Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 12, pp. 1275-1280, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00208-1

SYNTHESIS AND ANTIVIRAL ACTIVITY OF RIGID ACYCLONUCLEOTIDE ANALOGS

Patrick J. Casara*+, Jean-Michel Altenburger+¤, Debra L. Taylor#, A. Stanley Tyms#, Michael Kenny† and Jean-François Navé+

+*Marion Merrell Dow Research Institute, 16 rue d'Ankara, 67080 Strasbourg, France*

† *Marion Merrell Dow Inc., 10236 Marion Park Drive, Kansas City, MO, USA*

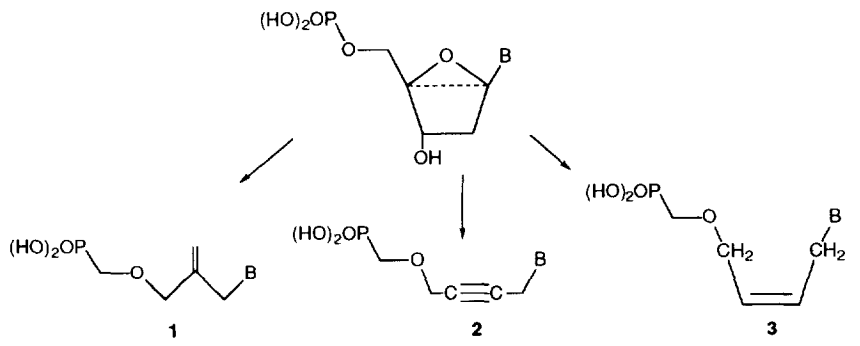
#*MRC, Collaborative Centre, 1-3 Burtonhole Lane, Mill Hill, London, UK*

¤*Present address : Synthelabo 92220 Bagneux, France*

Abstract : The synthesis, anti-HIV-1 and anti-herpesvirus activities of new rigid acyclonucleotide analogs are described. 9-[2-methylidene-3-(phosphonomethoxy)propyl]guanine **1a** exhibits *in vitro* anti-HIV-1 activity similar to that of the antiviral agent 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA). Compound **1a** is 9-fold less toxic to human T-lymphoid cells MT-4 than PMEA.

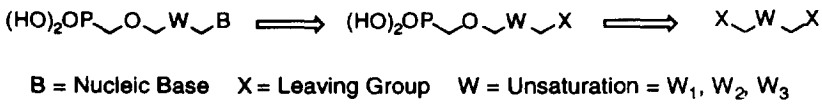
In the last years, several (phosphonomethoxy)alkyl derivatives of purines and pyrimidines have emerged as potent antiviral agents[1,2,3]. As mimics of nucleoside monophosphates, these acyclonucleotide analogs exert their antiviral effect following sequential activation by cellular kinases to their diphosphate derivatives[4,5] (nucleoside triphosphate analogs) which act as potent inhibitors of viral DNA polymerases. A selective inhibition for these enzymes as opposed to host cell DNA polymerases is critical for the potential use of such compounds as drugs. Various attempts to improve the selectivity index have led to acyclonucleotide analogs modified in their acyclic moiety by introduction of functional groups[6,7], very often with an asymmetric center[8,9,10]. Another approach towards more active and selective acyclonucleotide analogs would be to make more rigid the acyclic chain in order to mimic the conformation of the ribose ring. This can be achieved by the introduction of an unsaturation in the acyclic chain as depicted in scheme 1. In the proposed new chemical structures of type **1**, **2** and **3**, the unsaturation allows the maintenance of the nucleic base B at an appropriate distance from the phosphorus atom, similar to that in nucleoside-5'-monophosphates. The unsaturation is either superimposable to the ribose ring for structures of type **1** and **3**, or crosses it in the case of **2** (scheme 1).

