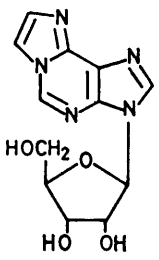
Synthesis of Certain Fluorescent Tricyclic Nucleosides Derived from Pyrazolo[3,4-*d*]Pyrimidine Nucleosides

By **Ganapati A. Bhat** and **Leroy B. Townsend,*** Department of Medicinal Chemistry, College of Pharmacy and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, U.S.A. and Department of Medicinal Chemistry and Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, U.S.A.

The synthesis of certain tricyclic nucleosides with a dihydroimidazole, imidazole, triazole, or tetrazole ring fused to the pyrazolo[3,4-*d*]pyrimidine ring system in an angular position (C-4 and N-5) has been accomplished. The 4-aziridinyl derivative (2) was prepared by a nucleophilic displacement of the chlorine atom of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (1) with ethylenimine. The nucleoside (2) was then treated with sodium iodide to furnish 7-(β-D-ribofuranosyl)-2,3-dihydroimidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidine (3). The reaction of (1) with lithium azide gave 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*e*]-tetrazolo[1,5-*c*]pyrimidine (5) and cyclization of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine with chloroacetaldehyde provided the tricyclic nucleoside 7-(β-D-ribofuranosyl)imidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidine (7). 2,4-Dinitrophenoxamine was treated with 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (6) to give an intermediate (8) which on cyclization with diethoxymethyl acetate gave 7-(2,3-*O*-methoxymethylene-β-D-ribofuranosyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (9). Acid-catalysed deblocking of (9) provided the desired tricyclic nucleoside (10). The reaction of trimethyl orthoformate with 4-hydrazino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine under different experimental conditions resulted in the formation of a mixture of diastereoisomers due to the 2',3'-*O*-methoxymethylene group. Treatment of (10) with alkali gave a ring-opened intermediate which on treatment with sodium nitrite and acetic acid cyclized to give an aza-derivative (16) of (10).

THE synthesis [1] and subsequent observation [2,3] that 3-(β-D-ribofuranosyl)imidazo[2,1-*i*]purine (1,N^6-ethenoadenosine, ε-adenosine) possessed fluorescent properties which could be used as a biological probe, generated a considerable amount of interest in this specific nucleoside and certain closely related 5 : 6 : 5 tricyclic nucleoside

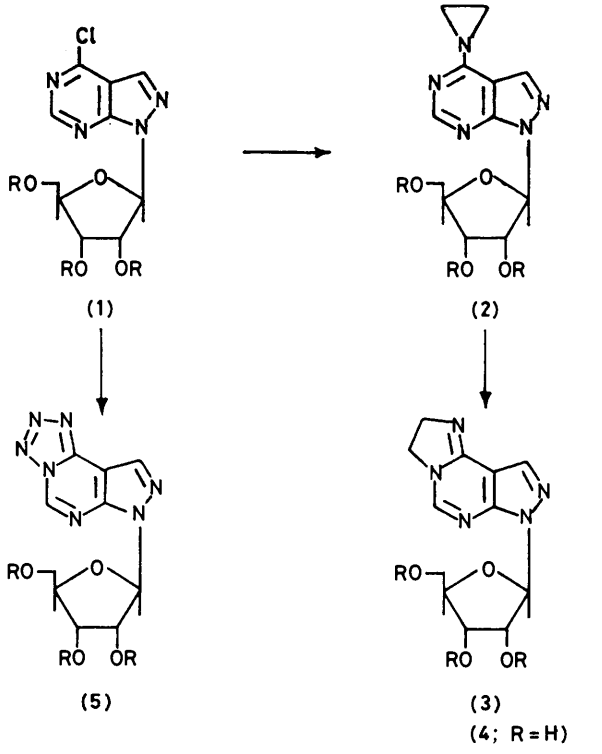


ε-adenosine

derivatives.[1-5] Several other types of tricyclic nucleosides have been synthesized and reported [6-9] from our laboratory with most of these tricyclic nucleosides exhibiting some significant *in vivo* antitumour activity.[10] This prompted us to initiate the synthesis of some tricyclic nucleosides structurally related to ε-adenosine but using bicyclic nucleosides, *e.g.* certain pyrazolo[3,4-*d*]pyrimidines,[11-13] with some previously demonstrated antitumour activity [13,14] as our starting material. Therefore, we wish to report the synthesis of some tricyclic nucleosides which use certain pyrazolo[3,4-*d*]-pyrimidine nucleosides as the starting material with the subsequent formation of a dihydroimidazole, imidazole, triazole or tetrazole ring fused to the C-4–N-5 position of the pyrazolo[3,4-*d*]pyrimidine ring system.

