SYNTHESIS OF A PHOSPHONATE ISOSTERE OF ACYCLOVIR MONOPHOSPHATE: A HERPESVIRUS ACTIVE PHOSPHONATE NUCLEOTIDE ANALOGUE

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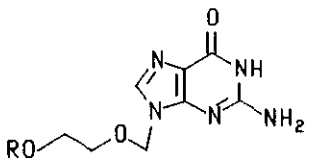
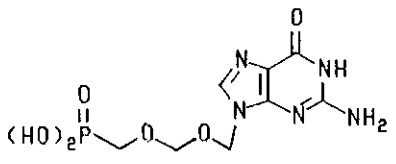
Abstract - A novel synthetic methodology for the acyclic acetal functionality was developed. The rationally designed phosphonate analogue (3) of acyclovir monophosphate was active against herpesviruses.

The discovery of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, 1) as a chemotherapeutically effective nucleoside for the treatment of the herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) has stimulated a great interest in the area of acyclic nucleoside analogues.[1] In cells, acyclovir is phosphorylated by the HSV induced thymidine kinase to the monophosphate (2); this is in turn phosphorylated further by cellular kinase to the di- and ultimately to the tri-phosphate, which is a potent inhibitor of viral DNA polymerase.[2,3] Metabolically and chemically stable phosphonate analogues,[4] which mimic the acyclovir monophosphate (2), thus bypassing the initial enzymatic phosphorylation could be highly promising as broad spectrum antiviral agents. In the series of (phosphonomethoxy)alkylpurine derivatives, the spacial location of the oxygen atom in the acyclic chain has been demonstrated to play a crucial role for anti-herpesvirus and anti-retrovirus activity.[5,6] *Our strategy to mimic the acyclovir* monophosphate as close as possible led to the discovery of the herpesvirus active phosphonate (3).



1 R=H

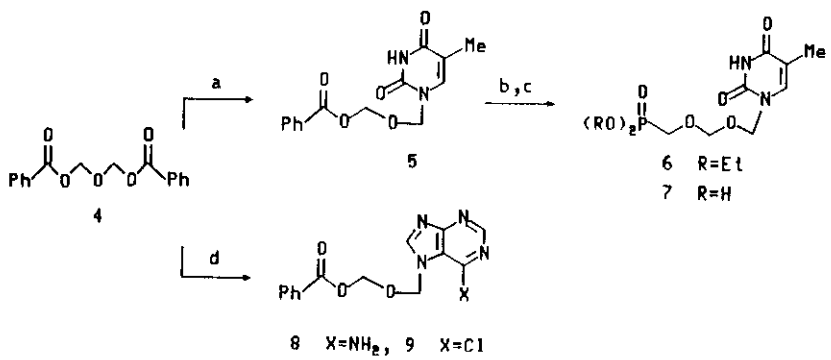
2 R=P(O)(OH)$_2$



3

First, we developed a new methodology to assemble the acyclic acetal functionality as shown in the synthesis of the thymine analogue (7) (Scheme I). The Vorbruggen type coupling[7] of bisbenzoyloxymethyl ether (4)[8] and silylated thymine in the presence of trimethylsilyl tri-fluoromethanesulfonate (0.2 equiv.) in CH_2Cl_2 at 0°C provided the acylacetal (5) (45%). Reaction of 5 with diethyl hydroxymethylphosphonate (1.2 equiv.) in the presence of trimethylsilyl trifluoromethanesulfonate (1.2 equiv.) in CH_2Cl_2 at 25°C afforded the phosphonate (6) (65%) which upon treatment with trimethylsilyl bromide (5 equiv.) in DMF at 25°C gave the acetal phosphonate (7) (75%). Unfortunately, when the above reaction sequence was applied to the synthesis of purine analogues, undesired N-7 isomers were formed exclusively. For example, coupling of 4 with silylated adenine or silylated 6-chloropurine in the presence of trimethylsilyl trifluoromethanesulfonate gave the N-7 isomers (8) (39%) and (9) (42%) only. The attachment of the side chain at the N-7 position in 8 (and 9) was confirmed by the ^{13}C-^{1}H two dimensional long-range heteronuclear correlation spectroscopy, in which the C_5 and the 1'-H exhibited a strong interaction. This regioselectivity was further ascertained by the X-ray crystallography as shown in Figure I. Although the highly regioselective kinetic formation of an N-7-(pentofuranosyl)guanine was reported,[10] the exclusive formation of 8 appeared to be the first case in the adenine nucleosidation.

