Synthesis and Enzymatic Resolution of Carbocyclic 2′-*Ara*-fluoro-Guanosine: A Potent New Anti-Herpetic Agent

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(±)-Carbocyclic-9-(2′-deoxy-2′-β-fluoroarabinofuranosyl) guanine (**8**) and the corresponding furanose compound (**12**) have been synthesised; the former compound [which was resolved by formation of the monophosphate (**20**) and enantioselective hydrolysis using a 5′-nucleotidase] is an extremely potent inhibitor of herpes simplex viruses types 1 and 2.

Nucleoside analogues have been extensively investigated in the search for agents effective in the treatment of herpes simplex virus (HSV) infections.[1] To date, the most potent anti-herpes activity has been displayed by certain acyclic guanine derivatives,[2] *e.g.* acyclovir (ACV) (**1**) and 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl} guanine (DHPG) (**2**), and by some 2′-*ara*-fluoropyrimidine nucleosides,[3] *e.g.* 9-(2′-deoxy-2′-fluoroarabinofuranosyl)-5-methyl uracil (FMAU) (**3**). However, since carbocyclic nucleosides benefit from greater metabolic stability than their furanose counterparts[4] we decided to prepare carbocyclic 2′-*ara*-fluoroguanosine (**8**).[5]

The racemic fluoroaminodiol (**4**)[6] was coupled with 2-amino-4,6-dichloropyrimidine to afford the crystalline diamine (**5**) (88%) (Scheme 1). Reaction of compound (**5**) with *p*-chlorophenyldiazonium chloride followed by reduction of the intermediate diazo compound gave the triamine (**6**) (60%). Cyclisation of the latter compound with triethyl *ortho*formate followed by treatment with dilute hydrochloric acid provided the 6-chloropurine (**7**) (75%) which was finally hydrolysed to give (±)-carbocyclic 2′-*ara*-fluoroguanosine (**8**) (76%). This carbocyclic nuceloside showed extremely high levels of activity against HSV-1 and HSV-2 in the plaque reduction assay and in the mouse systemic model.[7] For example, the nucleoside analogue (**8**) is about an order of magnitude more active than FMAU and about thirty times more active than ACV in the *in vitro* assay of HSV-1 in infected cells. In contrast, the compound shows no effect on uninfected cells even at very much higher concentrations.

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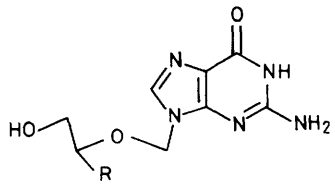
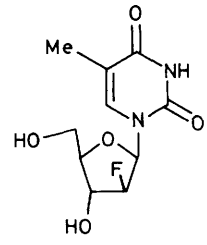


(**1**) R = H
(**2**) R = CH_2OH



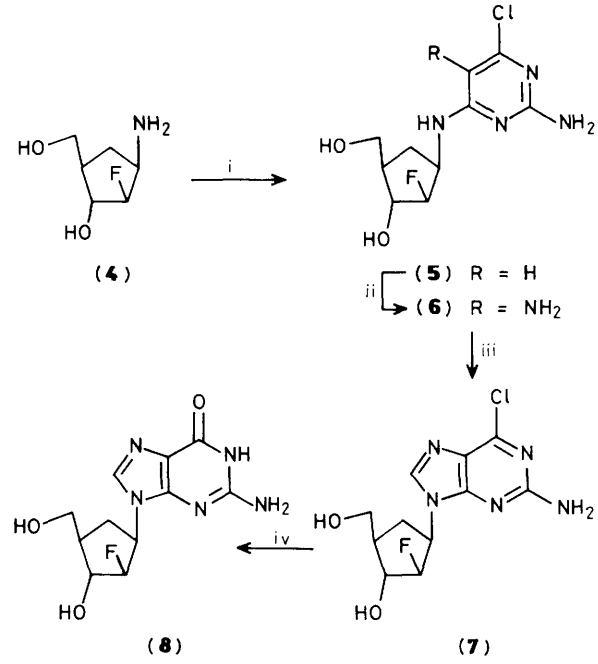
(**3**)

It is noteworthy that the fluorine atom in compound (**8**) contributes to the display of potent biological activity since the compound lacking this atom in the carbocyclic ring has been prepared recently by Shealy *et al.*[8] and is much less active against herpes simplex virus *in vitro*. As carbocyclic versions of anti-viral nucleosides have been found to be less active than their furanose parents[6,9] we considered that it would be prudent to synthesise the then unknown sugar analogue (**12**).