RESULTS AND DISCUSSION

We initiated our research in this area by treating 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo-[3,4-*d*]pyrimidine [15] (1) with lithium azide in dry acetone and molecular sieves (5A) at room temperature. This reaction furnished a good yield (71%) of the tetrazolo-tricyclic nucleoside 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-e]-1,2,3,4-tetrazolo[1,5-*c*]pyrimidine (5). An examination of the i.r. spectrum of (5) revealed no absorbance band in the 2 100 cm^{-1} region. The absence of an absorption band in this region would



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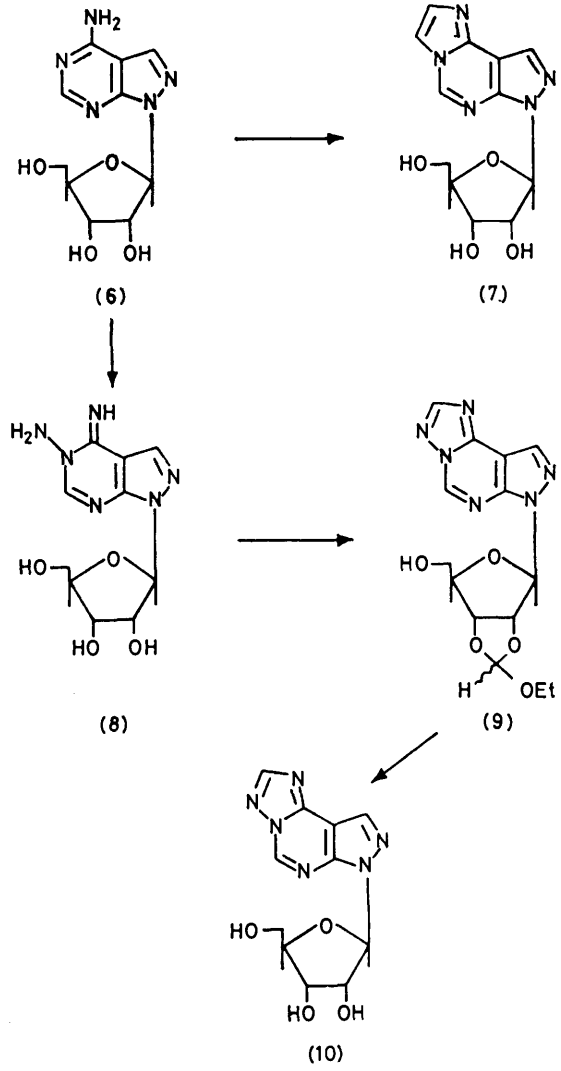
tend to exclude the possibility [15] that a simple nucleophilic displacement of the 4-chloro-group without subsequent ring closure had occurred to give only the 4-azidopyrazolo[3,4-*d*]pyrimidine derivative. All attempts to remove the acetyl blocking groups using standard conditions resulted in ring-opening or degradation reactions which prompted us to investigate the synthesis of ring systems which were not as electron-deficient as the tetrazole ring.

We then turned our attention to the synthesis of tricyclic nucleoside derivatives of imidazo[1,2-*c*]pyrazolo-[4,3-*e*]pyrimidine which are more closely related structurally to ε-adenosine than (5) and should also be more stable.

The reaction of (1) with ethylenimine (aziridine) in methylene chloride and anhydrous potassium carbonate at room temperature furnished the 4-aziridinyl derivative (2) in excellent yield. The nucleoside (2) was then heated in dry acetone [17] with sodium iodide for 1 h to effect a smooth rearrangement which furnished 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,3-dihydroimidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidine (3). The acetyl groups on (3) were removed with methanolic ammonia, as determined by ^{1}H n.m.r., to obtain 7-(β-D-ribofuranosyl)-2,3-dihydroimidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidine (4) in good yield. Several attempts to aromatize the dihydroimidazole ring and obtain the imidazo[1,2-*c*]pyrazolo-[4,3-*e*]pyrimidine riboside (7) were unsuccessful. The unsuccessful aromatization of (3) to obtain (7) prompted us to attempt the synthesis of compound (7) by an independent route. The adenosine analogue of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine[15-18,*] (6,4-APP riboside) was chosen as the starting material for this purpose. An aqueous solution of chloroacetaldehyde in the presence of sodium acetate buffer [19] (pH 4—4.5) was treated with (6) to provide the desired nucleoside, 1-(β-D-ribofuranosyl)imidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidine (7), in 64% yield. The structural assignment as depicted was established by ^{1}H n.m.r. spectroscopy and elemental analysis.

We next investigated the feasibility of forming a 1,2,4-triazole ring system (at the N-2–C-3 positions) in the angular position (C-4–N-5) of a pyrazolo[3,4-*d*]pyrimidine ribonucleoside. The route we envisaged for the synthesis of this tricyclic nucleoside involved the synthesis of an *N*-amino-intermediate followed by annulation. This was achieved by aminating 4-APP riboside (6) with 2,4-dinitrophenylhydroxylamine [20] in a dry dimethylformamide solution at room temperature for 5 days. This reaction furnished the required intermediate (8) (isolated as the hydrochloride salt) which underwent a smooth cyclization on heating with an excess of diethoxymethyl acetate [21] to furnish what we at first assumed was the desired tricyclic nucleoside 7-(β-D-ribofuranosyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10). However, ^{1}H n.m.r. spectroscopy revealed that instead of (10), we had actually obtained the tricyclic nucleoside 7-(2,3-*O*-ethoxymethylene-β-D-ribofuranosyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (9), probably as a mixture of diastereoisomers.[22] The



