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SYNTHETIC *C*-NUCLEOSIDES: 3-(α- AND β-D-ARABINOFURANOSYL)PYRAZOLO[4,3-*d*]PYRIMIDINE-5,7-DIONES*

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ABSTRACT

2,3,5-Tri-*O*-benzyl-D-arabinofuranosyl bromide (**4**) was converted into 2,5-anhydro-3,4,6-tri-*O*-benzyl-D-gluconononitrile (**5**), mixed with 20% of the D-*manno* epimer **6**. The mixture was reduced to the amine **7**, which *via* the *N*-nitrosoacetamide **10** afforded the 1-deoxy-1-diazo sugar **11**. Dipolar addition to dimethyl acetylenedicarboxylate afforded the *C*-nucleoside derivative, dimethyl 3-(2,3,5-tri-*O*-benzyl-α,β-D-arabinofuranosyl)pyrazole-4,5-dicarboxylate (**20**). Selective ammonolysis afforded the 4-ester-5-carboxamide **21**, which was separated chromatographically into the α-(minor) and β-(major) anomers. Hydrazinolysis and Curtius reaction of the pair of 4-acid hydrazides (α-**22** and β-**22**) afforded the anomeric 3-glycosyl-1*H*-pyrazolo-[4,3-*d*]pyrimidine-5,7-diones (α-**24** and β-**24**). Hydrogenolytic debenzylation yielded the β-D-*arabino* epimer (**1**) of oxoformycin B, and the α-D-*arabino* form **2**. These anomeric *C*-nucleosides were distinguished by circular dichroism spectra that showed the same relationship as α- and β-D-*arabino* anomers of normal purine nucleosides.

INTRODUCTION

Total syntheses have recently been reported for formycin B[1] and oxoformycin B[2], in the formycin series of naturally occurring *C*-nucleosides. Significant biological activity has not been reported for oxoformycin B, but recently the lower homolog **3**, obtained in model studies[1] that employed 2,3-*O*-isopropylidene-DL-erythrofuranose as the sugar, exhibited activity against leukemia L1210 in mice. The therapeutic importance of 1-β-D-arabinofuranosylcytosine[3] and of 9-β-D-arabinofuranosyladenine[4] provides a strong impetus for synthesizing the D-*arabino* epimers of natural D-ribonucleosides. This paper describes the synthesis of the D-*arabino* epimer (**1**) of oxoformycin B, 3-β-D-arabinofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7(4*H*,6*H*)-dione, and its α-anomer **2**.

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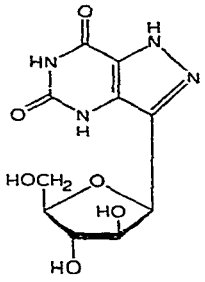

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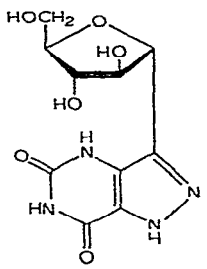

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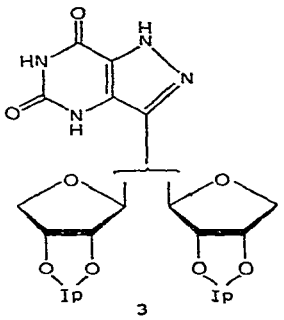

