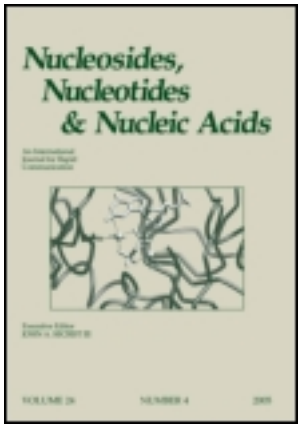

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THE SYNTHESIS AND ANTIVIRAL ACTIVITY OF 4-FLUORO-1-β-D-RIBOFURANOSYL-1H-PYRAZOLE-3-CARBOXAMIDE

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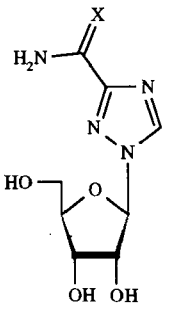
ABSTRACT

A novel fluoropyrazole ribonucleoside has been shown to have significant anti-influenza activity *in vitro*. The compound is compared and contrasted with the structurally-related compound ribavirin in attempts to identify factors having significant bearing on the mode of action of both compounds.

Introduction

Ribavirin (**1**), (Virazole : 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide) is a broad spectrum antiviral agent which exhibits potent activity both *in vitro* and *in vivo* against a wide range of DNA and RNA viruses.[1,2] Its spectrum of activity includes influenza A and B although it has been approved only for the therapy of severe infections in infants caused by respiratory syncytial virus.

The mode of action of ribavirin has been the subject of considerable research in attempts to find insights which would, in particular, aid the design of a more selective anti-influenza agent. A number of components of the mechanism of action of ribavirin which may contribute to its activity have been identified. It is known that ribavirin is readily phosphorylated to the monophosphate by adenosine kinase[3] and that other cellular kinases mediate further phosphorylation to the triphosphate. The monophosphate is a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH) which has the effect of reducing GTP levels.[4] This is a possible mechanism of toxicity in addition to an unproven contribution to activity. Indeed, in earlier work by our group we have shown[5] that carbocyclic analogues of ribavirin which are potent inhibitors of IMPDH did not possess antiviral activity. Ribavirin triphosphate competes with GTP responsible for initiation of capping of viral mRNA by inhibition of guanylyl transferase and N7 methyltransferase[6,7] which again is a host-cell process and may account for toxicity in addition to any possible contribution to activity. Finally, ribavirin triphosphate is an inhibitor of the RNA polymerases of influenza A and B.[6,8] A precise role for each of these actions or indeed the critical nature of a specific combination of them possibly together with other as yet undefined effects has not yet been elucidated.



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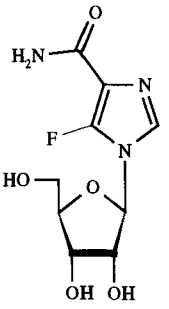
2 X=NH



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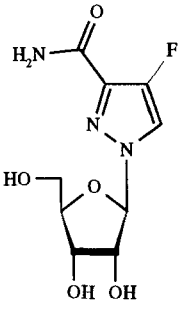

