Purines, Pyrimidines, and Imidazoles. Part 64.[1] Alkylation and Acylation of Some Aminoimidazoles Related to Intermediates in Purine Nucleotide *de novo* and Thiamine Biosynthesis

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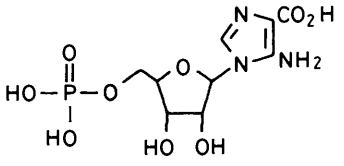
Treatment of ethyl 5-amino-1-benzylimidazole-4-carboxylate with butyl-lithium and methyl iodide gave the 5-*N*-methylamino derivative (**4b**) and the 3-methiodide (**5**) whereas ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate gave both the 5-*N*-methylamino (**6b**) and 2-methyl (**6d**) derivatives. Ethyl 5-amino-1-benzylimidazole-4-carboxylate with acetic anhydride or acetyl chloride gave various products, according to the conditions, including the 5-*N*-mono- and -*N*,*N*-di-acetylamino derivatives (**4d**) and (**4c**), respectively, and *N*,*N*'-dibenzyloxamide (**9**). The oxamide also arose from treatment of the imidazole (**4a**) with formaldehyde. 3-Cyanopropanimidate with ethyl α-amino-α-cyano acetate followed by benzylamine or 2,3-*O*-isopropylidene-D-ribosylamine afforded ethyl 5-amino-1-benzyl-2-(2-cyanoethyl)imidazole-4-carboxylate and ethyl 5-amino-1-(2,3-*O*-isopropylidene-α- and -β-D-ribofuranosyl)imidazole-4-carboxylates, respectively. Ethyl 5-amino-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2-ethoxycarbonylethylimidazole-4-carboxylate and the corresponding 2-ethoxyethyl nucleoside (**6i**) were similarly prepared. Oxidation of ethyl 5-amino-2-methylimidazole-4-carboxylate with *N*-chlorosuccinimide and potassium hydroxide led to ethyl 5-amino-1-benzyl-2-formylimidazole-4-carboxylate and oxidation of the protected 2-ethoxycarbonylethyl nucleoside (**6j**) with selenium dioxide produced the urea derivative (**6l**).

For a variety of reasons we have been interested in the preparation of certain types of 2-substituted 5-aminoimidazoles including nucleosides and nucleotides related to intermediates in the *de novo* biosynthetic pathway leading to purine nucleotides. Preliminary results[2] have indicated that such compounds may have potential as inhibitors of specific enzymes in the *de novo* pathway. In addition we hoped to synthesize similar imidazoles related to possible intermediates in thiamine biosynthesis which we believe involves 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5'-phosphate (CAIR) (**1**)



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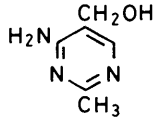

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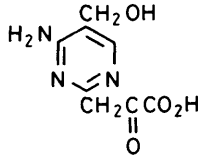
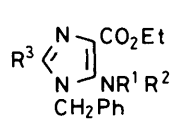


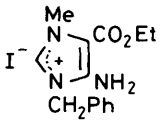
(**3**)

as a precursor of the pyrimidine moiety (**2**) of thiamine. It is not known whether the 2-methyl group in the pyrimidine is introduced at the imidazole or the pyrimidine stage, but there is evidence[3] that an oxidised three-carbon chain, probably derived from the ribosyl group in the nucleotide, is a precursor of the methyl group. In particular we wished to synthesize the oxo derivative (**3**) which has been proposed[4] as an intermediate in thiamine biosynthesis.

Accordingly, we have explored routes to 2-substituted imidazoles including 1-D-ribofuranosides in which the 2-substituent has up to three carbon atoms and is capable of being converted into suitable oxy and oxo alkyl derivatives which can be used in labelled form for appropriate biochemical incorporation studies. Apart from their intrinsic interest, the nucleoside derivatives are also a potential source, by acid hydrolysis, of the corresponding aglycones.

Substitution of the 2-position in 5-aminoimidazole-4-carboxylic acid derivatives may be compared with substitution at the analogous 8-position in purines. We have recently[5] measured the rate of hydrogen exchange at C-2 in ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate and this may be compared to exchange at C-8 in analogous purines. Direct alkylation at C-8 of a variety of purines *via* their lithium derivatives in high yields has recently been reported[6] and the electrophiles used included a wide variety of alkyl halides and aldehydes. We have examined similar reactions with some analogous amino imidazoles. Ethyl 5-amino-1-benzylimidazole-4-carboxylate (**4a**), prepared from ethyl α-amino-α-cyanoacetate, triethyl orthoformate, and benzylamine, was converted into a lithium derivative with butyl-lithium in THF and then treated with methyl iodide at −45 °C. Three compounds were produced and two were identified as the *N*-methyl derivative (**4b**) and the quaternary salt (**5**); the latter was also prepared by direct alkylation of (**4a**) with methyl iodide. In contrast, a similar reaction of the analogous isopropylidene nucleoside (**6a**)[7] with butyl-lithium followed by methyl iodide at −45 °C gave, in addition to starting material (46%), two monomethyl derivatives namely the *N*-methyl- and 2-methyl-nucleosides (**6b**) (11%) and (**6d**) (26%), respectively.



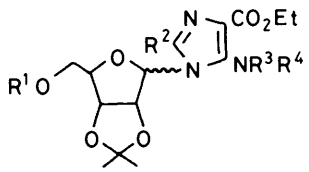


(5)

(4) a; $R^1 = R^2 = R^3 = H$
b; $R^1 = Me$, $R^2 = H$, $R^3 = H$
c; $R^2 = R^2 = Ac$, $R^3 = H$
d; $R^1 = Ac$, $R^2 = R^3 = H$
e; $R^1 = R^2 = H$, $R^3 = Me$
f; $R^1 = R^2 = H$, $R^3 = (CH_2)_2CN$
g; $R^1 = R^2 = H$, $R^3 = CHO$

The structure assigned to the latter compound was confirmed by comparison with a sample prepared by an alternative method involving reaction of the imidate (**7**) with 2,3-*O*-isopropylidene-D-ribosylamine (**8**)[7] and separation of the resulting α- and β-anomeric nucleosides (**6c**) and (**6d**),[8] respectively. An attempt to react the lithium derivative of nucleoside (**6a**) with benzaldehyde however resulted in recovery of starting material in high yield. The structure of the former compound (**6b**) was confirmed by its negative reaction in the Bratton–Marshall[9] test for aromatic amines implying that the introduced methyl group was attached to the 5-amino group.

