Studies on the Synthesis of 2-Bromomethylimidazole Nucleosides Related to AICA-riboside [5-Amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide] †

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The synthesis of 2-methyl- and 2-bromomethyl-imidazole nucleoside derivatives, related to AICA-riboside, is described. Ethyl *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)acetimidate and ethyl *N*-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)acetimidate, obtained from 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine and 2,3-*O*-isopropylidene-D-ribofuranosylamine respectively, reacted with ethyl α-amino-α-cyanoacetate and α-amino-α-cyanoacetamide to give ethyl 5-amino-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- and ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate (3) and (5) and 5-amino-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- and 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2-methylimidazole-4-carboxamide (4) and (6), respectively. Treatment of 5-amino-2-methylimidazole glucosides (3) and (4) with Ac_2O and H_3PO_4 as catalyst followed by reaction with *N*-bromosuccimide (NBS) gave 5-(*N*,*N*-diacetylamino)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-bromomethylimidazole-4-carboxylate and the *N*-acetyl 4-carboxamide analogue, (18) and (17) respectively. Deisopropylidenation of the 5-amino-2-methylimidazole ribosides (5) and (6) followed by acetylation with Ac_2O in the presence of 4-dimethylaminopyridine gave 5-(*N*,*N*-diacetylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate and -4-carboxamide (12) and (10). Treatment of (12) with NBS afforded ethyl 5-(*N*,*N*-diacetylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-bromomethylimidazole-4-carboxylate (16). Structural assignments were made on the basis of ^1H n.m.r. spectra.

In earlier parts of this series we have reported the synthesis, cytostatic activity, and mode of action of a variety of nucleosides of halogenomethylpentaheterocycles as a new type of alkylating agent.[1-4] The design of these nucleosides as potential antineoplastic drugs was based on the use of the alkylating benzylic-type halogenomethyl group as the active moiety of such compounds. Efforts to increase the specificity of the biological action of the alkylating agents led various authors to attempt the preparation of compounds in which the alkylating group is attached to carrying-structures normally involved in cell growth.[5]

Some of the alkylating derivatives related to naturally occurring nucleosides have been reported to show anticancer activity;[6,7] 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide-5′-phosphate is a biosynthetic precursor of purine nucleotides,[8] and 4-(3,3-dimethyl-1-triazeno)-1-(β-D-ribofuranosyl)imidazole-5-carboxamide is an alkylating nucleoside which shows antileukaemic activity.[9] These facts led us to investigate the synthesis of nucleosides in which the chemically alkylating bromomethyl group is attached to carriers related to 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside).

The preparation of certain bromomethylpyrazole and 4- and 5-bromomethylimidazole nucleosides by reaction of the corresponding methyl substituted nucleosides with *N*-bromosuccinimide (NBS), has been reported.[10,11] We have now attempted to extend this procedure to the preparation of nucleosides of 5-amino-2-bromomethylimidazole-4-carboxylate and 5-amino-2-bromomethylimidazole-4-carboxamide. The precursors, 2-methyl substituted nucleosides, namely 5-amino-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-methylimidazole-4-carboxylate and -4-carboxamide (3) and (4), and 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate and -4-carboxamide (5) and (6), were prepared by reaction of the hitherto unknown ethyl *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-acetimidate (1) and ethyl *N*-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)acetimidate (2) with ethyl α-amino-α-cyanoacetate [12] or α-amino-α-cyanoacetamide,[13] following the Shaw method for the synthesis of 5-amino-1-glycosylimidazoles from glycosylamines *via* *N*-glycosylimidates [14,15] (Scheme 1). It is interesting to note that the previously described condensation of 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate with ethyl formimidate hydrochloride gave a mixture of α- and β-ribosylformimidates which reacted with α-amino-α-cyanoacetate to provide an anomeric mixture of 5-aminoimidazole ribosides.[14,15] An identical mixture of aminoimidazole α- and β-ribosides was also obtained by reaction of the same ribofuranosylamine with ethyl *N*-[alkoxy-carbonyl or -carbamoyl(cyano)methyl]formimidates.[14,15] In our case, reaction of ethyl acetimidate hydrochloride with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine [16] and with 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate [15] gave, in each case, the corresponding β-glycosylacetimidates (1) and (2) as the only reaction products. The structure assigned to the β-glucosylacetimidate (1) was established by elemental analysis and ^1H n.m.r. spectroscopy. Although an analytically pure sample of the β-ribofuranosylacetimidate (2) could not be obtained, since it decomposed during chromatography, evidence for its structure came from its ^1H n.m.r. spectrum which showed the presence of a single anomeric form and from its subsequent reaction with α-amino-α-cyanoacetate and with α-amino-α-cyanoacetamide to give, in each case, a single 5-amino-1-ribofuranosyl-2-methylimidazole (5) and (6) respectively. Assignment of the β-anomeric configuration to the *N*-ribofuranosylacetimidate (2) was made by applying Imbach's criterion [17] to the 5-aminoimidazole ribosides (5) and (6), obtained from (2). Thus, the ^1H n.m.r. spectra of (5) and (6) showed a difference in the δ values of the isopropylidene methyl signals of 0.21 and 0.23 p.p.m. respectively, consistent only with a β configuration (Table 1).