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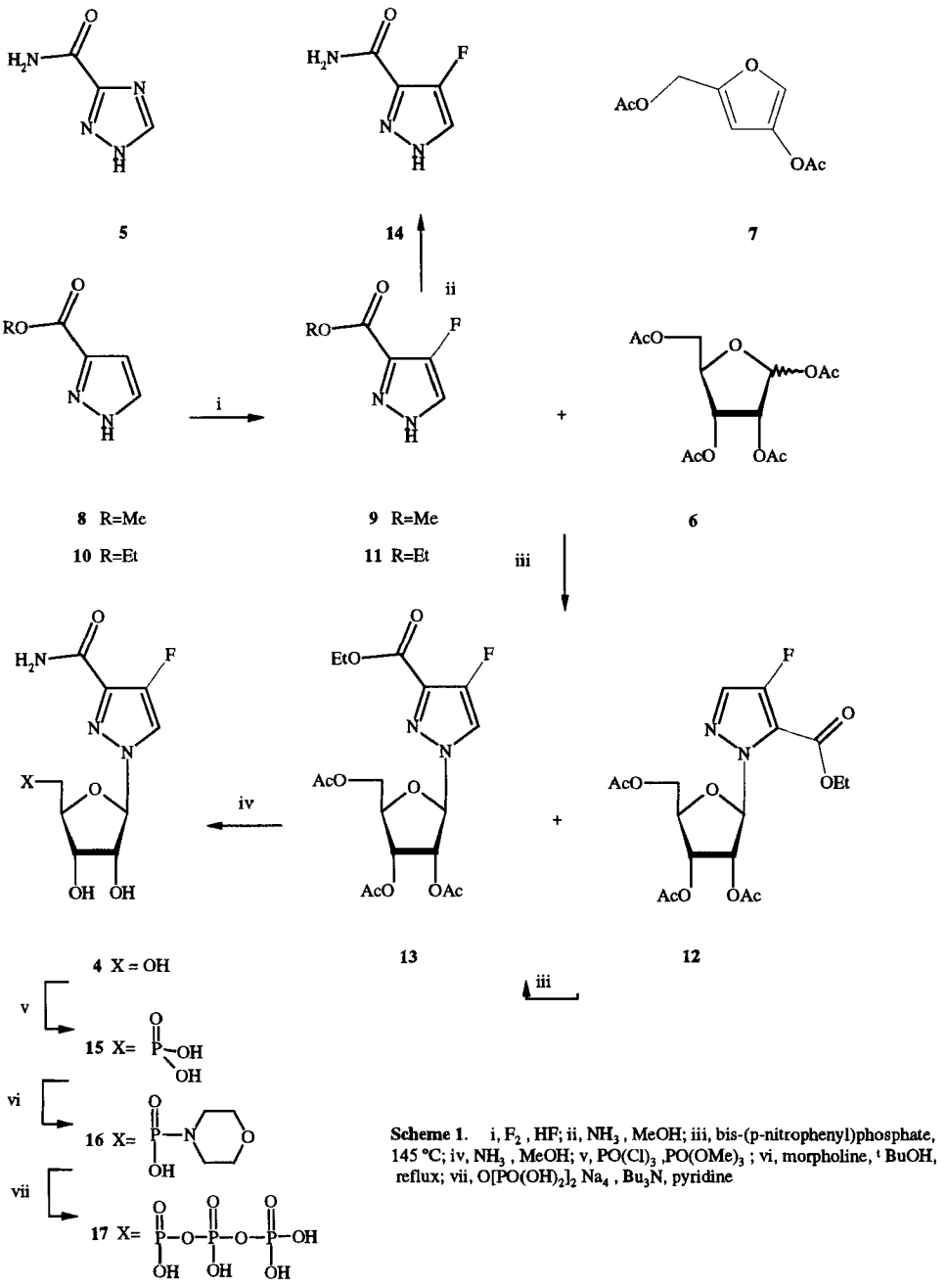
A wide variety of modifications to the structure of ribavirin have been described and the resulting compounds have been found to have either no, or in a very few cases, significantly reduced antiviral activity. Even fairly modest changes such as replacement of oxygen in the sugar ring by sulphur[9] or replacement of the carboxamide by the

corresponding thioamide[10] have not been well tolerated. The carboxamidine (**2**) has been reported to retain some of the activity of the parent although in this case it has not been rigorously established whether the compound is serving as a prodrug for the carboxamide or has a distinct antiviral mechanism of action in its own right.[11] Interestingly, a compound which has been reported to retain broad spectrum antiviral activity is that in which N2 has been replaced by C-F (**3**).[12] We noted that this modification had not been described for the N4 position of ribavirin (**4**). A variety of related 4-deaza 4-substituted analogues of ribavirin have been described previously although none has been reported to have antiviral activity.[13,14]