We have also examined the acylation of some amino-imidazoles under a variety of conditions. In particular ethyl 5-amino-1-benzylimidazole-4-carboxylate (**4a**) with acetic anhydride at 100 °C over 1 h gave the 5-*N*,*N*-diacetyl derivative (**4c**) (26%) but the main product formed was *N*,*N*′-dibenzyloxamide (**9**) (46%). A similar reaction of (**4a**) with acetyl chloride in hot dry pyridine over 1.4 h gave mainly starting material (66%) together with the oxamide (**9**) (22%) and the diacetyl derivative (**4c**) (13%). In contrast (**4a**), when boiled with a mixture of acetic anhydride and acetic acid for 1 h, produced the *N*-monoacetylimidazole (**4d**) (58%) as the major



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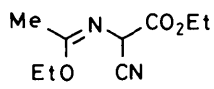

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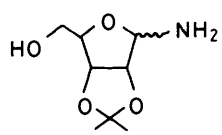

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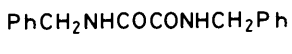
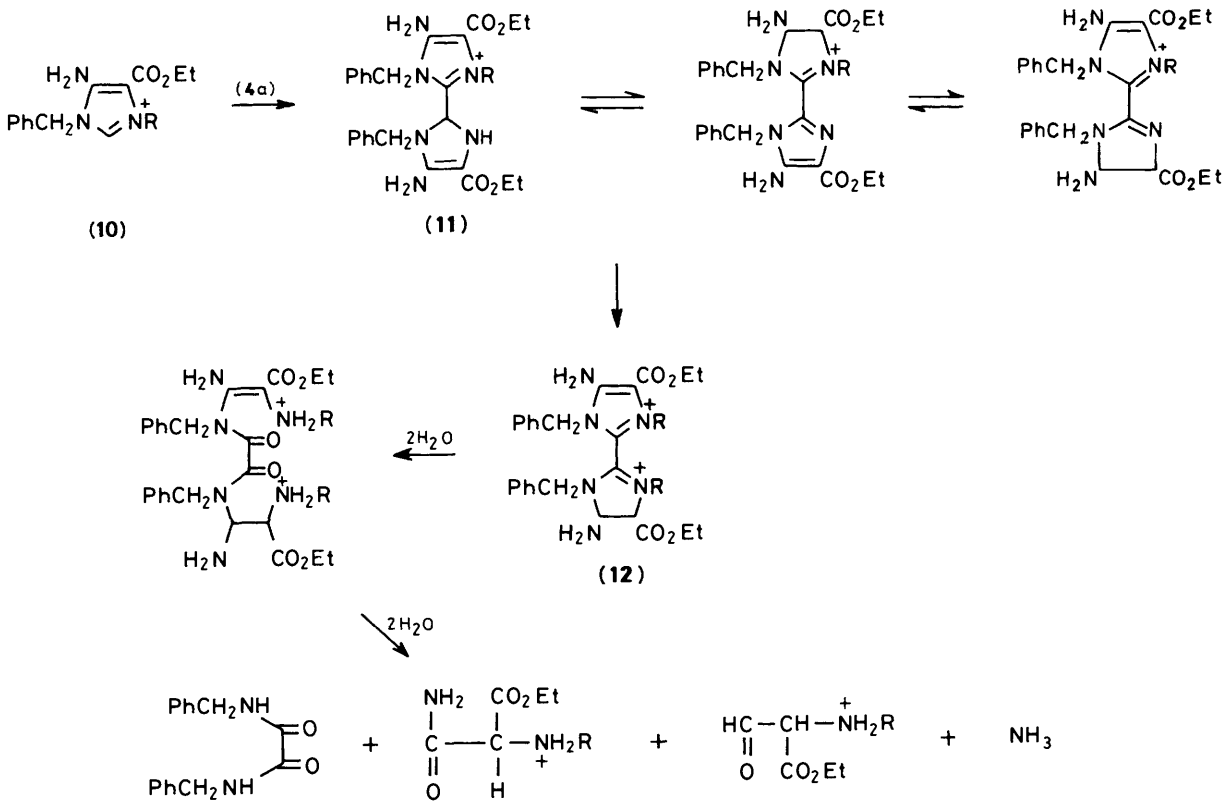


(9)

a; $R^1 = R^2 = R^3 = R^4 = H$, β-anomer
b; $R^1 = R^2 = R^3 = H$, $R^4 = Me$, β-anomer
c; $R^1 = R^3 = R^4 = H$, $R^2 = Me$, α-anomer
d; $R^1 = R^3 = R^4 = H$, $R^2 = Me$, β-anomer
e; $R^1 = R^3 = R^4 = H$, $R^2 = (CH_2)_2CN$, α-anomer
f; $R^1 = R^3 = R^4 = H$, $R^2 = (CH_2)_2CN$, β-anomer
g; $R^1 = R^3 = R^4 = H$, $R^2 = (CH_2)_2CO_2Et$, α-anomer
h; $R^1 = R^3 = R^4 = H$, $R^2 = (CH_2)_2CO_2Et$, β-anomer
i; $R^1 = R^3 = R^4 = H$, $R^2 = (CH_2)_2OEt$, β-anomer
j; $R^1 = COBu^t$, $R^2 = (CH_2)_2CO_2Et$, $R^3R^4 = =CHNMe_2$, β-anomer
k; $R^1 = COBu^t$, $R^2 = CH_2COCO_2Et$, $R^3R^4 = =CHNMe_2$, β-anomer
l; $R^1 = COBu^t$, $R^2 = (CH_2)_2CO_2Et$, $R^3 = H$, $R^4 = CONMe_2$, β-anomer.
m; $R^1 = H$, $R^2 = (CH_2)_2CO_2Et$, $R^3R^4 = =CHNMe_2$, β-anomer

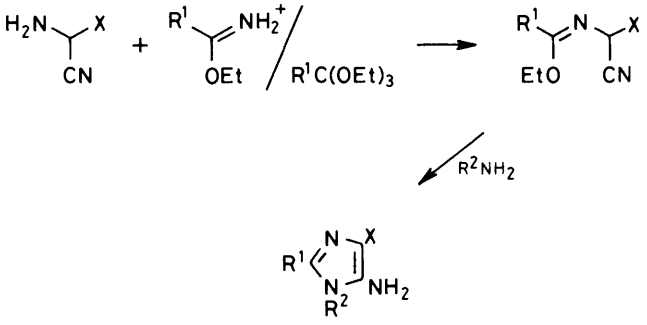
product together with the oxamide (22%). The oxamide was also obtained from the reaction of the aminoimidazole (**4a**) with hot aqueous formaldehyde. Both acetyl derivatives (**4c**) and (**4d**) gave negative tests with the Bratton–Marshall reagents.