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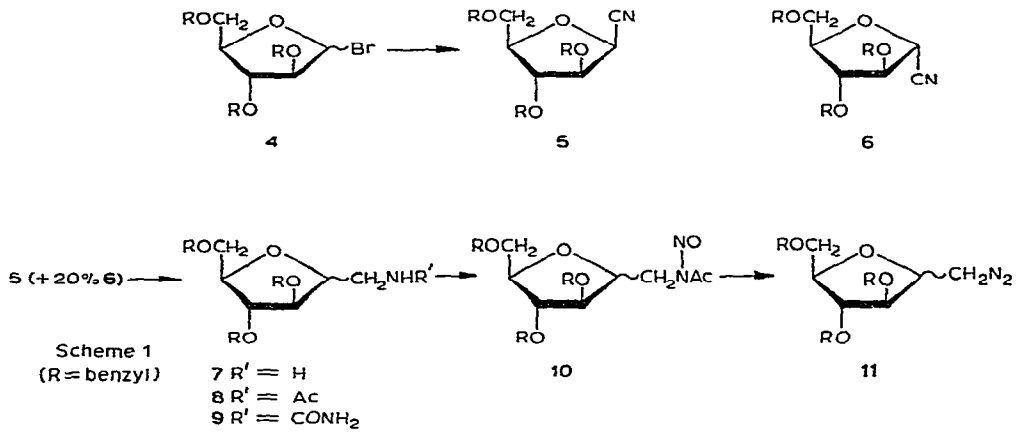
RESULTS AND DISCUSSION

As in the ribose series[1,2], the synthesis of **1** and **2** began with the conversion of a glycosyl halide into a nitrile, to establish the C–glycosyl bond. 2,3,5-Tri-*O*-benzyl-D-arabinofuranosyl bromide[5] (**4**, Scheme I) in benzene solution was treated with mercuric cyanide to form 2,5-anhydro-3,4,6-tri-*O*-benzyl-D-gluconononitrile (**5**), having the configuration desired for a "β-D-arabinofuranosyl cyanide." The product showed no detectable nitrile absorption in the infrared, like some other glycosyl nitriles[6,7], and it was difficult to identify and characterize. The nitrogen content showed that the product was mostly nitrile, with about 15% of a non-nitrogenous sugar contaminant. In practice, the nitrile **5** was best characterized by the presence in the p.m.r. spectrum in benzene-d_6 solution of a sharp, two-proton doublet at δ 3.48 (J 6.0 Hz). A weak doublet at δ 3.38 (J 5.5 Hz) disclosed the presence of the epimeric* mannononitrile **6**. These doublets were assigned to 6-H_2 of the nitriles. The ratio of **5** to **6** was measured as about 4:1 by ^{13}C n.m.r., from the ratio of singlets for CN at δ_c 117.2 and 118.2 p.p.m , respectively. This ratio reflected in reverse the β:α ratio (3:17) in the bromide **4**, and it seemed likely that the epimeric nitriles were formed by direct displacement of bromide. Choice of conditions to form **5** was critical. Use of 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride[8] in nitromethane solution[6,7,9,10] with mercuric cyanide gave only about 20% of nitrile (based on nitrogen content)†. The main product was a nitrogen-free sugar displaying a characteristic doublet for the 6-H_2 resonance at δ 3.62 (J 6.0 Hz) in benzene-d_6; it neither showed OH absorption in the infrared nor any alkenic p.m.r. signals suggestive of elimination, and it may have been a disaccharide having the constitution of an arabinosyl arabinoside. The bromide **4** in nitromethane afforded nearly equal amounts of the non-nitrogenous sugar and the two nitriles **5** and **6**. Resolution by t.l.c. was poor, but a portion of the faster-moving mannonitrile **6** could be separated and characterized. Treatment of **4** in xylene solution with silver cyanide gave a product containing isonitrile, as judged

*Though epimers by systematic nomenclature, **5** and **6** may be informally thought of as anomeric α- and β-arabinofuranosyl cyanides.

†We are indebted to Mr. G. L. Tong and Dr. W. W. Lee for the initial observation.

from an i.r. band at 4.7 μm; on refluxing in xylene this product disappeared with formation of **5** and **6**.



Scheme I

An independent synthesis of the glucononitrile **5** was achieved by starting from 2,5-anhydro-D-gluconamide[11] (**14**, the amide of "chitaric acid," Scheme II), but side reactions encountered in the tri-*O*-benzylation of **14** made it impractical as an alternative approach. Crude tri-*O*-benzyl amide **16** was dehydrated with *p*-toluenesulfonyl chloride in pyridine, and chromatographic purification of the product afforded in low yield a nitrile fraction that was identical to the previous sample of **5**.