Scheme 1



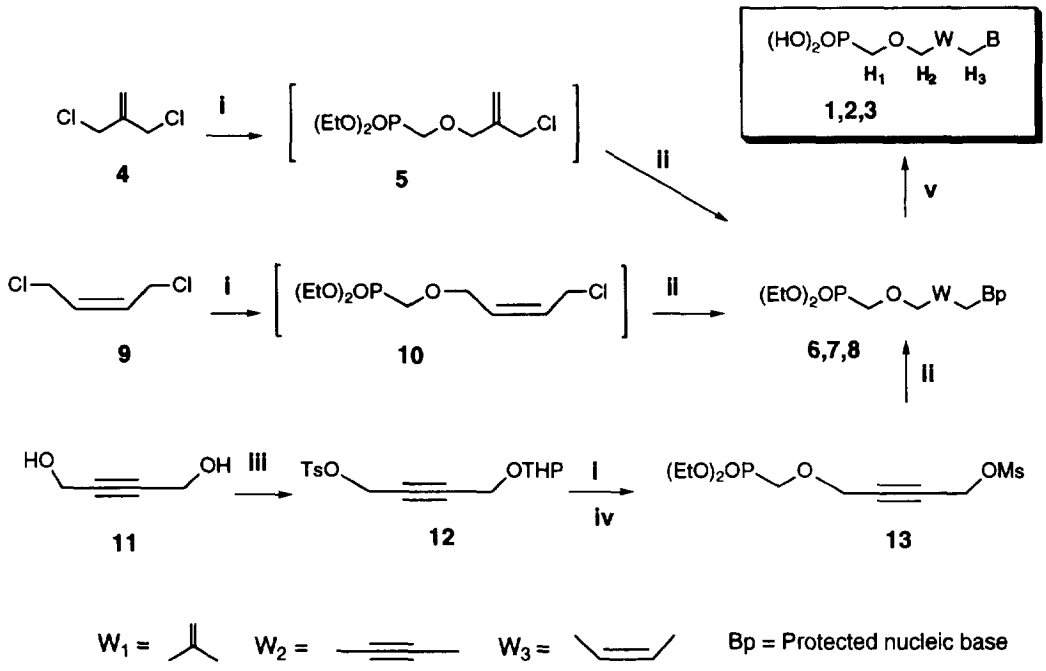
A retrosynthetic analysis for these new classes of compounds showed that they could be obtained from symmetrical allyl or propargyl synthons provided that we could introduce sequentially the phosphonomethoxy moiety and the nucleic base as shown in scheme 2.

Scheme 2



When W = W_1 or W_3, this approach was successfully applied directly from the dichloro derivatives **4** and **9** (scheme 3) since in these cases the mono alkylation by the diethylphosphonomethylalcoholate could be controlled. However, when W = W_2, the bis propargylalcohol **11** was selectively protected in order to control the monoalkylation. Then, the introduction of a nucleic base in an adequately protected form (Bp) was possible. Finally, the diethylphosphonate esters were hydrolyzed by treatment with trimethylsilyl bromide followed by alkaline treatment in order to cleave the nucleic base protective groups when necessary (scheme 3).

Scheme 3



i : $(EtO)_2OPCH_2OH$, LDA, THF. **ii** : Bp, K_2CO_3, CH_3CN. **iii** : a) DHP, PPTS, CH_2Cl_2 b) TsCl, NEt_3. **iv** : a) HCl 1N, b) MsCl, NEt_3. **v** : a) TMSBr, CH_3CN, b) NH_4OH, when Bp = N-AcCyt b) NaOH, when Bp = 6-ClGua.

The chemical yields (%) and the ^1H NMR (δ, ppm; (J, Hz)) characteristics of each product are summarized in Table 1.

Table 1.

