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DESIGN AND SYNTHESIS OF BIO-ISOSTERES OF THYMIDINE TRIPHOSPHATE

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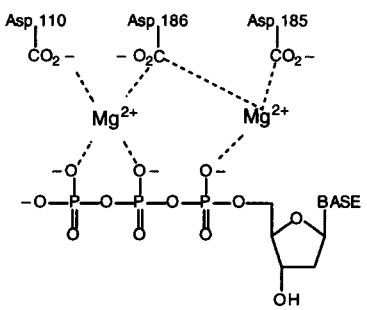
Abstract: This paper describes the design and synthesis of potential isosteres of triphosphates which should show enhanced metabolic stability and lipophilicity compared to triphosphates. The triphosphate isosteres were then linked to nucleosides and evaluated for their inhibitory activity against HIV infection.

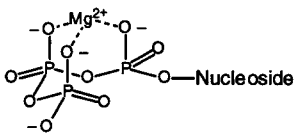
Introduction

Nucleoside triphosphates are important metabolites in many cellular processes, and analogues of these compounds have therapeutic potential in a number of diseases. However, triphosphates are very poor drug candidates owing to chemical and enzymatic instability, low bioavailability and poor cellular uptake. It has been found that some nucleoside analogues can be phosphorylated intra-cellularly by kinases to the nucleoside triphosphates to give therapeutically useful agents. Examples include the anti-HIV compounds AZT, d4T, ddC, ddI and 3TC, and the anti-herpes agent acylovir. However many nucleoside analogues are not well recognised by kinases and cannot be activated intracellularly to pharmacologically active agents.

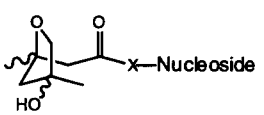
We have a programme to design and synthesise lipophilic and chemically and enzymatically stable isosteres of triphosphates which can be attached to nucleosides.[1,2,3] Attachment of triphosphate isosteres to nucleosides should prevent the need for intracellular activation and should increase the variety of nucleoside analogues which can be used for anti-HIV treatment.



Proposed complex of the nucleoside triphosphate in the active site of reverse transcriptase



Magnesium ions complexed to a nucleoside triphosphate in X-ray structures



X = O, NH

Isostere (1)

Figure 1

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Intracellularly, triphosphates are co-ordinated to magnesium ions and in addition crystallographic data suggests that in the case of HIV reverse transcriptase the nucleoside triphosphate is complexed to two magnesium ions in the active site (Figure 1).[4] It is likely that the nucleoside triphosphates are complexed to magnesium ions in the active site of many enzymes. Otoski and Wilcox[5] designed an isostere (**1**) of the triphosphate based on the crystal structure of a complex between ATP and metal ions (Figure 1).[6] The isostere is a substituted tetrahydrofuranyl ring, and is designed to mimic the shape generated by the complex of a magnesium ion with the β and γ phosphates. Such a structure is believed to exist in the active site of HIV reverse transcriptase.[4] We previously reported the attachment of these isosteres to nucleosides and known anti-HIV nucleoside analogues in an attempt to develop inhibitors of HIV reverse transcriptase which would not require intracellular activation (**1**) (nucleosides used included AZT, d4T, thymidine, 5'-amino-d4T).[1] Unfortunately the ester analogues (X=O) were hydrolysed to yield the nucleoside analogues whilst the amide analogues were inactive (X=NH).

Modelling

In order to investigate this further we carried out modelling studies. The methyl ester of the isostere **2** was energy minimised using Monte-Carlo Energy Minimisation with the MM3* forcefield in MACROMODEL[7] using a correction for water (Figure 2). The resulting structures were then compared with the crystal structure of ATP complexed to magnesium ions. This showed the ester group in a pseudo-equatorial conformation relative to the tetrahydrofuranyl ring. In order to obtain good overlap with the crystal structure of ATP the ester linkage must be in a pseudoaxial position, but no low energy conformation was found in which this was the case. This barrier for conformational change may explain the lack of activity of the isosteres.

