Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 20, pp. 2567-2572, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10048-8

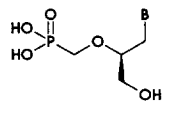
SYNTHESIS AND ANTI HCMV ACTIVITY OF 3,4-DISUBSTITUTED TETRAHYDROFURAN DERIVED NUCLEOSIDES AND NUCLEOTIDES: A TETHERED SERIES OF PME DERIVATIVES

Wei Wang, Haolun Jin, Nancy Fuselli, and Tarek S. Mansour*

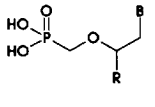
BioChem Therapeutic Inc., 275 Armand Frappier Blvd., Laval, Quebec, Canada, H7V 4A7

Abstract: The synthesis of a novel series of 3,4-disubstituted tetrahydrofuran derived nucleosides and nucleotides analogues was achieved by a linear approach starting from azido intermediates **9** and **13**. The *trans* cytosine nucleoside **19** emerged with good activity (IC_{50} = 3 μg/mL) against HCMV in vitro with a selectivity index of 33. © 1997 Elsevier Science Ltd.

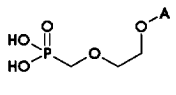
Acyclic nucleoside phosphonate analogues are an important class of broad-spectrum antiviral agents.[1] The 2-phosphonomethoxypropyl (PMP) and 2-phosphonomethoxyethyl (PME) series represent subclasses from which HPMPC, PMEA, PMPA and FPMPA have emerged with potent antiviral activities (Figure 1).[2,3] A structurally related class of 2-phosphonomethoxyethoxy nucleotides have been recently reported from which the adenine analogue **5** (Figure 1) had potent and selective activity against retroviruses (HIV-1 and HIV-2).[4]



1; HPMPC; B = cytosin-1-yl



2; PMEA R = H; B = adenin-9-yl
3; PMPA R = CH_3; B = adenin-9-yl
4; FPMPA R = CH_2F; B = adenin-9-yl



5

Figure 1

Considerable efforts in this area have focused on unraveling the structure–activity relationships in the acyclic series. In an attempt to explore further nucleotide analogues that maintain the stable phosphonate bond, we envisaged the preparation of cyclic analogues obtained by tethering C1' and C2' carbons with a - CH_2OCH_2- moiety which would lead to rigid analogues (Figure 2).[5] Herein, we report the synthesis and anti- HCMV activity in vitro of nucleotide analogues **6** (Base = uracil-1-yl, cytosin-1-yl, adenin-9-yl) represented in both *cis* and *trans* relative stereochemistry from 2,5-dihydrofuran.

Retrosynthetic analyses indicated that the synthesis of the desired analogues can be achieved by a direct ring opening of a 3,4-epoxy intermediate or substitution of a suitable 3,4-diol derivative with the nucleobase (Figure 3). However, experiments with nucleobases in both approaches were somewhat problematic.

Fortunately, ring opening or substitution reactions could be readily achieved with NaN_3 or LiN_3. The resultant azido compounds would serve as building blocks for the preparation of the target nucleotides as well as the nucleosides counterpart.

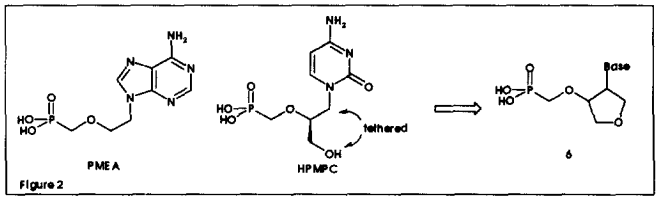


Figure 2

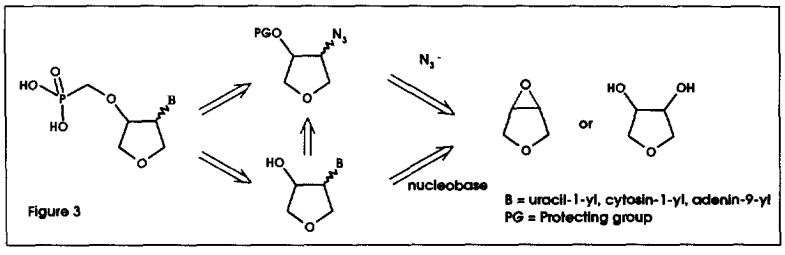
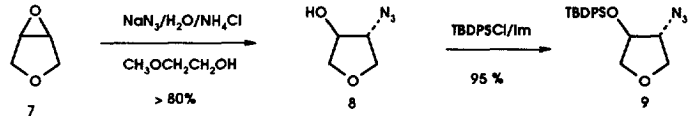


