Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 15, pp. 1741-1744, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00294-4

SYNTHESIS OF RACEMIC 2-PHOSPHONOMETHYL-1,3-DIOXOLANE NUCLEOSIDE ANALOGUES AS POTENTIAL ANTIVIRAL AGENTS

Krzysztof Bednarski, Dilip M. Dixit,* Tarek S. Mansour
Susan G. Colman,[¥] Sarah M. Walcott[¥] and Clare Ashman[¥]

BioChem Therapeutic Inc., 275 Armand-Frappier Blvd., Laval (Quebec) CANADA H7V 4A7

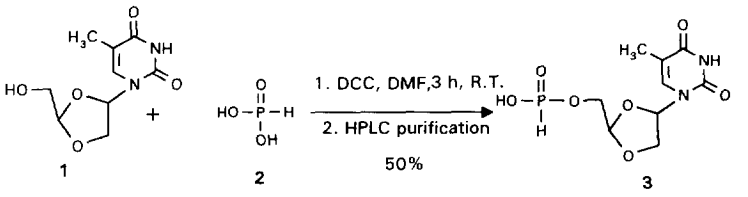
[¥]Department of Virology, Glaxo Wellcome Ltd.,
Gunnels Wood Road, Stevenage, Hertfordshire, United Kingdom, SG1 2NY

Abstract: 2-Phosphonomethyl-1,3-dioxolane nucleosides containing appropriately linked pyrimidine and purine bases at C-4 position were prepared as biomimetic analogues of antiviral 2',3'-dideoxynucleoside monophosphates. The key coupling intermediate **10** was prepared by a cyclocondensation of diethylacetal **6** and diol **7** followed by hydrolysis and lead tetra-acetate oxidative decarboxylation.

Several years ago novel analogues of 2',3'-dideoxynucleosides in which the 3'-methylene moiety was replaced by a heteroatom such as oxygen and sulphur were first reported.[1] Amongst these nucleosides (-)-2'-deoxy-3'-thiacytidine (lamivudine; 3TC™) is now in advanced stages of clinical trials in humans for HIV and hepatitis B infections and potent antiviral activities were also reported with (-)-FTC,[2] dioxolane-G [3,4] and dioxolane-C.[5,6]

In early studies, racemic *cis*-2'-hydroxymethyl-4'-thymin-1-yl-1',3'-dioxolane **1** (dioxolane-T) exhibited varied anti-HIV-1 activity depending on the cell line used for the assay. While this nucleoside was quite potent in PBM cells,[7] it had moderate activity in ATH-8 cell line,[8] showed no activity in CEM or MT-4 cells and was not cytotoxic in the above cell lines. Furthermore, following resolution, the (-) enantiomer of **1** exhibited moderate anti-HIV-1 activity in MT-4 cells of in agreement with the report of Chu and co-workers in PBM cells.[7] As nucleosides require activation by kinases before inhibiting viral polymerases, we postulated that nucleoside **1** was poorly phosphorylated in MT-4 cells. Herein we report on the synthesis of phosphonate derivatives of 1,3-dioxolanes **4** with thymine, cytosine and guanine bases. Such analogues incorporate a monophosphonate as a biomimetic of the first stage of kinase activation and may have more comprehensive cellular antiviral activity.

To test the hypothesis, we first prepared the bioisostere of the monophosphate of **1** (Scheme 1) by forming the phosphorus acid monoester **3** upon DCC mediated condensation of **1** with **2**.[9] To our satisfaction, nucleotide **3** was a moderate inhibitor of HIV-1 replication (EC_{50} = 51 μM, CD_{50} = 342.5 μM) in



Scheme 1.

MT-4 cells where **1** was inactive, confirming that phosphorus acid monoester **3** is stable towards enzymatic hydrolysis, probably caused by 5'-nucleotidase. At this stage, the synthesis of other nucleotide phosphonate derivatives **4** was undertaken which would be enzymatically and hydrolytically more stable analogues than the correponding monophosphates. They could be viewed as constrained analogues of PMEA **5** which is currently in clinical trials against AIDS.[10] Homophosphonate analogues of the oxathiolane nucleoside BCH-189 and its *trans* isomer have also been described in the literature.[11]

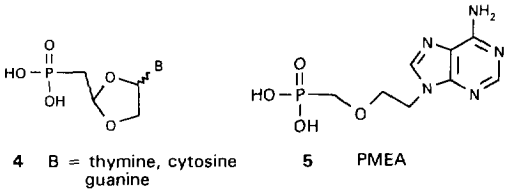


Figure 1.