Scheme 1.

A possible mechanism for the formation of *N,N'*-dibenzyloxamide in these various reactions involves prior formation of a quaternised (at N-3) imidazole (**10**) which under the hot conditions reacts with a second molecule of the imidazole to produce an intermediate imidazolyl–imidazoline (**11**) which, perhaps after further quaternisation to give (**12**), would be expected to undergo ready hydrolysis to produce the oxamide and appropriate malonic acid derivatives (Scheme 1). In support of such a mechanism it should be noted that N-3 is the preferred site for protonation and alkylation in aminoimidazoles of these types.[10] Also quaternised imidazoles of this type are very susceptible to ring opening in aqueous solution[10] and the aminoimidazoles react readily at C-2 with electrophiles, *e.g.* bromine.[8]

The problems encountered in the various attempts to introduce a 2-substituent directly into the aminoimidazole ring system prompted us to return to the synthesis of 2-substituted imidazoles from acyclic intermediates and recorded in earlier publications[8,11] in this series. This, the Shaw method,[11] involves the reaction of an α-amino-α-cyanoacetic acid derivative (ester, amide, nitrile, *etc.*) with either an imidate or an ortho ester followed by a primary amine (Scheme 2).

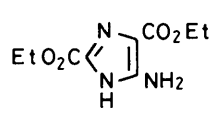


R^1 = alkyl or aryl
R^2 = alkyl, aryl, or glycosyl
X = CO_2R^1, $CONH_2$, $CSNH_2$, or CN

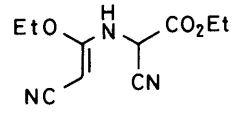
Scheme 2.

Although the reaction has earlier been shown to work well both in model systems and with nucleosides using alkanoic acid imidates or ortho esters, an attempt to react ethyl α-amino-α-cyanoacetate with the imidate derived from ethyl cyanoacetate only produced the aminoimidazole diester (**13**), a compound which we have earlier shown[12] to form by heating ethyl α-amino-α-cyanoacetate, or by leaving it at room temperature for some weeks. Similar results were obtained using imidates derived from aminomalononitrile suggesting that the intermediate imidate produced in these reactions adopts the carbamate-like structure (**14**) in which the alkoxy group would be less likely to react readily with a primary amine. In support of this, and in direct contrast to the above results, reaction of ethyl α-amino-α-cyanoacetate with the monoimidate derived from the next homologue of malononitrile namely succinonitrile, followed by benzylamine gave the crystalline ethyl 5-amino-1-benzyl-2-(2-cyanoethyl)imidazole-4-carboxylate (**4f**) in modest yield (31%). 2,3-*O*-Isopropylidene-D-ribosylamine (**8**) with ethyl 3-cyanopropanimidate similarly gave a mixture of the α- and β-nucleoside esters (**6e**) and (**6f**) which were separated by chromatography on silica gel. Similarly, the imidate from ethyl 2-cyanopropionate produced the α- and β-imidazole nucleosides (**6g**) and (**6h**), respectively.

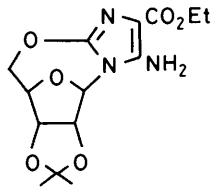
However, only the β-anomer (**6i**) resulted from the treatment of 3-ethoxypropanimidate with the ribosylamine (**8**) followed by ethyl α-amino-β-cyanoacetate. Oxidation of ethyl 5-amino-2-



(**13**)



(**14**)



(**15**)

methylimidazole-4-carboxylate (**4e**) with *N*-chlorosuccinimide and potassium hydroxide gave the corresponding 2-formyl derivative (**4g**). In contrast to this reaction we have shown earlier[13] that oxidation of the 2-unsubstituted 5-aminoimidazole nucleoside (**6a**) with *N*-chlorosuccinimide leads to the cyclonucleoside (**15**). Accordingly, to avoid this type of reaction, we protected the amino group in (**6h**) by reaction with dimethylformamide dimethyl acetal to produce (**6m**) which, with pivalic anhydride, gave (**6j**). Attempts to produce an oxo derivative of type (**6k**) by treatment of (**6j**) with selenium dioxide in acetic anhydride gave the urea (**6l**) the structure of which is assigned on the basis of n.m.r. evidence (see Experimental section). Notable points are (*i*) the absence of absorptions for the formamidino proton and carbon atoms (expected at δ_H 8.2 and δ_C 159 p.p.m., respectively); (*ii*) the NMe_2 group has δ_H 2.11 and 2.50, and δ_C 25.2 and 26.4 were more in keeping with an amide group than an amidino group [*cf.* data for (**6j**)].

Experimental

Evaporations were carried out with a Büchi rotary evaporator under water pump vacuum with a flask temperature <40 °C unless otherwise stated. U.v. absorption spectra were measured with Unicam SP 800 and Varian DMS 90 spectrophotometers, i.r. spectra with Perkin-Elmer 307 and 1320 spectrophotometers, n.m.r. spectra with JEOL-MH-100, JEOL 270 MHz FT NMR, and JEOL-FX-90Q instruments and mass spectra with an A.E.I. MS 902 spectrophotometer. Silica gel (0.05—0.20 mm, 315—70 mesh) from Machery Nagel and Co. was used for column chromatography and silica gel $60F_{254}$ 0.25 mm pre-coated glass plates from Merck were used for t.l.c. Compounds were detected on t.l.c. plates by u.v. absorbance or by the Bratton–Marshall test,[9] for primary aromatic amines.