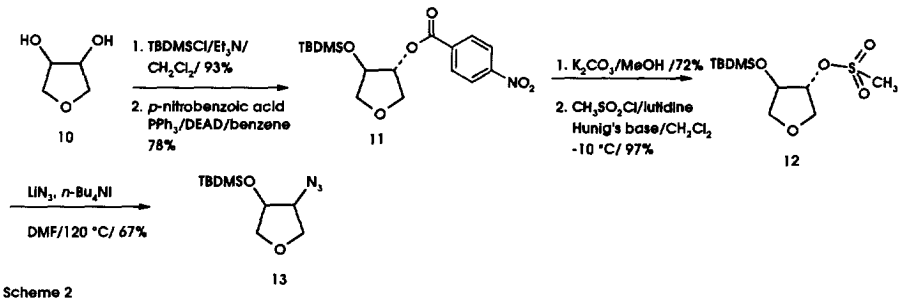
Figure 3

The synthesis of the azido intermediates in both *trans* and *cis* relative stereochemistry is shown in Schemes 1 and 2. 3,4-Epoxytetrahydrofuran (**7**), readily available from 2,5-dihydrofuran, underwent a nucleophilic ring opening reaction with sodium azide to afford **8** in good yield. Silylation of the hydroxyl group of **8** with *tert*-butyldimethylsilyl chloride under the standard conditions gave the *trans* azido intermediate **9** in a excellent yield. The synthesis of the *cis* azido intermediate **13** was accomplished from commercially available 1,4-anhydroerythritol (**10**). Monosilylation of diol **10** with *tert*-butyldimethylsilyl chloride proceeded in excellent yield to give the desired alcohol, which was then subjected to a Mitsunobu reaction with 4-nitrobenzoic acid to afford benzoate **11** in good yield. Benzoate **11** was then hydrolyzed with potassium carbonate in methanol and the resultant alcohol was converted to mesylate **12** which proved to be a stable intermediate for further transformations. The desired azide **13** was obtained from **12** by a substitution reaction with lithium azide in DMF assisted by tetra-*n*-butylammonium iodide.[6]

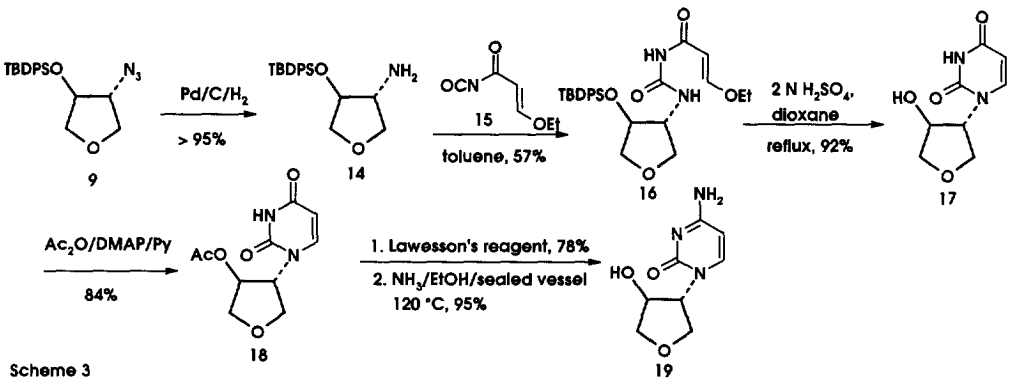


Scheme 1

The synthesis of the uracil and cytosine nucleoside analogues is shown in Scheme 3. Hydrogenation of azide **9** gave the amino alcohol **14**, which was further converted to the uracil derivative **17** according to the literature procedure via the urea intermediate **16** derived from isocyanate **15**.[7] Acetylation of the uracil derivative **17** followed by a thionation reaction with Lawesson's reagent led to the 4-thiouracil nucleoside which was further converted to the cytosine derivative **19** by reaction with ethanolic ammonia in a sealed flask.