Scheme I



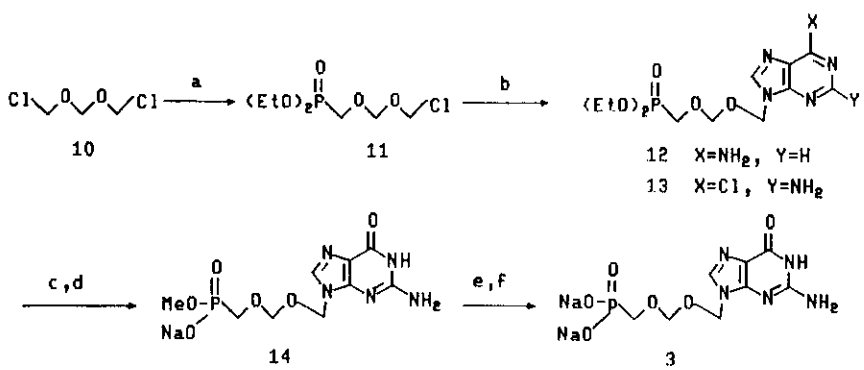
(a) silylated thymine, $CF_3SO_3SiMe_3$; (b) $(EtO)_2P(O)CH_2OH$, $CF_3SO_3SiMe_3$; (c) Me_3SiBr_3; (d) silylated adenine (for 8) or silylated 6-chloropurine (for 9), $CF_3SO_3SiMe_3$.

In contrast to the Vorbruggen type coupling, the S_N2 displacement reaction of the purine sodium salt on the chloromethyl ether (11) produced only N-9 isomers as illustrated in Scheme II. Addition of bischloromethoxymethane (10)[11] to the solution of sodium diethyl phosphite in THF at -70°C produced the chloromethyl ether (11) which was used promptly in the subsequent transformations. Reaction of 11 with 2-amino-6-chloropurine sodium salt in DMF at 25°C to give the N-9 isomer (13) (42% from 10), saponification with sodium methoxide followed by sodium hydroxide to form 14 (85%), and deprotection with trimethylsilyl bromide provided the phosphonate (3) (68%).[12] Likewise, the N-9 adenine phosphonate (12) was also prepared in 47% yield. The side chain attachment at th N-9 position in 3 was ascertained by its ^{13}C nmr (δ 118.194 for the C_5 signal) and uv (λmax 252 and 274 nm) spectra which were consistent with the published data of N-9 alkylated guanine derivatives.[13]

In antiviral tests carried out in Vero (for HSV) and MRC-5 for (HCMV, human cytomegalovirus) cells the IC_{50}'s (50% inhibitory concentration) for inhibition of the replication of HSV-1 and 2 of HCMV were 2.6, 11 and 5.0 μg/ml for 3 (cf. 0.5, 0.5 and 40 μg/ml for acyclovir).

In conclusion, a novel route has been developed for the acyclic acetal functionality and has been used in the synthesis of a phosphonate isostere of acyclovir monophosphate, which has exhibited a good anti-herpesvirus activity.

Scheme II



(a) $(EtO)_2\overset{O}{\overset{\|}{P}}Na$; (b) adenine sodium salt (for 12) or 2-amino-6-chloropurine sodium salt (for 13);(c) MeONa; (d) 1N-NaOH; (e) Me_3SiBr; (f) $NaHCO_3$.

