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Synthesis of 4- and 5-amino-1-(2-deoxy-D-*erythro*-pentofuranosyl)imidazole nucleosides by chemical and biotransformation methods*

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ABSTRACT

(2-Deoxy-D-*erythro*-pentofuranosyl)imidazole nucleosides have been synthesised by glycosylation of the sodium salt of ethyl 5-aminoimidazole-4-carboxylate with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride. Glycosylation of ethyl 5-aminoimidazole-4-carboxylate with 2-deoxy-β-D-*erythro*-pentofuranose was also achieved enzymically using *E. coli*, immobilised by ionotropic gelation in an alginate gel, with 2'-deoxyuridine as glycosyl donor. 5-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazole-4-carboxylic acid 5'-phosphate and 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazole-5-carboxylic acid 5'-phosphate were synthesised by phosphorylation of the respective nucleosides and examined as inhibitors of phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21) and phosphoribosylaminoimidazolesuccinocarboxamide synthetase (EC 6.3.2.6), which are involved in the *de novo* biosynthesis of purine nucleotides.

INTRODUCTION

(2-Deoxy-*erythro*-pentofuranosyl)imidazole nucleosides were first prepared[1] by the fusion method. In particular, 4-amino-1-(2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)imidazole-5-carboxamides were obtained by condensation of methyl 5-nitroimidazole-4-carboxylate with 1,3,5-tri-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranose at 145°, followed by deacylation, and catalytic reduction of the nitroimidazole formed. Methyl 1-(3,5-di-*O*-*p*-chlorobenzoyl-2-deoxy-α-D-*erythro*-pentofuranosyl)-4-nitroimidazole-5-carboxylate was produced[2,3] in low yield by reaction of the corresponding mercury or silver derivatives of methyl 4-nitroimidazole-5-carboxylate with 3,5-di-*O*-*p*-chlorobenzoyl-2-deoxy-α-D-*erythro*-pentofuranosyl chloride.

* Dedicated to Professor Grant Buchanan on the occasion of his 65th birthday.
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More recently[4], silylation of methyl 5-(cyanomethyl)imidazole-4-carboxylate, followed by condensation with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride under various conditions gave a mixture of anomeric and positional isomers. Increasing the amount of stannic chloride used in these reactions (at ambient temperature) to 1.4 mol equivalents favoured the formation of methyl 5-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)imidazole-4-carboxylate over the corresponding methyl 4-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)imidazole-5-carboxylate (ratio 2:1). Each reaction gave an excess of the β anomer. More conveniently, glycosylation[5] of the sodium salt of methyl 5-(cyanomethyl)imidazole-4-carboxylate with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride gave exclusively the β anomers in good yield. This stereospecific sodium-salt-glycosylation procedure[5-7] has been adapted to the synthesis of 2′-deoxy analogues of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5′-phosphate (CAIR), as discussed below.

Early biochemical approaches to the synthesis of 2′-deoxyimidazole nucleosides employed either cell-free extracts of bacteria[8] or suspensions of bacteria[9]. The glycosylation of 5-aminoimidazole-4-carboxamide (AICA) to the corresponding 2-deoxy-D-*erythro*-pentofuranose nucleoside or nucleotide was observed[9] during studies of purine requiring mutants of *E. coli*. Transfer of the 2-deoxy-D-*erythro*-pentofuranosyl moiety from one purine or pyrimidine to another was achieved[9] using a dialysed enzyme preparation from *Lactobacillus helveticus*. Formation of the 2′-deoxy analogue of AICA riboside was also observed[8] when pyrimidine 2-deoxy-D-*erythro*-pentofuranosides were used as glycosyl donors. Enzyme preparations from two other bacteria (*Lactobacillus debrueckii* and *Thermobacterium acidiphilus* R 26) that require 2-deoxy-D-*erythro*-pentofuranoside also catalysed the transfer. However, attempts to demonstrate the biotransformation in cells of *Leuconostoc citrovorum* were unsuccessful. Recently[10], nucleoside transferases (EC 2.4.2.6) from *Lactobacillus leichmanii* have been found to catalyse the transfer of 2-deoxy-D-*erythro*-pentofuranose from thymidine to AICA to produce 5-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazole-4-carboxamide as the sole product.