Considerable efforts have been applied to studying the conformations of ribavirin.[15] It has been suggested that an important feature of ribavirin is the ability of the base to mimic both adenine or guanine by rotation about the single bond which attaches the carboxamide moiety to the triazole ring. Energy calculations suggested that there was little difference in energy (1.9 Kcal./mol.) between the guanine conformer and the more favoured adenine conformer. Despite this, in the crystal structure of ribavirin which has two conformers V1 and V2 in the unit cell, in each of these it is the guanine conformer which is present. Calculations on the compound (**4**) bearing the replacement of C-F for N4 were interesting in that they suggested it would be the guanine-like conformation which was more stable by 1.15 Kcal./mol. although that figure again suggested that both conformers would be populated.[16] The fact that there is a change-over between the predicted lowest energy forms could manifest itself in more guanine-like mimicry in the fluoropyrazole (**4**). The potential to effect such a subtle difference in a series so intolerant of structural modifications prompted us to attempt a synthesis of (**4**). In the event that the compound proved to have an interesting level of activity, we hoped to be able to examine the importance of the various mechanistic effects in a detailed comparison with ribavirin.

Chemistry

The target structure (**4**) represented a novel class of nucleoside analogues. We had previously developed a synthesis of ribavirin itself which allowed incorporation of the readily available triazole carboxamide base (**5**)[17] as an intact unit. The method employed



Scheme 1. i, F_2 , HF; ii, NH_3 , MeOH; iii, bis-(p-nitrophenyl)phosphate, 145 °C; iv, NH_3 , MeOH; v, $PO(Cl)_3$,$PO(OMe)_3$; vi, morpholine, ^{t} BuOH, reflux; vii, $O[PO(OH)_2]_2$ Na_4 , Bu_3N, pyridine

condensation of the base with ribose tetraacetate (**6**) in the presence of *bis*-(p-nitrophenyl)phosphate at 150° under reduced pressure as the key step. At higher temperatures the known furan derivative (**7**) derived from ribose tetraacetate by a double elimination was the only isolable product. We anticipated that it would be possible to use a 4-fluoro-1H-pyrazole in a similar condensation with ribose tetraacetate as a key stage in the synthesis of our target structure.

It had been reported that direct fluorination of 3-methoxycarbonylpyrazole[18] (**8**) with molecular fluorine in acetic acid as solvent at 20° led to a modest conversion to the 4-fluoro derivative[19] (**9**). Our attempts to repeat this procedure or to employ trifluoroacetic acid as an alternative led to violent reactions from which no product could be isolated. However, when a stream of elementary fluorine diluted in nitrogen was passed slowly into a solution of 3-methoxycarbonylpyrazole in anhydrous HF as solvent at -20°, a controlled reaction afforded the required product (**9**) in good yield.[20] Physical data were consistent with the required structure and agreed with those reported by the Russian workers.[19] Fluorination of the corresponding ethyl ester (**10**) under similar conditions also worked well to yield (**11**) and allowed us to take advantage of the ease of preparation of (**10**) relative to (**8**).

The coupling reaction described above was applied to the pyrazole ester (**11**) and afforded a mixture of the regioisomeric products (**12**) and (**13**) in which the required N1 product (**13**) was predominant in an approximate ratio of 2:1. The products were separated chromatographically. Further treatment of the undesired N2 isomer (**12**) with *bis*-(p-nitrophenyl)phosphate at 145° for 15 min. afforded a 68% conversion to the desired N1 isomer in giving a similar equilibrium mixture of (**12**) and (**13**) to that obtained initially. Further similar examples of N2 to N1 isomerisation were observed in related pyrazole work and will be described in later papers. Treatment of (**13**) with ammonia in methanol resulted in conversion of the ester to the carboxamide and also effected removal of the acetate groups to afford a good yield of the required product (**4**). Treatment of the pyrazole ester (**11**) with ammonia gave the corresponding carboxamide (**14**). In view of the success of the above procedure, coupling of (**14**) which would have required one additional step overall was not attempted.

TABLE 1

Activity I_{50}(μg/ml) in cell based assays for inhibition of influenza A and B.