Preparation of Imidates.—The appropriate nitrile (0.5 mol) in either dry ether or THF (100 cm^3) with EtOH (0.5 mol) was cooled to 0 °C and hydrogen chloride (0.5 mol) added until the theoretical increase in weight resulted. The solution was left for 24—28 h at 4 °C and then the crystalline product was collected and used without further purification. The following results were obtained for $RC(OEt)\cdot NH_2^+Cl^-$ (R, yield, m.p., spectra): Me, 80%, 112—114 °C; $CNCH_2CH_2$, 76%, 198—201 °C; $\nu_{max.}$ 2 250 cm^{-1} (CN); δ_H(60 MHz; $CDCl_3$) 1.25 (3 H, t, *J* 7 Hz, Me), 4.2 (2 H, q, *J* 7 Hz, OCH_2), 2.53—2.63 (4 H, m, CH_2CH_2), and 12.0 (2 H, s, NH_2^+); $EtO_2CCH_2CH_2$, 86%, 89—90 °C; δ_H(60 MHz, $CDCl_3$) 1.20 and 1.30 (each 3 H, t, Me × 2), 2.73—3.33 (4 H, m, CH_2CH_2), 4.20 and 4.58 (each 2 H, q, OCH_2), and 11.5 (2 H, s, NH_2^+); $EtOCH_2CH_2$, 48%, 202—204 °C.

Ethyl 5-Amino-1-benzylimidazole-4-carboxylate (**4a**).—A mixture of ethyl α-amino-α-cyanoacetate (5.6 g) and triethyl

orthoformate (7 g) in acetonitrile (40 cm^3) was boiled under reflux for 45 min. The solution was cooled and benzylamine (5 g) added to produce a red solution which was set aside at room temperature overnight to give a precipitate of the aminoimidazole (**4a**) (5.7 g, 58%) which recrystallised from ethanol as needles, m.p. 156 °C (Found: C, 63.5; H, 6.25; N, 17.2%; M^+, 245. $C_{13}H_{15}N_3O_2$ requires C, 63.65; H, 6.15; N, 17.15%; *M*, 245); δ_H(60 MHz; $CDCl_3$) 1.32 (3 H, t, *J* 7 Hz, CH_2CH_3), 4.08—4.41 (2 H, q, *J* 7 Hz, CH_2CH_3), 4.90 (4 H, br s, CH_2Ph and NH_2, latter exch. with D_2O), 6.90 (1 H, s, N=CH), and 6.90—7.4 (5 H, m, Ph); δ_C(270 MHz, $CDCl_3$) 14.5 (Me), 47.4 (CH_2Ph), 59.7 (OCH_2), 112.3 (C-4), 126.7, 128.5, 129.2, and 134.3 (Ph), 131.4 (C-2), 145.1 (C-4), and 164.7 p.p.m. (CO). The compound gave a positive Bratton–Marshall test.

Alkylation and Acetylation of Imidazoles (**4a**) *and* (**6a**).—(a) *With butyl-lithium and methyl iodide.* Ethyl 5-amino-1-benzylimidazole-4-carboxylate (**4a**) (0.50 g, 0.002 mol) was dissolved in peroxide-free THF (50 cm^3) in a 3-necked flask fitted with a condenser, calcium chloride drying tube, and septum and the mixture cooled to −45 °C in a slush obtained from chlorobenzene and liquid nitrogen. BuLi in cyclohexane (2 cm^3, 0.02 mol) was added and the reaction mixture left for 2 h. The reaction was then quenched with methyl iodide (4 cm^3) and the reaction mixture allowed to reach room temperature. T.l.c. examination (chloroform–methanol, 9:1) of the pale yellow solution showed the presence of two compounds (R_F 0.76 and 0.50); the former gave a negative and the latter a positive Bratton–Marshall reaction. The solution was evaporated to a gum (0.63 g) and chromatographed on a silica gel column (2.5 × 60 cm) with EtOH–$CHCl_3$ (1:99). The first eluted component was *ethyl 1-benzyl-5-methylaminoimidazole-4-carboxylate* (**4b**), as a gum (70 mg, 13%) which was homogeneous on t.l.c. (R_F 0.76) (Found: M^+, 259. $C_{14}H_{17}N_3O_2$ requires *M*, 259); δ_H(60 MHz; $CDCl_3$) 1.32 (3 H, t, *J* 7 Hz, CH_2CH_3), 2.90 (3 H, br s, $NHCH_3$), 4.18—4.42 (2 H, q, *J* 7 Hz, CH_2CH_3), 4.70 (1 H, br s, NH exch. with D_2O), 4.90 (2 H, s, CH_2Ph), 6.90—7.40 (5 H, m, ArH), and 7.45 (1 H, s, 2-H). It gave a negative Bratton–Marshall test. The second eluted component was *5-amino-1-benzyl-4-ethoxycarbonyl-3-methylimidazolium iodide* (**5**) (0.36 g, 46.5%) (R_F 0.50) which crystallised from ethanol as needles, m.p. 142 °C (Found: C, 43.3; H, 4.6; N, 10.7%; M^+, 260. $C_{14}H_{18}IN_3O_2$ requires C, 43.45; H, 4.7; N, 10.8%; *M*, 260 + 127); $\lambda_{max.}$(MeOH) 273 nm; δ_H(60 MHz, $CDCl_3$) 1.43 (3 H, t, *J* 7 Hz, CH_2CH_3), 4.0 (3 H, s, NCH_3), 4.46 (2 H, q, *J* 7 Hz, 2 H, CH_2CH_3), 5.70 (2 H, s, CH_2Ph), 6.43 (2 H, br s, NH_2 exch. with D_2O), 7.30—8.70 (5 H, m, ArH), and 9.42 (1 H, s, 2-H). It gave a positive Bratton–Marshall test. Compound (**5**) was also obtained as follows. A suspension of imidazole (**4a**) (1.0 g) and methyl iodide (1.3 g) in acetonitrile (10 cm^3) was heated in a sealed tube at 100 °C for 1 h. Evaporation of the solvent gave a gum which afforded the *imidazole quaternary salt* (1.1 g) which separated from ethanol as needles, m.p. 142 °C, identical (mixed m.p., n.m.r, and mass spectra) to the compound prepared above.

Ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylate (**6a**) (1.0 g) and butyl-lithium were allowed to react as above. T.l.c. examination (chloroform–dimethyl ether–methanol, 5:5:1) of the pale yellow solution showed three products (R_F 0.39, 0.31, and 0.20) the lower of which corresponded to the starting material and gave a positive Bratton–Marshall test. The remaining compounds gave negative Bratton–Marshall tests. The solution was evaporated to a gum and chromatographed on a silica gel column with $CHCl_3$–Et_2O–EtOH (50:50:1). The first eluted component (R_F 0.39) was *ethyl-5-methylamino-1-(2,3-O-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate* (**6b**) (110 mg, 11%, obtained as a gum but homogeneous on t.l.c. examination in several solvents) (Found: M^+, 341. $C_{15}H_{23}N_3O_6$ requires *M*, 341); $\lambda_{max.}$(MeOH) 268 nm; δ_H(90 MHz; $CDCl_3$) 1.37 and 1.60 (each 3 H, s, CMe_2), 1.37 (3 H, t, *J* 7 Hz, CH_2CH_3), 2.0—3.5 (2 H, br s, OH, NH exch. with D_2O), 2.94 (3 H, s, $NHCH_3$), 3.85—3.92 (2 H, m, 5′-H), 4.29—4.44 (2 H, q, *J* 7 Hz, CH_2CH_3), 4.30—4.42 (1 H, m, 4′-H), 4.80—4.90 (2 H, m, 2′- and 3′-H), 5.83 (1 H, d, *J* 3 Hz, 2′-H), and 7.69 (1 H, s, 2-H). It gave a negative Bratton–Marshall test. The second eluted component was ethyl 5-amino-2-methyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylate (**6d**) (270 mg, 26%) (R_F 0.31) which crystallised from ethanol as needles, m.p. and mixed m.p. 167 °C (lit.,[8] 167 °C) (Found: C, 52.7; H, 6.95; N, 12.1. Calc. for $C_{15}H_{23}N_3O_6$: C, 52.8; H, 6.8; N, 12.3%) and a third component was the starting material (460 mg, 46%), m.p. and mixed m.p. 154 °C (lit.,[6] 153—154 °C) (Found: C, 51.5; H, 6.7; N, 12.7. Calc. for $C_{14}H_{21}N_3O_6$: C, 51.35; H, 6.45; N, 12.85%).

(b) *With acetic anhydride.* Ethyl 5-amino-1-benzylimidazole-4-carboxylate (**4a**) (1.0 g, 4 mmol) and acetic anhydride (5 cm^3) were heated at 100 °C in a sealed tube for 1 h. T.l.c. examination (chloroform–methanol, 11:1) showed the presence of three compounds (R_F 0.75, 0.67, and 0.46). This mixture was chromatographed on silica gel with $CHCl_3$–EtOH (99:1). The first eluted component (R_F 0.75) was *N,N′*-dibenzyloxamide (450 mg, 41%) which crystallised from ethanol as needles, m.p. 203 °C (Found: C, 71.2; H, 6.0; N, 10.2%; M^+, 268. Calc. for $C_{16}H_{16}N_2O_2$: C, 71.65; H, 6.0; N, 10.45%; *M*, 268); δ_H(60 MHz; $CDCl_3$/[$^2H_6$]DMSO) 4.38 (4 H, d, *J* 6 Hz, CH_2NH × 2), 7.13 (10 H, m, ArH), and 8.10 (2 H, br t, *J* 6 Hz, NH × 2). The compound was identical with an authentic sample prepared as follows. (*i*) Oxalic acid (1 g, 0.014 mol), benzylamine (2.8 cm^3, 0.028 mol), and ammonium chloride (0.15 g) were heated together under reflux for 1 h. The mixture was cooled and then shaken with water (10 cm^3) to give *N,N′*-dibenzyloxamide which crystallised from ethanol as needles, m.p. 203 °C (Found: C, 71.6; H, 6.0; N, 10.45%; M^+, 268). (*ii*) Oxalic acid (1.0 g, 0.014 mol), benzylamine (2.8 cm^3, 0.28 mol), and DCC (5.7 g) in acetonitrile (50 cm^3) were shaken together for 15 min and the solution left for 1 h when the oxamide separated.

The second eluted component was *ethyl 5-diacetylamino-1-benzylimidazole-4-carboxylate* (**4c**) isolated as a gum (220 mg, 26%) homogeneous on t.l.c. (R_F 0.67) (Found: M^+, 329. $C_{17}H_{19}N_3O_4$ requires *M*, 329); $\nu_{max.}$ 1 695 ($CONR_2$) and 1 710 cm^{-1} (CO_2R); δ_H(60 MHz, $CDCl_3$) 1.30 (3 H, t, *J* 7 Hz, OCH_2CH_3), 2.02 [6 H, s, $N(COCH_3)_2$] 4.78 (2 H, s, CH_2Ph) 6.98—7.40 (5 H, m, ArH), and 7.48 (1 H, s, N=CH). The compound gave a negative Bratton–Marshall test.

(c) *With acetyl chloride.* Acetyl chloride (0.4 g, 4.6 mmol) was added to a suspension of the imidazole (**4a**) (1.0 g, 4 mmol) in anhydrous pyridine (75 cm^3) and the mixture heated at 55 °C for 3 h with exclusion of moisture. T.l.c. examination ($CHCl_3$–MeOH, 11:1) indicated the presence of the two products (R_F 0.67 and 0.75). The pyridine solution was cooled and mixed with dichloromethane (150 cm^3) and the mixture washed successively with 2M sulphuric acid (2 × 100 cm^3), saturated aqueous sodium hydrogen carbonate (2 × 20 cm^3), and water (1 × 20 cm^3) and dried (Na_2SO_4). Evaporation gave a gum which was chromatographed on a silica gel column (2.5 × 5 cm) with EtOH–$CHCl_3$ (1:99). The first eluted component was *N,N′*-dibenzyloxamide (0.12 g, 22%) (R_F 0.75), m.p. and mixed m.p. 204 °C and the second component was ethyl 5-diacetylamino-1-benzylimidazole-4-carboxylate (**4c**) (100 mg, 13%) (R_F 0.67) identical with compound obtained above.