^{1}H n.m.r. spectrum of (9) showed peaks which could be assigned to three aromatic protons, one signal at δ 9.8 (H-3 or H-6 of the pyrazolopyrimidine part of the ring system), and a signal (two protons) at δ 8.76 [one proton being H-3 or H-6 and the other being the aromatic proton (H-5) of the triazole part of the ring system]. The anomeric proton was observed as a broad singlet † at δ 6.73 possibly due to the diastereoisomeric mixture). Another peak was observed as a broad singlet at δ 6.23 and was assigned to the ethoxymethylene proton (CH). Peaks for the ethoxy-group were observed as a triplet at δ 1.3 (OCH_2Me) and a multiplet at δ 3.64 (OCH_2Me) as well as the pattern of peaks usually observed in the δ 3.5—5.0

* The nucleoside (3) was stirred with activated MnO_2 in a dry methylene chloride solution for several days, but only starting material was recovered. The use of dichlorodicyanoquinone (DDQ) resulted in tar formation.

† However, the ^{1}H n.m.r. spectrum of (9) in $CDCl_3$ showed a peak for the anomeric proton at δ 6.94 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1'), orthoformate proton at δ 6.1.

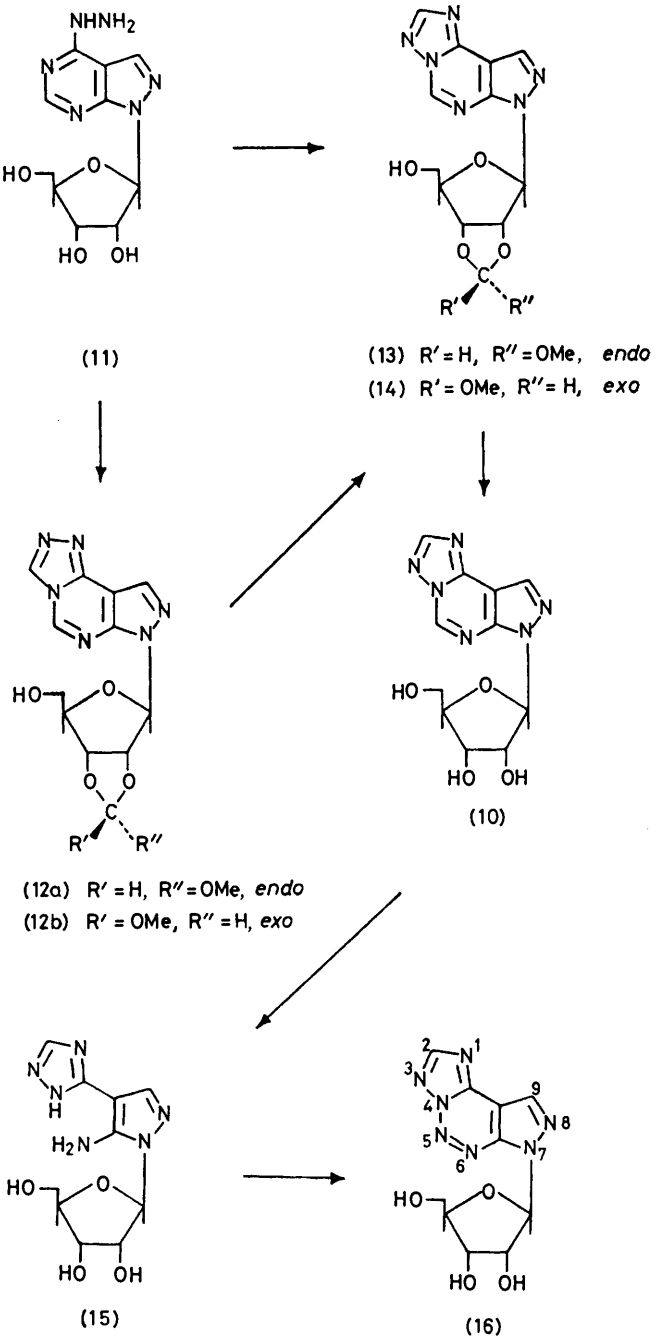
region for the carbohydrate moiety.[23] Treatment of (9) with warm aqueous acetic acid readily removed the ethoxymethylene group to provide the desired tricyclic nucleoside 7-(β-D-ribofuranosyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10) as a pure crystalline compound.

We then initiated a synthesis of the isomeric tricyclic triazolo-nucleoside 7-(β-D-ribofuranosyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidine. We selected 4-hydrazino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine [24] (11) as our starting material.