	Influenza A Singapore		Influenza B Victoria
	Direct FLISA	Plaque Reduction	Plaque Reduction
Ribavirin	10	3.2	3.2
Compound (4)	3	0.4	0.2

The 5′-monophosphate (**15**) was obtained using a procedures similar to that we have described previously[21] and the triphosphate (**17**) was prepared *via* the morpholidate (**16**).

Biology

In vitro activity

The fluoropyrazole nucleoside (**4**) was found to have excellent activity against influenza A and B *in vitro.* (Table 1). To our knowledge, this compound is the first analogue of ribavirin which has been found to display selective *in vitro* activity superior to ribavirin itself against influenza. The triazole carboxamide base (**5**) of ribavirin shows some antiviral activity *in vitro* which is presumed to result from conversion into ribavirin by a phosphoribosyltransferase. The corresponding fluoropyrazole carboxamide (**14**) did not show any activity and is presumably not a substrate for for such an enzyme.

Compound (**4**) was evaluated *in vitro* against a range of other RNA and DNA viruses including HIV and Herpes Simplex type I but it displayed no activity. Initial cytotoxicity studies with (**4**) in a range of cell lines indicated lack of overt toxicity and superior profile to ribavirin (Table 2).

In vivo activity

Preliminary studies with the pyrazole (**4**) in the mouse model indicated weak anti-influenza activity at 12.5 mg/kg using intranasal administration but the compound was toxic

TABLE 2

Cytotoxicity evaluation in different cell lines (I_{50}μg/ml).

	Cell Line			
	MDCK	L1210	Vero	CEM
Ribavirin	2	1.5	300	2
Compound (4)	>50	15	>100	100

at 25 mg/kg. It was found to be inactive at 20 mg/kg when administered by the i.p. or p.o. routes. Further *in vivo* studies were carried out to investigate anti-influenza A activity in the ferret model. Intranasal instillation of influenza A (Singapore strain) was made while the animals were under isoflurane sedation. Ferrets were nasal-washed once daily on days 0-7 to determine the nasal wash viral titres which were measured by an ELISA method. The ferrets were dosed by the i.p. route at 24 hrs and 2 hrs pre-infection, 6hrs post-infection and on days 1-5 twice daily with the pyrazole (**4**) or ribavirin at 25 or 12.5 mg/kg. Control animals were dosed with distilled water. Whilst ribavirin showed good activity at both doses, the pyrazole (**4**) was not active at either dose and higher doses were not tolerated.

We examined the extent to which the compounds were absorbed by investigating urinary recoveries of both compounds. Ferrets were given ribavirin at 100 mg/kg i.p. or p.o. or the pyrazole at 20 mg/kg s.c. or p.o. Urinary recoveries for each compound by each route of administration were comparable and in the range 30-43% showing good bioavailability although the availability of compound at the site of infection was obviously not determined.

Biochemical studies

We conducted further studies to try and determine the extent to which the above results could be explained by findings from investigation of the mechanism of the compound in comparison with ribavirin. We first investigated the extent to which each was 'phosphorylated' by adenosine kinase using the procedure indicated earlier.[5] The pyrazole

(**4**) proved to be an excellent substrate for adenosine kinase. Whilst ribavirin was found to be phopshorylated to the extent of 112% compared with adenosine, the corresponding figure for the (**4**) was 228%.

Our next study was to measure the extent to which the monophosphates of ribavirin and the fluoropyrazole inhibited IMPDH. Two assays developed to measure this were indicated in an earlier paper.[5] The first employed IMPDH purified from E. coli and required synthesis of the appropriate monophosphates whilst a later mouse L1210 cell-based assay allowed direct evaluation of the nucleosides. Results confirmed our earlier reported findings that ribavirin monophosphate is a very potent inhibitor of the isolated enzyme (IC_{50} 0.48μM) and that ribavirin shows activity in the whole cell assay (IC_{50} 8.5μM). Under identical conditions, although (**4**) showed some activity in the whole cell assay (IC_{50} 55μM), its monophosphate (**14**) was essentially inactive in the enzyme assay with (IC_{50} 0.7mM). Thus the pyrazole (**4**) proved to be significantly less potent than ribavirin as an inhibitor of IMPDH clearly demonstrating support for the view that potent inhibition of that enzyme is not a prerequisite for antiviral activity. This result is complementary to our earlier findings with some carbocyclic analogues of ribavirin where we identified compounds which showed no antiviral activity despite being potent inhibitors of this enzyme.

