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PHOSPHORAMIDATE DERIVATIVES OF 2',3'-DIDEHYDRO-2',3'-DIDEOXYADENOSINE [d4A] HAVE MARKEDLY IMPROVED ANTI-HIV POTENCY AND SELECTIVITY

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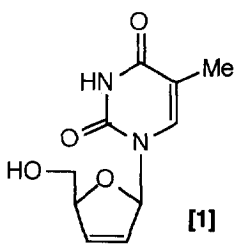
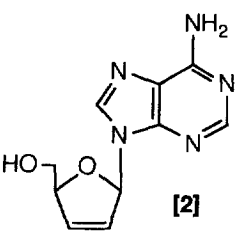
Abstract. New 5'-phosphate derivatives of the nucleoside analogue d4A were prepared as potential membrane-soluble prodrugs of the free nucleotide. The anti-viral potency and selectivity of the derivatives is markedly increased by comparison to the parent nucleoside analogue. The new analogues show particular promise for further pre-clinical development.

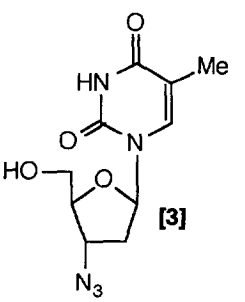
There has been much interest in 2',3'-dideoxynucleosides as inhibitors of HIV-1, the causative agent of AIDS.[1] The 2',3'-dideoxy-2',3'-didehydro analogue of thymidine (d4T) (**1**) has been found to be a very potent inhibitor of HIV.[2,3] These, and other nucleoside analogues suffer from an absolute 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,[4] and others[5,6] 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 two particularly efficacious phosphate derivatives of d4A (**2**), designed to act as an intracellular source of the free d4A 5'-monophosphate (d4AMP). It is known that the kinetics of the three phosphorylation steps from the nucleoside analogue to the potentially bio-active 5'-triphosphate differ for d4A, by comparison to AZT (**3**) and other 3'-modified nucleoside analogues. In particular, the rate-limiting step for AZT appears to be the conversion of mono- to di-phosphate, whereas the conversion of nucleoside to monophosphate may well be rate-limiting for d4A, as is the case with ddA.[6,7] It could follow that the intracellular delivery of pre-formed d4AMP may be more useful than the delivery of AZTMP.



[1]



[2]

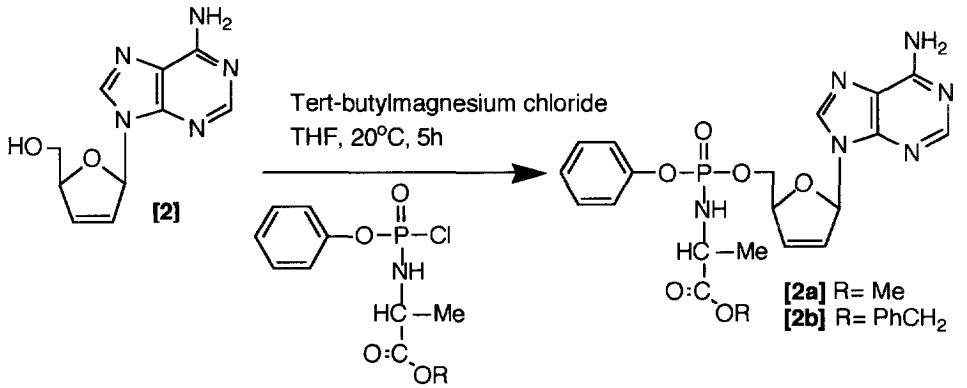


[3]

We have recently noted that simple dialkyl phosphate triesters of d4T are inactive as anti-viral

agents, whereas bis(trihaloethyl) phosphates are active.[8] We have also previously noted the anti-HIV activity of phosphoramidate derivatives of AZT[9] and of the 3'-fluorothymidine analogue (FLT).[10] Thus, we were interested to apply the phosphoramidate technology to the purine analogue d4A. D4A was prepared from adenosine using Mattock's bromide followed by reduction using a Zn/Cu couple.[11] Then, phenyl methoxyalaninyl phosphorochloridate was allowed to react with d4A using THF / tert-butyl magnesium chloride,[12] to give compound (**2a**) in reasonable yield (63%). As anticipated, this material displayed two closely spaced signals in the ^{31}P NMR (δ_P ca. 4.8 ppm),[13] corresponding to the presence of diastereoisomers, resulting from mixed stereochemistry at the phosphate centre. Similar diastereomeric splitting, and phosphorus coupling where appropriate, were also noted in the H-decoupled ^{13}C spectrum.[14] The presence of diastereoisomers was also apparent from ^{1}H NMR spectroscopy.[15]

We have also recently noted the importance of the carboxyl ester terminus for the activity of analogous phosphoramidate derivatives of AZT and d4T; in both cases a benzyl ester terminus was found to be the most efficacious ester group.[16,17] Thus, phenyl benzylalaninyl phosphorochloridate was allowed to react with d4A as above, to give compound (**2b**) in good yield; (**2b**) displayed spectroscopic and analytical data fully confirming the structure and purity, and closely resembling that of (**2a**).