W	Product	Bp	Yield	Product	B	Yield	$2H_1$	$2H_2$	$2H_3$	$2H_w$	$1(2)H_B$
W_1	6a	6-ClGua	20	1a	Gua	67	d, 3.61(9)	s, 4.16	s, 5.41	s, 4.88 s, 5.08	s, 8.46
W_1	6b	Ade	25	1b	Ade	73	d, 3.47(9)	s, 4.10	s, 4.86	s, 4.98 s, 5.32	s, 8.27 s, 8.28
W_1	6c	N-AcCyt	43	1c	Cyt	92	d, 3.48(9)	s, 4.10	s, 4.51	s, 4.90 s, 5.28	d, 6.04 (7) d, 7.67 (7)
W_2	7a	6-ClGua	32	2a	Gua	50	d, 3.59(9)	s, 4.27	s, 4.97	/	s, 8.16
W_2	7b	Ade	40	2b	Ade	40	d, 3.53(9)	s, 4.30	s, 5.10	/	s, 8.24 s, 8.26
W_2	7c	N-AcCyt	38	2c	Cyt	90	d, 3.52(9)	s, 4.32	s, 4.65	/	d, 6.02(7) d, 7.78(7)
W_3	8a	6-ClGua	36	3a	Gua	56	d, 3.57(9)	d, 4.35(6)	d, 4.82(6)	m, 5.80 m, 5.93	s, 7.80
W_3	8b	Ade	21	3b	Ade	36	d, 3.54(9)	d, 4.33(6)	d, 4,97(6)	m, 5.84 m, 5.94	s, 8.19 s, 8.26
W_3	8c	N-AcCyt	30	3c	Cyt	50	d, 3.51(8)	d, 4.26(7)	d, 4.50(7)	m, 5.66 m, 5.85	d, 6.00(7) d, 7.64(7)

The anti-HIV-1 activity of the acyclonucleotide analogs **1a,b,c**, **2b,c**, **3a,b,c** was evaluated in the human T-lymphoid cell lines MT-4[11] and C-8166[12] infected with the HIV-1-RF strain (Table 2). In both cell types, 9-[2-methylidene-3-(phosphonomethoxy)propyl]guanine **1a** emerged as a good inhibitor of HIV-1 replication, being as potent as the promising acyclonucleotide analog 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA)[2,13]. In spite of its lower anti-HIV activity than 3'-azido-3'-deoxythymidine (AZT) *in vitro* [14], PMEA has proved to be a much more potent antiretroviral agent against acute simian immunodeficiency virus infection in monkeys[13] than AZT which had only marginal activity under the same experimental conditions[15]. Interestingly, **1a** was about 9-fold less cytotoxic to MT-4 cells than PMEA (Table 2), making this compound more selective than PMEA[16]. It is of note that compound **1b**, the adenine analog of **1a**, was devoid of anti-HIV-1 activity (Table 2). Its is also noticeable that **1a** is much less toxic to cells than PMEG, the guanine analog of PMEA. PMEA was reported to show anti-HIV-1 activity similar[2] (MT-4 cells) or higher[9] (CEM cells) than PMEA. Yet, PMEG was also found to be 6-to 30-fold more toxic[2,9] than PMEA.

The acyclic phosphonate derivative of adenine **3b** also showed anti-HIV-1 activity but was 8- and 22-fold less potent than PMEA, in MT-4 and C-8166 cells, respectively. Very recently, Shirokova *et al* [17] reported the preparation of compounds **3a**, **3b** and **3c** using a different synthetic approach. In agreement with our results, **3b** was found to be active against HIV-1 in MT-4 cells whereas **3a** and **3c** were inactive.

Table 2. Anti-HIV-1 activity of the acyclonucleotide analogs

compound	EC_{50} (μM) [a,b]		CC_{50} (μM) [c]
	MT-4 cells	C-8166 cells	MT-4 cells
AZT	0.035 (± 0.004)	0.03 (± 0.03)	113 (± 36)
PMEA	22 (± 10)	1.7 (± 0.9)	187 (±135)
1a	25 (± 10)	1.9 (± 0.3) *	1800 (± 0) *
3a	> 100	> 100	> 1000
1b	> 100	> 100	> 1000
2b	> 100	> 100	800
3b	170	38 (± 7) *	> 1000
1c	> 100	> 100	> 100
2c	> 100	> 100	> 100
3c	> 100	> 100	> 100

Some data are mean values of at least three independent experiments (± SD); data marked with * are mean of two independent experiments.

[a] Concentration required to inhibit HIV-1-induced cythopathic effect by 50% in MT-4 cells.

[b] Concentration required to inhibit p24 viral antigen production by 50% in C-8166 cells.

[c] Concentration required to reduce the viability of MT-4 cells by 50%.