Ribavirin triphosphate is only a weak inhibitor of the influenza A virus RNA polymerase (Ki ~85-200μM) and its antiviral activity may well result from the high levels of triphosphate which accumulate in cells. The triphosphate (**17**) of the fluoropyrazole nucleoside proved to have a similar level of potency as an inhibitor of the polymerase (75-130μM) but it is possible that it does not accumulate to the same extent as does ribavirin triphosphate and that this is the reason for the lack of activity observed *in vivo*.

Experimental

4-Fluoro-1H-pyrazole-3-carboxylic acid methyl ester (9)

A stream of elementary fluorine diluted (1:1) with nitrogen (1.5L/hr) was passed into a solution of pyrazole (**8**) (10g) in anhydrous hydrogen fluoride (50ml) cooled at -20° for 4

hrs. The mixture was allowed to attain room temperature over 12 hrs during which time all excess hydrogen fluoride evaporated to leave a pale yellow solid. Recrystallisation from methanol / water afforded the title product (5.6g, 49 %) m.p. 169-171^0 (lit. [19] 172-173^0). Physical data agreed well with those reported.[19]

1H-Pyrazole-3-carboxylic acid ethyl ester (10)

A solution of diazomethane (prepared by the Diazald method) (ca. 0.11 mol) in ether (300ml) was stirred at room temperature whilst ethyl propiolate (9.77g, 0.099 mol) in ether (50ml) was added dropwise. The colourless precipitate that rapidly formed was allowed to stand overnight. The solid was collected by filtration and dried to afford the title compound (11.66g, 100%) as a colourless solid. m.p. 163-164^0 (lit.[22] m.p. 158°); ^{1}H NMR (D_6-DMSO) δ 7.85 (1H, br s, CH-5), 6.75 (1H, s, CH-4), 4.3 (2H, q, J7, OCH_2), 1.3 (3H, t, J7, CH_3). Found: C, 51.65; H, 5.96; N, 20.03. $C_6H_8N_2O_2$ requires: C, 51.42; H, 5.75; N, 19.99%.

4-Fluoro-1H-pyrazole-3-carboxylic acid ethyl ester (11)

Fluorination of the ethyl ester was carried out in an identical fashion to that described above for the methyl ester. The product was obtained in a yield of 52% as a white solid m.p. 183-184°; ^{1}H NMR (D_6-DMSO) δ 7.91 (1H, br s, CH-5), 4.3 (2H, q, J7, OCH_2), 1.3 (3H, t, J7, CH_3); MS calc for $C_6H_7FN_2O_2$:158.1, found:159 (MH^+), 176(MNH_4^+)

4-Fluoro-1-(β-D-tri-O-acetylribofuranosyl)-1H-pyrazole-5-carboxylic acid ethyl ester (12) and 4-Fluoro-1-(β-D-tri-O-acetylribofuranosyl)-1H-pyrazole-3-carboxylic acid ethyl ester (13)