The nucleoside analogues (**2**) and (**3**), and the phosphates (**2a-b**) were tested for their ability to inhibit the replication of HIV, as previously described,[9] and the results obtained using HIV-1 (III_B) or HIV-2 (ROD) -infected CEM cells are displayed in the Table. Data are also included for the inhibitory effect of the test compounds against MSV [moloney sarcoma virus]- induced transformation of C3H cells.

It is most notable that the phosphate derivatives (**2a-b**) are far (1000- to 4000-fold) more active against HIV than the parent nucleoside analogue d4A (**2**). Moreover, although the phosphoramidates are also (ca. 30-fold) more cytotoxic than the free nucleoside analogue, their selectivity indices (200 to 700) are at least two orders of magnitude in excess of that for d4A, which barely has any antiviral selectivity. Indeed, it is reasonable to regard the phosphoramidate derivatisation of d4A as a further

example of what we have termed "kinase bypass", wherein an inactive nucleoside which is active as its triphosphate form may be activated by judicious 5'-phosphorylation.[18] The data for the d4A derivatives herein reported are very similar to that we recently noted for the poorly effective analogue ddU and its corresponding phosphoramidates,[19] although the activities are far higher in the present case.

Table. Antiviral activity for compounds **[2]**, **[2a-b]**, and **[3]**.[20]

Compound	EC_{50} HIV-1 (CEM) μM	EC_{50} HIV-2 (CEM) μM	CC_{50} (CEM) μM	Selectivity Index (SI) (CC_{50}/EC_{50})	MIC MSV (C3H) μM	CC_{50} MSV (C3H) μM
2 [d4A]	20	20	91	4.5	31	>100
2a	0.006	0.018	3.8	633	9.4	>100
2b	0.008	0.008	1.9	237	0.2	>4
3 [AZT]	0.005	0.008	>100	>20,000	0.02	>20

Furthermore, the masked d4A phosphates (**2a-b**) retain virtually full activity in thymidine kinase-deficient cells [CEM TK^-] confirming their independence from thymidine kinase (data not shown); this is in contrast to the situation with AZT (**3**) which is virtually inactive against HIV-2 in this cell line.

In MSV-infected C3H cell cultures, the increased antiviral activity of (**2a**) and (**2b**) was also evident as compared to (**2**), but the improved inhibitory efficacy was less pronounced, particularly for (**2a**) against MSV as compared to HIV-1 and -2. By comparison to our earlier studies on d4T, the benzyl derivative (**2b**) did not show an increase in anti-HIV activity over the methyl analogue (**2a**); the two compounds have very similar profiles of activity. It is interesting to wonder if other parameters, such as the choice of the amino acid, or the stereochemistry at the phosphate or amino acid sites, also differ here by comparison to the case of d4T.

The most obvious mechanism of action consistent with the antiviral activity of the phosphoramidates we herein report is that of intracellular delivery of the free nucleotide d4AMP by intracellular chemical and / or enzymatic hydrolysis of the prodrugs (**2a-b**), and further phosphorylation to generate the active metabolite d4ATP. Further experiments are underway in our laboratories to confirm this hypothesis.

In conclusion, the masked phosphate derivatives of d4A herein described show very marked in vitro advantage over the parent nucleoside analogue d4A. We suggest intracellular nucleotide delivery to be responsible for the marked antiviral potency of the phosphoramidates of d4A and cite this as a further example of our "kinase bypass" approach.

