Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No 20, pp. 2411-2416, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00443-X

LIPOPHILIC BIOISOSTERES OF NUCLEOSIDE TRIPHOSPHATES

Alastair O. Goldring[a], Ian H. Gilbert[a]*, Naheed Mahmood[b], and Jan Balzarini[c]

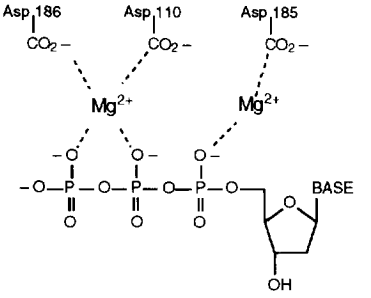
[a]*Welsh School of Pharmacy, University of Wales Cardiff, Redwood Building, King Edward VII Avenue, Cardiff, CF1 3XF, UK.*
[b]*MRC Collaborative Centre, 1-3 Burtonhole Lane, Mill Hill, London, NW7 1AD, UK.*
[c]*Rega Institute, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.*
e-mail: gilbertih@cardiff.ac.uk fax +44 1222 874 180

Abstract: This paper describes the design and synthesis of lipophilic bioisosteres of nucleoside triphosphates as potential inhibitors of HIV reverse transcriptase. The bioisostere is designed to mimic the conformation of the nucleoside triphosphate when complexed to a metal ion such as magnesium.

Introduction

Anti-HIV nucleosides such as AZT, d4T, ddI, ddC and 3TC are activated intracellularly by phosphorylation to give the nucleoside triphosphates, which then act at the virus encoded enzyme reverse transcriptase as either competitive inhibitors or as chain terminators of DNA synthesis. This intracellular activation requires human kinases and is often the rate limiting step for conversion of the nucleoside analogue to its active metabolite.[1] Poor intracellular phosphorylation reduces the efficacy of some nucleosides and can be so slow that the nucleoside has no anti-HIV activity, although the nucleoside triphosphate is known to inhibit reverse transcriptase *in vitro*.[2]

Use of the nucleoside triphosphates themselves would bypass this requirement for intracellular activation. However, they are poor drug candidates due to chemical and enzymatic instability, impermeability to the cell membrane and poor bioavailability. We have an ongoing programme to design enzymatically and chemically stable lipophilic triphosphate bioisosteres.[3] These can then be attached to a variety of nucleosides to produce compounds that should no longer require intracellular activation. This methodology may allow new nucleoside analogues to be used for anti-HIV treatment and should be applicable in other viral diseases such as herpes simplex virus and human hepatitis B virus.



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

Figure 1

Inside the active site of reverse transcriptase it is thought that the triphosphate group is complexed to two magnesium ions:- one which co-ordinates to the α-phosphate and the other to the β- and γ-phosphates (Figure

1).[4] Therefore, we decided to produce a lipophilic bioisostere of the triphosphate which would mimic the conformation of the triphosphate group whilst it is complexed to magnesium at the β- and γ-phosphates. When linked to an appropriate nucleoside such an bioisostere should mimic the nucleoside triphosphate bound at the active site of reverse transcriptase. By choosing a nucleoside known to be selective for HIV it should be possible to design selective inhibitors of reverse transcriptase. Otoski and Wilcox[5] have reported a lipophilic triphosphate bioisostere in which the shape produced by the magnesium ion and the β- and γ-phosphates is maintained by a five membered ring (Figure 2). The phosphorus atoms are replaced by carbon atoms and the oxygens by either oxygen or carbon. To design this bioisostere they used information from X-ray crystal structures[6] of complexes of nucleoside triphosphates with metal ions, in which the metal ions are complexed to the α-, β- and γ-phosphates giving rise to two diastereomers.