A mixture of tetraacetylribofuranose (**6**) (8.75g, 0.0275 mol), *bis*-(p-nitrophenyl)phosphate (100mg) and fluoropyrazole (**11**) (4.0g, 0.025 mol) in toluene (50ml) was heated (in an oil-bath at 145^0) to separate the solvent by distillation. The residue was evaporated (15mm) *in situ* for 5 mins. After cooling, the residue was taken up in chloroform, filtered and the filtrate absorbed onto silica gel. The products were purified by column chromatography on elution with mixtures of cyclohexane: ethylacetate: acetic acid (40:20:1,

increasing polarity to 30:30:1). The first product to be eluted, gave after evaporation, the title product (**12**) (2.87g, 27.6%) as a pale yellow oil which crystallized on standing. A portion was recrystallized from toluene: cyclohexane to afford an analytical sample m.p. 85-86.5° $[\alpha]^{22}_{D}$ -30.0°(c=1, $CHCl_3$) λ max (EtOH) 222, 235 (inf); ^1H NMR ($CDCl_3$) δ 7.51 (1H, d, J5, H-5), 6.88 (1H, d, J2, H-1′) 5.93 (1H, dd, J 5,2, H-2′), 5.73 (1H, t, J5) 4.40 (4H, m, OCH_2CH_3, H-4′ and H-5′), 4.11 (1H, dd, J5), 4.40 (4H, m, OCH_2CH_3, H-4′ and H-5′), 4.11 (1H, dd, J5), 2.14, 2.11 and 2.04 (9H 3xS, CH_3CO x3), 1.41 (3H, t, J 7, CH_3). Found: C, 49.22; H, 5.14; N, 6.71. $C_{17}H_{21}FN_2O_9$ requires: C, 49.15; H, 5.09; N, 6.75%.

Subsequent elution of the second major product afforded the title compound (**13**) (4.85g, 47%) as a pale yellow oil that did not crystallize. γ_{max} ($CHBr_3$) 1746 (w), 1584, 1499, 1443, 1371, 1229, cm^{-1} ; ^1H NMR ($CDCl_3$) δ 7.60 (1H, d, J5, H-5), 5.90 (1H, d, J4, H-1′), 5.70 (1H, t, J4 H-2′), 5.50 (1H, t, J4, H-3′), 4.41 (4H, m, CH_2, H-4′ and H-5′), 4.28 (1H, dd, J8,3, H-5′), 2.18, 2.10 (9H, 2x5, CH_3COx3) 1.40 (3H, t, J7, CH_3) ppm. MS $[MH^+]$ 416.4, $[MNa]^+$ 438.5 Found: C, 48.60; H, 5.11; N, 6.20: $C_{17}H_{21}FN_2O_9$ requires 49.15, H, 5.09; N, 6.75%.

Isomerisation of compound (12) to (13)

The N-2 pyrazole ribofuranose (**12**) (2.87g, 6.91 mmol) and *bis*-(p-nitrophenyl)phosphate (57mg) was stirred at 145^0 for 15 min. The mixture was purified by column chromatography (on silica gel, 250g) eluting with cyclohexane: ethylacetate: acetic acid (30:15:0.75) to afford the N-1 product (**13**) (1.97g, 68%) as a pale yellow oil having spectral data identical to those described above.

4-Fluoro-1H-pyrazole-3-carboxamide (14)

A solution of pyrazole ester (**11**) (0.34g, 2.36 mmol) in saturated methanolic ammonia (150ml) was sealed and allowed to stand at room temperature for 4 days. The solvent was evaporated and the residue co-evaporated with diethyl ether. The resultant colourless solid was subjected to column chromatography (50g) eluting with

dichloromethane:methanol (15:1) to afford the title product (0.29g, 95%) as a colourless solid. m.p. 185-90^0 λ_{max} (EtOH) 227.8 cm^{-1};^{1}H NMR (D_6-DMSO) δ13.2 (1H, br s, NH-1), 7.91 (1H, s, H-5), 7.4 (2H, S, NH_2). Found: C, 37.17; H, 3.06; N, 32.48. $C_4H_4N_3FO$ requires 37.20; H, 3.12; N, 32.55%.