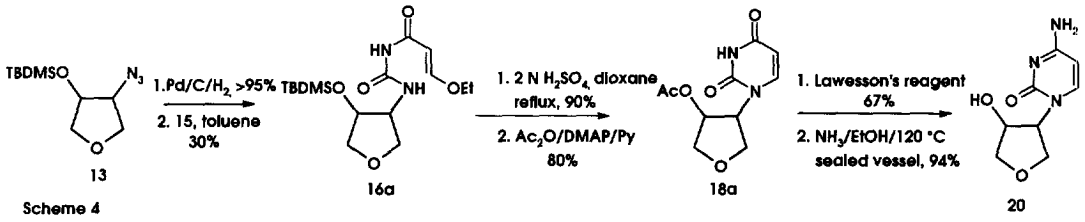


Scheme 2



Scheme 3

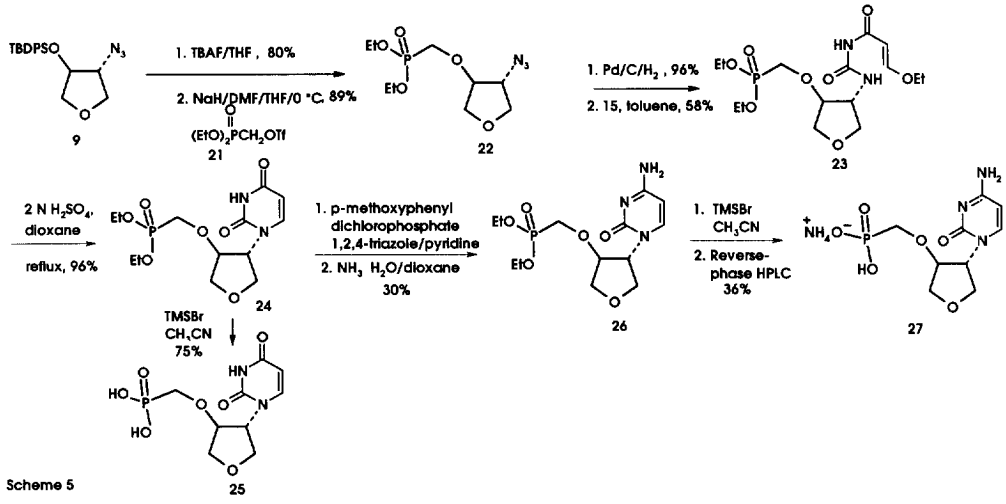
The same synthetic route was applied successfully to the preparation of the *cis* cytosine derivative **20** starting from the corresponding azido intermediate **13** (Scheme 4). With **19** and **20** in hand, the synthesis of the corresponding phosphonate derivatives was attempted by alkylation of the secondary hydroxyl group of a N-BOC protected **19** with diethylphosphonomethyltriflate (**21**). Unfortunately, this route did not afford the desired nucleotide presumably due to the interference of the amino group on cytosine. Therefore, an alternative strategy based on *O*-alkylation of the secondary hydroxyl group was considered to introduce the phosphonate moiety prior to the construction of the nucleobase.



Scheme 4

The synthesis of methylphosphonate derivative of **19** was initiated from **9** (Scheme 5). Unmasking of the hydroxyl group was accomplished in good yield by stirring **9** with tetra-*n*-butyl ammonium fluoride in tetrahydrofuran. The resultant azido alcohol was treated with sodium hydride in a DMF-THF mixture at 0 °C and then *O*-alkylated with triflate **21** at this temperature to afford phosphonate **22**. The azido functionality in **22** was reduced to the corresponding amino compound which upon treatment with isocyanate **15** and cyclization

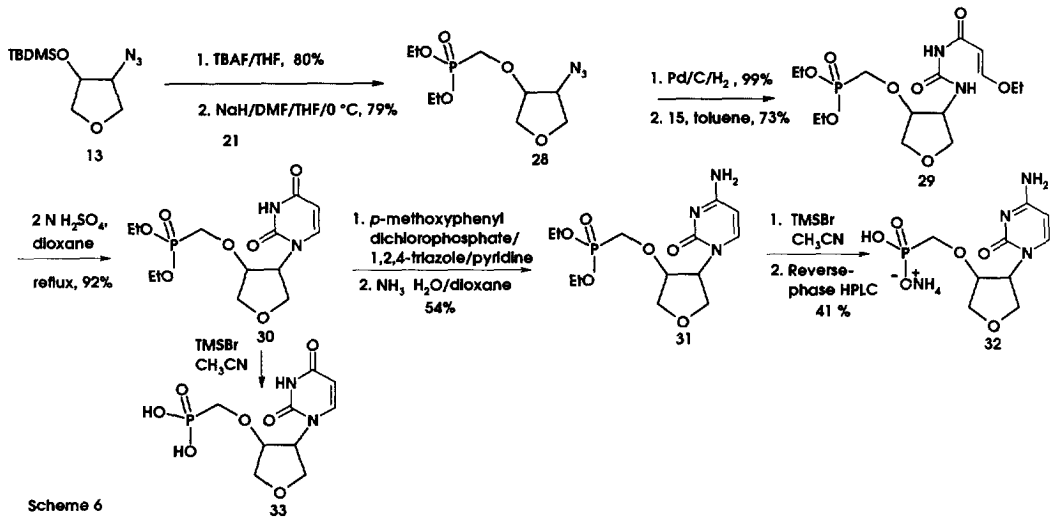
with 2 N H_2SO_4 provided the key uracil nucleotide **24** in good yield.[7] Attempted conversion of **24** to **26** via the 4-thio intermediate failed because the phosphonate moiety was not compatible with Lawesson's reagent. However, **26** was synthesized from **24** via the N^4-1,2,4-triazolyl intermediate (Scheme 5). Hydrolysis of nucleotides **24** and **26** using bromotrimethylsilane in acetonitrile afforded the desired nucleotide **25** and **27**, respectively,[8] which were purified by reverse phase HPLC techniques on C18 column (YMC ODS, 120 °A or Delta Pak 100 °A) using acetronitrile - 0.01 M ammonium acetate (pH 6) as eluent.