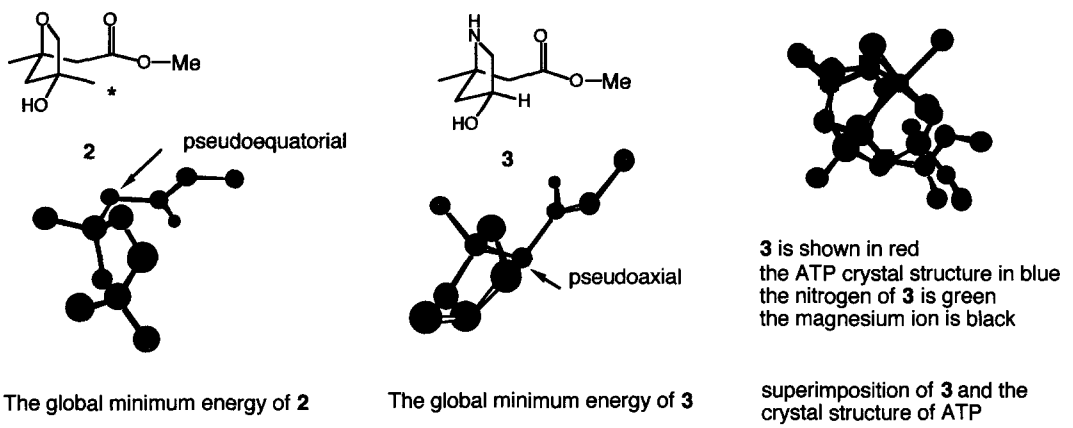


Figure 2

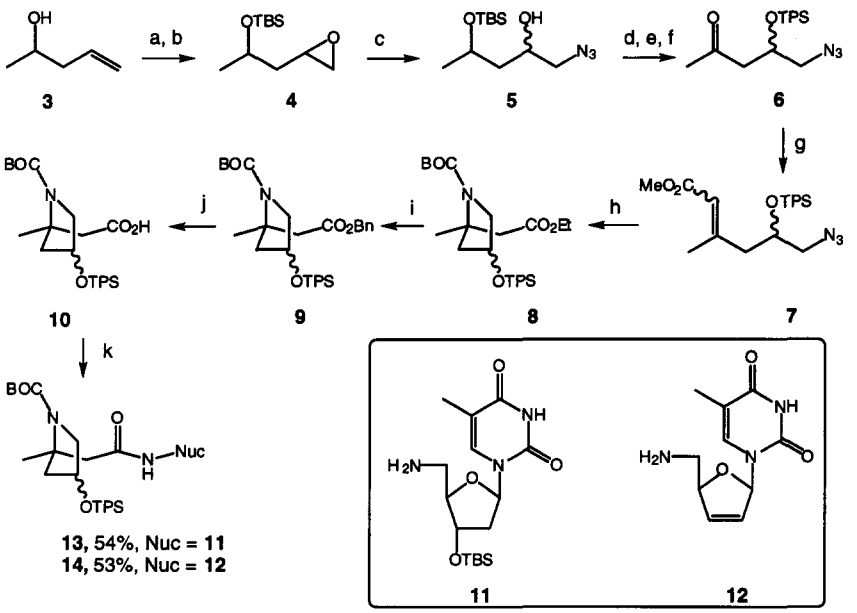
To improve the isosteric model, two changes were envisaged: (a). removal of the methyl group (*) (**2**, Figure 2) which should reduce the energy barrier as it would remove diaxial interactions with the ester chain. (b). replacement of the tetrahydrofuranyl ring with a pyrrolidine or piperidine ring. At physiological pH the nitrogen should be protonated which would give strong interaction with the aspartic acid residues in the active site (Figure 1).