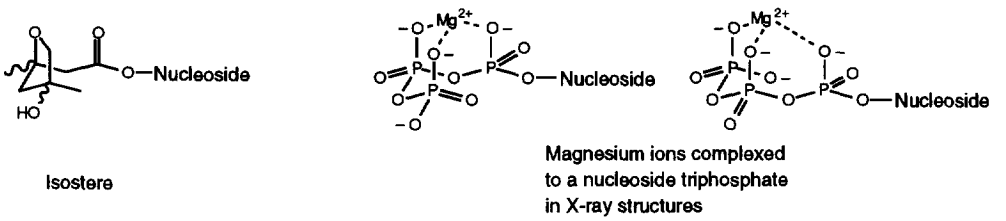
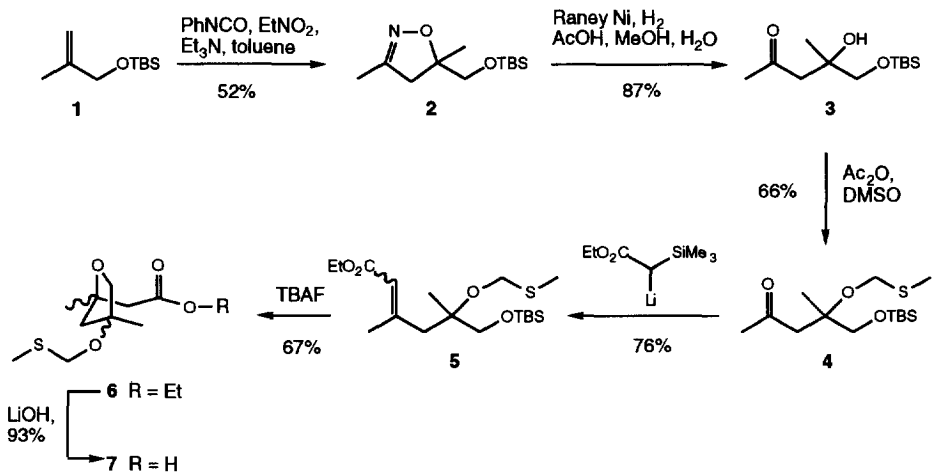


Figure 2

Chemistry

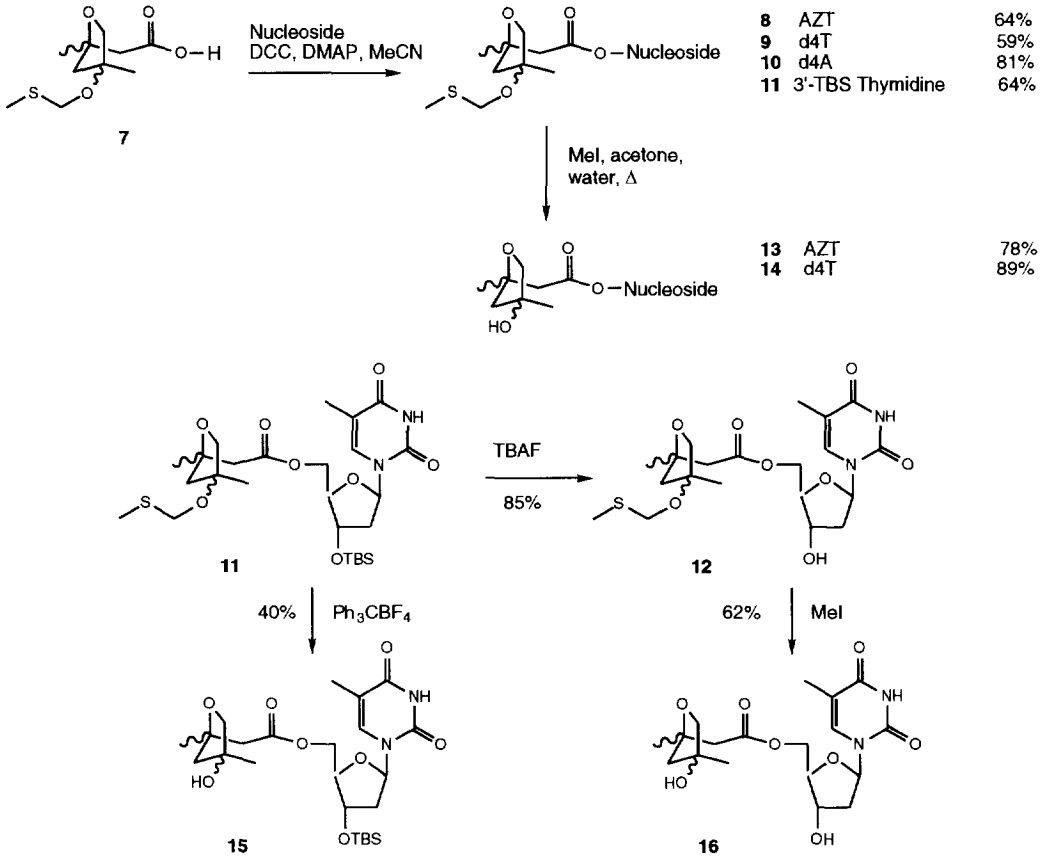
The basic bioisostere (**7**) was synthesised according to the methodology of Otoski and Wilcox (Scheme 1) as a mixture of diasteromers.[5,7]