The acyclonucleotide analogs were also tested for activity against the herpes simplex viruses of type 1 (HSV-1, strain HF) and 2 (HSV-2, strain G) grown in vero cells[18] and against human cytomegalovirus (HCMV, strain AD-169) grown in MRC-5 cells[18] (Table 3). None of the compounds tested were active against HSV-2. Compounds **3a** and **1b** exhibited anti-HSV-1 activity but at concentrations 6-fold higher than that of acyclovir. Compound **1a** showed anti-HCMV activity similar to that of PMEA which itself is a modest anti-HCMV agent compared to compounds such as ganciclovir[3] or the acyclonucleotide analog (S)-HPMPC[3]. Compound **1a** was not toxic to Vero and MRC-5 cells at least up to 250 μM.

Intracellular phosphorylation of the 5'-monophosphates of the acyclic nucleosides acyclovir and ganciclovir by guanylate kinase is considered as a mandatory step[19,20] in the pathway leading to the antivirally active forms of these drugs. Phosphorylation by guanylate kinase has been demonstrated for various 9-phosphonomethoxyalkyl derivatives of guanine and it is likely that this enzyme plays a prominent role in their activation. Compound **1a** was phosphorylated by guanylate kinase with an efficiency[21] of 0.6 % of that of GMP.

Table 3. Anti-herpesvirus activity of the acyclonucleotide analogs

Compound	EC_{50} (μM) [a]			CC_{50} (μM) [b]	
	HSV-1	HSV-2	HCMV	Vero cells	MRC-5 cells
Acyclovir	6	3	18	> 100	≥ 100
PMEA	58	69	36	≥ 250	> 250
1a	> 250	> 250	34	> 250	> 250
3a	37	> 250	> 250	> 250	> 250
1b	36	> 250	> 250	> 250	> 250
2b	> 250	> 250	> 250	≥ 250	> 250
3b	> 250	> 250	> 250	≥ 250	> 250
1c	> 250	> 250	> 250	> 250	> 250
2c	> 250	> 250	> 250	> 250	> 250
3c	> 250	> 250	> 250	> 250	> 250

[a] Concentration required to inhibit HSV-1-, HSV-2- or HCMV-induced cytopathic effect by 50%.

[b] The lowest concentration of compound producing overt cytotoxicity in virus-free cultures.

The Michaelis-Menten constants were 18 ± 1 μM (n=4) and 238 ± 12 μM (n = 4) for GMP and **1a**, respectively. Vmax of **1a** was 8 ± 1 % (n=4) of that of GMP. In contrast, compound **3a** tested at 1.3 mM in the presence of a high concentration of enzyme[22] was not phosphorylated by guanylate kinase.

In conclusion, in a series of novel unsaturated acyclonucleotide analogs, we have identified 9-[2-methylidene-3-(phosphonomethoxy)propyl]guanine **1a** as a good inhibitor of HIV-1 replication. In view of its low cellular toxicity, this compound clearly deserves to be further evaluated *in vivo*.

Acknowledgements : We wish to thank A. Eggenspiller, A. Eschbach, J. Dulworth, T. Brennan for technical assistance and C. Reeb for typing this manuscript.

