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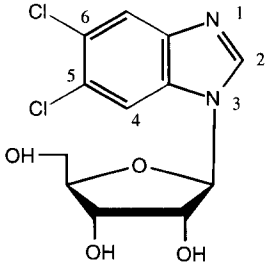
3-[(3'-HYDROXYMETHYL)-4'-HYDROXYBUTYL]IMIDAZO[4,5-*b*]PYRIDINES—NOVEL ANTIVIRAL AGENTS

Darren J. Cundy,* George Holan, Michelle Otaegui, and Gregory W. Simpson

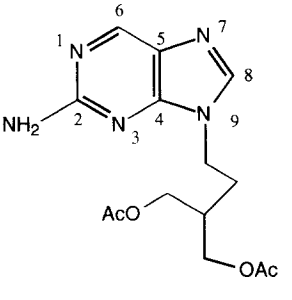
CSIRO: Division of Chemical and Polymers, Private Bag 10, South Clayton MDC, Clayton Victoria 3122 Australia

Abstract. Derivatives of 3- and 1-(4'-hydroxy-3'-(hydroxymethyl)butyl)-imidazo[4,5-*b*]pyridine were prepared in several steps from 2-amino-5-chloropyridine. Selected compounds were evaluated against human cytomeglovirus (HCMV), herpes simplex virusus (HSV1/HSV2) and varicella zoster virus (VZV). Details of their synthesis and biological activities are presented.

Synthetic nucleosides have been investigated as antiviral agents over the past 40 years. These studies have demonstrated[1] that the stability of these materials toward the major pathways of nucleoside inactivation, e.g., deamination by adenosine deaminase and glycosidic cleavage by nucleoside phosphorylases, is an important factor in the design of therapeutic agents. For these reasons, benzimidazole based nucleosides have been prepared and evaluated[2,3] as antiviral drugs. Derivatives of 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB) (**1**), for example, have been screened against viral pathogens such as human cytomegalovirus (HCMV) and herpes simplex viruses 1 and 2 (HSV1 and HSV2).[1,4] However in many cases their low in vivo activity and/or levels of cytotoxicity have diminished their usefulness in the treatment of viral infection. The acyclonucleoside famciclovir (**2**) has, however, demonstrated excellent antiviral activity against the hepatitis B (HBV) and HSV viruses.[5] This antiviral possesses a 2-aminopurine heterocycle and a 4'-acetoxy-3'-(acetoxymethyl)butyl residue. We postulated that hybrid compounds, which included structural features of compounds **1** and **2** would be interesting candidates for screening against a spectrum of DNA viruses.



1



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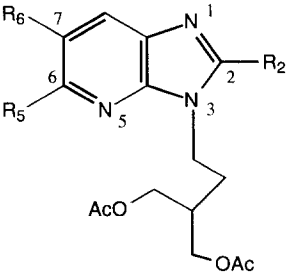


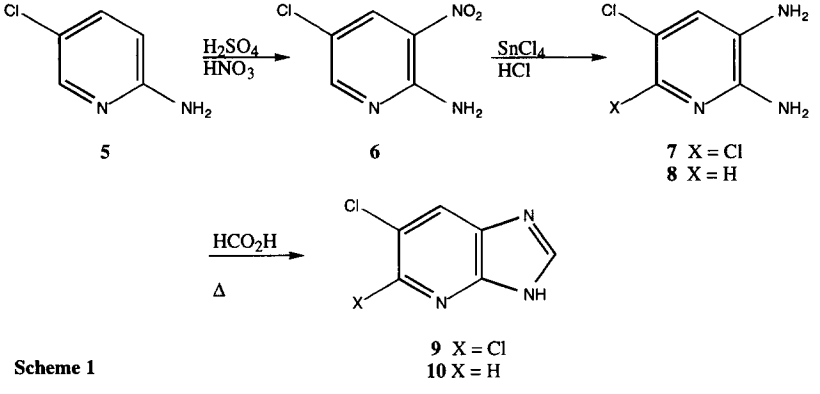
3 : $R_5 = R_6 = Cl$, $R_2 = H$
4 : $R_5 = NH_2$, $R_6 = Cl$, $R_2 = CF_3$

Synthetic nucleosides containing the 7-amino-imidazo[4,5-*b*]pyridine nucleus (i.e., the 1-deazapurines) have already been employed in numerous chemotherapeutic applications.[6] However, the less accessible

glycosides and acyclonucleoside derivatives of halo-substituted imidazo[4,5-*b*]pyridines (which lack an amino substitutent in the 7-position) are reported[7] much less frequently. Thus, we undertook to prepare the *N*-alkylated imidazo[4,5-*b*]pyridines (**3** and **4**), as these targets incorporated the halogen and/or the α-amino substituents present in heterocyclic components of **1** or **2** and the modified glycone residue of famciclovir.