(d) *With acetic anhydride and acetic acid.* Ethyl 5-amino-1-benzylimidazole-4-carboxylate (0.5 g, 2 mmol), acetic anhydride (5 cm^3), and acetic acid (5 cm^3) were boiled under reflux for 1 h. T.l.c. examination (chloroform–methanol, 11:1) showed the presence of two products (R_F 0.75 and 0.55), each of which gave a negative Bratton–Marshall spray test. The solvent was

evaporated and the resulting gum chromatographed on a silica gel column (2.5 × 60 cm) with EtOH–$CHCl_3$ (1:99) to afford *N,N'*-dibenzyloxamide (0.12 g, 22%) (R_F 0.75) and a second component (R_F 0.55), identified as *ethyl 5-acetylamino-1-benzylimidazole-4-carboxylate* (**4d**) (290 mg, 58%) which crystallised from ethanol as needles, m.p. 174 °C (Found: C, 62.7; H, 5.9; N, 14.4%; M^+, 287. $C_{15}H_{17}N_3O_3$ requires C, 62.7; H, 5.95; N, 14.6%; *M*, 287); $\nu_{max.}$ 1 680 ($CONR_2$) and 1 710 cm^{-1} (CO_2R); δ_H(60 MHz; $CDCl_3$) 1.32 (2 H, t, *J* 6 Hz, $CO_2CH_2CH_3$), 2.10 (3 H, s, $COCH_3$), 4.10—4.45 (3 H, q, *J* 6 Hz, $CO_2CH_2CH_3$), 5.10 (2 H, s, CH_2Ph), 6.90—7.45 (6 H, m, ArH and N=CH), and 8.10 (br, HNCO exch. with D_2O). The compound gave a negative Bratton–Marshall test.

(e) *With formaldehyde.* Ethyl 5-amino-1-benzylimidazole-4-carboxylate (0.5 g, 2.0 mmol) and a solution of formaldehyde (20 cm^3) were heated under reflux for 1 h. The cooled solution gave a crystalline precipitate of *N,N'*-dibenzyloxamide (0.15 g) and t.l.c. examination of the remaining solution revealed only starting material.

Ethyl 5-Amino-1-benzyl-2-methylimidazole-4-carboxylate (**4e**).—A suspension of ethyl aminocyanoacetate (5.2 g, 0.03 mol) and ethyl acetimidate hydrochloride (4.0 g, 0.03 mol) in acetonitrile (60 cm^3) was shaken until the crystalline solids had been replaced by a precipitate of ammonium chloride (approximately 1 h). The solution was cooled at 4 °C for 30 min and filtered. Benzylamine (4 cm^3, 0.03 mol) was added to the filtrate and the solution left at room temperature overnight to give a precipitate of the *imidazole* (**4e**) (3.9 g, 43%), which crystallised from ethanol as needles, m.p. 223 °C (Found: C, 64.95; H, 6.6: N, 16.3%; M^+, 259. $C_{14}H_{17}N_3O_2$ requires C, 64.85; H, 6.6; N, 16.2%; *M*, 259); $\lambda_{max.}$(MeOH) 272 nm; δ_H(60 MHz; $CDCl_3$) 1.37 (3 H, t, *J* 7 Hz, CH_2CH_3), 2.30 (3 H, s, 2-Me), 4.15—4.50 (2 H, q, *J* 7 Hz, CH_2CH_3), 4.70 (2 H, br s, NH_2 exch. with D_2O), 4.92 (2 H, s, CH_2Ph), and 6.90—7.40 (5 H, m, ArH). The compound gave a positive Bratton–Marshall test.

Ethyl 5-Amino-1-benzyl-2-(2-cyanoethyl)imidazole-4-carboxylate (**4f**).—A suspension of ethyl aminocyanoacetate (5.2 g, 0.03 mol) and ethyl 3-cyanopropanimidate hydrochloride in acetonitrile (60 cm^3) was treated as above, and then treated with benzylamine (4 cm^3, 0.03 mol) to give the *imidazole* (**4f**) (2.5 g, 31%) which crystallised from ethanol as needles, m.p. 196 °C (Found: C, 64.6; H, 6.2; N, 18.9%; M^+, 298. $C_{16}H_{18}N_4O_2$ requires C, 64.4; H, 6.1; N, 18.8%; *M*, 298); $\lambda_{max.}$(MeOH) 272 nm; δ_H(60 MHz; $CDCl_3$) 1.32 (3 H, t, *J* 7 Hz, CH_2CH_3), 2.80 (4 H, s, CH_2CH_2CN), 4.22—4.50 (2 H, q, *J* 7 Hz, CH_2CH_3), 4.80 (2 H, br s, NH_2 exch. D_2O), 4.96 (2 H, s, CH_2Ph), and 6.85—7.45 (5 H, m, ArH). The compound gave a positive Bratton–Marshall test.

Ethyl 5-Amino-1-benzyl-2-formylimidazole-4-carboxylate (**4g**).—A solution of ethyl 5-amino-1-benzyl-2-methylimidazole-4-carboxylate (0.39 g, 1.5 mmol) and *N*-chlorosuccinimide (0.42 g, 3.0 mmol) in peroxide-free THF with 1M potassium hydroxide (2.35 cm^3, 4.5 mol) was set aside at room temperature for 3 days. The mixture was evaporated to a gum which was chromatographed on a silica gel column (2.5 × 60 cm) with toluene–ethyl acetate (3:7) to yield the *formylimidazole* (**4g**) (0.12 g, 30%) which crystallised from ethanol as needles, m.p. 154 °C (Found: C, 61.6; H, 5.6; N, 15.5%; M^+, 273. $C_{14}H_{15}N_3O_3$ requires C, 61.5; H, 5.5; N, 15.4%; *M*, 273); $\lambda_{max.}$(MeOH) 257 and 326 nm; δ_H(60 MHz; $CDCl_3$) 1.4 (3 H, t, *J* 7.1 Hz, OCH_2CH_3), 4.3 (2 H, q, *J* 7.1 Hz, OCH_2CH_3), 5.2 (2 H, br s, NH_2), 5.5 (2 H, s, CH_2Ph), 7.0—7.4 (5 H, m, ArH), and 9.5 (1 H, s, CHO); δ_C 14.50 (OCH_2CH_3), 47.2 (OCH_2CH_3), 60.8 (CH_2Ph), 115.7 (C-4), 126.8, 128.6, 129.4, and 133.9 (Ph), 137.2 (C-2), 148.6 (C-5), 164.1 (CO_2Et), and 181.3 p.p.m. (CHO). The compound gave a positive Bratton–Marshall test.