The nucleoside (11) and a large excess of trimethyl orthoformate were stirred at 70 °C for 45 min to give a crystalline compound. This nucleoside was assigned the structure 7-(2,3-*O*-methoxymethylene-β-D-ribofuranosyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidine (12) on the basis of the n.m.r. spectra and elemental analysis. For the nucleoside (12), there were two peaks observed at δ 9.43 and one at δ 8.63, which is in direct contrast to the nucleosides (9) and (10) which each showed one peak (one proton) in the δ 9.8 region (H-5 or H-9) and one peak (two protons) in the δ 8.76 region [with one signal being either H-5 or H-9, and the other signal being assigned to H-2, respectively]. However, when the nucleoside (12) was refluxed in ethylene glycol monomethyl ether, a facile isomerization to the tricyclic nucleoside 7-(2,3-*O*-methoxymethylene-β-D-ribofuranosyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (13) occurred. The n.m.r. spectrum of (13) showed one peak at δ 9.73 and another peak (two protons) at δ 8.66 [comparable to the spectra obtained for (9)]. Similarly, if compound (11) was treated with trimethyl orthoformate at reflux for 12 h (instead of 45 min with stirring at 70 °C) the thermodynamically more stable isomer [25] [nucleoside (13)] was isolated instead of (12). A study of the n.m.r. spectrum of (12) revealed a major peak for the anomeric proton at δ 6.63 (and a very small peak at δ 6.55), a major peak for the orthoformate proton at δ 6.1 (and a very small peak at δ 6.23), which indicated that one of the isomers had been formed in a marginally more abundant quantity.

We then found that if the nucleoside (11) was treated with trimethyl orthoformate using toluene-*p*-sulphonic acid as a catalyst (room temperature), only the thermodynamically more stable isomer (13) was obtained with an n.m.r. spectrum which was very similar in the aromatic region to that previously observed for (9). The anomeric proton of (13) was assigned to the major peak in the n.m.r. spectrum at δ 6.4 (minor peak at δ 6.56), the orthoformate proton to the major peak at δ 6.1 (minor peak at δ 6.27) and the methoxy-protons to the major peak at δ 3.41 (minor peak at δ 3.31). A comparison of the integration for the major peak to the corresponding minor peak would suggest that the ratio of major isomer : minor isomer was *ca.* 5 : 2.

Based on an earlier report,[22] the major isomer of (13) has been assigned the *endo*-configuration in which the orthoformate proton residing *exo* to the ribose ring is observed at δ 6.1 (at higher field). The minor isomer was then assigned as the *exo*-compound since the orthoformate proton (*endo* to the ribose ring) was observed at δ 6.27 (lower field), due to a deshielding effect. By analogy, the major isomer of (12) was assigned the *endo*-configuration (isomer ratio 7 : 1 based on integration).



Compound (11) was treated with an excess of trimethyl orthoformate at reflux, using toluene-*p*-sulphonic acid as a catalyst, to give (14) as the major isomer.

The peak for the anomeric proton was observed as a doublet at δ 6.56 (minor peak at δ 6.63), a major peak for the orthoformate proton at δ 6.23 (minor peak at δ 6.1), and a major peak for the methoxy-protons at

TABLE 1

U.v. spectral data [λ/nm (ε × 10^{-3})]

Compound	$\lambda_{max.}$ (pH 1)	$\lambda_{min.}$ (pH 1)	$\lambda_{max.}$ (MeOH)	$\lambda_{min.}$ (MeOH)	$\lambda_{max.}$ (pH 11)	$\lambda_{min.}$ (pH 11)
(2)	264.5 (13.75)	242 (7.13)	266 (11.1) 278sh (10.4)	240.5 (4.6)	266 (11.74) 279sh (10.5)	241 (6.2)
(4)	266 (11.73)	245 (6.74)	276sh (8.21) 267 (9.53)	252 (7.03)	276sh (8.8) 268 (9.97)	251 (7.04)
(5)	269 (7.76)	245 (5.03)	269 (5.87)	244 (3.98)	266 (5.24)	
(7)	263sh (5.53) 231 (24.9)		278 (2.47) 265sh (4.07) 256 (4.22) 237.5sh (21.55) 230 (28.11) 225sh (26.79)	273.5 (2.33) 251.5 (4.08)	278sh (3.05) 265sh (5.24) 257 (5.68) 237 (19.37) 230 (23.15)	250 (5.47)
(8)	257 (13.49)	239 (8.57)	276 (13.68) 260 (12.40)	265 (11.85) 238 (5.84)	274.5 (14.59) 259.5 (13.13)	263 (12.77) 236 (6.38)
(9)	264 (7.38)	242 (5.4)	266 (6.97)	243 (4.35)	252 (7.49)	240 (6.97)
(10)	265 (6.9)	245 (5.26)	267 (6.72)	244 (4.23)	248 (9.99)	239 (9.88)
(12)	267 (5.62)		266 (5.35)	244 (4.34)	246 (8.52)	240 (8.35)
(13)	265 (7.35)	245 (5.85)	266 (7.19)	242 (4.68)	247 (8.69)	238 (8.35)
(14)	264 (5.28)	245 (5.01)	266 (5.62)	242 (3.54)	247 (7.02)	240 (6.68)
(15)	261 (9.46) 239 (8.18)	226 (7.07)	246 (10.72)	223 (5.92)	244 (10.85)	
(16)	239sh (1.46) 292—279 (5.718)	251 (1.17)	330sh (1.31) 227sh (4.98) 282 (5.57)	252 (0.73)	229sh (1.17) 295—278 (5.28)	249 (0.15)

δ 3.3 (minor peak at δ 3.4), respectively. The ratio of major isomer : minor isomer was estimated by integration of the appropriate peaks in the n.m.r. spectra to be 10 : 3. The major isomer in this case had the *exo*-configuration. Both the tricyclic nucleosides (13) and (14) on treatment with warm aqueous acetic acid gave the desired compound (10) in good yield. At this point it is important to note that due to the non-stereospecificity of the orthoester exchange reaction, the optical rotation and melting points of compounds (9), (12), (13), and (14) are likely to vary.