Synthesis

Although the preparation of 5,6-dichloroimidazo[4,5-*b*]pyridine (**9**) had not been previously described, we expected that cyclization of the appropriate 2,3-diaminopyridine with formic acid would give us access to the required heterocycle. Following the general method of Vaughan et al.[8] we treated 2-amino-5-chloropyridine (**5**) with a mixture of sulfuric and nitric acid and obtained 2-amino-5-chloro-3-nitropyridine (**6**) in 57% yield. A one-pot reductive chlorination procedure described by Israel and Day[9] yielded a mixture containing mostly 2,3-diamino-5,6-dichloropyridine (**7**), as well as smaller amount of 2,3-diamino-5-chloropyridine (**8**). The cyclization of **8** with formic acid had been reported[8] to proceed at room temperature, however we found that both **7** and **8** only yielded the corresponding imidazo[4,5-*b*]pyridines (**9**[10] and **10**[8]) after an extended period of reflux. (Scheme 1).



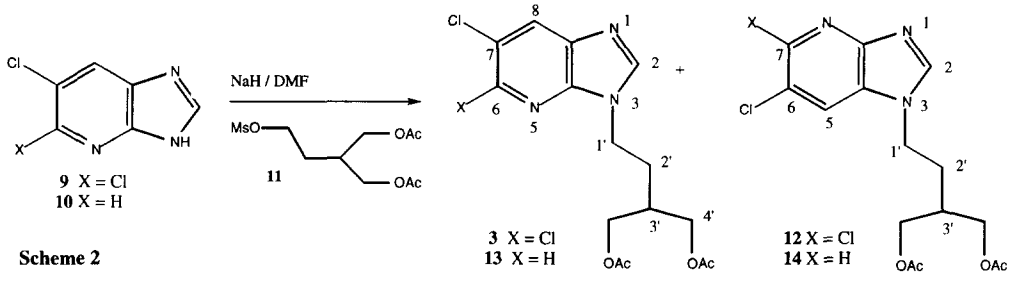
5 **6** **7** X = Cl, **8** X = H

9 X = Cl, **10** X = H

Scheme 1

Alkylation of similarly substituted benzimidazoles with the 4-acetoxy-3-(acetoxymethyl)butyl moiety had previously been accomplished[11] by treatment of the heterocycle with the mesylate (**11**) in DMF in the presence of potassium carbonate. However, we found that the reactions of **9** and **10** with **11** proceeded poorly under those conditions and found it necessary to follow a protocol[12] that first deprotonated the heterocycle with sodium hydride. Under these alkylation conditions, the tautomeric nature of the deprotonated imidazopyridine nucleus led to a mixture of regioisomers as product. Thus, alkylation of **9** yielded a mixture of compounds **3**[13] and **12**[13] while **10** afforded the regioisomers **13**[14] and **14**[14] (Scheme 2). The downfield steric effect of *N*-alkylation observed for H-5 (δ = 8.25 ppm) in the ^{1}H NMR spectrum of compound **12** was used to assign the regiochemistry. The corresponding hydrogen atom in compound **3**, H-8, (being more remote from the site of alkylation) appearing at δ = 8.10 ppm. Under the described conditions, the yield of compound **3** was approximately three times that of compound **12**. Based on a similar outcome for the alkylation reaction of

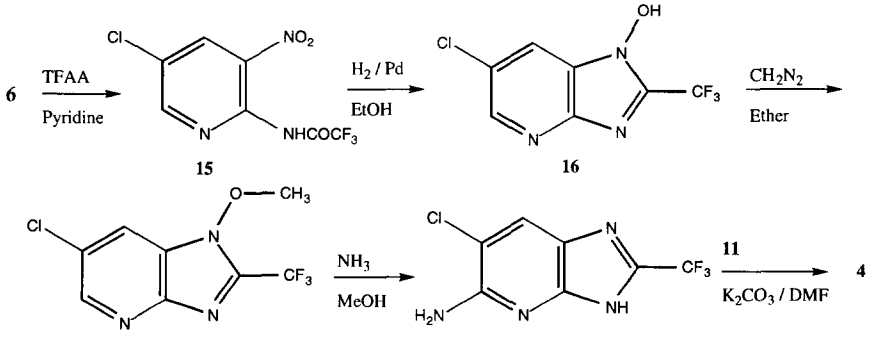
compound **10**, the major product was assigned structure **13**, and the minor component designated as compound **14**, however in each case the regiochemistry was not rigorously determined.



