Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 22, pp. 2787-2790, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00514-8

ENZYMATIC SYNTHESIS AND ANTIVIRAL ACTIVITY OF 2'-DEOXY-2'-FLUORO-RIBAVIRIN

Martin J.Slater[#]*, Clare Gowrie[#], G. A. Freeman[@], and S. A. Short[@].

[#]*Glaxo Wellcome Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY UK*
[@] *Glaxo Wellcome Research, Five Moore Drive, RTP, North Carolina, USA, 27709*

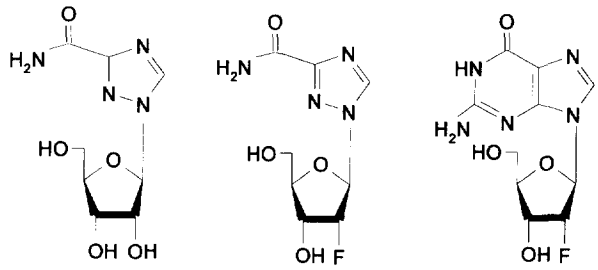
Abstract: 2'-Deoxy-2'-fluoro-ribavirin has been efficiently synthesised from 2'-deoxy-2'-fluorouridine using nucleoside transferase as the key step, and evaluated for its antiviral properties in cell culture. An efficient synthesis of 3,5-di-O-p-chlorobenzoyl-2-deoxy-2-fluoro-β-D-ribofuranose is also described.

Influenza virus infections cause considerable mortality and morbidity[1]. Frequent epidemic and occasional worldwide pandemics have posed major health problems for decades. Currently, the only useful prophylactic and therapeutic treatments are Amantidine and Rimantadine. However, these are limited to influenza virus A infections[2,3], and give rise to the rapid emergence of infectious resistant strains[4].The broad spectrum antiviral agent ribavirin has been evaluated clinically against influenza A, though the results were not encouraging[5,6]. Recently, the anti-influenza activities of 2'-deoxy-2'-fluoropurine nucleosides were reported[7]. Most notable was the activity of 2'-deoxy-2'-fluoroguanosine (**2**), effective *in vitro*, and *in vivo* in the mouse model of influenza[8].

As the α-fluoro atom in 2-deoxy-2-fluoro-β-D-ribofuranose confers a ribose-like conformation on the pentofuranose[9], and ribavirin structurally resembles guanosine by x-ray analysis[10], the synthesis and antiviral properties of 1-(2-deoxy-2-fluoro-β-D-ribo-furanosyl)-1,2,4-triazole-3-carboxamide (2'-deoxy-2'-fluoro-ribavirin, **1**) were of major interest. Herein we report the synthesis and antiviral activity of **1**.

2 was prepared from 2'-deoxy-2'-fluorouridine (**3**)[11] using the combined thymidine phosphorylase (TPase)/purine nucleoside phosphorylase (PNPase) system[7]. The role of TPase is to produce the 1-α-phosphate of 2-deoxy-2-fluoro-β-D-ribofuranose (**4**). PNPase then transfers guanine to **4**. As 1,2,4-triazole-3-carboxamide[12] was not a substrate for PNPase, alternative approaches were sought (scheme 1).

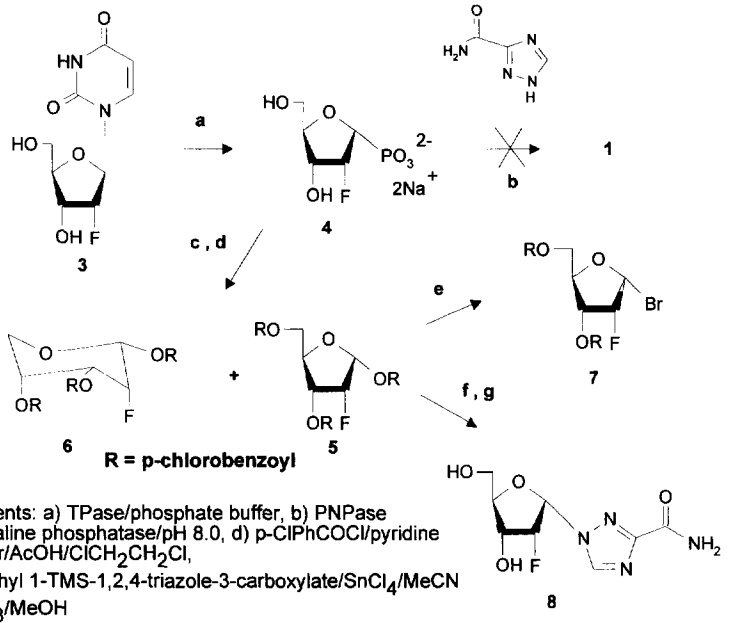
Fax : +44 1438 763616 e-mail: MJS40312@ggr.co.uk