It was of considerable interest that when compound (10) was stirred in dilute sodium hydroxide solution, we observed an opening of the pyrimidine ring [26] to give the interesting pyrazole nucleoside intermediate, 5-amino-1-(β-D-ribofuranosyl)-4-(1,2,4-triazol-3-yl)pyrazole (15) in good yield. Subsequent cyclization of compound (15) with acetic acid and sodium nitrite furnished the new tricyclic nucleoside 7-(β-D-ribofuranosyl)pyrazolo-[3,4-*d*]-1,2,4-triazolo[3,2-*f*]-[1,2,3]-triazine (16) in 63% yield. That ring-closure had occurred as shown was

TABLE 2

Technical fluorescence data [a]

Compound	pH	Fluorescence [Emission [b]] $\lambda_{max.}$/nm	Fluorescence [Excitation/ nm [c]]
(4)	MeOH	345, 435	286
	pH 1	334, 412	290
	pH 11	428	317
(5)	MeOH	350	291
	pH 1	354	285
	pH 11		
(7)	MeOH	421	313
	pH 1	430	275
	pH 11	436	310
(10)	MeOH	360	282
	pH 1	360	272
	pH 11	362	295
(12)	MeOH	354	286
	pH 1	354	278
	pH 11	352	292
(16)	MeOH	345, 415	300
	pH 1	340, 418	308
	pH 11	350, 420	295

[a] In methanol and uncorrected. [b] Fluorescence emission spectra were taken by fixing on the fluorescence excitation maximum. [c] Fluorescence excitation spectra were taken by fixing on the fluorescence emission maximum.

established by the n.m.r. spectrum of (16) which showed a peak which could be assigned to two aromatic protons at δ 8.93 and a peak for the anomeric proton at δ 6.6.

All the tricyclic nucleosides reported above possessed fluorescent properties which are reported in Table 2.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover apparatus and were uncorrected. U.v. absorption spectra were measured on a Beckman Acta CIII spectrometer. Fluorescence spectra were taken on Aminco-Bowman spectrofluorophotometer. ^{1}H N.m.r. spectra were obtained on a EM-390 90-MHz spectrometer using $SiMe_4$ as internal standard; chemical shifts are from $SiMe_4$. The optical rotations were taken with a Perkin-Elmer Model 141 automatic digital readout polarimeter. T.l.c. was run on glass plates coated (250-μ) with SilicAR 7 GF (Mallinckrodt). Solvents were removed *in vacuo* at low temperature on a water bath (*ca.* 40—50 °C), unless specifically stated otherwise. The i.r. spectra were recorded on a Beckman IR-8 spectrometer in pressed potassium bromide disks.

4-(*Aziridin-1-yl*)-1-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)-*pyrazolo*[3,4-d]*pyrimidine* (2).—4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine [27] (1) (1.24 g, 3 mmol) was dissolved in methylene chloride (40 ml). Anhydrous potassium carbonate (1.1 g, 8 mmol) was then added and the mixture stirred at room temperature. To this mixture was added a solution of ethylenimine (0.52 g, 12 mmol) in methylene chloride (10 ml), dropwise during 30 min. After stirring for an additional 3 h, more ethyleneimine (0.26 g, 6 mmol) was added and stirring was continued at room temperature for 18 h. The reaction mixture was filtered, the residual solid was washed with methylene chloride (2 × 25 ml), and the combined filtrates were washed with cold water (50 ml). After drying over sodium sulphate, the solvent was evaporated *in vacuo* to yield 1.2 g (95%) of a clear oil which soon formed a solid foam, $[\alpha]_D^{24}$ −30° (*c* 1, MeOH); δ([$^2H_6$]DMSO) 8.2 and 8.65 (2 s, 2 H, H-3 and H-6), 6.60 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1'), and 2.1 (br d, 9 H, OCOMe) (Found: C, 51.3; H, 5.1; N, 16.7. Calc. for $C_{18}H_{21}N_5O_7$: C, 51.55; H, 5.06; N, 16.70%).