Energy minimisation calculations were undertaken. These showed that the global minimum energy of the pyrrolidine isostere **3** placed the ester substituent in the required pseudoaxial orientation and showed very good overlap with the crystal structure of ATP (Figure 2). In addition, the pyrrolidine isostere showed some conformational flexibility (the conformation with the ester substituent pseudoequatorial was just 2.9KJmol^{-1} higher in energy) which should allow adaptation to the active site. The global minimium energy structure of **3** and the ATP crystal structure gave excellent overlap. Therefore, the selected targets were the nucleoside

derivatives of the pyrrolidine isosteres. It was decided to link the isosteres through amide rather than ester bonds owing to hydrolysis of the ester linkages occuring in other isosteres.[1]

Chemistry

Initial studies showed that the methodology for synthesis of the tetrahydrofuranyl isosteres[1] could not be adapted. Therefore a new synthetic strategy was devised.[8]



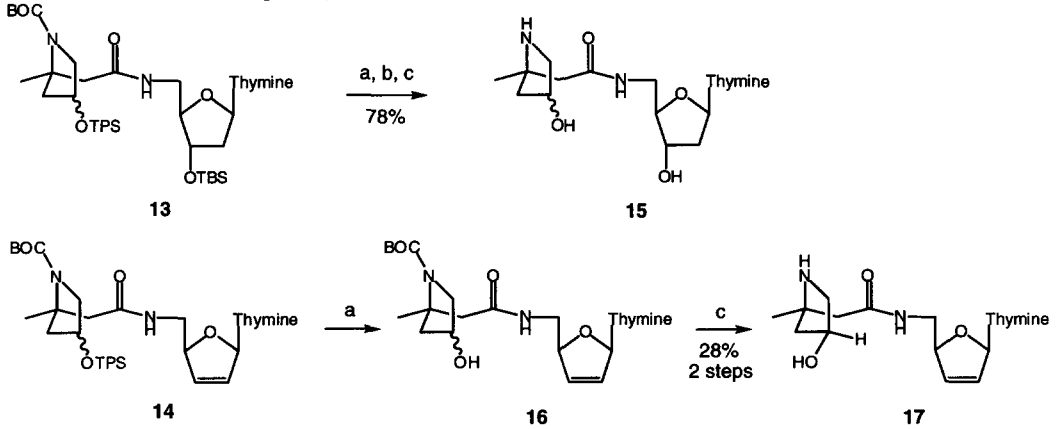
a). TBDMSiCl, imidazole, DMF, 93%. **b**). mCPBA, CH_2Cl_2, 95%. **c**). NaN_3, $LiOSO_2CF_3$, CH_3CN, Δ, 67%. **d**). TBDPSiCl, imidazole, DMF, 91%. **e**). acetic acid:THF:H_2O, Δ, (3:1:1), 84%. **f**). PCC, CH_2Cl_2, celite, 96%. **g**). $EtO_2CCH_2SiMe_3$, LDA, THF, -78°C, 60%. **h**). i. H_2, Lindlar's catalyst, EtOH. ii. $(BOC)_2O$, 65%. **i**). $(i\text{-}PrO)_4Ti$, $PhCH_2OH$, **j**). H_2, Pd/C, EtOH, 70% (2 steps). **k**). DCC, DMAP, CH_3CN **11** or **12**.

Scheme 1

4-Penten-2-ol (**3**) was silylated and epoxidised and the resulting epoxide opened with sodium azide in the presence of lithium triflate to give the azido-alcohol **5** as a mixture of diastereoisomers. The alcohol was protected as the *tert*-butyldiphenylsilyl ether and the *tert*-butyldimethylsilyl protecting group was selectively removed. The alcohol was subsequently oxidised and then a Petersen olefination was carried out to give **7**. The azide **7** was reduced and underwent a concomitant Michael addition to form the isostere ring. It was necessary to protect the secondary amine for the coupling reaction to the nucleoside analogues which was acheived *in situ* with di-*tert*-butyl-dicarbonate to give the BOC protected derivative **8**. Hydrolysis of the ethyl ester **8** in preparation for coupling to the nucleosides proved problematic due to low yields and deprotection of the tert-butyldiphenylsilyl protecting group. Therefore the ethyl ester was transesterified to the benzyl ester **9**. The ester group was then readily removed by hydrogenolysis to give the free acid **10**, which was then attached to nucleoside analogues by a DCC coupling. Two nucleoside analogues were selected: 3'-TBS aminothymidine (**11**) and amino d4T[3] (**12**). Compounds **13** and **14** were isolated as mixtures of 4 diastereomers.[9]