Coupling to the bromo-compound (**9**)[10] with the silylated chloropurine (**10**)[11] gave the crystalline amine (**11**) (27%) which was separated by column chromatography from a small amount of the α-anomer (11%). Hydrolysis of (**11**) using aqueous sodium hydroxide gave the nucleoside (**12**) {$[\alpha]_D^{24}$ 41.6° (*c* 0.31, methanol)}. This method of preparation of compound (**12**) is more efficient than the alternative procedure that was published recently.[12] Surprisingly, compound (**12**) was found to be *ca.* 1000-fold *less* active than the carbocycle (**8**) *in vitro* against both HSV-1 and HSV-2. Compound (**8**) represents, therefore, the first example of a carbocyclic analogue of an unnatural nucleoside to exhibit greater anti-herpes activity than its furanose parent.

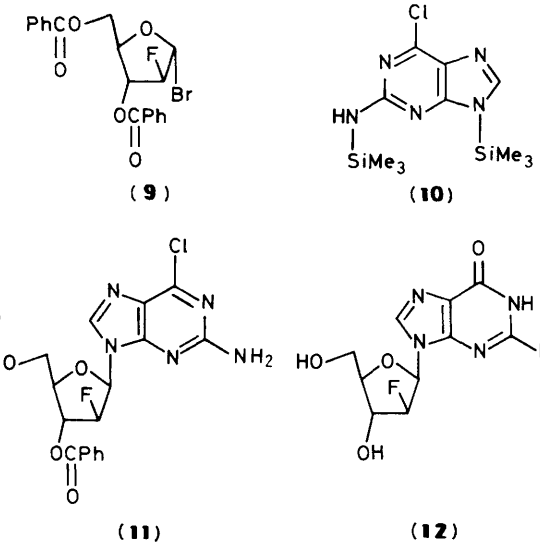
The unprecedented biological activity of compound (**8**) led us to investigate a second, more convergent synthesis of the substance starting from the diol (**13**).[13] Selective tritylation of the primary hydroxyl group followed by oxidation with t-butylhydroperoxide under V^V catalysis furnished the epoxide (**14**) (73%) (Scheme 2). Benzylation of the free hydroxyl group gave the oxirane (**15**) (60%) which underwent reaction with potassium hydrogen difluoride to afford the alcohol (**16**) in 30% yield after purification by chromatography. The trityl group appears to be lost fairly rapidly under the reaction conditions and the lability of this protecting group is probably a major factor accounting for the low yield of (**16**) obtained in this reaction. Differentiation and activation of the requisite hydroxyl group was accomplished without any further problem to give the tosylate (**17**) [56% from (**16**)]. Displacement of the tosylate moiety with the chloropurine (**18**) gave the coupled product (**19**) (18%) which was hydrolysed and deprotected to give the carbocyclic nucleoside (**8**).

Finally it was of interest to determine whether the observed biological activity of compound (**8**) was due to one enantiomer; we report an expeditious enzyme-controlled resolution process for the production of optically active material. Herpes simplex virus type 1 thymidine kinase (TK) was purified from HSV-1 infected Vero cells.[14] The racemic compound (**8**) was phosphorylated using adenosine triphosphate (ATP) under TK catalysis in the appropriate reaction mixture.‡ The starting material and desired monophosphate (**20**) were extracted and separated by ion exchange h.p.l.c.