7-(2,3,5-*Tri*-O-*acetyl*-β-D-*ribofuranosyl*)-2,3-*dihydroimidazo*[1,2-c]*pyrazolo*[4,3-e]*pyrimidine* (3).—Sodium iodide (1.5 g, 6 mmol) was added to a solution of 4-(aziridin-1-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (2) (3.35 g, 8 mmol) in dry acetone (150 ml), and the mixture was gently refluxed with stirring for 1 h. The solvent was removed *in vacuo* at room temperature, the resulting residue was extracted with methylene chloride (5 × 100 ml), the combined extracts washed with water (50 ml), dried (anhydrous magnesium sulphate), and the solvent evaporated *in vacuo* at room temperature to give an oil (3.0 g). The oil was dissolved in chloroform (15 ml) and applied to the top of a silica gel column (SilicAR CC-7, 80 g, 60 × 1.25 cm packed in chloroform) and then eluted first with chloroform (200 ml). The fraction which eluted with chloroform–methanol (49 : 1 v/v) (500 ml) was collected, filtered, and evaporated to dryness *in vacuo* to afford a solid foam (2.8 g, 83%); $[\alpha]_D^{24}$ −26° (*c* 1.038, methanol); δ($CDCl_3$) 7.93 and 7.73 (2 s, 2 H, aromatic protons), 6.3 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1'), 4.06 (br s, 4 H, CH_2CH_2 of dihydroimidazole ring), 2.1 (br d, 9 H, 3 × OAc) (Found: C, 51.7; H, 5.35; N, 16.85. Calc. for $C_{18}H_{21}N_5O_7$: C, 51.55; H, 5.05; N, 16.7%).

7-(β-D-*Ribofuranosyl*)-2,3-*dihydroimidazo*[1,2-c]*pyrazolo*[4,3-e]*pyrimidine* (4).—The nucleoside (3) (2.1 g, 5 mmol) was dissolved in methanolic ammonia (100 ml, saturated at −5 °C) and set aside at 4 °C for 24 h in a sealed pressure bottle. After removal of the solvent, the resulting residue was crystallized from ethanol to furnish (4) (1.0 g 68%), m.p. 201—202 °C; $[\alpha]_D^{24}$ −67° (*c* 1.032, MeOH); δ([$^2H_6$]-DMSO) 8.06 (br d, 2 H, aromatic protons), 6.05 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), and 4.06 (m, 4 H, CH_2CH_2 of dihydroimidazole ring) (Found: C, 49.35; H, 5.3; N, 23.7. Calc. for $C_{12}H_{15}N_5O_4$: C, 49.14; H, 5.16; N, 23.85%).

7-(2,3,5-*Tri*-O-*acetyl*-β-D-*ribofuranosyl*)*pyrazolo*[4,3-e]-*tetrazolo*[1,5-c]*pyrimidine* (5).—Lithium azide (1.3 g) and molecular sieve (5A, 30 g) were added to a solution of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (1) (1.24 g, 3 mmol) in dry acetone (100 ml), and the mixture stirred at room temperature for 3.5 h. It was then filtered, the residue washed with dry acetone (2 × 25 ml), and the combined filtrate and washings evaporated to dryness *in vacuo* at room temperature. The resulting oil was dissolved in chloroform (150 ml), washed with water (25 ml), dried over sodium sulphate, and the solvent was evaporated *in vacuo*. The oil was dissolved in chloroform (10 ml) and placed on a silica gel column (SilicAR CC-7, 80 g, 60 cm × 1.25 cm packed in chloroform) and eluted with chloroform (400 ml). The eluate from chloroform–methanol (99 : 1 v/v) (500 ml) was collected, filtered, and evaporated to dryness *in vacuo* at room temperature to give (5) as a foam (0.9 g, 71%) (5); $[\alpha]_D^{26}$ −28° (*c* 1, DMF), δ([$^2H_6$]DMSO) 10.06 and 8.88 (2 s, 2 H, aromatic protons); 6.65 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1'), and 2.03 and 2.15 (br d, 9 H, 3 × OAc); i.r. showed no absorption band in the 2 100 cm^{-1} region [6] (Found: C, 45.55; H, 3.8; N, 23.2. Calc. for $C_{16}H_{17}N_7O_7$: C, 45.83; H, 4.08; N, 23.38%).

7-(β-D-*Ribofuranosyl*)*imidazo*[1,2-c]*pyrazolo*[4,3-e]*pyrimidine* (7).—4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (6) (1.0 g, 4 mmol), water (15 ml), freshly distilled chloroacetaldehyde (10 ml), and sodium acetate (1.2 g) were mixed and then stirred at 80 °C for 3 h. The pH of the reaction mixture was maintained at 4.5 by adding acetic acid as needed. The solvent was then evaporated to dryness *in vacuo*, and the residue was co-evaporated with aqueous ethanol (3 × 25 ml). The resulting tan solid was dissolved in methanol (20 ml) and placed on the top of a silica gel column (SilicAR CC-7, 80 g, 60 × 1.25 cm packed in chloroform). The fraction which eluted with methanol–chloroform (15%, v/v) was collected, evaporated to dryness *in vacuo*, and the solid recrystallized from methanol to furnish (7) (0.7 g, 64%), m.p. 227—228 °C; $[\alpha]_D^{28}$ −73° (*c* 1.002, DMF); δ([$^2H_6$]DMSO) 9.41, 8.57, 8.2, and 7.68 (4 s, 4 H, aromatic protons), and 6.3 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1') (Found: C, 49.45; H, 4.55; N, 24.15. Calc. for $C_{12}H_{13}N_5O_4$: C, 49.48; H, 4.5; N, 24.04%).