References and notes

1. De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holy, A. *Antiviral Res.* **1987**, 8, 261.
2. Pauwels, R.; Balzarini, J.; Schols, D.; Baba, M.; Desmyter, J.; Rosenberg, I.; Holy, A.; De Clercq, E. *Antimicrob. Agents Chemother.* **1988**, 32, 1025.
3. Snoeck, R.; Sakuma, T.; De Clercq, E.; Rosenberg, I.; Holy, A. *Antimicrob. Agents Chemother.* **1988**, 32, 1839.
4. Votruba, I.; Bernaerts, R.; Sakuma, T.; De Clercq, E.; Merta, A.; Rosenberg, I.; Holy, A. *Mol. Pharmacol.* **1987**, 32, 524.
5. Balzarini, J.; Hao, Z.; Herdewijn, P.; Johns, D.G.; De Clercq, E. *Proc. Natl. Acad. Sci. USA* **1991**, 88, 1499.
6. Kim, C.U.; Misco, P.F.; Luh, B.Y.; Hitchcock, M.J.M.; Ghazzouli, I.; Martin, J.C. *J. Med. Chem.* **1991**, 34, 2286.
7. Perkins, R.M.; Immelmann, A.; Elphick, L.; Duckworth, D.M., Harnden, M.R.; Kenig, M.D.; Planterose, D.N.; Brown, A.G. *Antiviral Res.* **1992**, 17, Suppl. 1, 59.
8. Balzarini, J.; Holy, A.; Jindrich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. *Antimicrob. Agents Chemother.* **1993**, 37, 332.
9. Yu, K.L.; Bronson, J.J.; Yang, H.; Patick, A.; Alam, M.; Brankovan, V.; Datema, R.; Hitchcock, M.J.M.; Martin, J.C. *J. Med. Chem.* **1992**, 35, 2958.
10. Yu, K.L.; Bronson, J.J.; Patick, A.; Alam, M.; Brankovan, V.; Datema, R.; Hitchcock, M.J.M.; Martin, J.C. *J. Med. Chem.* **1993**, 36, 2726.
11. The anti-HIV-1 activity of compounds in MT-4 cells was determined using the MTT assay, essentially as described by Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P. *J. Virol. Methods* **1988**, 20, 309. The toxicity of compounds was evaluated after a 6-day incubation of uninfected cells with varying concentrations of test compounds by measurement of cell viability using the MTT assay.
12. C-8166 cells were collected in exponential growth phase, washed and infected with HIV-1 (1h, RT, multiplicity of infection = 0.001). After washing 3 times in RPMI, 100 µl (10^5 cells) aliquots of cell suspension were distributed into wells of 96-well cell culture plates filled with 100 µl of growth medium without or with test compounds at various concentrations. After 3 days of incubation at 37°C, the cell culture medium was assayed for levels of p24 viral core antigen using the ELISA method of Moore, J.P.; Mc Keating, J.A.; Weiss, R.A.; Sattentau, Q.J. *Science* **1990**, 250, 1139.
13. Tsai, C.-C.; Follis, K.E.; Sabo, A.; Grant, R.F.; Bartz, C.; Nolte, R.E.; Benveniste, R.E.; Bischofberger, N. *J. Infect. Dis.* **1994**, 169, 260.
14. Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D.G.; De Clercq, E. *Proc. Natl. Acad. Sci. USA* **1989**, 86, 332.
15. Tsai, C.-C.; Follis, K.E.; Grant, R.F.; Nolte, R.E.; Bartz, C.R.; Benveniste, R.E.; Sager, P.R. *J. Acquir. Immune Defic. Syndr.* **1993**, 6, 1086.
16. PMEA and compound **1a** were found not to be toxic to C-8166 cells at least up to 1000 µM (measurement of cell viability using the MTT assay).
17. Shirokova, E.A.; Tarussova, N.B.; Shipitsin, A.V.; Semizarov, D.G.; Krayevsky, A. A. *J. Med. Chem.* **1994**, 37, 3739.
18. Cytopathic effect (CPE) inhibition assays were performed using Vero or MRC-5 cell monolayers propagated in 96-well microtiter plates. The lowest concentration of compound inhibiting virus-induced CPE by 50 % or more in replicate cultures was considered the EC_{50}. The lowest concentration of compound producing overt cytotoxicity in virus-free cultures was considered the CC_{50}.
19. Miller, W.H. ; Miller, R.L. *J. Biol. Chem.* **1980**, 255, 7204.
20. Ashton, W.T.; Karkas, J.D.; Field, A.K.; Tolman, R.L. *Biochem. Biophys. Res. Comm.* **1982**, 108, 1716.
21. Guanylate kinase (hog brain) was assayed according to Navé, J.F.; Eschbach, A.; Halazy, S. *Archiv. Biochem. Biophys.* **1992**, 295, 253. Efficiency is defined as $[(Vmax/Km)_{compound}/(Vmax/Km)_{GMP}] \times 100$.
22. At enzyme concentration 20-fold higher than that used for GMP, substrate activity of compounds phosphorylated at rates equal to 0.2 % of the maximal velocity calculated for GMP under similar conditions could be detected.

(*Received in Belgium* 21 *February* 1995; *accepted* 28 *April* 1995)