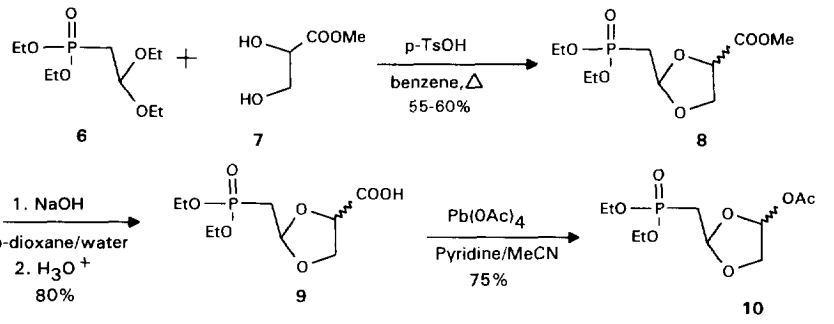
Diethyl phosphonoacetaldehyde diethylacetal **6** was condensed with methyl glycerate **7**[8] in benzene using a catalytic amount of p- TsOH to give dioxolane **8** in good yields after purification. Compound **8** was hydrolysed in aqueous NaOH to produce carboxylic acid **9** which was subjected to oxidative decarboxylation with lead tetraacetate to give an epimeric mixture (1:2 ratio) of **10** in 75% yield (Scheme 2).

Glycosylation of **10** with persilylated thymine or cytosine in the presence of trimethylsilyltriflate as a Lewis acid[12] furnished an inseparable mixture of epimers (1.2:1 ratio) in relatively low yields which was deprotected with bromotrimethylsilane in acetonitrile and further purified by reverse phase HPLC[13] to give the thymine and cytosine phosphonate derivatives **11**, **12**, **13** and **14**, respectively (Scheme 3).

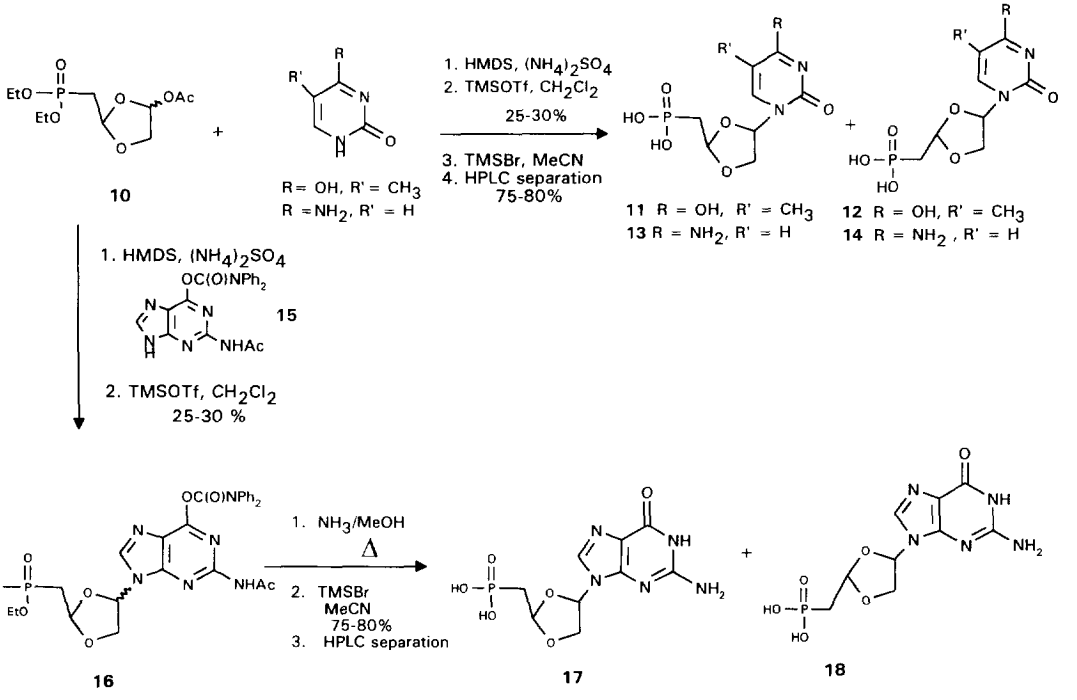
In a similar manner persilylated 2-acetamido-4-diphenylcarbamoyloxypurine **15**[14] was glycosylated with **10** using trimethylsilyltriflate catalysis to produce a mixture of phosphonates **16** with only the N-9 regioisomers present. The mixture of *cis* and *trans* epimers was deprotected with methanolic ammonia

followed by treatment with bromotrimethylsilane and then subjected to reverse phase HPLC to produce *cis* and *trans* epimers **17** and **18**, respectively.