The preparative scale biosynthesis of pyrimidine or purine 2-deoxy-β-D-*erythro*-pentofuranonucleosides by thymine-dependent *E. coli* mutant cells immobilised by ionotropic gelation in alginate gel[11] was applied to the biosynthesis of 2′-deoxy analogues of CAIR, as reported below, in order to ascertain the stereo- and regio-specificity of the enzymically catalysed transfer of 2-deoxy-D-*erythro*-pentofuranose from 2′-deoxyuridine to ethyl 5-aminoimidazole-4-carboxylate.

RESULTS AND DISCUSSION

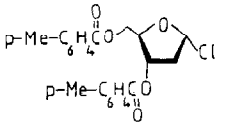
Ethyl 5-amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazole-4-carboxylate has been synthesised[12] by direct condensation of ethyl 5-aminoimidazole-4-carboxylate (**1a**) with 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride in hot acetonitrile containing triethylamine. Under similar conditions, the reaction of **1a** with

TABLE I

Effect of reaction conditions on the synthesis of **3a–6a**

Reaction conditions	*Base reacted (%)*	*Yields (%)*				*Ratios*	
		3a	**4a**	**5a**	**6a**	*N-3:N-1*	*β:α*
NEt_3 in MeCN reflux	35	8	2	19	6	2.5:1	1:3.8
NaH (50% in oil) room temperature	15	3	2	7	3	2.0:1	1:2.4
NaH (anhydrous) room temperature	39	0	10	1	28	2.9:1	29:1

1a $R^1 = CO_2Et$, $R^2 = NH_2$
1b $R^1 = CONH_2$, $R^2 = NH_2$
1a $R^1 = CN$, $R^2 = OH$



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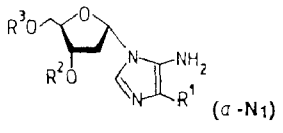
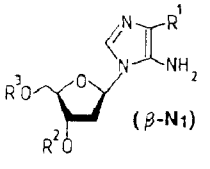


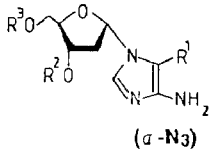
(α-N1)

3a $R^1 = CO_2Et$, $R^2 = R^3 = COC_6H_4$-*p*-Me
3b $R^1 = CO_2Et$, $R^2 = R^3 = H$



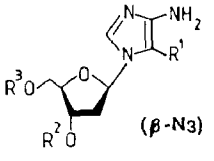
(β-N1)

4a $R^1 = CO_2Et$, $R^2 = R^3 = COC_6H_4$-*p*-Me
4b $R^1 = CO_2Et$, $R^2 = R^3 = H$
4c $R^1 = CO_2H$, $R^2 = H$, $R^3 = PO(OH)_2$



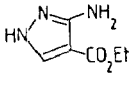
(α-N3)

5a $R^1 = CO_2Et$, $R^2 = R^3 = COC_6H_4$-*p*-Me
5b $R^1 = CO_2Et$, $R^2 = R^3 = H$



(β-N3)

6a $R^1 = CO_2Et$, $R^2 = R^3 = COC_6H_4$-*p*-Me
6b $R^1 = CO_2Et$, $R^2 = R^3 = H$
6c $R^1 = CO_2H$, $R^2 = H$, $R^3 = PO(OH)_2$



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2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**2**) gave (n.m.r. data) a mixture of the α,β-N-1 (**3a** and **4a**) and the α,β-N-3 (**5a** and **6a**) isomers (Table I). This mixture was resolved by chromatography on silica gel into two fractions that contained α,β-N-1 and α,β-N-3 isomers respectively.