(**4**) (**5**) R = H (**6**) R = NH_2 (**8**) (**7**)

Scheme 1. *Reagents and conditions:* i, 2-amino-4,6-dichloropyrimidine, Bu^nOH, Et_3N, heat; ii, *p*-chlorophenyldiazonium chloride, HOAc, NaOAc, H_2O, then Zn–HOAc, EtOH, heat; iii, $(EtO)_3CH$, dimethylformamide (DMF), conc. HCl, then 2 M HCl, heat; iv, 1 M HCl, heat.

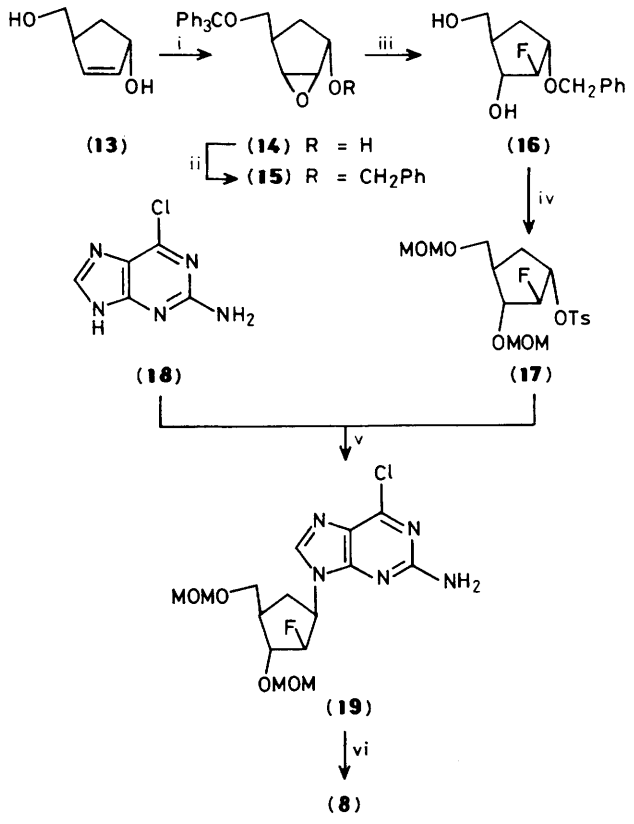


(**9**) (**10**) (**11**) (**12**)

The enzymic phosphorylation showed low enantioselectivity. The monophosphate (**20**) was incubated with 5′-nucleotidase (EC 3.1.3.5) from *Crotalus atrox* venom.§ After 15 min, the starting material and product were extracted and separated by reverse phase h.p.l.c. to give optically active carbocyclic nucleoside (**8**) {$[\alpha]_D^{20}$ +48° (*c* 3.57, water)} and recovered monophosphate. The nucleotidase catalysed reaction is enantioselective; presumably the enantiomer corresponding to the natural sugar (guanosine) is hydrolysed more rapidly under the reaction conditions. The recovered monophosphate was

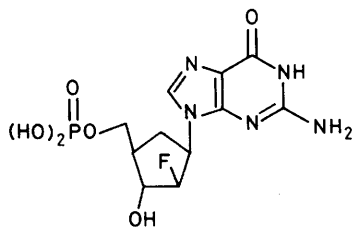
‡ The reaction mixture (1 ml) contained compound (**12**) (2 mg), $(CH_2OH)_3NMe(Tris)$–HCl (pH 7.5, 50 mM), bovine serum albumen (BSA) (1 mg), ATP (5 mM), $MgCl_2$ (5 mM), dithiothreitol (1 mM), phosphocreatine (10 mM), creatine phosphotransferase (12.5 U), and NaF (2.5 mM)

§ The reaction mixture contained glycine (70 mM, pH 9), $MgCl_2$ (20 mM), and 1 mg monophosphate/35 U 5′-nucleotidase or 1.3 mg monophosphate/2.85 U alkaline phosphatase.