Ethyl 5-Amino-2-methyl-1-(2,3-O-isopropylidene-α- and -β-D-ribofuranosyl)imidazole-4-carboxylates (**6c**) *and* (**6d**).—The following is an improved preparation of the β-nucleoside. A suspension of 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate[7] (7.2 g, 0.02 mol) and ethyl acetimidate (2.4 g, 0.02 mol) in acetonitrile (60 cm^3) was stirred in a stoppered flask for 5 min. Triethylamine (2.8 cm^3, 0.02 mol) was then added and the mixture stirred for a further 1 h. The precipitate was filtered off and the filtrate evaporated to a gum which was then dissolved in chloroform (60 cm^3). The solution was washed with water (2 × 10 cm^3), dried (Na_2SO_4), and re-evaporated to give a pale yellow gum (4.5 g) which was treated with ethyl aminocyanoacetate (2.4 g, 0.02 mol) in acetonitrile (50 cm^3) under reflux for 20 min. T.l.c. examination ($CHCl_3$–MeOH, 9:1) showed the presence of one major Bratton–Marshall positive compound (R_F 0.62). The reaction mixture was evaporated to a gum which was dissolved in chloroform (60 cm^3) and the solution washed with 2M sodium hydroxide (2 × 10 cm^3). The organic phase was dried (Na_2SO_4) and evaporated to a gum (3.2 g) which was chromatographed on a silica gel column (2.5 × 60 cm) with EtOH–$CHCl_3$ (1:49). The β-imidazole nucleoside (**6d**) (R_F 0.62) eluted first (2% ethanolic chloroform) and crystallised from ethyl acetate, then ethanol as needles (2.0 g, 30%), m.p. 167 °C (lit.,[8] 167 °C) (Found: C, 52.7; H, 6.7; N, 12.3%; M^+, 341. Calc. for $C_{15}H_{23}N_3O_6$: C, 52.8; H, 6.8; N, 12.3%; *M*, 341); $\lambda_{max.}$(MeOH) 273 mm; δ_H(200 MHz; $CDCl_3$) 1.35 and 1.59 (each 3 H, s, CMe_2), 1.36 (3 H, t, *J* 7 Hz, OCH_2CH_3), 2.35 (3 H, s, 2-Me), 3.96—4.06 (2 H, m, 5'-CH_2), 4.19—4.21 (1 H, m, 4'-H), 4.31 (2 H, q, *J* Hz, OCH_2CH_3), 4.99—5.09 (2 H, m, 2'-H), 5.67 (1 H, d, *J* 4 Hz, 1'-H), and 5.85 (3 H, br s, OH, NH_2 exch. D_2O). A second eluted component, probably the *α-imidazole nucleoside* (**6c**) was obtained as a gum (140 mg, 2%) homogeneous on t.l.c. (Found: M^+, 341. $C_{15}H_{23}N_3O_6$ requires *M*, 341); δ_H($CDCl_3$) 6.12 (1 H, d, *J* 4 Hz, 1'-H).

Ethyl 5-Amino-2-(2-cyanoethyl)-1-(2,3-O-isopropylidene-α- and -β-D-ribofuranosyl)imidazole-4-carboxylates (**6e**) *and* (**6f**).—A suspension of 2,3-*O*-isopropylidene-D-ribofuranosylamine -sulphonate (7.2 g, 0.02 mol) and ethyl 3-cyanopropanimidate hydrochloride (2.6 g, 0.02 mol) was allowed to react as above and the product treated similarly with ethyl aminocyanoacetate (2.4 g, 0.02 mmol). T.l.c. examination of the reaction mixture ($CHCl_3$–MeOH, 9:1) showed the presence of two Bratton–Marshall positive compounds (R_F 0.69 and 0.44). The reaction mixture was worked up and chromatographed as above. The β-*imidazole nucleoside* (**6f**) (R_F 0.69) eluted first with EtOH–$CHCl_3$ (1:49) and crystallised from ethyl acetate then EtOH as colourless needles (1.1 g, 14%), m.p. 208 °C (Found: C, 53.4; H, 6.3; N, 14.7%; M^+, 380. $C_{17}H_{24}N_4O_6$ requires C, 53.7; H, 6.35; N, 14.7%; *M*, 380); $\lambda_{max.}$(MeOH) 273 nm; δ_H(90 MHz; $CDCl_3$) 1.35 and 1.60 (each 3 H, s, CMe_2), 2.43—3.35 (4 H, m, CH_2CH_2), and 5.6 (1 H, d, *J* 3 Hz, 1'-H). A second eluted component, the *α-imidazole nucleoside* (**6e**) (0.2 g, 3%), was homogeneous on t.l.c. (Found: M^+, 380. $C_{17}H_{24}N_4O_6$ requires *M*, 380); $\nu_{max.}$ 2 250 cm^{-1} (CN); δ_H(60 MHz; $CDCl_3$) 1.35 and 1.45 (each 3 H, s, CMe_2) and 5.90 (1 H, d, *J* 7 Hz, 1'-H).

Ethyl 5-Amino-2-(2-ethoxycarbonylethyl)-1-(2,3-O-isopropylidene-α- and -β-D-ribofuranosyl)imidazole-4-carboxylates (**6g**) *and* (**6h**).—A suspension of 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate (7.2 g, 0.02 mol) and ethyl 2-ethoxycarbonylpropanimidate hydrochloride (4.19 g, 0.02 mol) were allowed to react as above and the product treated similarly with ethyl α-amino-α-cyanoacetate (2.4 g, 0.02 mol). T.l.c. examination ($CHCl_3$–MeOH, 9:1) showed the presence of

one major Bratton–Marshall positive compound (R_F 0.68). The reaction mixture was worked up and chromatographed as above. The β-*imidazole nucleoside* (**6h**) (2.5 g, 29%) eluted first ($CHCl_3$–EtOH, 45:1) and crystallised from ethanol as prisms, m.p. 200 °C (Found: C, 53.2; H, 6.85; N, 9.7%; M^+, 427. $C_{19}H_{29}N_3O_8$ requires C, 53.4; H, 6.85; N, 9.8%; *M*, 427); $\lambda_{max.}$(MeOH) 272 nm; δ_H(200 MHz; $CDCl_3$) 1.25 and 1.35 (each 3 H, t, *J* 6.9 Hz, OCH_2CH_3 × 2), 1.35 and 1.59 (each 3 H, s, CMe_2), 2.7—3.1 (4 H, m, CH_2CH_2), 3.9—4.3 (m, ribose), 4.17 and 4.31 (each 2 H, q, *J* 6.9 Hz, OCH_2CH_3 × 2), 5.06 (2 H, m, ribose), 5.69 (1 H, d, *J* 4.6 Hz, 1′-H), and 6.0 (2 H, br s, NH_2); δ_C (67.8 MHz, $CDCl_3$) 14.2 and 14.7 (CH_3 × 2), 22.6 (CH_2CO_2Et), 25.3 and 27.3 (CMe_2), 32.3 (CH_2–imidazole), 58.7, 60.7, and 61.3 (OCH_2 × 3), 79.9, 81.5, 84.1, and 90.7 (OCH × 4), 109.5 (C-4), 115.0 (CMe_2), 140.0 (C-2), 146.8 (C-5), and 164.8 and 172.4 p.p.m. (CO_2Et × 2). A second eluted minor component, probably the α-imidazolenucleoside (**6g**), was obtained as a gum (0.2 g) homogeneous on t.l.c. (Found: M^+, 427. $C_{19}H_{29}N_3O_8$ requires *M*, 427); δ_H(60 MHz, $CDCl_3$) 1.32 and 1.45 (each 3 H, s, CMe_2) and 5.95 (1 H, d, *J* 8 Hz, 1′-H).