Scheme 2

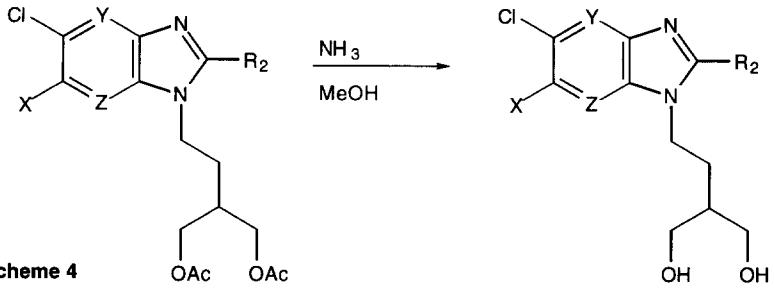
The synthesis of **4** first required the preparation of 5-amino-6-chloro-2-(trifluoromethyl)-imidazo[4,5-*b*]pyridine (**18**). The synthesis of **18** has been described in the patent literature.[15] Thus, **6** was treated with trifluoroacetic anhydride in pyridine to give the corresponding amide (**15**) in 83% yield. In an interesting one-pot reductive cyclisation, hydrogenation of **15** with palladium on carbon gave the *N*-hydroxy intermediate (**16**) in 67% yield. Reaction of **16** with diazomethane gave the *O*-methyl adduct (**17**), which could be rearranged with concomitant introduction of the amino group[16] into the 5-position to afford the required heterocycle (**18**) in 75% yield.

Treatment of **18** with sodium hydride followed by quenching with **11** did not, however, yield the alkylation adduct but rather, a poor recovery of starting materials. Reverting to the original alkylation conditions, reaction of **18** with **11** in the presence of potassium carbonate afforded **4**.[17] Notably neither alkylation of the free amino group nor formation of a second regioisomer was detected. (Scheme 3).



Scheme 3

In the case that compounds **3**, **12**, or **4** possesed intrinsic activity it is likely they would behave as prodrugs to their corresponding hydrolysis products. In order to obviate the requirement for cellular esterases in the screening assays, the diacetoxy groups were hydrolysed prior to evaluation. Reaction of the respective compounds with methanolic ammonia, followed by high vacuum distillation of the acetamide by-product afforded the diols (**19**,[18] **20**,[19] and **21**[20]) in good yield. (Scheme 4).



Scheme 4

3 Z = N, Y = C, X = Cl, R_2 = H
12 Z = C, Y = N, X = Cl, R_2 = H
4 Z = N, Y = C, X = NH_2, R_2 = CF_3

19 Z = N, Y = C, X = Cl, R_2 = H
20 Z = C, Y = N, X = Cl, R_2 = H
21 Z = N, Y = C, X= NH_2, R_2 = CF_3

Biological Results and Discussion

The diacetoxy compound **3** was only evaluated for activity against HCMV, whereas the diols **19–21** were screened separately. The antiviral activities are presented in Table 1.

Table 1. Antiviral Activity

	HSV-1 CPE Inhib.			HSV-1 Plq. Red'n			HSV-2 CPE Inhib.			HSV-2 Plq. Red'n			HCMV CPE Inhib.			VZV Plq. Red'n		
	E	C	SI	E	C	SI	E	C	SI	E	C	SI	E	C	SI	E	C	SI
3[a]													132.2					
19[b]	>100	>100	0				>100	>100	0				>100	>100	0	11.7	>100	>8.5
20[b]	>100	>100	0				>100	>100	0				>100	>100	0	49.8	>100	>2.0
21[b]	1.2	>100	>83	>100	>100	0	>100	>100	0	>100	>100	0	>100	>100	0			
ACV	0.06			0.3			0.2			1.1						0.6		
GCV													0.1					

[a]Evaluated at ViroMed Laboratories Minneapolis; [b]Evaluated at the University of Birmingham Alabama; **HSV-1** = Herpes Simplex Virus 1; **HSV-2** = Herpes Simplex 2; **HCMV** = Human Cytomeglovirus; **CPE Inhib.** = Cytopathetic Effect Inhibition; **Plq red'n** = Viral Plaque reduction; **E** = $[EC]_{50}$ Effective Concentration (μg/mL) required to inhibit virus proliferation by 50%; **C** = $[CC]_{50}$ Cytotoxic Concentration (μg/mL) required to reduced human embryonic lung cells by 50%; **SI** = Selectivity index =CC_{50}/EC_{50}; **ACV** = Acyclovir; **GCV** = Gancyclovir.