MOM = CH_2OMe

Scheme 2. *Reagents and conditions:* i, Bu^tO_2H, toluene, $VO(acac)_2$; ii, NaH, THF, N_2, then $PhCH_2Br$, Bu^n_4NI; iii, KHF_2, $(CH_2OH)_2$, 150—160 °C; iv, $MeOCH_2Cl$, Pr^i_2NEt, CH_2Cl_2, then Pd–C, H_2, EtOAc, H^+ then toluene-*p*-sulphonyl chloride, Et_3N, dimethylaminopyridine (DMAP); v, K_2CO_3, dimethylsulphoxide (DMSO), 80 °C; vi, 1 M HCl, heat.



(20)

hydrolysed with the more catholic enzyme alkaline phosphatase[16] to give (−)-(**8**) {$[\alpha]_D^{20}$ −68° (*c* 1.93, water)}. We believe that this is the first example of the resolution of an unnatural compound using 5′-nucleotidase.[15] The dextrorotatory enantiomer (**8**) was twice as active as the racemate in the HSV-1 plaque reduction assay while the laevorotatory enantiomer was at least two orders of magnitude less active.

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References

1 G. Streissle, A. Paessens, and H. Oediger, *Adv. Virus. Res.*, 1985, **30**, 83.
2 J. L. Kelley, M. P. Krochmal, and H. J. Schaeffer, *J. Med. Chem.*, 1981, **24**, 472, 1525; J. L. Kelley, J. E. Kelsey, W. R. Hall, M. P. Krochmal, and H. J. Schaeffer, *ibid.*, p. 753; R. J. Remey and J. A. Secrist, *Nucleosides, Nucleotides*, 1985, **4**, 411; J. L. Kelley, J. W. T. Selway, and H. J. Schaeffer, *J. Pharm. Sci.*, 1985, **74**, 1302; M. R. Harnden and R. L. Jarvest, *Tetrahedron Lett.*, 1985, **26**, 4265.
3 Anon., *Drugs of the Future*, 1986, **11**, 332, 618; T. L. Su, K. A. Watanabe, R. F. Shinazi, and J. J. Fox, *J. Med. Chem.*, 1986, **29**, 151.
4 V. E. Marquez and M.-I. Lim, *Med. Res. Rev.*, 1986, **6**, 1.
5 A. D. Borthwick, D. N. Evans, B. E. Kirk, K. Biggadike, and L. Stephenson, Eur. Pat. Appl., 0 212 956, 1987.
6 K. Biggadike, A. D. Borthwick, D. N. Evans, A. M. Exall, B. E. Kirk, S. M. Roberts, L. Stephenson, P. Youds, A. M. Z. Slawin, and D. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1987, 251.
7 A. C. Ericson, A. Larsson, F. Y. Aoki, W.-A. Yisak, N.-G. Johansson, B. Oberg, and R. Datema, *Antimicrob. Agents Chemother.*, 1985, **27**, 753.
8 Y. F. Shealy, C. A. O'Dell, W. M. Shannon, and G. Arnett, *J. Med. Chem.*, 1984, **27**, 1416.
9 Y. F. Shealy, C. A. O'Dell, W. M. Shannon, and C. Arnett, *J. Med. Chem.*, 1983, **26**, 156; 1986, **29**, 79; P. Herdewijn, E. De Clercq, J. Balzarini, and H. Vanderhaeghe, *ibid.*, 1985, **28**, 550.
10 C. H. Tann, P. R. Brodfuehrer, S. P. Brundidge, C. Sapino, and H. G. Howell, *J. Org. Chem.*, 1985, **50**, 3644.
11 M. J. Robins and P. W. Hatfield, *Can. J. Chem.*, 1982, **60**, 547.
12 J. A. Montgomery, A. T. Shortnack, D. A. Carson, and J. A. Secrist, III, *J. Med. Chem.*, 1986, **29**, 2389.
13 H. Paulsen and U. Maass, *Chem. Ber.*, 1981, **114**, 346.
14 Y. C. Cheng and M. Ostrander, *J. Biol. Chem.*, 1976, **251**, 2605; 1978, **253**, 8721.
15 *Cf.* P. Herdewijn, J. Balzarini, E. De Clercq, and H. Vanderhaeghe, *J. Med. Chem.*, 1985, **28**, 1385.