2,3-*O*-Isopropylidene protected β-ribosides (5) and (6) were

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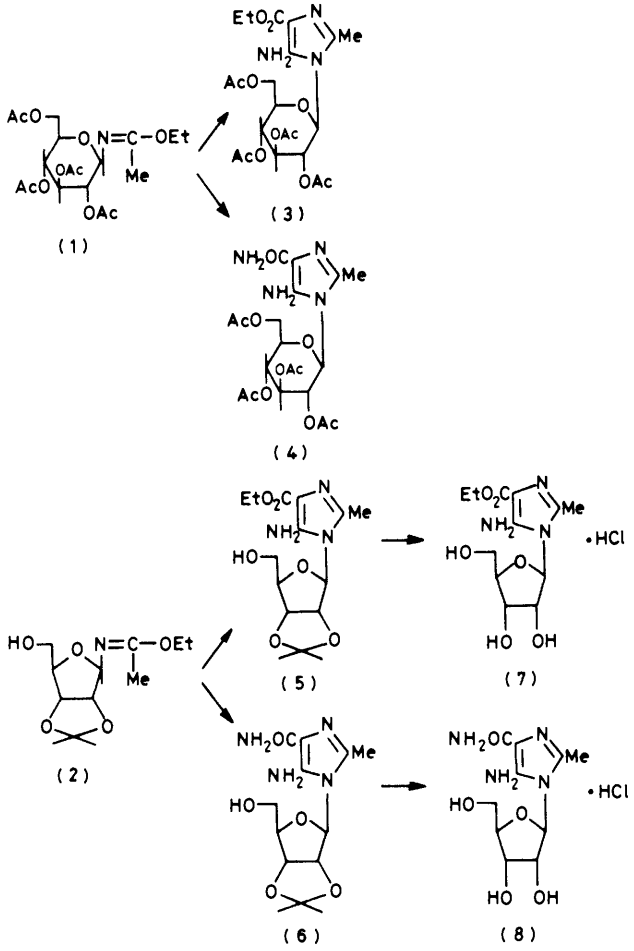
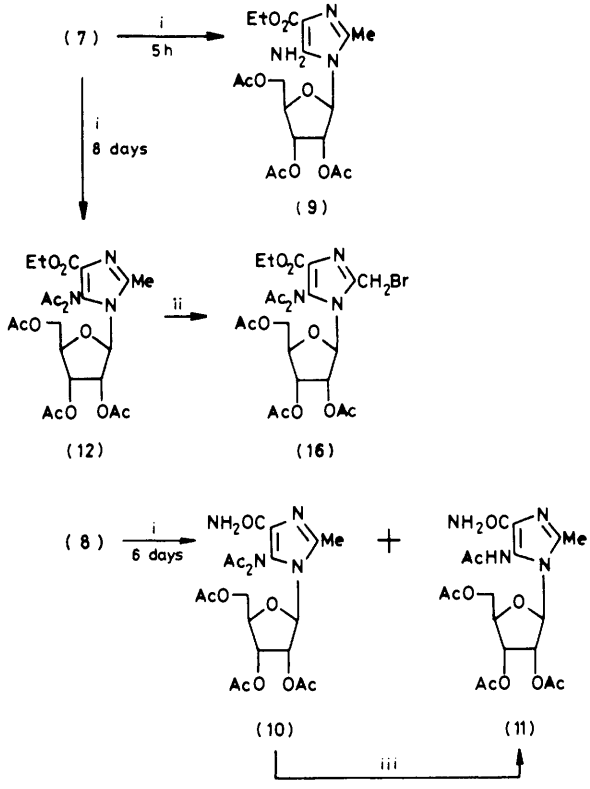
Table 1. ^{1}H N.m.r. data of 5-amino-2-methylimidazole nucleoside derivatives recorded at 90 MHz. Chemical shifts (δ) and coupling constant (Hz)