Discussion

In was anticipated that the substitution of an additional nitrogen in the ring in a position concordant with the N^1-nitrogen of purine bases might aid the incorporation of the target compounds into viral DNA. However, the decrease of antiviral activity (particularly against HCMV) of the *N*-alkylated imidazopyridines suggests that this was not effective. However there is also the possibility that phosphokinases failed to recognise the glycosyl unit, precluding phosphorylation and incorporation. Overall then, this may have decreased recognition and outweighed the activity enhancement which might have been expected from the additional ring nitrogen. It is also noteworthy that the activity of compound **19** against VZV was unexpected.

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10. **5,6-Dichloroimidazo[4,5-*b*]pyridine (9)**. A mixture of formic acid (98%, 2.5 mL) and **7** (0.50 g, 2.80 mmol) was heated at reflux overnight. Excess formic acid was removed in vacuo and the residue crystallized from aqueous methanol to yield a pale-yellow solid. This was further purified by column chromatography on silica (ethyl acetate) and the appropriate fractions concentrated to afford a colourless powder (0.20 g, 38%). mp 273 °C, ^{1}H NMR (DMSO-d_6) δ 8.40, (s, 1H, ArH), 8.60 (s, 1H, NH). MS CI(+ve) *m/z* 188, 190, 192 ($M+H^+$).
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13. **3-[4′-Acetoxy-3′-(acetoxymethyl)butyl]-6,7-dichloroimidazo[4,5-*b*]pyridine (3) and 1-[4′-acetoxy-3′-(acetoxymethyl)butyl]-6,7-dichloroimidazo[4,5-*b*]pyridine (12)**. Sodium hydride (60% disp. in oil) (0.055 g, 1.37 mmol) was added in a single portion to **9** (0.235 g, 1.25 mmol) in dry DMF (7 mL). After effervescence had ceased (20 min) **11** (0.71 g, 2.50 mmol) in DMF (3 mL) was added and the mixture stirred overnight. The mixture was concentrated in vacuo and the residue partitioned between chloroform (50 mL) and water (30 mL). The organic layer was washed with brine (30 mL) dried over $CaCl_2$ and concentrated in vacuo to an oil. This was purified by HPLC to yield **3** (120 mg, 26%), ^{1}H NMR ($CDCl_3$) δ 1.90 (m, 3H, $C\underline{H}_2C\underline{H}(CH_2)_2$), 2.00 (s, 6H, 2×C(O)$CH_3$), 4.05 (d, J = 5.0 Hz, $(OC\underline{H}_2)_2CH$), 4.30 (t, J = 6.2 Hz, 2H, $NC\underline{H}_2CH_2$), 8.05 (s, 1H, NCHN), 8.10 (s, 1H, H-8); MS CI(+ve) *m/z* 374, 376, 378 ($M+H^+$) and **12** (38 mg, 8%). ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 3H, $C\underline{H}_2C\underline{H}(CH_2)_2$), 2.05 (s, 6H, 2×C(O)$CH_3$), 4.10 (m, $(OC\underline{H}_2)_2CH$), 4.30 (t, J = 6.3 Hz, 2H, $NC\underline{H}_2CH_2$), 7.90 (s, 1H, NCHN), 8.25 (s, 1H, H-5).
14. **3-[4'-Acetoxy-3'-(acetoxymethyl)butyl]-7-chloroimidazo[4,5-*b*]pyridine (13) and 1-[4'-acetoxy-3'-(acetoxymethyl)butyl]-6-chloroimidazo[4,5-*b*]pyridine (14)**. A solution of **10** (0.115 g, 0.75 mmol) was treated with sodium hydride (0.036 g, 0.90 mmol) and **11** (0.212 g, 1.5 mmol) as in the preceding example to yield an oil that was purified by HPLC to yield **13** (56 mg, 22%) as an oil, ^{1}H NMR ($CDCl_3$)

δ 1.99 (m, 3H, C<u>H</u>C<u>H</u>$_2$), 2.02 (s, 6H, 2×C(O)C<u>H</u>$_3$), 4.11 (d, J = 4.0 Hz, 4H, 2×OC<u>H</u>$_2$), 4.41 (t, J = 6.8 Hz, 2H, NC<u>H</u>$_2$), 8.07 (s, 1H, NCHN), 8.30 (s, 1H, ArH), 8.35 (s, 1H, H-8) MS CI(+ve) *m/z* 340, 342, ($M+H^+$) and **14** (8 mg, 3%).

