 (5-$\underline{C}H_3$). FAB MS (Argon, MNOBA Matrix) 633 (MNa^+ (2 x ^{37}Cl), 2); 631 (MNa^+ (1 x ^{37}Cl), 4); 629 (MNa^+, 1); 615 ((MH^+ (4 x ^{37}Cl), 3); 613 (MH^+ (3 x ^{37}Cl), 5); 611 (MH^+ (2 x ^{37}Cl), 9); 609 (MH^+ (1 x ^{37}Cl), 13); 607 (MH^+, 7); 485 (4); 483 (6); 481 (3); 444 (6); 442 (14); 440 (16); 438 (9) 376 (7); 374 (16); 372 (23); 370 (12); 127 (27). FAB MS (HR-Argon, PEG-MNOBA Matrix) 606.9138 (MH^+ {$C_{14}H_{18}N_6O_6P_1Cl_6$ Requires 606.9156}). Analytical HPLC Retention Time 26.52 min (ACS quarternary system, using an Ultratech ODS2 (5 μM (250 x 4.6 mm) column, eluent A = water, B = acetonotrile, linear gradient conditions 82%A 0 min, 82%A 10 min, 20%A 30 min, 20%A 45 min, Flow Rate 1.00 cm^3/min).

18. Y. C. Cheng and W. H. Prusoff, Biochemistry, **1974**, 13, 1179.

19. J. P. Neenan and W. Rohde, J. Med. Chem., **1973**, 16, 580.

20. Y. C. Cheng and W. H. Prusoff, Biochemistry, **1973**, 12, 2612.

(*Received in Belgium* 26 *July* 1996; *accepted* 19 *September* 1996)