Thus, 2-deoxy-2-fluoro-β-D-ribofuranose was prepared enzymatically from **3** using a TPase-alkaline phosphatase enzyme system. This provided a crude mixture of 2-fluorosugars, which were per-acylated to give 2-deoxy-2-fluoro-1,3,5-tri-O-p-chlorobenzoyl-α-D-ribofuranose (**5**) in 55% yield after column chromatography[13], along with 5% of 2-deoxy-2-fluoro-1,3,5-tri-O-p-chlorobenzoyl-α-D-ribopyranose (**6**). This synthesis of 2-deoxy-2-fluororibose in 4 steps from uridine compares favourably with the multistep procedures reported recently[14]. Unfortunately, using either **5** or the relatively stable glycofuranosyl bromide (**7**), very poor yields of nucleoside mixtures were obtained on condensation with methyl 1-TMS-1,2,4-triazole-3-carboxylate[15] under a variety of standard conditions (TMSOTf or $SnCl_4$ in MeCN and CH_2Cl_2). In the most successful procedure (bromide **7**/$SnCl_4$/MeCN/24 hrs), it was possible to isolate the major nucleoside product (**8**)[16] after deprotection (NH_3/MeOH) and HPLC purification (10% overall yield from **7**). It was not possible to assign the stereochemistry using ^{1}H NOE difference spectroscopy. The α configuration was assigned on the basis of coupling constants ($J_{H1'-F}$=0 Hz, $J_{H1'-H2'}$=9Hz) and confirmed on comparison with the β-anomer. The regiochemistry was assigned on the basis of the chemical shifts of the H5 proton (9.1ppm) and the C5 carbon (144ppm) which compare well with literature values for attachment at N1[17]. Recent biochemical studies with nucleoside 2'-deoxyribosyltransferase (EC 2.4.2.6. transferase) showed this enzyme to have broad substrate specificity [18] which has been exploited for nucleoside analog synthesis [19]. 1,2,4-triazole-3-carboxamide and **3** were found to be substrates for transferase, thus providing an efficient, stereospecific synthesis of the target molecule (**1**), (scheme 2). Purification of **1** from the crude reaction mixture was difficult due to its polar nature, even by HPLC. Following acetylation of the crude mixture, purification as the di-acetate (**9**) was straightforward by column chromatography.

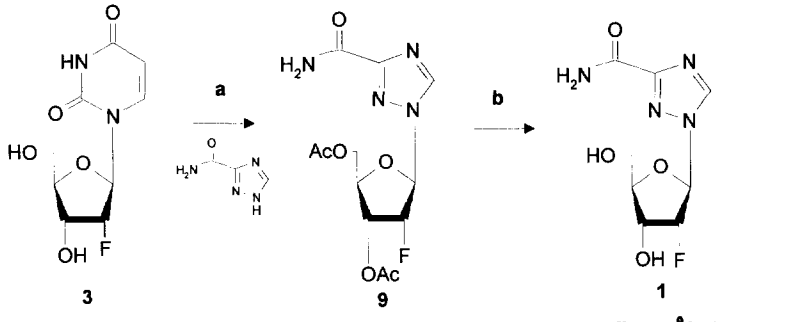
Exposure of **9** to NH_3/MeOH provided **1**[20] as the pure β-anomer in 25% overall yield. Coupling constants are typical for a 2'-deoxy-2'-fluoro-β-D-ribonucleoside ($J_{H1'-F}$= 15Hz, $J_{H1'-H2'}$ = 2Hz)[7].

Scheme 1



Neither **1** nor **8** were active against influenza A and B viruses up to 100μM in MDCK cells. They also had no antiviral activity against HCMV, HSV-2, VZV, and HIV-1, and are presumably not substrates for viral or mammalian kinases.

Scheme 2



Acknowledgements : We wish to thank Dr Margaret Tisdale, Dr Stuart Russell and John Selway for the biological results, and the Department of Physical Sciences for analytical data, especially Mrs Jan Gillam for her excellent NMR assistance.