Compound	Solvent	1′-H	$J_{1',2'}$	Me	Others
(3)	$CDCl_3$–D_2O	5.68	9	2.34	
(4) [a]	$CDCl_3$–D_2O	5.66	9	2.32	
(5)	Me_2SO–D_2O	5.68	3	2.33	1.31, 1.52 (CMe_2)
(6)	Me_2SO–D_2O	5.63	4	2.23	1.30, 1.53 (CMe_2)
(7) [b]	Me_2SO–D_2O	5.80	7	2.56	
(8) [b]	Me_2SO–D_2O	5.65	7	2.56	
(9)	$CDCl_3$	5.30—5.76 [c]		2.36	5.36 (NH_2)

[a] As picrate. [b] As hydrochloride. [c] Multiplet including 1′-, 2′-, and 3′-H.



Scheme 1.



Scheme 2. *Reagents:* i, Ac_2O–DMAP; ii, NBS, *hν*; iii, 1 month, room temp.

transformed into 2,3,5-tri-*O*-acetyl-β-ribosides, since it had been previously demonstrated that one of the most important aspects in conditioning the cytostatic activity of alkylating *N*-glycosylhalogenomethylpentaheterocycles is the protecting group of the sugar hydroxy groups, the acetyl group being the most suitable of those investigated.[1] 5-Amino-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-methylimidazole derivatives could not be directly prepared from 2,3,5-tri-*O*-acetyl-β-D-ribofuranosylamine, since our attempts to obtain this starting material by catalytic hydrogenation of 2,3,5-tri-*O*-acetyl-β-D-ribofuranosylazide [18] yielded mixtures of unstable compounds. This was not unexpected since the relative instability of the acyl derivatives of β-D-ribofuranosylamine had been previously reported.[15] Removal of the isopropylidene protecting group in the ribosides (5) and (6) with ethanolic HCl afforded ethyl 5-amino-1-(β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate (7) and 5-amino-1-(β-D-ribofuranosyl)-2-methylimidazole-4-carboxamide [2-methyl AICA-riboside (8)] as hydrochlorides. When (7) and (8) were treated with acetic anhydride and pyridine at 10 °C,[19] the formation of the various reaction products was detected by analytical t.l.c. Better results were found on treatment of (7) and (8) with an excess of acetic anhydride containing 4-dimethylaminopyridine at room temperature (Scheme 2). In the case of the 4-carboxylate substituted riboside (7), the tri-*O*-acetyl derivative, namely ethyl 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate (9) was formed in almost quantitative yield within 5 h. However, in a similar experiment with the 4-carboxamide substituted riboside (8), no reaction was observed in this time and 6 days were required for its complete transformation. After this time, 5-(*N*,*N*-diacetylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxamide (10) was isolated in 48% yield along with a minor amount (28%) of 5-acetamido-1-(2,3,5-tri-*O*-

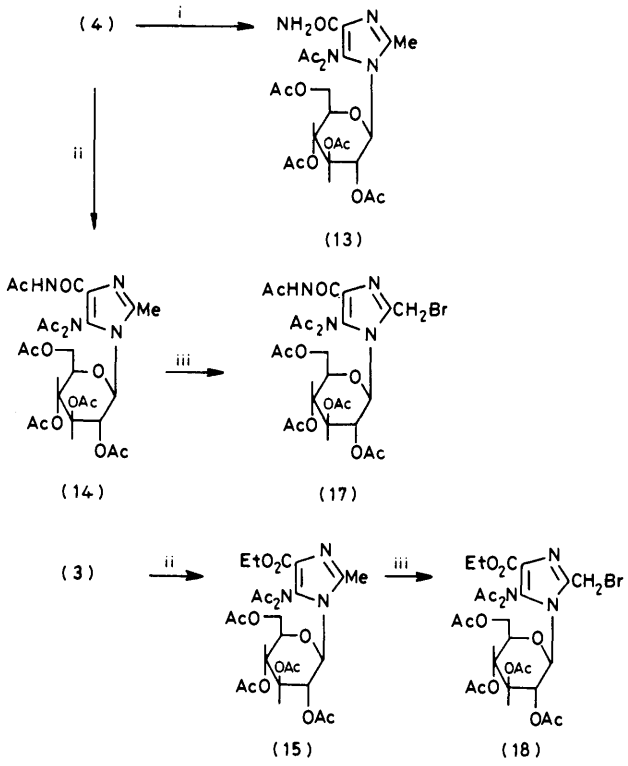
Table 2. ^{1}H N.m.r. data of amino-protected 2-methyl- and 2-bromomethyl-imidazole nucleoside derivatives recorded at 90 MHz. Chemical shifts (δ) and coupling constants (Hz)