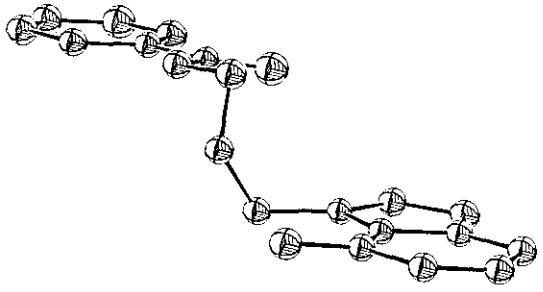


Figure I Ortep Drawing Of Compound (8)

REFERENCES AND FOOTNOTES

1. See C. K. Chu and S. J. Cutler, J. Heterocycl. Chem., 1986, 23, 289 for a recent review.
2. G. B. Elion, P. A. Furman, J. A. Fyfe, P. De Miranda, L. Beauchamp, and H. J. Shaeffer, Proc. Natl. Acad. Sci. USA, 1977, 74, 5716.
3. H. J. Shaeffer, "Nucleosides, Nucleotides and Their Biological Applications", eds. J. L. Rideout, D. W. Henry, and L. M. Beauchamp, pp. 1-17, Academic Press, New York (1983).
4. Some phosphonate analogues which mimic the acyclovir monophosphate have been reported. (a) W. Streicher, G. Werner, and B. Rosenwirth, Chemical Scripta, 1986, 26, 179 (b) E. J. Reist, P. A. Sturm, R. Y. Pong, M. J. Tanga, and R. W. Sidwell, "Nucleotide Analogues as Antiviral Agents", ed. J. C. Martin; ACS Symposium Series 401, American Chemical Society, Washington, DC., 1989, p. 73.
5. (a) E. De Clercq, A. Holy, I. Rosenberg, T. Sakuma, J. Balzarini, and P. C. Maudgal, Nature, 1986, 323, 464. (b) A. Holy, E. De Clercq, and I. Votruba, "Nucleotide Analogues as Antiviral Agents", ed. J. C. Martin; ACS Symposium Series 401, American Chemical Society, Washington, DC., 1989, p. 51.
6. C. U. Kim, B. Y. Luh, P. F. Misco, J. J. Bronson, M. J. M. Hitchcock, I. Ghazzouli, and J. C. Martin, Nucleosides & Nucleotides, 1989, 8, (5 & 6), 927.
7. H. Vorbruggen and G. Hofle, Chem. Ber., 1981, 114, 1256.
8. Compound (4) was prepared in quantitative yield by reaction of bischloromethyl ether with sodium benzoate (2 equiv.) in DMF at 100°C.
9. D. P. Phillion and S. S. Andrews, Tetrahedron Letters, 1986, 27, 1477.
10. M. J. Robins, R. Zou, F. Hansske, D. Madej, and D. L. J. Tyrrell, Nucleosides & Nucleotides,1989, 8, (5 & 6), 725.
11. P. R. Stapp, J. Org. Chem., 1969, 34, 1143.
12. The phosphonate (3) was quite acid stable, thus a pH 2 solution of 3 showed no sign of degradation after 24 h as evidenced by hplc and nmr analysis.
13. (a) J. Zemlicka, Nucleosides & Nucleotides, 1984, 3, 245. (b) J. A. Montgomery and C. Temple, Jr., J. Am. Chem. Soc., 1961, 83, 630. (c) J. Kjellbert and N. G. Johansson, Tetrahedron, 1986, 42, 6544. (d) J. Kjellbert and N. G. Johansson, Nucleosides & Nucleotides, 1989, 8, 225.
14. Compound (3) (as sodium salt): Uv (H_2O) λmax 252 nm (ε 12,113), 274 nm (ε 8,201); ^1H nmr (D_2O) δ 3.525 (d, J=8.9 Hz, 2H), 4.766 (s, 2H), 4.766 (s, 2H), 5.539 (s, 2H), 7.892 (s, 1H); Anal. Calcd for $C_8H_{10}N_5O_6Na_2P \cdot 3H_2O$: C, 23.84; H, 4.01; N, 17.37. Found: C,23.94; H, 3.92; N, 17.21.

Received, 6th June, 1990