Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 20, pp. 2405-2410, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00444-1

ISOSTERES OF NUCLEOSIDE TRIPHOSPHATES

Richard Weaver[a], Ian H. Gilbert[a]*, Naheed Mahmood[b], 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

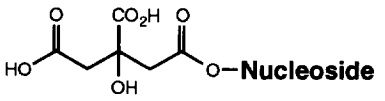
Abstract: This paper describes the design and synthesis of lipophilic isosteres of nucleoside triphosphates as potential inhibitors of HIV reverse transcriptase. The isosteric replacement of the triphosphate group is a modification of the citrate group. Copyright © 1996 Elsevier Science Ltd

Introduction

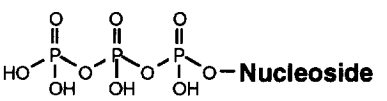
All current antiviral nucleosides, such as AZT, d4T, acyclovir and BVDU are activated intracellularly by phosphorylation to the bioactive triphosphate form. The activation uses either the human kinases (*e.g.* in the case of HIV) or viral kinases (*e.g.* in the case of HSV). In some cases this intracellular activation is slow because the nucleosides are not good substrates of the kinases. For example in the case of d4T the phosphorylation of the nucleoside is the rate limiting step, whilst for AZT phosphorylation of AZT monophosphate is the rate limiting step as AZT monophosphate inhibits thymidine monophosphate kinase.[1] The rate of phosphorylation of a given nucleoside is also dependent on the type of cell and state of the cell cycle.[2] The poor intracellular phosphorylation reduces the efficacy of the drugs and in some cases prevents potential nucleosides having antiviral activity. In the case of HIV infection, the nucleoside triphosphate then acts at the enzyme reverse transcriptase either by acting as a competitive inhibitor of the enzyme or by acting as a chain terminator of DNA synthesis as they lack a 3'-OH.

The nucleoside triphosphates themselves would bypass this requirement for intracellular activation. However they are poor drug candidates due to chemical and enzymatic instability, impermeabilitiy to the cell membrane and poor bioavailability. Therefore, to bypass the intracellular phosphorylation of nucleosides, we sought to make a chemically and enzymatically stable isostere of the triphosphate which could be attached to a variety of nucleosides. The isostere should have the same shape and charge distribution in order to mimic the triphosphate at the enzymatic target (*e.g.* reverse transcriptase). In addition the isostere should have a greater lipophilicity which should increase cellular uptake and improve bioavailability. Such an isostere should have application in a number of different therapeutic areas, increasing the efficacy of existing nucleoside analogues and allowing new nucleoside analogues to be considered. An example is dideoxyuridine. This nucleoside is inactive in cell culture against HIV. However, dideoxyuridine triphosphate is very active against reverse transcriptase. Therefore, an isostere of dideoxyuridine triphosphate may be an effective anti-HIV agent.[3]

The triphosphate isostere that we chose to develop was the citrate functionality (Figure 1).



citrate isostere

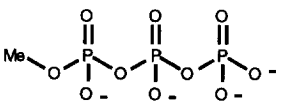


nucleoside triphosphate

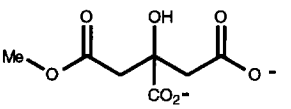
Figure 1

Molecular Modelling

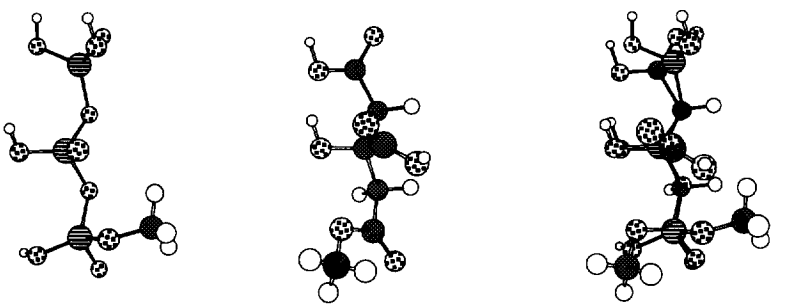
Molecular modelling showed that the citrate molecule should be a good mimic of the triphosphate. The monomethyl esters of both triphosphate and citrate were energy minimised using MM2 calculations and conformational searching was applied to find the global minimum, using the programme MACROMODEL. These calculations used a factor to account for water as a solvent. The global minima were superimposed (Figure 2). The structures are almost entirely superimposeable, with the carboxylate and hydroxyl group oxygens of the citrate being in the same position of the phosphate oxygens. Simple charge distribution calculations gave a similar charge distribution for both the citrate and the triphosphate.