Acknowledgements

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References and Notes

1. Mitsuya, H.; Broder, S. Proc. Natl. Acad. Sci.USA **1986**, 83, 1911.
2. Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E.; Desmyter, J.; Vandeputte, M. Biochem. Biophys. Res. Commun. **1987**, 142, 128.
3. Balzarini, J.; Kang, G.-J.; Dalal, M.; Herdewijn. P.; De Clercq, E.; Broder, S.; Johns, D.G. Mol. Pharmacol. **1987**, 32, 162.
4. McGuigan, C.; Tollerfield, S.M.; Riley, P.A. Nucleic Acids Res. **1989**, 17, 6065; Jones, B.C.N.M.; McGuigan, C.; Riley, P.A. ibid **1989**, 17, 7195.
5. Farrow, S.N.; Jones, A.S.; Kumar, A.; Walker, R.T.; Balzarini, J.; De Clercq, E. J. Med. Chem. **1990**, 33, 1400.
6. Perigaud, C.; Aubertin, A.M.; Peliano, H.; Giradet, J.L.; Maury, G.; Gosselin, G.; Kim, A.; Imbach, J.L. Biochem. Pharmacol. **1994**, 48, 11.
7. Cooney, D.A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johnson, M.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D.G. Biochem. Pharmacol. **1987**, 36, 1765.
8. McGuigan, C.; Sheeka, H.M.; Mahmood, N.; Hay, A. Bioorg. Med. Chem. Lett. **1993**, 3, 1203.
9. McGuigan, C.; Pathirana, R.N.; Balzarini, J.; De Clercq, E. J. Med. Chem. **1993**, 36, 1048.
10. McGuigan, C.; Jones, B.C.N.M.; Devine, K.G.; Nicholls, S.R.; O'Connor, T.J.; Kinchington, D. Bioorg. Med. Chem. Lett. **1991**, 1, 729.
11. Talekar, R.R.; Coe,P.L.; Walker, R.T. Synthesis, **1993**, 303.
12. Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. J. Org. Chem. **1993**, 58, 373.
13. Mark, V.; Dungan, C.H.; Crutchfield, M.M.; Van Wazer, J.R. *Compilation of 31P NMR data;* Grayson, M.; Griffith, E.J. Ed.; Topics in Phosphorus Chemistry, vol. 5, Wiley, New York, 1969.
14. McGuigan, C.; Pathirana, R.N.; Mahmood, N.; Devine, K.G.; Hay, A.J. Antiviral Res., **1992**, 17, 311.
15. Selected data for (**2a**): δ_P (DMSO) 4.92, 4.78; δ_H (DMSO) 8.14 (1H, s, H8), 8.06 (1H, d, H2), 7.07-7.40 (7H, m, Ar, NH_2), 6.93 (1H, s, H1'), 6.47 (1H, m, H3'), 6.21 (1H, m, H2'), 5.96 (1H, m, NH ala), 5.11 (1H, m, H4'), 4.10 (2H, m, H5'), 3.5-4.83 (1H, m, CH ala), 3.52 (3H, d, OMe), 1.08 (3H, 2d, CH_3 ala); δ_C (DMSO)172.91-172.82 (CO ala), 154.66 (C-2), 152.24 (C-6), 149.52-149.44 (Ar-ipso), 148.78 (C-4), 138.00-137.91 (C-8), 132.29-132.21 (C-3'), 128.62 (Ar-meta), 125.38-125.21 (Ar para), 123.93 (C-2'), 119.07-119.00 (Ar ortho), 118.50 (C-5), 87.31-87.06 (C-1'), 84.49-84.37 (C-4'), 66.09-65.32 (C-5'), 51.48-51.43 (OMe), 49.11-48.99 (C-H ala), 19.90-19.59 (Me ala); FAB m/e: 475.15 [MH^+, calculated 475.149].
16. McGuigan, C.; Pathirana, R.N.; Choi, S.S.-M.; Kinchington, D.; O'Connor, T.J. Antiviral Chem. Chemother. **1993**, 4, 97.
17. McGuigan, C.; Cahard, D.; Sheeka, H.M.; De Clercq, E.; Balzarini, J. BioMed. Chem. Lett. **1996**, 6, 1183.
18. McGuigan, C.; Nicholls, S.R.; O'Connor, T.J.; Kinchington, D. Antiviral Chem. Chemother. **1990**, 1, 25.
19. McGuigan, C.; Bellevergue, P. ; Sheeka, H.; Mahmood, N.; Hay A.J. FEBS Lett. **1994**, 351, 11.
20. EC_{50} is the compound concentration in μM required to reduce virus-induced cytopathicity by 50%; CC_{50} is the drug concentration which reduces the viability of uninfected cells by 50%. MIC is the drug concentration which causes a microscopically visible alteration of cell morphology. Data are given for HIV-1 and HIV-2- induced cytopathicity in CEM cells and MSV [moloney murine sarcoma virus]- induced transformation of murine embryo fibroblast C3H cells.

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