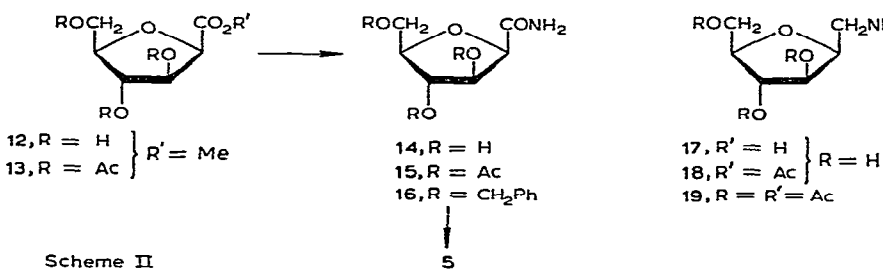
Reduction of **5** with borane in tetrahydrofuran[12] was the method of choice to prepare the amine **7**. Lithium aluminum hydride was first tried for the reduction of **5**, but additional impurities were produced (notably the alcohol resulting from C–N cleavage[12]) and the isolation was less convenient. The amine **7** could not be extracted into M hydrochloric acid, nor be precipitated from ether as a hydrochloride salt, and so it was not readily separated from non-basic impurities. Chromatographic purification was accomplished with **7**, but was more practical with the acetamide **8**, where there was better resolution from the impurities (mainly the nitrogen-free sugar carried through from **5**). All attempts to obtain these *O*-benzyl sugars as crystalline solids were unsuccessful. With dinitrogen tetraoxide in acetic acid–carbon tetrachloride[13], the amide was converted in nearly quantitative yield into a reasonably stable nitrosoacetamide **10**, as evidenced by diagnostic shifts of the i.r. C=O band from 6.0 to 5.75 μm and of the p.m.r. *N*-acetyl singlet from δ 1.82 to 2.70. Conversion of **10** with base into the 1-diazo sugar **11**, followed by 1,3-dipolar addition of **11** to dimethyl acetylenedicarboxylate to form a pyrazole *C*-nucleoside (**20**) were key steps in the synthesis. This approach to formycins was first devised in a model system[14] and then applied[1,2,15], to 1-diazo sugars.

The acetamide **8** was much superior to the corresponding urea **9** as a source of the 1-diazo sugar. The urea **9** was a syrup obtained in nearly quantitative yield from

the amine **7** by treatment with acid and potassium cyanate in aqueous 1,2-dimethoxyethane, but nitrosation of **9** gave an impure nitrosourea low in nitrogen content. There was probably attack at both ureido nitrogen atoms, giving an unstable isocyanate, together with the desired 1-substituted-1-nitrosourea; weak i.r. absorption at 4.4 μm (N=C=O) was observed in addition to the C=O band at 5.77 μm (nitrosourea).

The diazo sugar **11** was most efficiently formed when nitrosoamide **10** in ether–dimethoxyethane was treated with aqueous 30% potassium hydroxide. The presence of 1,2-dimethoxyethane was necessary to adjust the partition coefficient so that decomposition of **10** proceeded at a practical rate. Appearance of a diazo band in the i.r. at 4.78 μm with concomitant loss of the C=O band at 5.75 μm could be monitored on aliquots of the reaction mixture. Inexplicably, complete loss of the carbonyl band was never observed, even after several h by which time very strong diazo bands were always attained. Nevertheless, good yields were obtained repeatedly in the dipolar addition with dimethyl acetylenedicarboxylate.