References and Notes :

1. Murphy, B.R; Webster, R,G., Virology; Fields,B.N.,Ed; Raven Press, New York.**1985**, pp. 1179-1239.
2. Van Voris, L.P; Betts, R.F; Hayden, F.G; Christmas,W.A; Douglas, Jr., R.G., *J.Amer. Med.Assoc.*, **1981**, 245, 1128.
3. Hayden, F,G; Cote, K.M; Douglas, Jr., R.G., *Antimicrob. Agents Chemotherapy*, **1980**, 17, 865.
4. Hayden, F.G; Belshe, R.D; Clover, A.J; Oakes, M.G; Soo,W., *N. Engl. J. Med.*, **1989**, 321, 1696.
5. Smith, C.B; Charette, R.B; Fox, J.P; Cooney, M.K; Hall, C.B., *J. Infect. Dis.* **1980**, 141, 548.
6. Toto, Y; McCracken, E.A., *J. Infect. Dis.*, **1976**, 133 (suppl), A109.
7. Tuttle, J.V; Tisdale, M; Krenitsky, T.A., *J. Med Chem.*, **1993**, 36, 119.
8. Tisdale, M; Appleyard, G; Tuttle, J.V; Nelson, D.J; Nusinoff-Lehrman, S; Al Nakib, W; Stables, J.N; Purifoy, D.J.M; Powell,K.L; Darby, G., *Antiviral Chem and Chemotherapy,* **1993**, 4, 281.
9. Ikehara, M,. *Heterocycles*, **1984**, 21, 75.
10. Prusiner, P; Sundaralingam, M., *Acta Cryst.*, **1976**, B32, 419.
11. Codington, J.F; Doerr, I.L; Fox, J.J., *J. Org Chem.*, **1964**, 29, 558
12. Prepared quantitatively from methly 1,2,4-triazole-3-carboxylate using NH_3/MeOH, m.p. >300° C.
13. Selected data for **5**: m.p.190-191° C, ^{1}H NMR($CDCl_3$) 8.0(4H,2xd,Ph), 7.9(2H,d,Ph), 7.5-7.3(6H,m,Ph), 6.6(1H,2xd,H1, $J_{H\text{-}F}$=6Hz, $J_{H1\text{-}H2}$=2Hz), 5.7(1H,2xd,H3,$J_{H3\text{-}F}$= 26Hz, $J_{H2\text{-}H3}$=2Hz), 5.6(1H,m,H4), 4.95(1H,3xd,H2, $J_{H2\text{-}F}$=48Hz, $J_{H2\text{-}H3}$= 2Hz, $J_{H2\text{-}H1}$=2Hz), 4.2(2H,m,H5). NOE-irradiation of H1 showed positive enhancement of one H5 proton.
14. Thomas, H.J; Tiwari, K,N; Clayton, S.J; Secrist III, J.A; Montgomery, J.A., *Nucleosides and Nucleotides*, **1994**, 13, 309. Mikhailopuol, I.A; Grigorii, G.S; Poopeiko, N,E; Khripach, N.B., *Carbohydrate Research*, **1995**, 71.
15. Witkowski J.T; Robins R.K; Sidwell R.W; Simon L.N., *J.Med. Chem.*, **1972**,1150.
16. Selected data for **8**: m.p.126-127°C, ^{1}H NMR(d_6-DMSO) 9.1(1H,s,H5), 8.4(1H,bs,NH) 8.0(1H,bs,NH), 6.6(1H,d,H1', $J_{H1'\text{-}F}$~0Hz,$J_{H1'\text{-}H2'}$=9Hz), 5.6(1H,bs,OH), 5.2(1H,bs,OH), 5.0(1H,3xd,H2', $J_{H2'\text{-}F}$=57Hz, $J_{H2'\text{-}H3'}$=2Hz), 4.25(1H,m, H3', $J_{H3'\text{-}F}$=10Hz), 3.7(3H,m,H4' and H5'). ^{13}C NMR(d_6-DMSO) 158(C=O), 146(C3), 144 (C5), 90(C2'), 78(C1'), 68.5(C3'), 66(C4'), 65.5(C5').
17. Kreishman, G.P; Witkowski, J.T; Robins, R.K; Schweizer,M.P., *J. Amer. Chem. Soc.*, **1972**, 94, 5894.
18. Porter, D.J.T; Short, S.A., *J.Biol. Chem.* **1995**, 270, 15557.
19. Freeman, G.A; Shaver, S.R; Rideout, J.L; Short, S.A; *Bioorganic and Medicinal Chemistry,* **1995** , 3, 447.
20. Selected data for **1**: m.p. 162-163° C, ^{1}H NMR(d_6-DMSO) 8.9(1H,s,H5), 7.9(1H,bs,NH), 7.65(1H,bs,NH), 6.3(1H,2xd,H1', $J_{H1'\text{-}F}$=15Hz, $J_{H1'\text{-}H2'}$=2Hz), 5.7(1H,d,3'-OH), 5.2(1H,3xd,H2',$J_{H2'\text{-}F}$=54Hz, $J_{H2'\text{-}H3'}$=5Hz, $J_{H1'\text{-}H2'}$=2Hz), 5.0(1H,t,5'-OH), 4.45(1H,m,H3'), 4.0(1H,m,H4'), 3.6(2H,m,H5').

(*Received in Belgium* 31 *July* 1996; *accepted* 21 *October* 1996)