Scheme 1

The bioisostere was then coupled to various nucleoside analogues, initially through an ester linkage (Scheme 2). The coupling was achieved by a standard DCC procedure to give the methylthiomethyl (MTM) protected nucleoside triphosphate bioisosteres (**8**, **9**, **10**, **11**). The bioisosteres were isolated as approximately

1:1:1:1 mixtures of the 4 possible diastereomers. In the case of 3'-*tert*-butyldimethylsilyl thymidine (**11**), the *tert*-butyldimethylsilyl group was removed using TBAF to give the thymidine derivative (**12**).



Scheme 2

The MTM group was removed with methyl iodide[8] or triphenylcarbenium tetrafluoroborate.[9] The fully deprotected thymidine bioisostere (**16**) was obtained by deprotection of compound **12** and the 3'-*tert*-butyldimethylsilyl thymidine bioisostere (**15**) by deprotection of compound **11**.

At this stage it proved possible to separate the diastereomers into pairs by flash chromatography (Figure 3). The stereochemistry of the diastereomers was assigned as follows. It was found that on standing in $CDCl_3$ one of the pairs of diastereomers of d4T decomposed to give the lactone **17**. This must be due to diastereomers C and D, where the hydroxyl of the bioisostere is cis to the ester linkage allowing intramolecular displacement of the nucleoside in the slightly acidic medium. This reaction could be observed by appearance of the parent nucleoside by TLC, and in mass spectrometry both the lactone and nucleoside could be detected. No such reaction was detected for the other pair of isomers.

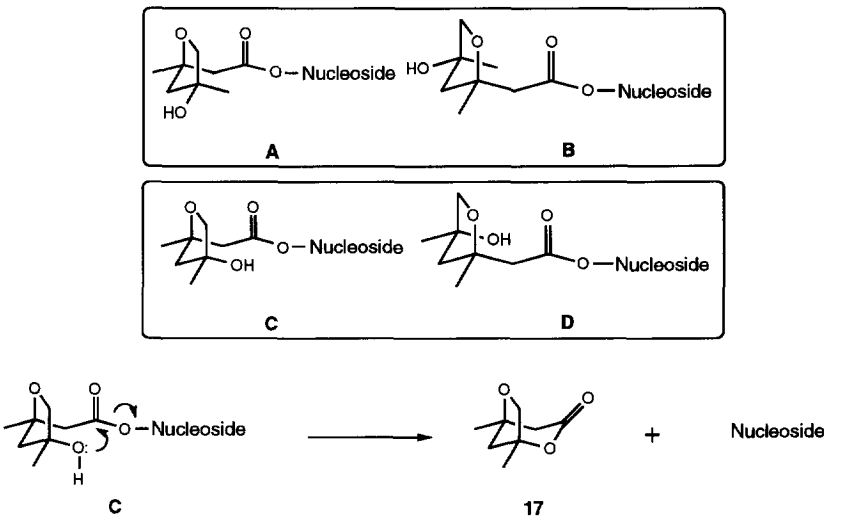
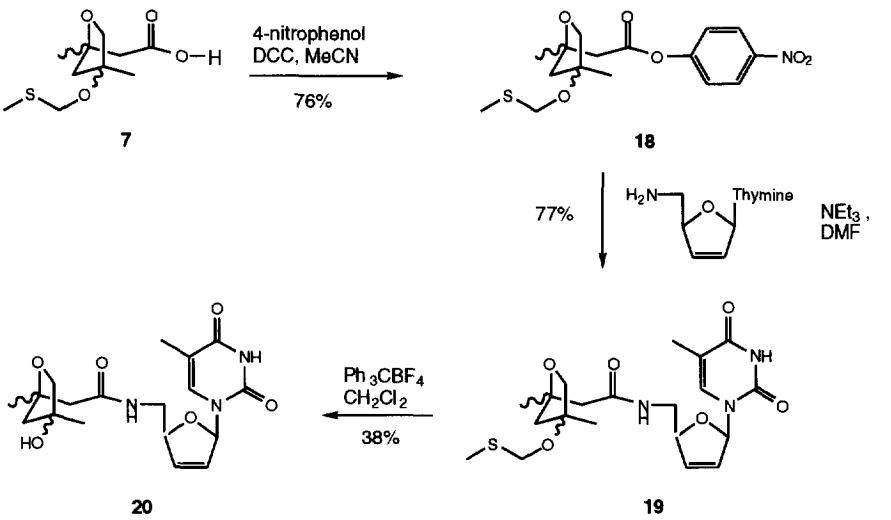