4-Fluoro-1-(β-D-ribofuranosyl)-1H-pyrazole-3-carboxamide (4)

A solution of ester (**13**) (4.98g, 0.012 mol) in methanol (750ml) at -10^0 was saturated with ammonia and placed in a Parr stainless steel bomb. The solution was allowed to stand at room temperature for two days. Evaporation of the solution afforded a yellow gum which was crystallized from isopropyl alcohol to afford the title compound (**4**) (2.046g, 65.3%) as colourless crystals. m.p. 159-62^0 [α] d^{22} - 31.0^0 (c 1.08, H_2O) ν_{max} (nujol) 3405-2800 (OH and NH), 1657 (CO), 1600, 1579, 1384, 1301, 1060 cm^{-1}. ^{1}H NMR (D_6 -DMSO) δ 8.21 (1H, d, J_{HF}5, H-5), 7.50 (1H, bs, NH), 7.39 (1H, bs, NH), 5.56 (1H, d, J 5, H-1′), 5.51 (1H, d, J5.5, OH), 5.16 (1H, d, J5, OH), 4.97 (1H, t, J 5, H-5′ OH), 4.28 (1H, q, J 5, H-2′), 4.10 (1H, q, J5, H-3′), 3.91 (1H, q, J5, H-4′), 3.57 (2H, m, H-5′x2) ppm. Peaks at 7.50, 7.39, 5.51, 5.16 and 4.97 ppm disappeared upon addition of D_2O. C^{13}NMR(D_6-DMSO) δ 161.25 (d, J^3_{CF} 3.4, C=0), 147.54 (d, J^1 253.6, C-4) 132-47 (d, J^2_{CF} 4.5, C-3), 117.00 (d, J^2 27.7, C-5) 94.56 (C-1′), 85.26 (C-4′), 74.37 (C-2′), 69.88 (C-3′), 61.08 (C-5′) ppm. Found: C, 41.29; H, 4.68; N, 15.91; F,7.1: $C_9H_{12}F_1N_3O_5$ requires: C, 41.38; H, 4.63; N, 16.09; F, 7.27 %.

The filtrate from the crystallization was purified by silica gel column chromatography to afford more product (390mg) that was crystallized (IPA) to afford further product (260mg) bringing the total yield to73%.

4-Fluoro-1-(β-D-ribofuranosyl)-1H-pyrazole-3-carboxamide 5′-phosphate ammonium salt (15)

The nucleoside analogue (**4**) (0.4g, 1.52 mmol) was added to a solution of phosphoryl chloride (0.48ml, 5.28 mmol) in trimethyl phosphate (10ml) at 0^0 under nitrogen, and the

mixture stirred for 6 h. The resultant solution was poured into ice-water (15ml) and the pH adjusted to 2.5 with 2N sodium hydroxide. This solution was washed with chloroform (3x) and then applied to a charcoal (8g) column eluting first with water and subsequently with an ethanol: water: ammonia (10:10:1) mixture. Elution was monitored by t.l.c. and appropriate fractions (rf 0.4, MeCN: 0.1M ammonium chloride (7:3); silica gel) were combined and evaporated to leave a brown foam (413mg, 76%). Purification was effected by elution on a Sephadex A-25 (HCO_3 form, 20g) column using a gradient of 0 to 0.4M ammonium bicarbonate. Appropriate fractions were combined, concentrated and freeze-dried to afford the title product (**14**) (351mg, 85% recovery) as a white foam. ν_{max} (nujol) 3700-2700 (OH), 1669, 1459, 1376 cm^{-1}. λmax (H_2O) 227.2), ; ^1H NMR (D_2O) δ 8.14 (1H, d, J_{HF}5,H-5), 5.80 (1H, d, J6 , H-2′), 4.45 (1H, t, J6, H-3′) 4.31 (1H, m, H-4′), 3.95 (2H, m, H-5′ x 2) ppm. Found: C, 26.23; H, 5.52; N, 16.70; F, 4.3. $C_9H_{13}F_1N_3O_8P$, $2NH_3$. $2H_2O$ requires C. 26.28; H, 5.64; N, 17.03; F, 4.62 %.

4-Fluoro-1-(β-D-ribofuranosyl)-1H-pyrazole-3-carboxamide-5′-phosphoromorpholidate, triethylamine salt (16).