Compd.	Solvent	1′-H	$J_{1',2'}$	Me or CH_2Br	NAc	Exchangeable protons
(10)	Me_2SO	5.76	5.0	2.46	2.26, 2.13	7.40, 7.20 ($CONH_2$)
(11)	Me_2SO	5.13—5.66 [a]		*b*	2.40	9.60 (5-NH), 7.06 ($CONH_2$)
(11)	$CDCl_3$	5.50—5.70 [c]		2.45	2.14	8.71 (5-NH), 6.92, 5.90 ($CONH_2$)
(12)	Me_2SO	5.84	5.5	*b*	2.32, 2.18	
(12)	$CDCl_3$	5.45	2.0	2.60	2.36, 2.30	
(13)	$CDCl_3$	4.96—5.73 [d]		2.66	2.50, 2.13	6.96, 6.00 ($CONH_2$)
(14)	$CDCl_3$	4.90—5.66 [d]		2.63	2.46, 2.43, 2.08	9.37 (4-CONH)
(15)	$CDCl_3$	4.90—5.66 [d]		2.67	2.42, 2.11	
(16)	Me_2SO	6.04	4.5	4.27	2.32, 2.16	
(16)	$CDCl_3$	5.75	6.0	4.66	2.46, 2.18	
(17)	$CDCl_3$	5.03—5.43 [d]		4.60	2.46, 2.36, 2.26	9.33 (4-CONH)
(18)	$CDCl_3$	5.57	9.0	4.67	2.45, 2.20	

[a] Multiplet including 1′-, 2′-, and 3′-H. [b] Signal including Me and Me_2SO. [c] Multiplet including 1′- and 2′-H. [d] Multiplet including 1′-, 2′-, 3′-, and 4′-H.

acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxamide (11); this was probably formed by partial *N*-deacetylation of (10), since when pure (10) was allowed to stand at room temperature for 1 month, conversion into the 5-monoacetyl derivative (11) in 72% yield took place. A similar prolonged acetylation (8 days) of the deblocked 4-carboxylate substituted riboside (7) also led to diacetylation of the 5-amino group to provide ethyl 5-(*N*,*N*-diacetylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate (12) in 74% yield. The structures of the acetylated ribosides (9), (10), (11), and (12) were assigned on the basis of their elemental analyses and ^{1}H n.m.r. spectra (Tables 1 and 2). Thus, the ^{1}H n.m.r. spectra of (10) and (12) showed, besides the signals corresponding to the three *O*-acetyl groups, two singlets at δ 2.26 and 2.13, and δ 2.32 and 2.18, corresponding to two *N*-acetyl groups (Table 2). These two singlets were not present in compound (9), but there were two exchangeable amino protons at δ 5.36 (Table 1). In the case of the 5-monoacetylamino riboside (11), the ^{1}H n.m.r. spectrum in $CDCl_3$ showed three NH protons at δ 8.71, 6.92, and 5.90, respectively, and, in the acetyl region, four signals corresponding to three *O*-acetyl and one *N*-acetyl groups (Table 2).

When bromination of the poly-*O*-acetylated nucleosides (3), (4), (9), and (10) with NBS, under irradiation, was attempted, complex mixtures of compounds which decomposed on t.l.c. were obtained. This was not very surprising, especially in the case of the unprotected 5-amino derivatives (3), (4), and (9), since alkylating nucleosides of halogenomethyl substituted five-membered ring heterocycles bearing free OH or NH groups had been previously observed to be highly unstable,[1,4] probably owing to the reaction of these nucleophilic functions with the CH_2X group. These results led us to protect, as much as possible, the NH groups of (3), (4), and (10) (Schemes 2 and 3) and to use the *N*-diacetylamino riboside (12), instead of (9), in the next bromination experiment. Acetylation of the 4-carboxamide substituted glucoside (4) with acetic anhydride in the presence of 4-dimethylaminopyridine for 8 days led to diacetylation of the 5-amino group providing 5-(*N*,*N*-diacetylamino)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-methylimidazole-4-carboxamide (13) in 60% yield. Since the reaction of the 4-carboxamide substituted riboside (10) with NBS did not lead to the desired 2-bromomethyl derivative, different acetylation conditions were attempted in order to also acetylate the 4-carboxamide group in nucleosides (4) or (13) and (10). Treatment of the glucoside (4) with acetic anhydride in the presence of phosphoric acid as catalyst at reflux