Figure 3

The amide linked d4T derivative (**20**) was also synthesised (Scheme 3). The bioisostere was activated as the para-nitrophenol ester[5] and then reacted with 5'-amino-d4T[10] to produce the product (**19**) as a 1:1:1:1 mixture of diastereomers. The MTM group could then be removed using triphenylcarbenium tetrafluoroborate. Separation of pairs of diastereomers was again possible.



Scheme 3

Biological Results

The compounds were assayed for their activity against HIV in a variety of cell lines (C8166, JM, MT2, CEM/0 cells and thymidine kinase deficient CEM/TK- cells). The results for the MTM derivatives are presented in Table 1 and for the fully deprotected compounds in Table 2.

Table 1: Anti-HIV Activity of MTM Derivatives Against Various HIV-Infected Cell Lines and in Enzyme Assays Against HIV-1 Reverse Transcriptase (RT).

	C8166 HIV-1		JM HIV-1		CEM/0 HIV-1	CEM/0 HIV-2	CEM/TK⁻ HIV-2	CEM/0	RT
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	IC_{50} (μM)
8	0.32	500	100	500	0.20	0.14	>250	233	>100
9	16	400	200	400	6.0	3.1	>250	>250	>100
10	80	400	100	100	50	40	N.D.	86	>100
11	16	80	16	80	>10	>10	N.D.	36	>100
12	200	1000	200	400	200	88	>250	>250	>100
7	>1000	>1000	>1000	>1000	>250	>250	>250	>250	>100
AZT	0.016	>1000	200	>1000	0.003	0.006	>250	>250	>100
d4T	0.32	400	40	500	0.2	0.12	55±42	130±77	>100
TBS-T	16	400	20	400					

EC_{50} The concentration of compound required to protect cells against the cytopathogenicity of HIV by 50%.
CC_{50} The concentration of compound required to reduce cell viability by 50%.
IC_{50} The concentration of compound required to inhibit HIV-1 reverse transcriptase by 50%.
TK^- Thymidine Kinase Deficient Cells.
TBS-T 3'-*tert*-Butyldimethylsilyl-thymidine.

Table 2: Anti-HIV Activity of Deprotected Bioisosteres Against Various HIV-Infected Cell Lines.

	C8166 HIV-1		JM HIV-1		MT2 HIV-2	
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
13	0.04	500	200	1000		
13 AB	8	>1000	400	400	4	>1000
13 CD	0.064	>1000	100	>1000	0.064	>1000
14	8	>500	40	>500	4	>1000
15 AB	20	200	20	200	20	200
15 CD	10	200	10	200	10	200
16	1000	>1000	500	>1000	1000	>1000
19	400	>1000	500	>1000	200	>1000
20 AB	>500	>500	500	>500	>500	>500
20 CD	500	>500	200	>500	500	>500

Discussion

Compounds **8-12**, which are MTM protected nucleoside triphosphate bioisosteres, all showed activity against HIV infected C8166 and CEM cells (Table 1). This activity appears to be related to that of the parent nucleoside. For example, the AZT bioisostere **8** shows the most potent anti-HIV activity and AZT is the most potent anti-HIV compound used in these studies. Compounds **8-12** showed no activity against HIV-infected thymidine kinase deficient CEM cells and much reduced activity against HIV-infected JM cells. Should the compounds be active directly against reverse transcriptase, it would be expected for them to show significant activity in these cell lines. In addition, the compounds showed no appreciable activity against reverse transcriptase in enzyme assay experiments. This lead us to the conclusion that the compounds were being hydrolysed to give the nucleoside, and the nucleoside was then being phosphorylated by cellular enzymes to the triphosphate and acting against reverse transcriptase. In support of this is the data from compound **12**. Compound **12** is a thymidine bioisostere and will only show activity if it is acting directly against reverse transcriptase. Should

hydrolysis to the nucleoside occur, then compound **12** will be inactive as the nucleoside released is thymidine. The results from compound **12** show only slight anti-HIV activity, suggesting that the compound is acting only weakly directly against reverse transcriptase or another viral or cellular target.