Reaction of the sodium salt of **1a**, produced *in situ* from the imidazole and sodium hydride in anhydrous acetonitrile with **2** under nitrogen, gave the β isomers (**4a** and **6a**) with the β-N-3 isomer (**6a**) obtained in higher yield (Table I). The β-N-1 (**4a**) and β-N-3 (**6a**) isomers crystallised readily following chromatography on silica gel, and the deblocked nucleosides β-N-1 (**4b**) and β-N-3 (**6b**) were obtained following treatment with ethanolic ammonia. When sodium hydride in oil was used in the above reaction, only modest yields were detected (Table I). N.m.r. analysis of fractions from column chromatography indicated poor selectivity towards the β anomers **4a** and **6a**.

H.p.l.c. of the mixture of deprotected nucleosides **3b–6b** resulted in the isolation of each isomer. The order of elution was N-1 before N-3 and α before β.

The specificity of enzymes present in encapsulated cells of *E. coli* capable of the biosynthesis of 2′-deoxyimidazole nucleosides was examined. The biological catalyst was auxotrophic thymine-dependent *E. coli* immobilised by ionotropic gelation in a permeable alginate gel, and the glycosyl donor was 2′-deoxyuridine. Such conditions were found to glycosylate **1a** to give a mixture of the β-N-1 (**4b**, 18%) and β-N-3 (**6b**, 35%) isomers, isolated by h.p.l.c. No reaction was observed with AICA (**1b**), 4-cyano-5-hydroxyimidazole (**1c**), and ethyl 3-aminopyrazole-4-carboxylate (7). The ambiguity observed in the site of deoxyribosylation by this method of biotransformation contrasts with results obtained with pyrimidines and purines[11] in which substitution takes place regiospecifically at N-1 and N-9, respectively.

5-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazole-4-carboxylic acid 5′-phosphate (**4c**) and 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazole-5-carboxylic acid 5′-phosphate (**6c**) were prepared by treatment of **4b** and **6b**, respectively, with phosphoryl chloride in triethyl phosphate, followed by hydrolysis and anion-exchange chromatography. The nucleotides were stored at −20°, prior to use in enzyme kinetic studies.

The effect of substrate analogues on the activity of phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21). — The kinetic plot (Fig. 1) for the 2′-deoxy-β-N-1 CAIR analogue **4c** shows a maximum inhibition of 27% at 80μM CAIR and 24% at 40μM. At low concentrations of substrate, a slight enhancement in the rate of the reaction was observed, perhaps associated with the allosteric characteristics of the enzyme[16].

The v_0 versus [S] plot (Fig. 2) for the 2′-deoxy-β-N-3 CAIR analogue **6c** showed no significant inhibition. The substrate and inhibition curves were essentially superimposable. Presumably, COOH-4 and NH_2-5 of the imidazole ring in CAIR are orientated specifically with respect to the sugar moiety in the active site of the enzyme, and interchange of the ring substituents prevents **6c** from entering the active site. However, the 2′-deoxy-β-N-3 CAIR analogue **6c** significantly inhibited phosphoribosylaminoimidazolesuccinocarboxamide synthetase (EC 6.3.2.6). This difference in the inhibition characteristics of the enzyme pair might be explained if the active sites for each of the activities are spatially distinct.