Compounds **11** , **12**, **13**, **14**, **17** and **18** were tested for anti-HIV-1 activites in MT-4 cell and found to be



Scheme 2.



Scheme 3.

inactive (EC_{50} > 332 μM). They were also not cytotoxic at that concentration (IC_{50} > 332 μM). The N-acetyl derivative of **13** exhibited weak activity (EC_{50} = 312 μM). In further assays these analogues did not show any antiviral activity against HSV-1 and HSV-2 in Vero cells or against HCMV in Flow 2002 cells. It is possible

that these modified nucleotides are not efficiently phosphorylated further by cellular kinases to yield the corresponding bioactive triphosphate analogues.

In conclusion, we have conveniently synthesized 2-phosphonomethyl-1,3-dioxolane nucleotides containing thymine, cytosine and guanine bases. Dioxolane **10** is stable and suitable for coupling under Vorbrüggen conditions for the preparation of more analogues for further investigation.[15]

Acknowledgements:

The authors wish to thank Ms. L. Bernier, J. Dugas and M. DiMarco of the Bioanalytical division of BioChem Therapeutic for HPLC separations. We also thank Dr. J.W. Gillard for the encouragement and revision of this manuscript.

References and notes:

1. Belleau, B. R.; Dixit, D. M.; Nguyen-Ba, N; Kraus, J. L., *Abstracts of Papers*, 5th International Conference AIDS, Montreal, Quebec, Canada, **1989;** Abstract TC01.
2. Paff, M. T.; Averett, D. R.; Prus, K. L. ; Miller, W. H.; Nelson, D. J. *Antimicrob. Agents Chemother.* **1994**, *38*, 1230.
3. Kim, H. O.; Schinazi, R. F.; Nampalli, S.; Shanmuganathan, K.; Canon, D. L.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Chu, C. K. *J. Med. Chem.* **1993**, *36*, 30.
4. Siddiqui, M. A.; Brown, W. L.; Nguyen-Ba; N. Dixit, D. M.; Mansour, T. S.; Hooker, E.; Viner, K. C.; Cameron, J. M. *Bioorg. Med. Chem. Lett.* **1993**, *8*, 1543.
5. Kim, H. O.; Shanmuganathan, K.; Alves, A.. J.; Jeong, L. S.; Beach, J. W.; Schinazi, R. F.; Chang, C. N.; Chang, Y.-C.; Chu, C. K. *Tetrahedron Lett.* **1992**, *33*, 6899.
6. Belleau, B. R.; Evans, C. A.; Tse, H. L. A.; Jin, H.; Dixit, D. M.; Mansour, T. S. *Tetrahedron Lett.* **1992**, *33*, 6949.
7. C. K.; Ahn, S. K.; Kim, H. O.; Beach, W.; Alves, A. J.; Jeong, L. S.; Islam, Q.; Van Roey, P.; Schinazi, R. F. *Tetrahedron Lett.* **1991**,*31*, 3791. Anti-HIV-1 activity of (-1)-**1** in MT-4 cells EC_{50} = 123 123 μM relative to AZT EC_{50} of 0.1 μM.
8. Norbeck, D. W. ; Spanton, S.; Broder, S.; Mitsuya, H. *Tetrahedron Lett.* **1989**,*30*, 6263.
9. Dyatkina, N. B.; Arzumanov, A. A.; Tarussova, N. B. *Nucleosides Nucleotides* **1991**,*10*, 731.
10. De Clercq, E.; Holý, A. ; Rosenberg, I.; Sabuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464.
11. Kraus, J. L.; *Nucleosides Nucleotides* **1993**, *12*, 157.
12. Vorbrüggen, H.; Krolikewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.
13. These nucleotide analogues were purified by reverse phase HPLC column using Delta Pak C18 100Å (30 mm x 300 mm) column at r.t. (99% purity) eluting with a gradient mixture of acetonitrile and 0.01 M ammonium acetate aqueous solution at pH 6. Satisfactory spectral data and analyses were obtained.
14. Zou, R.; Robins, M. J. *Can. J. Chem.* **1987**, *65*, 1436.
15. Details of the antiviral assays are given in the following: Coates, J. V.; Cammack, N.; Jenkinson, H. J.; Mutton, I. M.; Pearson, B. A.; Storer, R.; Cameron, J. M; Penn, C. R. *Antimicrob. Agents Chemother.* **1992**, *36*, 202.

(*Received in USA* 15 *May* 1995; *accepted* 30 *June* 1995)