For success in the 1,3-dipolar addition, it was essential that the 1-diazo sugar be generated in solution in a water-immiscible solvent that could be washed free of the aqueous alkali. This factor emphasized the necessity of using organic-soluble sugar derivatives with protecting groups—such as *O*-benzyl—that are stable to base. This necessity was demonstrated in further attempts to use 2,5-anhydro-D-gluconamide (**14**) as the precursor, because of its purity as a single epimer and its availability from 2-amino-2-deoxy-D-glucose and -D-gluconic acid. The amide, as the tri-acetate **15**, was reduced with borane–tetrahydrofuran to 1-amino-2,5-anhydro-1-deoxy-D-glucitol (**17**, the unprotected form of **7**), which was converted into **18**. Both the acetamide **18** and its triacetate **19** were readily nitrosated and converted into a 1-diazo sugar by alcoholic alkoxide. There was, however, no way to separate these

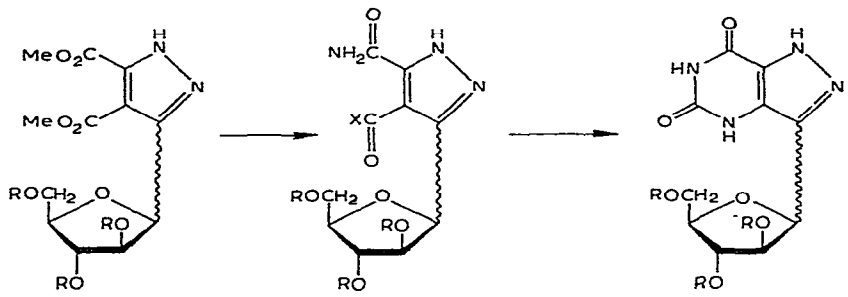


Scheme II

diazo sugars from the base that generated them. If dimethyl acetylenedicarboxylate was then added, it was largely consumed by the excess base in the medium, and little or no pyrazole *C*-nucleoside was formed. Consequently it was always impractical to use **14** as a source of D-arabinofuranosyl *C*-nucleosides, despite the promise of β-anomeric purity, because of the shortcomings of *O*-acyl protecting groups, and the side reactions and impurities encountered in the alternative *O*-benzylation of **14**.

The 1,3-dipolar addition of **11** with dimethyl acetylenedicarboxylate was

complete in one h at 0°. The pyrazole diester **20** (Scheme III) could be purified chromatographically to give a 52% yield, but it was convenient to delay chromatographic purification until after the next step. As in analogous sequences[2,14,15], ammonolysis in methanol at room temperature selectively afforded the 5-amide **21**, and conversion into the 4-hydrazide-5-amide **22** occurred with anhydrous hydrazine at 25°. The Curtius reaction was conducted on **22** with aqueous nitrous acid in *N*,*N*-dimethylformamide[2]. Rearrangement of the intermediate azide **23** with boiling toluene spontaneously afforded the pyrazolo[4,3-*d*]pyrimidinedione **24**, presumably through a non-isolable[1] pyrazole-4-isocyanate. These tri-*O*-benzyl *C*-nucleosides were obtained as syrups and glasses, and purification had to be accomplished by chromatography. The *β*:*α* anomeric ratio was still expected to reflect the 4:1 ratio of epimeric nitriles **5** and **6** encountered in the first step of the synthesis.



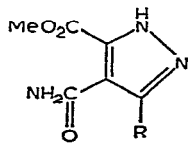
Scheme III
(R = Benzyl)

As predicted, when the *C*-nucleoside **24** was catalytically debenzylated, the product (a mixture of **1** and **2**) showed two p.m.r. doublets for H-1′. The doublet from the predominant *β*-anomer **1** was the more downfield (*δ* 5.28 *vs* 5.02 for **2**), in agreement with the observation[16] that H-1 in furanose rings resonates further downfield when it is *cis* to H-2 than when it is *trans*. The product could be crystallized and the anomeric ratio in various crops ranged from 2.2:1 to 3.5:1, but the anomers could not be resolved more completely.