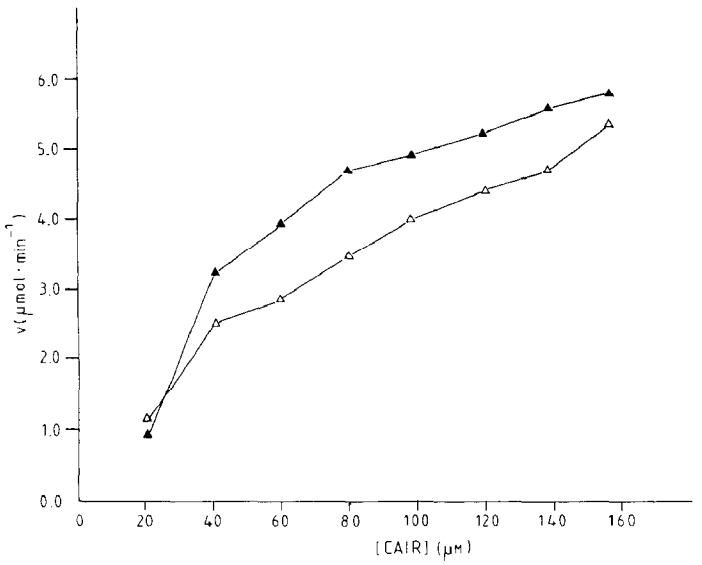

Fig. 1. Effect of **4c** on the activity of phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21): —▲—, CAIR only; —△—, in the presence of **4c** at a fixed concentration of 95μM.

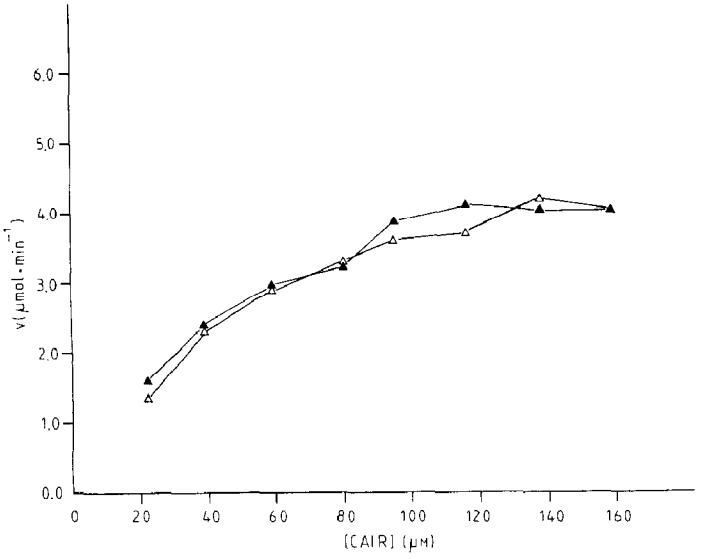

Fig. 2. Effect of **6c** on the activity of phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21): —▲—, CAIR only; —△—, in the presence of **6c** at a fixed concentration of 97μM.

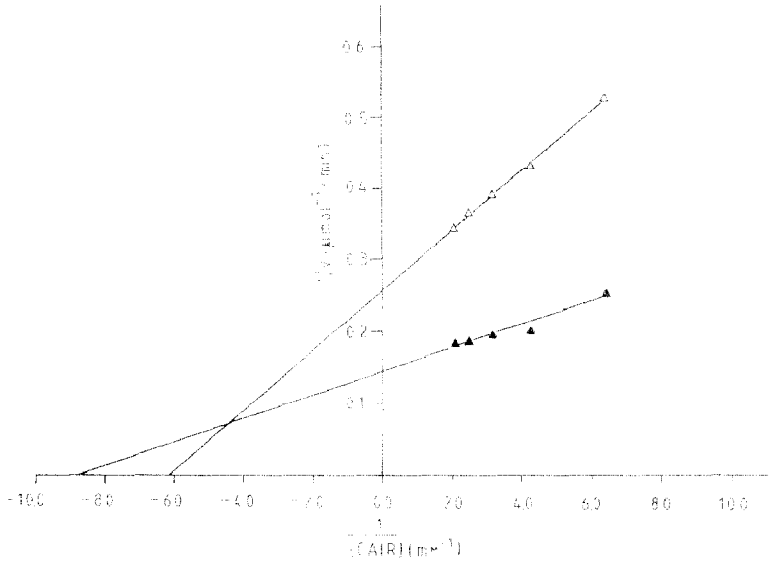

Fig. 3. Effect of **4c** on the activity of phosphoribosylaminoimidazolesuccinocarboxamide synthetase (EC 6.3.2.6): —▲—, CAIR only; - -△- -, in the presence of **4c** at a fixed concentration of 150μM.