Scheme 3. *Reagents:* i, Ac_2O–DMAP, 8 days; ii, Ac_2O–PO_4H_3; iii, NBS, *hν*

temperature for 1 h gave *N*-acetyl-5-(*N*,*N*-diacetylamino)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-methylimidazole-4-carboxamide (14) in 67% yield. Unfortunately, in the case of the riboside (10), these same conditions led to the complete cleavage of the ribosidic bond because of the acidic medium and no reaction took place when the experiment was carried out at room temperature. Finally, reaction of the glucoside (3) with refluxing acetic anhydride in the presence of phosphoric acid gave ethyl 5-(*N*,*N*-diacetylamino)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-methylimidazole-4-carboxylate (15) in 78% yield. Structural assignments of the 5-(*N*,*N*-diacetylamino) glucosides (13), (14), and (15) were made on the basis of their elemental analyses and ^{1}H n.m.r. spectra (Table 2). Thus, the diacetylation of the 5-amino

group of compounds (13) and (15) was shown to have occurred by the presence of six signals in the acetyl region, two of them at δ 2.50 and 2.13, and 2.42 and 2.11, respectively, corresponding to the *N*-acetyl groups. That the 4-carboxamide function of (14) had been monoacetylated was demonstrated by the presence of seven acetyl signals, three of them at δ 2.46, 2.43, and 2.03, attributed to the *N*-acetyl groups. At the same time, compound (14) only showed one exchangeable proton at δ 9.37 corresponding to the partially protected 4-carboxamide group, instead of two exchangeable protons as in compound (13). Slow conversion of the 5-(*N*,*N*-diacetylamino) derivatives (12), (13), (14), and (15) into more stable compounds could be observed by analytical t.l.c. Although these compounds were not identified, the transformation should be due to partial deacetylation of the 5-(*N*,*N*-diacetyl) group to a 5-acetamide group, as demonstrated in the case of the riboside (10).

Bromination of the amino and sugar hydroxy-protected nucleosides (12), (14), and (15) with NBS, under irradiation from a 200-W lamp, gave in each case the corresponding 2-bromomethylimidazole derivative namely, ethyl 5-(*N*,*N*-diacetylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-bromomethylimidazole-4-carboxylate (16), *N*-acetyl-5-(*N*,*N*-diacetylamino)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-bromomethylimidazole-4-carboxamide (17), and ethyl 5-(*N*,*N*-diacetylamino)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-bromomethylimidazole-4-carboxylate (18), in 60, 45, and 50% yields respectively (Schemes 2 and 3). The elemental analyses and ^{1}H n.m.r. spectra of compounds (16), (17), and (18) were in agreement with these structures (Table 2). However, analytical t.l.c. showed that all these 5-(*N*,*N*-diacetylamino)-2-bromomethylimidazole nucleosides were unstable compounds which decomposed on standing to give various spots. This was not very surprising since, on the one hand, partial deacetylation of the 5-(*N*,*N*-diacetylamino) group is known to occur (see above), and, on the other, several 4- and 5-bromomethylimidazole nucleosides, previously reported,[11] were also unstable compounds.

The 2-bromomethylimidazole nucleosides (16), (17), and (18) were evaluated as cytotoxic agents against HeLa cell cultures following the described procedures,[20] and none of them showed activity. The lack of activity is probably due to instability, since the ineffectiveness as cytostatics of unstable nucleosides of halogenomethyl substituted five-membered ring heterocycles is a well known fact.[1,11]

Experimental

M.p.s were measured with a Kofler hot-stage apparatus. ^{1}H N.m.r. spectra were recorded with a Varian EM-390 spectrometer operating at 90 MHz, with Me_4Si as internal standard. Analytical t.l.c. was performed on aluminium sheets coated with a 0.2-mm layer of silica gel $60F_{254}$ (Merck), and preparative layer chromatography was performed on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF_{254} (Merck). Compounds were detected with u.v. light (254 nm) or by spraying the plate with ethanol–sulphuric acid (3 : 1) and heating.

Ethyl N-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*acetimidate* (1).—2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylamine [16] (3.47 g, 10 mmol) in dry acetonitrile (50 ml) was treated with ethyl acetimidate hydrochloride (1.85 g, 15 mmol) and triethylamine (1.4 ml, 10 mmol) and the solution was refluxed for 1 h and then evaporated to dryness. The residue was dissolved in $CHCl_3$ (20 ml) and the solution was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The solid residue was crystallized from ethanol to give the acetimidate (1) (3.75 g, 90%), m.p. 110—111 °C (Found: C, 52.0; H, 6.9; N, 3.1. Calc. for $C_{18}H_{27}NO_{10}$: C, 51.8; H, 6.50; N, 3.35%); $\delta_H(Me_2SO)$ 5.37 (d, 1 H, 1′-H, $J_{1',2'}$ 9 Hz) and 2.05 (s, 3 H, Me).