Compound A



Compound B



Compound A | Compound B | Compounds A and B superimposed

Figure 2

It is likely that the triphosphate is complexed to magnesium ions in solution and in the active site of enzymes such as HIV reverse transcriptase.[4] Simple "ball and stick" models indicated that citrate would complex well to magnesium ions. Indeed the citrate – magnesium ion complex has been well characterised by NMR.[5]

By varying the nature of the substituents R^1 and R^2 on the citrate group (Figure 3) it should be possible to modify the physicochemical properties of the nucleoside triphosphate isostere, improving cellular uptake and bioavailability.

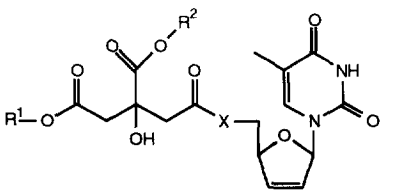
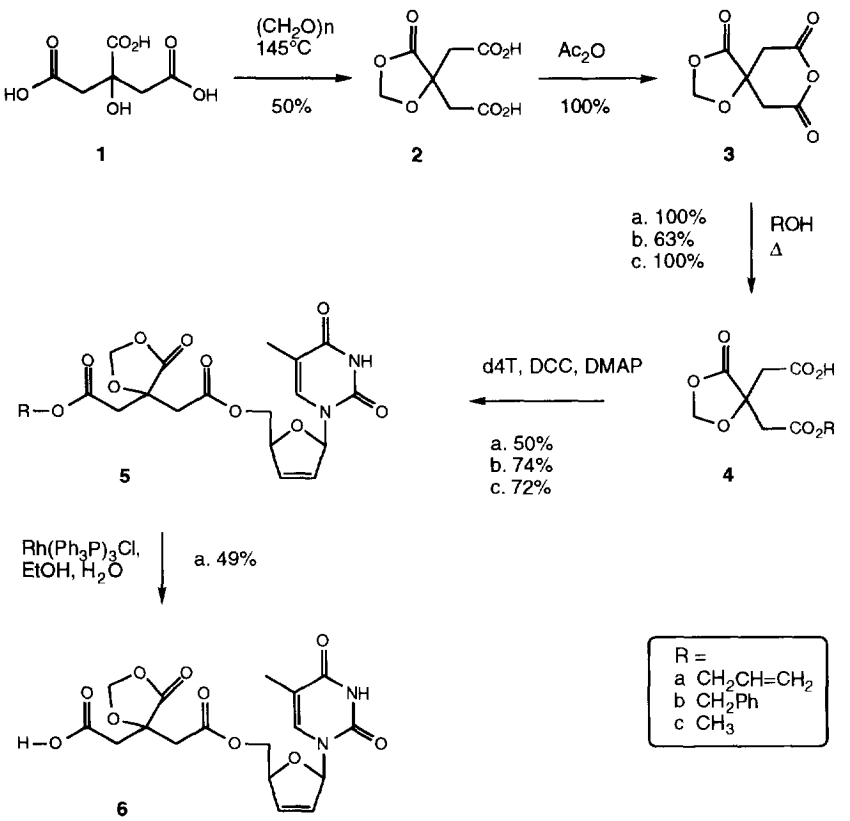


Figure 3

Chemistry

The target molecules are shown in Figure 3. We chose to look at anti-HIV compounds and selected d4T as the nucleoside. Citrate could be coupled to the nucleoside through either an ester or an amide bond (X=O, NH) and a variety of substituents could be placed on the citrate (R^1, R^2 = H, alkyl, aryl).