The deprotected compounds (**13-16**) show similar activities to the MTM protected bioisosteres (Table 2). The exception is the AZT triphosphate bioisostere (**13**) which shows a 10 fold increase in activity against both C8166 cells and CEM cells [CEM (HIV-1): EC_{50} = 0.01μM; CEM (HIV-2): EC_{50} = 0.01μM; CEM CC_{50} = >250μM]. However for compound **13** activity against both HIV infected JM cells and HIV 2 infected thymidine kinase deficient CEM/TK$^-$ cells was much reduced [CEM/TK$^-$: EC_{50} >250μM] and negligible activity against reverse transcriptase was observed [IC_{50} >100μM]. Therefore again it can be concluded that hydrolysis to the nucleoside is occurring and that the anti-HIV activity observed is due to intracellular phosphorylation of these nucleosides. This is also confirmed by the data from the separated isomers of compound **13**. The isomer where the hydroxyl group is *trans* to the ester (**13AB**) shows much lower activity than the *cis* isomer (**13CD**). In this latter compound, intramolecular cleavage of the ester group can occur to release the parent nucleoside (Figure 3). Similar results were obtained with the separated diastereomers of the 3'-*tert*-butyldimethylsilylthymidine triphosphate bioisostere (**15AB**, **15CD**). Again, the thymidine triphosphate bioisostere (**16**) showed very low activity suggesting only very weak activity directly against reverse transcriptase.

Finally, the amide linked bioisostere of d4T was synthesised (**20AB**, **20CD**). In these compounds the link between the bioisostere and the nucleoside should show greater chemical and enzymatic stability. These showed negligible activity against HIV infected cells again suggesting that the nucleoside triphosphate bioisostere is not acting directly against reverse transcriptase or another antiviral target.

In conclusion, although many of the bioisosteres synthesised showed good anti-HIV activity, the predominant mode of action of these compounds seems to be hydrolysis to give the parent nucleoside either intracellularly or in the culture medium prior to anabolism to its 5'-triphosphate derivative.

Acknowledgements. We wish to acknowledge the Welsh School of Pharmacy for funding a PhD studentship (AOG), and to Dr. Richard Weaver and Orson Wedgewood for donation of intermediates. We thank Mrs Ann Absillis and Mrs Lizette van Berckelaer for excellent technical help. This research was also supported by grants from the European Commission (Biomedical Research Programme and Human Capital and Mobility Programme).

1. Balzarini, J.; Herdewijn, P.; De Clercq, E., *J. Biol. Chem.*, **1989**, *264*, 6127. Gao, W.-Y.; Agbaria, R.; Driscoll, J. S.; Mitsuya, H., *J. Biol. Chem.*, **1994**, *269*, 12633.
2. Hao, Z.; Cooney, D. A., Farquhar, D.; Perno, C. F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N. R., Balzarini, J, Johns, D. G.; *Molecular Pharmacology*, **1990**, *37*, 157.
3. Weaver, R.; Gilbert, I. H.; Mahmood, N.; Balzarini, J., submitted to *Bioorg. Med. Chem. Lett.*
4. Patel, P. H.; Jacobo-Molina, A.; Ding, J.; Tantillo, C.; Clark, A. D.; Raag, R.; Nanni, R. G.; Hughes, S. H.; Arnold, E., *Biochemistry*, **1995**, *34*, 5351.
5. Otoski, R. M.; Wilcox, C. S., *Tetrahedron Letts.*, **1988**, *29*, 2615.
6. Cini, R.; Burla, M. C.; Nunzi, A.; Polidori, G. P.; Zanazzi, P. F., *J. Chem. Soc., Dalton Trans.*, **1984**, 2467.
7. Acknowledgement to C. S. Wilcox for supplying experimental details.
8. Pojer, P. M.; Angyal, S. J., *Aust. J. Chem.*, **1978**, *31*, 1031.
9. Niwa, H.; Migachi, Y., *Bull. Chem. Soc. Jpn*, **1991**, *64*, 716.
10. Weaver, R.; Gilbert, I. H.; Mahmood, N., manuscript in preparation.

(*Received in Belgium* 2 *August* 1996; *accepted* 16 *September* 1996)