Ethyl N-(2,3-O-*Isopropylidene*-β-D-*ribofuranosyl*)*acetimidate* (2).—To a solution of triethylamine (4.2 ml, 30 mmol) in dry acetonitrile (150 ml) were added 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate [15] (1.08 g, 30 mmol) and ethylacetimidate hydrochloride (4.08 g, 33 mmol). The mixture was stirred at room temperature for 2 h, then filtered and evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (60 ml), washed with H_2O, dried (Na_2SO_4) and evaporated to dryness to give the acetimidate (2) (3.9 g, 50%) as a homogeneous syrup which decomposed on preparative t.l.c. and was identified on the basis of its ^{1}H n.m.r. spectrum; $\delta_H(CDCl_3)$ 5.40 (d, 1 H, 1′-H, $J_{1',2'} < 2$ Hz), 2.05 (s, 3 H, Me), and 1.53 and 1.34 (s, 6-H, 2 Me, isopropylidene group).

Ethyl 5-*Amino*-1-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-2-*methylimidazole*-4-*carboxylate* (3).—A solution of compound (1) (4.17 g, 10 mmol) and ethyl α-amino-α-cyanoacetate [12] (2.56 g, 20 mmol) in dry acetonitrile (50 ml) was refluxed for 1 h, and then evaporated to dryness. The residue was chromatographed on preparative t.l.c. plates using EtOAc as eluant. The resulting compound crystallized from benzene to give (3) (3.13 g, 63%), m.p. 112—113 °C (Found: C, 47.3; H, 6.3; N, 7.9. Calc. for $C_{21}H_{29}N_3O_{11}\cdot 2H_2O$: C, 47.1; H, 6.15; N, 7.85%).

5-*Amino*-1-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-2-*methylimidazole*-4-*carboxamide* (4).—A solution of compound (1) (4.17 g, 10 mmol) and α-amino-α-cyanoacetamide [13] (1.50 g, 15 mmol) in dry acetonitrile (50 ml) was refluxed for 1 h and then evaporated to dryness. The residue was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 1) as solvent. The major band gave compound (4) (1.8 g, 38%) as a foam (Found: C, 46.5; H, 6.0; N, 11.2. Calc. for $C_{19}H_{26}N_4O_{10}\cdot H_2O$: C, 46.75; H, 5.75; N, 11.5%).

Ethyl 5-*Amino*-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)-2-*methylimidazole*-4-*carboxylate* (5).—A solution of compound (2) (6.0 g, 23.2 mmol) and ethyl α-amino-α-cyanoacetate [12] (3.08 g, 24.0 mmol) in dry acetonitrile (100 ml) was refluxed for 1 h and then evaporated to dryness. The residue was chromatographed on preparative t.l.c. plates using EtOAc as eluant. The resulting compound from the major band crystallized from benzene–light petroleum to give (5) (2.0 g, 24%); m.p. 164—166 °C (Found: C, 50.4; H, 6.8; N, 11.6. Calc. for $C_{15}H_{23}N_3O_6\cdot H_2O$: C, 50.15; H, 6.95; N, 11.7%).

5-*Amino*-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)-2-*methylimidazole*-4-*carboxamide* (6).—A solution of compound (2) (6.0 g, 23.2 mmol) and α-amino-α-cyanoacetamide [13] (2.5 g, 23.2 mmol) in dry acetonitrile (100 ml) was refluxed for 1½ h and then evaporated to dryness. The residue was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 1) as eluant. The resulting compound crystallized from methanol to give the *carboxamide* (6) (3.90 g, 64%); m.p. 258—259 °C (decomp.) (Found: C, 49.7; H, 6.3; N, 17.9. $C_{13}H_{20}N_4O_5$ requires C, 50.0; H, 6.4; N, 17.95%).

Ethyl 5-*Amino*-1-(β-D-*ribofuranosyl*)-2-*methylimidazole*-4-*carboxylate Hydrochloride* (7).—Compound (5) (1.34 g, 4 mmol) was treated with a saturated solution of HCl in EtOH (20 ml) and then evaporated to dryness. The residue was washed with acetone to give a solid which crystallized from propan-2-ol to provide compound (7) (1.10 g, 81.5%); m.p.