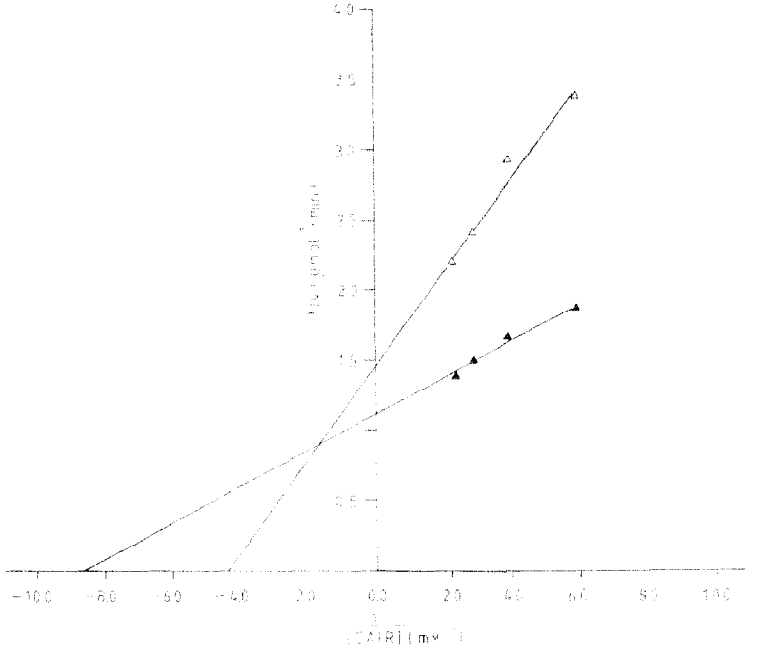

Fig. 4. Effect of **6c** on the activity phosphoribosylaminoimidazolesuccinocarboxamide synthetase (EC 6.3.2.6): —▲—, CAIR only; - -△- -, in the presence of **6c** at a fixed concentration of 155μM.

The effect of substrate analogues on the activity of phosphoribosylaminoimidazole-succinocarboxamide synthetase (EC 6.3.2.6). — The 2'-deoxy-β-N-1 CAIR analogue **4c** showed (Fig. 3) a maximum inhibition of 54% at ~30μM CAIR and 30% at 85μM. The K_m (Fig. 3) for CAIR was 0.115mM, and the apparent K_m in the presence of the inhibitor was increased 1.43 times, to 0.164mM. No substrate activity was observed.

The 2'-deoxy-β-N-3 CAIR analogue (**6c**) showed (Fig. 4) a maximum inhibition of 46% at 85μM CAIR. This result contrasts with the lack of inhibition exhibited by this analogue on phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21) activity. The K_m (Fig. 4) for CAIR was 0.116mM (in good agreement with the value obtained above), and the apparent K_m in the presence of the inhibitor was increased twofold to 0.233mM. No substrate activity was observed.