Deprotection of the isosteres **13** and **14** was undertaken in 2 steps (Scheme 2); firstly by removal of the silyl protecting groups and then by removal of the BOC protecting group. The isostere of thymidine (**15**) was isolated in 78% yield as a mixture of 4 diastereomeres as the trifluoroacetate salts. Separation of pairs of diastereomers was possible in the case of the d4T isomer after removal of the silyl protecting group (**16**), and these were assigned by comparison of the nmr spectra with analogous compounds.[1] The *trans* isostere of d4T

(**17**) was isolated as a mixture of 2 diastereomers in 28% yield.[9] Unfortunately the *cis* diastereomers of the amino d4T isostere were inseparably contaminated with an unknown compound.



a). TBAF, THF. b). Dowex 50WX2-200(H^+). c). CF_3CO_2H.

Scheme 2

Biological Assays

The compounds were assayed for their inhibitory activity against HIV-1 and HIV-2 in human lymphocyte CEM cells and thymidine kinase-deficient CEM cells and against HIV-1 reverse transcriptase using polyA.oligodT as the template/ primer and [^{3}H] dTTP as the radiolabelled substrate.[10,11] The nucleoside analogues synthesised to test the isostere were d4T and thymidine. D4T is known to have anti-HIV activity and to be selective as its triphosphate for reverse transcriptase over human DNA polymerases. Thymidine triphosphate is not an inhibitor of reverse transcriptase as it lacks DNA chain termination. However triphosphate isostere conjugates of thymidine should be metabolically stable and should bind to the enzyme. Unfortunately none of the compounds showed significant anti-HIV activity in cell culture (EC_{50} > 200μM) or against reverse transcriptase (IC_{50} > 500μM). This could be due to a number of reasons: the lack of charge on the isostere making it a much weaker binding ligand than the natural substrate or the crystal structure of ATP being an unreliable model for the conformation of compounds in the active site of reverse transcriptase.

Acknowledgements

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References

1. A.O. Goldring, I.H. Gilbert, N. Mahmood, J. Balzarini, *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2411.
2. R. Weaver, I. H. Gilbert, N. Mahmood, J. Balzarini, *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2405.
3. R. Weaver, I. H. Gilbert, *Tetrahedron*, **1997**, *53*, 5537.
4. P.H. Patel, A. Jacobo-Molina, J. Ding, C. Tantillo, A.D. Clark, R. Raag, R.G. Nanni, S.H. Hughes, E. Arnold, *Biochemistry*, **1995**, *34*, 5351.
5. R.M. Otoski, C.S. Wilcox, *Tetrahedron Letts.*, **1988**, *29*, 2615.
6. R. Cini, M.C. Burla, A. Nunzi, G.P. Polidori, P.F. Zanazzi, *J. Chem. Soc., Dalton Trans.*, **1984**, 2467.
7. Macromodel, Department of Chemistry, Columbia University.
8. All compounds were characterised by 1H and 13C nmr, mass spectrometry, ir and either elemental analysis or accurate mass spectrometry.
9. To simplify the diagrams only one stereoisomer of the quaternary carbon is shown.
10. J. Balzarini, A. Karlsson, S. Aquaro, C.-F. Perno, D. Cahard, L. Naesens, E. De Clercq, C. McGuigan, *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 7295.
11. J. Balzarini, M.-J. Perez-Perez, A. San-Felix, M.-J. Camarasa, I.C. Bathurst, P.J. Barr, E. De Clercq, *J. Biol. Chem.* **1992**, *267*, 11831.