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17. **3-[4′-Acetoxy-3′-(acetoxymethyl)butyl]-6-amino-7-chloro-2-(trifluoromethyl)imidazo[4,5-*b*]pyridine (4)**. The mesylate **11** (0.38 mg, 1.34 mmol) in dry DMF (3 mL) was added to a mixture of **18** (0.140 g, 0.68 mmol) and finely ground anhydrous potassium carbonate (187 mg, 1.34 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 48 h at which time more **11** (191 mg, 0.516 mmol) in DMF (0.5 mL) was added. After a total of 72 h the DMF was removed at high vacuum and the residue partitioned between ethyl acetate (30 mL) and water (30 mL). The organic layer was washed with brine (20 mL) then dried and concentrated in vacuo to yield 450 mg of a tan coloured oil. This was purified by HPLC to yield **4** (90 mg, 31%). ^{1}H NMR ($CDCl_3$) δ 1.80 (m, 3H, $CH_2C\underline{H}_2C\underline{H}$), 2.00 (s, 6H, 2×$COCH_3$), 4.10 (m, 4H, 2×$OCH_2$), 4.25 (m, 2H, NCH_2), 5.10 (s, 2H, NH_2), 7.90 (s, 1, ArH). MS CI(+ve) *m/z* 423, 424 ($M+H^+$).
18. **3-[4′-Hydroxy-3′-(hydroxymethyl)butyl]-6,7-dichloroimidazo[4,5-*b*]pyridine (19)**. Gaseous ammonia was bubbled into an ice-chilled solution of **3** (187 mg, 0.5 mmol) in dry methanol (25 mL). After the solution was saturated, the reaction was sealed and allowed to warm to room temperature overnight. The solvent was removed at reduced pressure and the solid warmed to 50 °C at ultra-high vacuum to remove acetamide. The residue could be crystallized from methanol to give a colourless crystals (45 mg, 35%). mp 160–161 °C. ^{1}H NMR ($CDCl_3$) δ 1.50 (m, 1H, $(CH_2)_2C\underline{H}$); 1.87 (m, 2H, $CHC\underline{H}_2CH_2$); 3.45 (m, 4H, $2C\underline{H}_2OH$); 4.05 (s, 2H, $2CH_2O\underline{H}$); 4.28 (m, 2H, NCH_2). MS CI(+ve) *m/z* 290, 292, 294 ($M+H^+$).
19. **1-[4′-Hydroxy-3′-(hydroxymethyl)butyl]-6,7-dichloroimidazo[4,5-*b*]pyridine (20)**. Gaseous ammonia was bubbled into an ice-chilled solution of **12** (103 mg, 0.27 mmol) in dry methanol (25 mL) and the reaction worked up as for compound **19** to yield **20** a colourless solid (30 mg, 42%). mp 139–142 °C. ^{1}H NMR, ($CDCl_3$) δ 1.57 (m, 1H, $(CH_2)_2C\underline{H}$), 1.77 (m, 2H, $CHC\underline{H}_2CH_2$), 3.39 (m, 4H, $2C\underline{H}_2OH$), 4.17 (m, 2H, NCH_2), 4.30 (s, 2H, $2CH_2O\underline{H}$).
20. **3-[4′-Hydroxy-3′-(hydroxymethyl)butyl]-6-amino-7-chloro-2-(trifluoromethyl)imidazo-4,5-*b*]pyridine (21)**. Gaseous ammonia was bubbled into an ice-chilled solution of **4** (57 mg, 0.13 mmol) in dry methanol (10 mL) and the reaction worked up as for compound **19** to yield **21** as a viscous oil (27 mg, 61%).^{1}H NMR ($CDCl_3$) δ ^{1}HNMR, ($CDCl_3$) δ 1.20 (m, 1H, $(CH_2)_2C\underline{H}$), 1.82 (m, 2H, $CHC\underline{H}_2CH_2$), 2.95 (bs, 2H, $2CH_2O\underline{H}$), 3.70 (m, 4H, $2C\underline{H}_2OH$), 4.35 (m, 2H, NCH_2), 5.20 (bs, 2H, NH_2). MS CI(+ve) *m/z* 339, 341, ($M+H^+$).

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