TABLE II

N.m.r. data for imidazole nucleosides **3a–6a** and **3b–6b**

	3a	**4a**	**5a**	**6a**	**3b**	**4b**	**5b**	**6b**
H-1'	6.00	5.98	6.60	6.62	5.93	5.96	6.35	6.36
H-2'a	2.95	3.02	2.95	2.4	2.65	2.39	2.56	2.16
H-2'b	2.89	2.60	2.57	2.91	2.12	2.14	2.03	2.28
H-3'	5.68	5.65	5.62	5.58	4.3	4.32	4.2	4.2
H-4'	4.6	4.54	4.87	4.60	4.02	3.82	4.1	3.78
H-5'a	4.6	4.71	4.56	4.67	3.4	3.55	3.4	3.58
H-5'b	4.6	4.62	4.56	4.67	3.4	3.55	3.4	3.52
H-2	7.36	7.12	7.60	7.68	7.42	7.35	7.67	7.83
NH_2	5.27	5.29	4.86	4.89	6.14	6.16	5.66	5.7
OCH_2	4.34	4.32	4.28 4.32	4.31 4.31	4.16	4.16	4.18 4.22	4.18 4.21
C-2	128.8	[a]	136.4	135.9	129.5	129.5	137.9	137.3
C-4	112.4	113.0	156.1	156.0	109.1	109.5	156.4	156.5
C-5	145.9	145.7	100.8	101.0	145.9	145.9	99.3	99.4
C-1'	84.9	85.4	89.2	87.5	88.0	87.4	89.1	87.2
C-2'	36.4	36.2	41.0	40.7	[a]	[a]	[a]	41.9
C-3'	74.5	74.4	74.9	74.7	70.6	70.6	70.7	69.2
C-4'	83.8	83.0	85.4	82.9	83.4	83.7	87.1	85.8
C-5'	63.8	63.9	64.3	64.0	61.5	61.3	61.7	60.8
CO	164.8	164.8	161.1	160.9	163.9	163.9	160.5	160.4
OCH_2	60.0	60.0	60.0	60.0	58.4	58.3	58.9	59.0
$J_{1',2'a}$	6.6	9.0	6.6	7.0	7.7	7.8	7.1	5.9
$J_{1',2'b}$	2.5	5.2	<1.0	5.8	3.4	6.1	1.9	6.1
$J_{2'a,2'b}$	−15.5	−14.1	−15.4	−14.1	−14.2	−13.2	−14.2	−13.3
$J_{2'a,3'}$	6.8	6.7	6.7	6.6	7.0	6.2	6.6	6.0
$J_{2'b,3'}$	2.4	1.8	<1.0	2.8	3.0	2.8	1.9	5.0
$J_{3',4'}$	2.3	2.6	1.0	3.3	3.0	2.8	2.6	3.8
$J_{4',5'a}$	[a]	3.6	4.2[b]	3.8[b]	4.5[b]	3.9[b]	[a]	4.0
$J_{4',5'b}$	[a]	3.0	4.2[b]	3.8[b]	4.5[b]	3.9[b]	[a]	4.4
$J_{5'a,5'b}$	[a]	−12.3	[a]	[a]	[a]	[a]	[a]	−13.9

[a] Data inacessible. [b] Average values.

EXPERIMENTAL

General methods. — Evaporations were carried out with a Buchi rotary evaporator Model RE 111, and a Cryocool trap (acetone), under oil pump vacuum at <40° (bath), unless otherwise stated. U.v. absorption spectra were measured with a Varian DMS 70 spectrophotometer and i.r. spectra with a Perkin Elmer 397 spectrophotometer. N.m.r. spectra were recorded with a JEOL GX270 spectrometer, using standard conditions with data point resolution of ~0.1 Hz. ^{1}H Chemical shifts were measured relative to that of Me_4Si and ^{13}C chemical shifts relative to that of $CDCl_3$ (77.1 p.p.m.) or Me_2SO (39.5 p.p.m.). The ^{1}H- and ^{13}C-n.m.r. data are given in Table II for compounds **3a**, **4a**, **5a**, and **6a** in $CDCl_3$ and compounds **3b**, **4b**, **5b**, and **6b** in Me_2SO. Column chromatography was performed on silica gel (230–400 mesh; Aldrich), and t.l.c. on Silica Gel 60 F_{254} (Merck) with detection by u.v. absorbance, the Bratton–Marshall test[13] for primary aromatic amines, and ethanolic sulphuric acid for sugars. H.p.l.c. was performed with a Gilson Gradient instrument, using a Dynmax semi-preparative ODS column. Optical rotations were obtained using an ETL-NPL automatic polarimeter.