Scheme 5

The generality of the approach described for **25** and **27** was demonstrated in the preparation of the corresponding analogues in the *cis* series. In this case, the *cis* phosphonate intermediate **28** was readily derived from **13** and converted to **30** in three steps (Scheme 6). Uracil **30**, in turn, afforded nucleotides **32** and **33**, respectively.

A similar strategy to pyrimidines (Schemes 3 and 4) was adopted for the preparation of adenine nucleosides **36** and **41**. Condensation of 5-amino-4,6-dichloropyrimidine with amine **14** followed by cyclization with diethoxymethyl acetate gave the 6-chloropurine derivative **34**. Deprotection of **34** with tetra-*n*-butyl ammonium fluoride in tetrahydrofuran produced the 6-chloropurine derivative **35** as a key intermediate in the synthesis of both adenine derivatives **36** and **38**. Thus, reaction of **35** with ethanolic ammonia in a sealed flask furnished the adenine nucleoside analogue **36** in good yield. However, contrary to the case of pyrimidines, alkylation of **35** with triflate **21** under sodium hydride conditions produced the desired phosphonate **37** in good yield. Further elaboration of **37** to **38** was achieved by displacement of the 6-chloro group with ammonia followed by hydrolysis mediated by bromotrimethylsilane in acetonitrile.[8] The synthetic route depicted in Scheme 7 was readily applied to the preparation of the isomeric *cis* isomers **41** and **42** via the 6-chloropurine intermediate **40** (Scheme 8). The latter is accessible in three steps from amino alcohol intermediate **39**, which was derived from *cis* azide **13**.