Ethyl 5-*Amino*-2-(2-*ethoxyethyl*)-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate* (**6i**).—A suspension of 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate (7.2 g, 0.02 mol) and ethyl 3-ethoxypropanimidate hydrochloride (5.15 g, 0.02 mol) was allowed to react as above and the product treated similarly with ethyl amino(cyano)-acetate (2.4 g, 0.02 mol). T.l.c. examination ($CHCl_3$–MeOH, 9:1) of the reaction mixture showed the presence of one major Bratton–Marshall positive compound (R_F 0.61). The mixture was worked up and chromatographed as above to give the *ethoxyethylimidazole nucleoside* (**6i**) which crystallised from ethanol as needles (0.9 g), m.p. 204 °C (Found: C, 54.0; H, 7.2; N, 10.4%; M^+, 399. $C_{18}H_{29}N_3O_7$ requires C, 54.1; H, 7.3; N, 10.5%; *M*, 399); $\lambda_{max.}$(MeOH) 272 nm; δ_H(60 MHz; $CDCl_3$) 1.23 (3 H, t, *J* 7 Hz, OCH_2CH_3), 1.37 (3 H, s, $CO_2CH_2CH_3$), 1.37 and 1.60 (each 3 H, s, CMe_2), 2.83 (2 H, t, *J* 6 Hz, OCH_2CH_2), 3.33—3.82 (4 H, m, CH_2OCH_2), 4.29—4.47 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), and 5.83 (1 H, d, *J* 3 Hz, 1′-H). There was no evidence of any α-nucleoside in the reaction mixture.

Ethyl 5-N,N-*Dimethylaminocarbonylamino*-2-(2-*ethoxy-carbonylethyl*)-1-(2,3-O-*isopropylidene*-5-O-*pivaloyl*-β-D-*ribo-furanosyl*)*imidazole*-4-*carboxylate* (**6l**).—To ethyl 5-amino-2-(2-ethoxycarbonylethyl)-1-(2,3-*O*-isopropylidene-β-D-ribo-furanosyl)imidazole-4-carboxylate (1.0 g, 2.3 mol) in dry acetonitrile (50 cm^3) was added dimethylformamide dimethyl acetal (0.56 g, 2.3 mmol) and the mixture refluxed for 1 h. The solution was cooled, filtered, and evaporated to a gum which was re-evaporated with acetonitrile (3 × 30 cm^3) using an oil pump. *Ethyl* 5-N,N-*dimethylaminomethylideneamino*-2-(2-*eth-oxycarboxyethyl*)-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)-*imidazole*-4-*carboxylate* (**6m**) was obtained as a solid foam (1.0 g, 91%) (Found: M^+, 482. $C_{22}H_{34}N_4O_8$ requires *M*, 482); $\nu_{max.}$ 1 695 (CO_2R), 1 725 cm^{-1} (CH=N); δ_H(60 MHz; $CDCl_3$) 3.03 (6 H, s, NMe_2) and 8.17 (1 H, s, N=CHN). The foregoing nucleoside (**6m**) was dissolved in the minimum amount of pivalic anhydride and left at room temperature overnight. Chloroform (50 cm^3) was added and the solution washed with water (10 cm^3), saturated aqueous sodium hydrogen carbonate (2 × 10 cm^3), and water (10 cm^3). The organic phase was dried (Na_2SO_4) and evaporated to afford a gum (0.9 g, 1.6 mol). The gum was dissolved in acetic anhydride (50 cm^3) and the solution refluxed with sublimed selenium dioxide (125 mg) for 30 min. The reaction mixture was cooled and the dark precipitate filtered off. The solution was evaporated and re-evaporated with water (10 cm^3) and cyclohexane (3 × 10 cm^3) to yield the *nucleoside* (**6l**) as a gum (400 mg) homogeneous on t.l.c. (Found: M^+, 582. $C_{27}H_{42}N_4O_{10}$ requires *M*, 582); δ_H(200 MHz; $CDCl_3$), 1.20 (9 H, s, Bu^t), 1.26 and 1.33 (each 3 H, t, *J* 7 Hz, OCH_2CH_3 × 2), 1.32 and 1.55 (each 3 H, s, CMe_2), 2.11 and 2.50 (each 3 H, s, NMe_2), 2.8—3.15 (4 H, m, $CH_2CH_2CO_2Et$), 4.15 and 4.32 (each 2 H, q, OCH_2CH_3 × 2), 4.1—4.4 (m, ribose-H), 4.5 and 4.9 (each 1 H, q, ribose-H), and 5.60 (1 H, d, *J* 3.8 Hz, 1′-H); δ_C (67.8 MHz, $CDCl_3$) 14.2 and 14.3 (OCH_2CH_3), 27.1 (Bu^t), 25.2 and 26.4 (NMe_2), 25.4 and 27.2 (CMe_2), 23.5 and 31.6 (CH_2CH_2), 60.8, 61.0, and 63.0 (OCH_2 × 3), 80.2, 81.7, 83.3, and 89.7 (OCH × 4), 116.4 (OCO), 127.3 and 131.6 (C-2 and C-4), 146.9 (C-5), 161.5 ($NHCONMe_2$), and 132.4, 172.7, and 173.1 p.p.m. (CO_2R × 3). The compound gave a negative Bratton–Marshall test.

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