*Ethyl 5-amino-1-(2-deoxy-3,5-di-*O*-p-toluoyl-β-*D*-erythro-pentofuranosyl)imidazole-4-carboxylate* (**4a**) *and ethyl 4-amino-1-(2-deoxy-3,5-di-*O*-p-toluoyl-β-*D*-erythro-pentofuranosyl)imidazole-5-carboxylate* (**6a**). — To a suspension of ethyl 5-aminoimidazole-4-carboxylate[14] (5 g, 32 mmol) in anhydrous acetonitrile (200 mL) was added NaH (1 g, 42 mmol). The mixture was stirred at ambient temperature under dry nitrogen for 30 min. Finely powdered 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[15] (12.5 g, 32 mmol) was added and the resulting suspension was stirred rapidly for 3 h, when the mixture darkened considerably. T.l.c. (toluene–ethyl acetate, 1:1) revealed two products (R_F 0.15 and 0.25). The mixture was filtered, then concentrated, and a solution of the foamy residue (16 g) in ethyl acetate–toluene (1:1) was applied to a column (100 × 3 cm) of silica gel, equilibrated with the same solvent. After the initial elution of sugar debris, fractions containing mostly N-3 substituents were collected, combined, and concentrated. Crystallisation of the residue (2.76 g, 17%) from ethyl acetate gave **6a** (1.0 g, 6%), m.p. 80–81°, $[\alpha]_D$ +8° (*c* 1.2, chloroform).

Anal. Calc. for $C_{27}H_{29}N_3O_7$ (507): C, 63.91; H, 5.72; N, 8.28. Found: C, 63.66; H, 5.48; N, 8.05.

The mother liquors contained a mixture (1.76 g) of **6a** (10%), and the α-N-3 isomer (**5a**, 1.1%).

Fractions that contained both N-1 and N-3 substituents were combined and concentrated. Crystallisation of the residue (3.63 g, 22%) from ethyl acetate gave **4a** (1.25 g, 8%), m.p. 172–173°, $[\alpha]_D$ −52° (*c* 0.6, chloroform).

Anal. Found: C, 63.73; H, 5.97; N, 8.36.

The remaining residue contained (n.m.r. data) a mixture (2.38 g) of **4a** (2.4%), **6a** (12%), and **5a** (0.5%). The overall base conversion was 39%.

*Ethyl 5-amino-1-(2-deoxy-β-*D*-erythro-pentofuranosyl)imidazole-4-carboxylate* (**4b**) *and ethyl 4-amino-1-(2-deoxy-β-*D*-erythro-pentofuranosyl)imidazole-5-carboxylate* (**6b**). — A solution of **4a** (0.5 g, 0.99 mmol) in dry methanol (100 mL) was cooled in a

salt/ice mixture and saturated with dry ammonia, kept at 4° for 15 h, then concentrated, and the residue was partitioned between chloroform and water. The aqueous phase was co-concentrated several times with ethanol, and the residue was crystallised from ethanol–ether–light petroleum to give **4b** (0.23 g, 85%), m.p. 143–144°, $[\alpha]_D$ +4° (*c* 2.9, methanol); λ_{max}^{MeOH} 270 nm.

Anal. Calc. for $C_{11}H_{17}N_3O_5 \cdot H_2O$ (271): C, 45.66; H, 6.62; N, 14.53. Found: C, 45.43; H, 6.84; N, 14.47%.

Similarly, **6a** was converted into **6b** (0.19 g, 71%), m.p. 152–153°, $[\alpha]_D$ +47° (*c* 4.6, methanol); λ_{max}^{MeOH} 278 nm.

Anal. Found: C, 45.90; H, 6.84; N, 14.36.

Isolation of **3b–6b** *by reverse-phase h.p.l.c.* — A solution of the mixture (3.0 g) of **3a–6a**, described above, in ethanol (200 mL) was saturated with anhydrous ammonia, kept overnight at 4°, and then concentrated. The residue was partitioned between chloroform and water, and the aqueous layer was concentrated. A solution of the residue in ammonium hydrogen carbonate buffer (50mM, pH 7.5, 4 mL) was filtered through a 0.25-μm membrane and applied (batchwise) to a semi-preparative ODS column (Dynamax, 30 × 2.5 cm, fitted with a 10 × 2.5 cm guard column) equilibrated with the same buffer. The flow rate was 7 mL/min. The column was developed with a linear methanol gradient 0–50%, during 1 h, at the same flow rate. The capacity factors were determined as α-N-1 (**3b**), 3.10; β-N-1 (**4b**), 3.29; α-N-3 (**5b**), 3.67; and β-N-3 (**6b**), 3.91 (*cf.* 0.99 for 2′-deoxyinosine).