163—165 °C (Found: C, 42.5; H, 6.0; N, 12.1. $C_{12}H_{20}ClN_3O_6$ requires C, 42.65; H, 5.65; N, 12.45%).

5-Amino-1-(β-D-ribofuranosyl)-2-methylimidazole-4-carboxamide Hydrochloride (8).—Compound (6) (1.50 g, 5 mmol) was treated with a saturated solution of HCl in EtOH (30 ml) and then evaporated to dryness. The solid residue was crystallized from MeOH–EtOAc to give compound (8) (1.51 g, 98%); m.p. 171 °C (decomp.) (Found: C, 39.0; H, 5.6; Cl, 11.75; N, 17.8. $C_{10}H_{17}ClN_4O_5$ requires C, 38.9; H, 5.5; Cl, 11.5; N, 18.15%).

Ethyl 5-Amino-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate (9).—A solution of compound (7) (0.40 g, 1.1 mmol) and 4-dimethylaminopyridine (0.14 g, 1.1 mmol) in acetic anhydride (10 ml) was allowed to stand at room temperature for 5 h. After this time, the solution was added to crushed ice (10 g), extracted with $CHCl_3$ (3 × 25 ml), and the combined extracts were successively washed with saturated aqueous $NaCO_3H$ (25 ml) and H_2O (25 ml), and dried (Na_2SO_4). Evaporation of the $CHCl_3$ left a residue which was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 3) as eluant. The major band gave compound (9) (0.44 g, 92%) as a syrup (Found: C, 48.2; H, 6.3; N, 9.5. $C_{18}H_{25}N_3O_9 \cdot H_2O$ requires C, 48.55; H, 6.1; N, 9.45%).

5-(N,N-Diacetylamino)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxamide (10) *and 5-Acetamido-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxamide* (11).—A solution of compound (8) (0.62 g, 2.0 mmol) and 4-dimethylaminopyridine (0.27 g, 2.2 mmol) in acetic anhydride (15 ml) was allowed to stand at room temperature for 6 days. After this time, the solution was added to crushed ice (20 g), extracted with $CHCl_3$ (3 × 50 ml) and the combined extracts were washed with saturated aqueous $NaCO_3H$ (2 × 25 ml) and H_2O (50 ml) and dried (Na_2SO_4). Removal of the $CHCl_3$ left a residue which was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 1) as eluant. Two major bands were observed. From the faster moving band *compound* (10) (0.46 g, 48%) was obtained as a syrup (Found: C, 46.6; H, 5.6; N, 10.7. $C_{20}H_{26}N_4O_{10} \cdot 2H_2O$ requires C, 46.35; H, 5.8; N, 10.8%).

From the slower moving band *compound* (11) (0.070 g, 8%) was obtained as a syrup (Found: C, 49.3; H, 5.7; N, 12.7. $C_{18}H_{24}N_4O_9$ requires C, 49.1; H, 5.45; N, 12.7%).

Compound (10) (0.24 g, 0.5 mmol) was allowed to stand at room temperature for 1 month. After this time, it was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 1) to give compound (11) (0.16 g, 72%).

Ethyl 5-(N,N-Diacetylamino)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-methylimidazole-4-carboxylate (12).—A solution of compound (7) (0.47 g, 1.3 mmol) and 4-dimethylaminopyridine (0.19 g, 1.6 mmol) in acetic anhydride (10 ml) was allowed to stand at room temperature for 8 days. After this time, the solution was added to crushed ice (10 g), extracted with $CHCl_3$ (3 × 25 ml) and the extracts were washed with saturated aqueous $NaCO_3H$ (25 ml), H_2O (25 ml), and dried (Na_2SO_4). Removal of the $CHCl_3$ left a residue which was chromatographed on preparative t.l.c. plates using EtOAc–hexane (3 : 1) as eluant. The resulting compound was crystallized from CCl_4 to give *compound* (12) (0.49 g, 74%), m.p. 136—138 °C (Found: C, 51.4; H, 5.7; N, 8.2. $C_{22}H_{29}N_3O_{11}$ requires C, 51.05; H, 5.6; N, 8.05%).