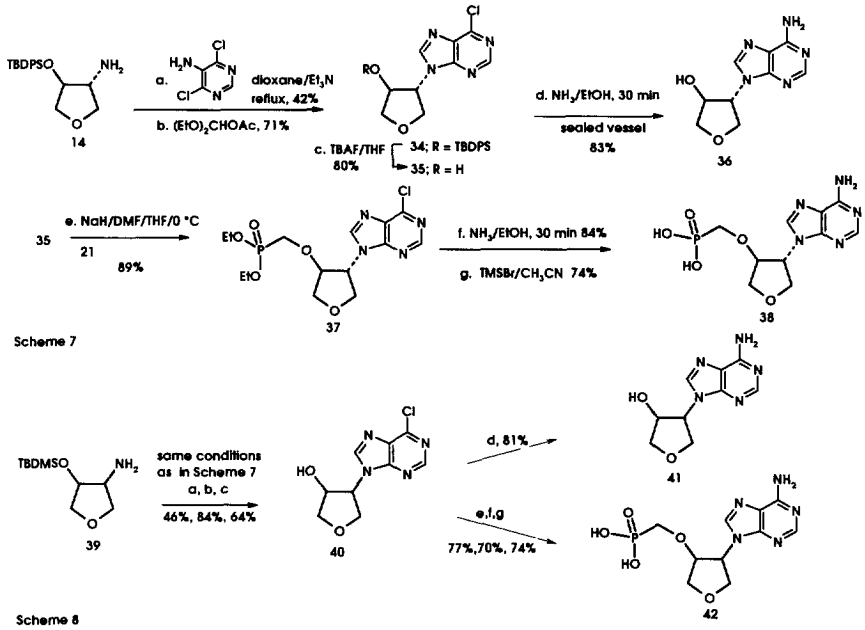
Scheme 6

The nucleotides **25**, **27**, **32**, **33**, **38**, and **42** were tested in plaque reduction assays against HCMV (WFI strain) in Flow 2002 (entries 1–4, 7, 8) or WI38 cells (entries 5, 6, 9) (Table 1). None of the nucleotides inhibited HCMV plaque formation, at concentration up to 100 μg/mL and were not cytotoxic (neutral red uptake), suggesting that incorporation of ring constrain in HPMPC and PMEA abolished the biological activity. Nucleosides **17**, **19**, **20**, **36**, and **41** were also tested in the HCMV assays and, to our surprise, the cytosine analogue **19** had relatively good activity being 30-fold weaker than the control ganciclovir, but with low selectivity (SI = 33). It is not clear yet whether **19** is phosphorylated to its triphosphate forms or exerts its activity in a manner similar to 5'nor- carbocyclic nucleosides.[9,10]

Table 1. Anti-HCMV activities of some selected nucleoside and nucleotide analogues

Entry	Compound #	IC_{50} (μg/mL)	CC_{50} (μg/mL)
1	**17**	>100	>100
2	**19**	3	100
3	**20**	>100	100
4	**25**	>100	100
5	**27**	>50	>100
6	**32**	>100	>100
7	**33**	>100	>100
8	**36**	>100	>100
9	**41**	>50	100
10	Ganciclovir	0.1 - 0.5	>100

In summary, azides **9** and **13** served as intermediates for twelve 3,4-disubstituted tetrahydrofuran nucleosides and nucleotides. The biological results indicated that all ring constrained analogues lack anti-HCMV activities but had relatively low cytotoxicities. However, nucleoside **19** emerged with some activity against HCMV in vitro.



Acknowledgment

The authors wish to thank the analytical and virology departments of BioChem Therapeutic and the virology department of GlaxoWellcome UK for the anti HCMV biological assay experiments and Thérèse Godbout for the preparation of this manuscript.

References

1. Naesens, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Neyts, J.; De Clercq, E. *Antiviral Chem. Chemother.* **1997**, *8*, 1.
2. Polis, M. A.; Spooner, K. M.; Baird, B. F.; Manischewitz, J. F.; Jatte, H. S.; Fisher, P. E.; Fallon, J.; Davey, R. T. Jr.; Kovacs, J. A.; Walker, R. E.; Whitcup, S. M.; Nussenblatt, R. B.; Lane, H. C.; Masur, H. *Antimicrob. Agents Chemother.* **1995**, *39*, 882.
3. Starrett, J. E. Jr.; Tortolani, D. R.; Russel, J.; Hitchcock, M. J. M.; Whiterock, V.; Martin, J. C.; Mansuri, M. M. *J. Med. Chem.* **1994**, *37*, 1857. Naesens, L.; Neyts, J.; Balzarini, J.; Bischofberger, N.; De Clercq, E. *Nucleosides Nucleotides* **1995**, *14*, 767.
4. Scrafinowska, H. T.; Ashton, R.; Bailey, S.; Harnden, M. R.; Jackson, S. M.; Sutton, D. *J. Med. Chem.* **1995**, *38*, 1372.
5. Bronson et al. have reported the preparation and anti-herpetic activity of a carbocyclic guanine analogue *see* Bronson, J.; Ferra, L.; Martin, J. C.; Mansuri, M. M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 685.
6. Borner, A.; Holz, J.; Kagan, H. B. *Tetrahedron Lett.* **1993**, *34*, 5273.
7. Shaw, G.; Warrener, R. N. *J. Chem. Soc.* **1958**, 157. Mansour, T. S.; Jin, H. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 757.
8. Yu, K.-L.; Bronson, J. J.; Yang, Y.; Patick, A.; Alam, M.; Brankovan, V.; Datema, R.; Hitchcock, M. J. M.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 2726 and references cited therein.
9. Patil, S. D.; Schneller, S. W. *J. Med. Chem.* **1992**, *35*, 3372. For a recent review see Mansour, T. S.; Storer, R. *Curr. Pharm. Design* **1997**, *3*, 227.
10. Satisfactory spectral data for all new compounds have been obtained. Presented at the 35th Organic Symposium in June 1997, San Antonio, Texas, USA. E-mail MansourT@BioChem-Pharma.com.

(*Received in USA* 8 *August* 1997; *accepted* 9 *September* 1997)