T.l.c. (1-butanol–acetic acid–water, 6:2:2) of the fractions showed the products to be homogenous, with R_F values **3b**, 0.34; **4b**, 0.34; **5b**, 0.43; and **6b**, 0.41.

Preparation of **4b** *and* **6b** *by transglycosylation.* — A solution of **1a** (1.02 g, 6.6 mmol) and 2′-deoxyuridine (3.0 g, 13.2 mmol) in ammonium acetate buffer (165 mL, 0.05M, pH 5.8) was stirred slowly for 20 h at 37° with 800 Ca^{2+} beads (diam. 5 mm) that each contained 8 × 10^8 cells of *E. coli* SPT[10]. The mixture was ultrafiltered, and the pellets were mechanically disintegrated in 200 mL of the same buffer, stirred for 2 h, and ultrafiltered. The combined filtrates were freeze-dried, a solution of the residue in water (40 mL) was filtered through Celite which was then washed with water (20 mL), and the filtrate was applied to a column (200 × 2.4 cm) of octadecylsilica gel (30 μm) in water. Elution with water at 3 mL/min afforded a fraction that contained 2′-deoxyuridine (1.6 g), followed by **1a** (0.25 g), and a mixture (1.3 g, 70%) of **4b** and **6b**.

This mixture was fractionated in 50-mg portions on a column (25 × 1.6 cm) of Separon SGX-C18 (10 μm) with triethylammonium hydrogen carbonate buffer (50mM, pH 7.5) that contained methanol (gradient 0–20%). The appropriate fractions were combined and concentrated *in vacuo* at 30° and 2 kPa. Ethanol was distilled twice from the residue, which was then crystallised from ethanol–light petroleum–ether to afford **4b** (340 mg, 19%), m.p. 143–144°, and **6b** (700 mg, 39.4%), m.p. 152–153°.

*5-Amino-1-(2-deoxy-β-*D-erythro-*pentofuranosyl)imidazole-4-carboxylic acid 5′-phosphate* (**4c**) *and 4-amino-1-(2-deoxy-β-*D-erythro-*pentofuranosyl)imidazole-5-carboxylic acid 5′-phosphate* (**6c**). — To a solution of the nucleoside **4b** or **6b** (0.1 g, 0.37 mmol) in triethyl phosphate (5 mL) at 0° was added phosphoryl chloride (0.075 mL, 0.8 mmol)

dropwise with stirring. The solution was kept overnight at 4°, then allowed to attain room temperature and poured into rapidly stirred anhydrous ether (200 mL). The precipitate was collected by centrifugation and washed with dry ether (50 mL), and a solution in water (2 mL) was adjusted quickly to pH 8 with 2M sodium hydroxide and applied to a column (1 × 15 cm) of Bio-Rad AG 1-X8 ($HCOO^-$) resin (200–400 mesh). The column was washed with water to remove unreacted nucleoside. The nucleotide was then eluted with a formic acid gradient (0 → 1.0M; total volume, 200 mL). The fractions that contained the main nucleotide peak were combined, and concentrated to dryness *in vacuo* (oil pump, bath < 35°). A solution of the residue in 0.5M sodium hydroxide (5 mL) was heated at 90° for 1–1.5 h, then decolourised with charcoal (pharmaceutical grade, acid free), and the pH was adjusted to 10 with 2M hydrochloric acid. The stock nucleotide solution was stored at −20° until required for enzyme kinetic studies.

The purification and assays for phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21) and phosphoribosylaminoimidazolesuccinocarboxamide synthetase (EC 6.3.2.6) will be reported elsewhere.

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