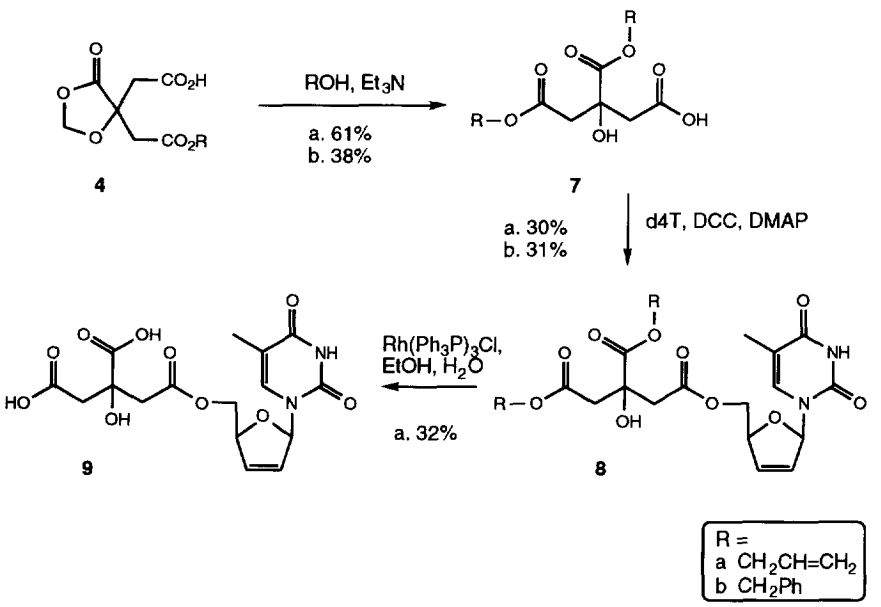


Scheme 1

Initially work concentrated on making the ester derivatives (X=O). The starting material was citric acid (**1**). This could be selectively protected by treatment with paraformaldehyde in a sealed tube to give the diacid (**2**) (Scheme 1).[6] It was not possible to directly couple the diacid to the nucleoside. Therefore, the diacid was

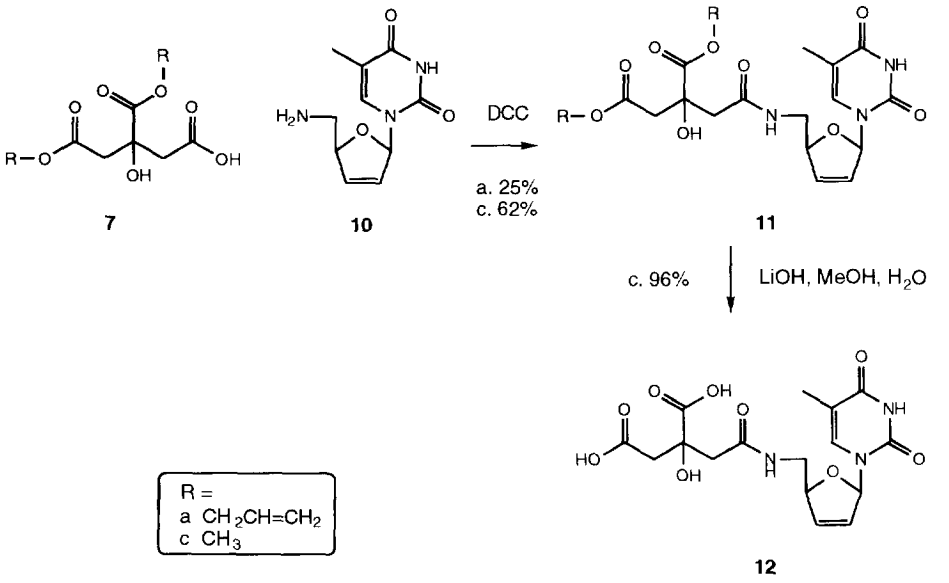
cyclised to form the anhydride (**3**). This anhydride proved remarkably stable to reaction with d4T. However, treatment with an alcohol gave the monoacid (**4**), without affecting the 1,3-dioxolan-4-one ring. The monoacid was then coupled to d4T using a standard DCC coupling to give the protected isostere (**5**) as a 1:1 mixture of diastereomers. The allyl protected ester (**5a**) was cleaved to give the free acid (**6**) using Wilkinson's catalyst.[7] Molecular modelling of this compound again showed that compound **6** should be a good mimic of the triphosphate, even with the dioxolanone ring present.