An aqueous (2ml) solution of monophosphate ammonium salt (**15**) (250mg. 0.07 mmol) was passed through a column of Amberlite IR-118 (H^+) (30ml) eluting with water. Appropriate fractions were combined and evaporated to yield a brown foam. The free acid was dissolved in water (9.4ml), t-butanol (9.4ml) and morpholine (0.24ml, 2.8 mmol) and the mixture was heated to reflux. Dicyclohexyl carbodiimide (0.57g, 2.8mmol) dissolved in t-butanol (13ml) was added dropwise over 2 hours, and reflux continued for a further 1.5 hours. The solution was evaporated *in vacuo* and the residue diluted with water (20ml). The resultant solid was separated by filtration and the filtrate washed with ether (3x20ml). Evaporation of the aqueous phase afforded a green-brown foam (575mg). The product was purified by passage through a column of Dowex -1 (HCO_3- form, 100 - 200 mesh, 50 ml) eluting with a gradient of 0 - 0.5M triethylammonium bicarbonate. Appropriate fractions were combined and freeze-dried to yield the title product (283mg, 79%) as a colourless foam; ^1H NMR (D_2O) δ 8.00 (1H, d, J 5 , H-5), 5.81 (1H, d, J 4.5 , H-1′) 4.70 (1H, t, J 4.5, H-2′), 4.52 (1H, t, J 4.5, H-3′), 4.30 (1H, m, H-4′), 3.98 (2H, m, H-5′x2), 3.60 (4H, m

, morpholine, CH_2O x 2), 2.95 (15H, m, morpholine CH_2N x 2, N $(CH_2CH_3)_3$) 1.15 (t, CH_3) ppm.

4-Fluoro-1-(β-D-ribofuranosyl)-1H-pyrazole-3-carboxamide-5′-triphosphate triammonium salt (17).

A solution of tetrasodium pyrophosphate decahydrate (0.94g, 2.12 mmol) in water (7.5ml) was passed through a column of Dowex 50W - X4 resin (pyridinium form, 60ml). The eluate (100ml) was collected and concentrated to ca. 10ml under reduced pressure). Pyridine (60ml) and tri-n-butylamine (2.12ml, 8.94 mmol) were added, and the resulting solution was evaporated to syrup which was dried by co-evaporation with toluene (2x10ml).

In a separate flask, the morpholidate (**16**) (273.4mg, 0.53 mmol) was dried by co-evaporation with pyridine (2x10ml) and toluene (2x10ml). The pyrophosphate tetra tri-n-butyl ammonium salt prepared above was transferred into the dried morpholidate (**15**) with dry DMSO (4x2.2ml) *via* a double-tipped needle. The resulting pale yellow solution was stirred under nitrogen for 3 days. Water (40ml) was added and the mixture passed through a sephadex A-25 column (HCO_3 form, 20g) with water washing. Elution with a gradient of 0-0.4M ammonium bicarbonate and freeze-drying gave the title product (106mg, 36%) as a colourless foam. ; ^1H NMR (D_2O) δ 8.00 (1H, d, J 5, H-5) 5.73 (1H, d, J 5.5 ,H-1′), 4.62 (1H, t, J5.5, H-2′), 4.49 (1H, t, J 5.5,H-3′), 4.29 (1H, m, H-4′), 4.12 (2H, m, H-5′ x 2) ppm δ^P (D_2O - 8.5 (8P), - 10.5 (αP), - 21.9 (βP)ppm. Found: C, 17.83; H, 4.98; N, 14.24 $C_9H_{13}FN_3O_8P_3$. $3NH_3$. $3H_2O$ requires C, 17.83; H, 4.99, N, 13.86%.

Conclusions

The work described herein demonstrates that it is possible to have potent *in-vitro* antiviral activity in the absence of potent inhibition of IMPDH. These results complement earlier findings that inhibition of IMPDH does not necessarily result in antiviral activity. The reasons for the poor level of *in vivo* activity in this case are not clear and more work will be required to investigate this. The synthesis, conformational studies and biological properties of further 4-substituted pyrazole nucleoside analogues in this series will be described in future papers.

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