5-*Amino*-4-*imino*-1-(β-D-*ribofuranosyl*)*pyrazolo*[3,4-d]*pyrimidine Hydrochloride* (8).—To a solution of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (6) (2.4 g, 9 mmol) in dry dimethylformamide (60 ml) was added 2,4-dinitrophenoxamine (2.0 g, 10 mmol) and the mixture was stirred at room temperature for 5 days. The mixture was then evaporated to dryness *in vacuo*, the resulting residue was treated with cold dilute hydrochloric acid solution (10%, 100 ml) and the solution filtered. The clear filtrate was evaporated to dryness *in vacuo*, and the residue was recrystallized from ethanol to afford (8) (2.0 g, 61%), m.p. 208—209 °C (decomp.); $[\alpha]_D^{24}$ −57° (*c* 0.927, MeOH);

δ([$^2H_6$DMSO) 8.6 and 8.53 (2 s, 2 H, H-3 or H-6), and 6.2 (d, 1 H, $J_{1',2'}$ 4.5 Hz, H-1') (Found: C, 39.75; H, 4.7; N, 22.99. Calc. for $C_{10}H_{15}ClN_6O_4 \cdot C_2H_5OH$: * C, 39.51; H, 4.9; N, 23.02%).

7-(2,3-O-*Ethoxymethylene*-β-D-*ribofuranosyl*)*pyrazolo*[4,3-e]-1,2,4-*triazolo*[1,5-c]*pyrimidine* (9).—A mixture of 5-amino-4-imino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine hydrochloride (8), (365 mg, 1 mmol) and diethoxymethyl acetate (10 ml) was refluxed for 3 h. The solvent was removed *in vacuo*, water (10 ml) was added to the residue, and this was followed by an extraction with chloroform (3 × 25 ml). After drying over sodium sulphate, the solvent was evaporated to dryness *in vacuo*. The residue was crystallized from chloroform–hexane to afford (9) (200 mg, 75%), m.p. 95—96 °C; $[\alpha]_D^{27}$ −123° (*c* 0.98, MeOH); δ($^2H_6$DMSO) 9.8 (s, 1 H, aromatic), 8.76 (br s, 2 H, aromatic), 6.73 (br s, 1 H, H-1'), 6.23 (br s, 1 H, orthorformate proton), 6.64 (m, 4 H, 5'-C*H*$_2$ and −OC*H*$_2$-Me), and 1.3 (t, 3 H, OCH$_2$*Me*) (Found: C, 48.2; H, 4.85; N, 24.35. Calc. for $C_{14}H_{16}N_6O_5$: C, 48.28; H, 4.63; N, 24.13%).

7-(β-D-*Ribofuranosyl*)*pyrazolo*[4,3-e]-1,2,4-*triazolo*[1,5-c]*pyrimidine* (10).—The nucleoside (9) (0.35 g, 1 mmol) and aqueous acetic acid (50%, 10 ml) were stirred at 80 °C for 1 h. The solvent was evaporated to dryness *in vacuo*, and the residue co-evaporated with aqueous ethanol (3 × 10 ml). Crystallization of the resulting residue from ethanol gave (10) (0.15 g, 51%) as a white solid, m.p. 179—180 °C; $[\alpha]_D^{27}$ −61° (*c* 1.006, DMF); δ([$^2H_6$]DMSO) 9.83 (s, 1 H, aromatic proton), 8.73 (s, 2 H, aromatic protons), and 6.4 (d, 1 H, $J_{1',2'}$ 4.5 Hz, H-1') (Found: C, 45.2; H, 4.35; N, 28.85. Calc. for $C_{11}H_{12}N_6O_4$: C, 45.20; H, 4.14; N, 28.71%).

7-(2,3-O-*Methoxymethylene*-β-D-*ribofuranosyl*)*pyrazolo*-[4,3-e]-1,2,4-*triazolo*[4,3-c]*pyrimidine* (12).—4-Hydrazino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine [26] (11) (0.85 g, 3 mmol) and trimethyl orthoformate (60 ml) were mixed and stirred at 70 °C for 45 min, by which time a clear solution was obtained. The solvent was evaporated to dryness *in vacuo*. The product was then treated with aqueous ethanol (1 : 1, v : v) (3 × 10 ml) and evaporated to dryness *in vacuo*. The resulting solid was crystallized from ethanol to furnish (12) (0.6 g, 60%), m.p. 179—182 °C; $[\alpha]_D^{25.5}$ −133° (*c* 1.0, DMF); δ([$^2H_6$]DMSO) 9.43 (d, 2 H, aromatic protons), 8.63 (s, 1 H, aromatic proton), 6.63 (d, 1, $J_{1',2'}$ 3 Hz, H-1'), 6.55 (small peak), 6.1 (s, 1 H, orthoformate proton), 6.23 (small peak), and 3.4 (m, 5 H, 5'-CH$_2$ and OMe) (Found: C, 46.48; H, 3.95; N, 25.35. Calc. for $C_{13}H_{14}N_6O_5$: C, 46.7; H, 4.22; N, 25.14%).