Various attempts at cleaving the dioxolanone ring were unsuccessful; therefore another approach was adopted to make the fully deprotected isostere (**9**). The monoacid (**4**) was treated with an alcohol in the presence of triethylamine (Scheme 2). This cleaved the dioxolanone ring to give the free acid (**7**). The monoacid (**7**) was then coupled to d4T using DCC to give the protected isostere (**8**). The diallyl isostere (**8a**) was then deprotected with Wilkinson's catalyst to give the fully de-protected isostere (**9**) as a 1:1 mixture of diastereomers.



Scheme 2

The amide analogues (X=NH) were synthesised using a similar methodology (Scheme 3). The monoacid (**7**) was coupled to 5'-amino-d4T (**10**)[8] using DCC to give the protected amide (**11**). It proved important not to use DMAP in the reaction as this lead to dehydration. Deprotection of the diallyl compound (**11a**) with Wilkinsons's catalyst proved problematic. However, the dimethyl compound (**11c**) was deprotected using lithium hydroxide to give the fully deprotected compound (**12**).



Scheme 3

Biological Results

Compounds were assayed for their anti-HIV activity against a variety of cell lines. The data is shown in the Table below.

	C8166 HIV-1		CEM/0 HIV-1	CEM/0 HIV-2	CEM/TK- HIV-2	CEM/0	HIV-1 RT
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	IC_{50} (μM)
5a	0.8	500	2.0	1.6	78±81	179±69	>100
5b	8	400	1.35	3.0	>250	92.8	
5c	0.8	400	0.95	2.5	110±121	100±31	>100
6	4	1000	3.47	4.5	>250	>250	>100
8a	1	500	0.7	0.55	72	69	>100
8b	1.6	500	1.6	0.55	87±17	64±11	>100
9	3.2	500	4	4	>250	209±55	>100
11a	400	>1000	>250	>250		>250	>100
11c	1000	>1000	>250	>250		>250	>100
12	>2500	>2500					>100
d4T	0.32	400	0.2	0.12	55±42	130±77	>100
AZT	0.016	>1000	0.003	0.006	>250	>250	>100

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%.

Discussion

The ester linked isosteres (compounds **5**, **6**, **8** and **9**) all show good activity against HIV infected C8166 and CEM cells. The activity does not seem to be dependent on the substituents on the carboxylic acid or on the

presence of the dioxolanone ring. The activity that the compounds are showing is of a similar order to that of d4T. The compounds showed poorer activity against thymidine kinase deficient cells and no significant activity against reverse transcriptase. Therefore we conclude the compounds are hydrolysed to d4T prior to further metabolism in the cell. The d4T is then the component which gives rise to the reported activities.

In order to test this hypothesis the ester linkage between the nucleoside and the isostere was replaced by an amide linkage. These compounds (**11**, **12**) should have much greater metabolic stability. However these compounds showed much lower activity against HIV infected cells and no appreciable activity against reverse transcriptase. This confirms the hypothesis that the activity of the isosterically modified compounds is caused by hydrolysis of the citrate group to release d4T.

In conclusion the citrate isostere is not a good replacement for the triphosphate group in this case. The activity of compounds is due to hydrolysis of the citrate group to release d4T.

Acknowledgements: We wish to acknowledge the Medical Research Council for funding (RW) and Dr. Jonathan Goodman (University of Cambridge) for help with the molecular modelling. 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.
2. Gao, W.-Y.; Agbaria, R.; Driscoll, J. S.; Mitsuya, H., *J. Biol. Chem.*, **1994**, *269*, 12633.
3. 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.
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. Lowenstein, A.; Roberts, J. D.; *J. Am. Chem. Soc.*, **1960**, *82*, 2705.
6. Nau, C. A.; Brown, E. B.; Bailey, J. R., *J. Am. Chem. Soc.*, **1925**, *47*, 2596.
7. Kunz, H.; Waldman, H., *Helv. Chim. Acta*, **1985**, *68*, 618.
8. Weaver, R.; Gilbert, I. H.; Mahmood, N., manuscript in preparation.

(*Received in Belgium* 14 *June* 1996; *accepted* 16 *September* 1996)