5-(N,N-Diacetylamino)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-methylimidazole-4-carboxamide (13).—A solution of compound (4) (0.10 g, 0.2 mmol) and 4-dimethylaminopyridine (0.01 g, 0.08 mmol) in acetic anhydride (3 ml) was allowed to stand at room temperature for 10 days. After this time, the solution was added to crushed ice (2 g), extracted with $CHCl_3$ (3 × 10 ml), and the extracts were washed with saturated aqueous $NaCO_3H$ (10 ml), H_2O (10 ml) and dried (Na_2SO_4). Removal of the $CHCl_3$ left a residue which was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 1) as eluant to give *compound* (13) (0.079 g, 64%) as a foam (Found: C, 50.2; H, 5.1; N, 10.0. $C_{23}H_{30}N_4O_{12}$ requires C, 49.85; H, 5.4; N, 10.1%).

N-Acetyl-5-(N,N-diacetylamino)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-methylimidazole-4-carboxamide (14).—To a solution of compound (4) (0.94 g, 2 mmol) in acetic anhydride (11 ml), phosphoric acid (1 drop) was added and the mixture was refluxed for 1 h. Then the mixture was evaporated to dryness to give a residue which was treated with crushed ice (10 g), extracted with $CHCl_3$ (20 ml), and the extract dried (Na_2SO_4). Removal of the $CHCl_3$ left a residue which was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 3) as eluant to provide *compound* (14) (0.8 g, 67%) (Found: C, 50.4; H, 5.7; N, 9.3. $C_{25}H_{34}N_4O_{13}$ requires C, 50.25; H, 5.55; N, 9.4%).

Ethyl 5-(N,N-Diacetylamino)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-methylimidazole-4-carboxylate (15).—To a solution of compound (3) (1.0 g, 2 mmol) in acetic anhydride (11 ml), phosphoric acid (1 drop) was added and the mixture was refluxed for 1 h. Then the mixture was evaporated to dryness to give a residue which was treated with crushed ice (10 g), extracted with $CHCl_3$ (20 ml), and the extract dried (Na_2SO_4). Removal of the $CHCl_3$ left a residue which was chromatographed on preparative t.l.c. plates using acetone–$CHCl_3$ (1 : 3) as eluant to give *compound* (14) (0.9 g, 78%) as a foam (Found: C, 51.5; H, 5.8; N, 7.0. $C_{25}H_{33}N_3O_{11}$ requires C, 51.45; H, 5.65; N, 7.2%).

Ethyl 5-(N,N-Diacetylamino)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-bromomethylimidazole-4-carboxylate (16).—To a suspension of compound (12) (0.40 g, 0.8 mmol) in CCl_4 (25 ml) was added *N*-bromosuccinimide (0.15 g, 0.85 mmol) and the mixture was irradiated for 1 h by suspending the flask 1 cm above a 200-W lamp. After this time, it was evaporated to dryness to give a residue which was chromatographed on preparative t.l.c. plates using EtOAc–hexane (3 : 1) as eluant to provide a solid which was crystallized from CCl_4 to give *compound* (16) (0.28 g, 60%), m.p. 143—144 °C (Found: C, 44.9; H, 5.0; Br, 13.4; N, 7.1. $C_{22}H_{28}BrN_3O_{11}$ requires C, 44.75; H, 4.75; Br, 13.55; N, 7.1%).

N-Acetyl-5-(N,N-diacetylamino)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-bromomethylimidazole-4-carboxamide (17).—To a solution of (14) (0.60 g, 1 mmol) in CCl_4 (25 ml), *N*-bromosuccinimide (0.27 g, 1.5 mmol) was added and the mixture was irradiated for 1½ h with a 200-W lamp as indicated above. Removal of the CCl_4 gave a residue which was chromatographed on preparative t.l.c. plates using $CHCl_3$–acetone (9 : 1) as eluant to provide *compound* (17) (0.30 g, 45%) as a foam (Found: C, 43.4; H, 4.8; Br, 11.3; N, 8.1. $C_{25}H_{32}BrN_4O_{13} \cdot H_2O$ requires C, 43.2; H, 4.6; Br, 11.5; N, 8.05%).

Ethyl 5-(N,N-Diacetylamino)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-bromomethylimidazole-4-carboxylate (18).—To a solution of compound (15) (0.58 g, 1 mmol) in CCl_4 (25 ml) was added *N*-bromosuccinimide (0.27 g, 1.5 mmol) and the mixture was irradiated for 1½ h with a 200-W lamp as indicated above. Removal of the CCl_4 gave a residue which

was chromatographed on preparative t.l.c. plates using $CHCl_3$–acetone (9 : 1) as eluant to give *compound* (18) (0.33 g, 50%) as a foam (Found: C, 41.6; H, 5.1; Br, 12.5; N, 6.2. $C_{25}H_{32}BrN_3O_{10}$ requires C, 41.4; H, 4.85; Br, 12.1; N, 6.35%).

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