7-(2,3-O-*Methoxymethylene*-β-D-*ribofuranosyl*)*pyrazolo*-[4,3-e]-1,2,4-*triazolo*[1,5-c]*pyrimidine* [(13) *and* (14)]. *Method A*. 4-Hydrazino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (11) (0.85 g, 3 mmol), trimethyl orthoformate (25 ml), and toluene-*p*-sulphonic acid (10 mg) were mixed and stirred at room temperature for 10 h. The solvent was then removed *in vacuo*. The residue was co-evaporated with aqueous ethanol (1 : 1, v/v) (2 × 10 ml) containing a few drops of triethylamine. The resulting residue was crystallized from ethanol to furnish (13) (0.5 g, 50%), m.p. 118—121 °C; $[\alpha]_D^{27}$ −138° (*c* 1.022, DMF); δ([$^2H_6$]-DMSO) 9.73 (s, 1, aromatic proton), 8.63 (s, 2, aromatic protons), 6.64 (d, 1 H, $J_{1',2'}$ 3 H, H-1'), 6.56 (minor peak), 6.1 (s, 1 H, orthoformate proton), 6.27 (minor peak), 3.41 (br s, 5 H, 5'-CH$_2$ and OMe), and 3.31 (minor peak) (Found: C, 46.5; H, 4.1; N, 25.4. Calc. for $C_{13}H_{14}N_6O_5$: C, 46.7; H, 4.22; N, 25.14%).

Method B. The same reagent in similar quantity (as described in Method A) were used. However, instead of stirring at room temperature, the reaction mixture was refluxed for 4 h. The crude product was crystallized from methanol to afford (14) (0.6 g, 60%), m.p. 164—167 °C (decomp.); $[\alpha]_D^{27}$ −86° (*c* 1.09, DMF); δ([$^2H_6$]DMSO) 9.68 (s, 1 H, aromatic proton), 8.65 (s, 2 H, aromatic protons), 6.56 (d, 1, $J_{1',2'}$ 3 Hz, H-1'), 6.63 (minor peak), 6.23 (s, 1 H, orthoformate proton), 6.1 (minor peak), 3.3 (m, 5, 5'-CH$_2$ and OMe), and 3.4 (minor peak) (Found: C, 47.0; H, 4.0; N, 25.0. Calc. for $C_{13}H_{14}N_6O_5$: C, 46.7; H, 4.22; N, 25.14%).

5-*Amino*-1-(β-D-*ribofuranosyl*)-4-(1,2,4-*triazol*-3-*yl*)-*pyrazole* (15).—A mixture of 7-(β-D-ribofuranosyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10) (0.88 g, 3 mmol) and aqueous sodium hydroxide (0.3N; 10 ml) was stirred at room temperature for 20 h. The clear solution was then cooled to 0 °C, and the pH adjusted to *ca.* 7 by the careful dropwise addition of concentrated hydrochloric acid. The white solid was collected by filtration and then crystallized from aqueous ethanol to give (15) (0.55 g, 65%), m.p. 194—195 °C (decomp.); $[\alpha]_D^{30}$ −83° (*c* 0.98, DMF); δ([$^2H_6$]-DMSO) 8.2 and 7.8 (2 s, aromatic protons), 6.5 (br s, 2 H 5-NH$_2$), 5.8 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1') (Found: C, 42.2; H, 5.15; N, 30.0. Calc. for $C_{10}H_{14}N_6O_4$: C, 42.55; H, 5.00; H, 29.77%).

7-(β-D-*Ribofuranosyl*)*pyrazolo*[4,3-e]-1,2,4-*triazolo*[2,3-c]-1,2,3-*triazine* (16).—5-Amino-1-(β-D-ribofuranosyl)-4-(1,2,4-triazol-3-yl)pyrazole (15), (0.845 g, 3 mmol) was dissolved in acetic acid (4 ml), and this solution was stirred and cooled to 10 °C. A solution of sodium nitrite (150 mg) in water (2 ml) was added at one time, and the reaction mixture was then stirred at room temperature for an additional 30 min. The solvent was removed *in vacuo*, and the residue was triturated with cold water (5 ml). The white crystals which had formed were collected by filtration, and recrystallized from ethanol to give (16) (0.55 g, 63%), m.p. 150—151 °C, $[\alpha]_D^{26}$ −80° (*c* 1.0, MeOH); δ([$^2H_6$]-DMSO) 8.93 (d, 2 H, aromatic protons), 6.6 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1') (Found: C, 40.7; H, 4.05; N, 33.4. Calc. for $C_{10}H_{11}N_7O_4$: C, 40.96; H, 3.78; N, 33.43%).

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* The presence of ethanol was corroborated in the ^1H n.m.r. spectrum of compound (8).

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