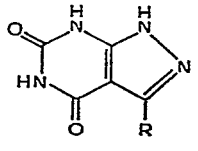
More-detailed investigation of intermediates in the synthesis revealed that anomers could be separated upon further chromatography of the amide ester **21**. Initial purification was effected on a column of silica gel in ether with ether–methanol as eluents. A second column of a finer grade of silica gel in acetone–benzene separated 11% of the material as homogeneous *α*-**21** and 50% as *β*-**21**, with an intermediate fraction of *α*,*β*-**21** that could be processed further. The pure anomers were nicely distinguished by the p.m.r. chemical shifts of the ester OCH_3 singlets (*δ* 3.63 for *β*-**21**; *δ* 3.78 for *α*-**21**, which was obscured in the *α*,*β*-mixture). Surprisingly, *β*-**21** and *α*-**21** showed H-1′ doublets identical in both chemical shift and coupling constant. At the subsequent stages in the series, conducted as before, none of the anomeric pairs could

be distinguished chromatographically. However, chemical shifts of the H-1′ doublets differentiated α-**24** and β-**24**, as well as **1** and **2**, and served to confirm their anomeric purity. Although several general procedures for hydrogenolytic debenzylation have been reported, we have found the procedure[8] using freshly prereduced palladium chloride to be the only method consistently effective.

The possibility had to be considered that the minor isomer separated from β-**21** was, instead of α-**21**, the 4-amide-5-ester (**a**). This might have resulted from competing ammonolysis of **20** at the 4-ester instead of at the 5-ester. Subsequently, **a** would have yielded the isomeric pyrazolo[3,4-*d*]pyrimidine-4,6-dione (**b**) in place of **2**. This possibility was excluded by the u.v. spectra. Spectra of the [3,4-*d*] system generally resemble those of the purine analogs, whereas the [4,3-*d*] isomers are distinguished by a shift to longer wavelength of 10–30 nm[17,18]. Diones **1** and **2** had nearly identical u.v. spectra that agreed with those of the previous analogs[1,2,14] (peaks at 284–288 nm in acid, at 297–307 in base). The u.v. spectrum of the 4,6-dione (**b**) has been reported only for the case when R = H[18,19] (peaks at 255 nm in acid, 268 in base); the purine nucleoside expected to resemble **b** (R = glycosyl) is xanthosine (peaks at 263 nm in acid, 277 in base). Clearly, **b** (R = glycosyl) would have differed from **1** by a wavelength shift of at least 20 nm. The absence of **a** and **b** upheld previous findings that ammonolysis of diesters such as **20** was completely selective at C-5.



a



b

In processing the final products, it was observed that **1** formed a highly crystalline tri-*O*-acetyl derivative. Its relative insolubility in organic solvents and ready deacylation made it extremely useful for the final purification of **1**. Thus, acetylation could even be used to separate **1** from mixtures of **1** and **2**, as the α-anomer **2** formed a triacetate of normally high solubility and the β-tri-acetate crystallized readily in its presence. This method was more practical for obtaining quantities of **1** than the separation of anomers based on chromatographic resolution of **21**. The α-anomer **2** had to be purified simply by crystallization, and no samples were obtained without solvent of crystallization. Its characterization was supported by the mass spectrum of its per(trimethylsilyl)ated derivative, which differed only slightly from the mass spectrum of per(trimethylsilyl)ated **1**. Mass spectra of α-**24** and β-**24** were, as expected, nearly identical; mass spectra are not very sensitive to stereochemical changes in the sugar. The spectrum of β-**24** was also nearly identical to that of its D-*ribo* analog*, except for the fact that β-**24** gave M + H and M + benzyl peaks. The spectra of **1**, α-**24**,

*We are indebted to Mr. K. J. Ryan for preparing a sample by the method in ref. 2.

and β-**24** showed the intense peak for the heterocyclic base+30 that has been reported[20] to be diagnostic for *C*-nucleosides; it is interesting that this peak was very weak in the spectrum of **2**.

The anomeric assignments of these *C*-nucleosides was further supported by circular dichroism. The c.d. spectra of **1** and **2** were clearly distinct (Fig. 1). The β-anomer **1** exhibited a negative Cotton effect and the α-anomer **2** a positive one, at the wavelength (285 nm) of the absorption maximum. The tri-*O*-benzyl derivatives β-**24** and α-**24** showed similar spectra but with stronger Cotton effects. This is the same

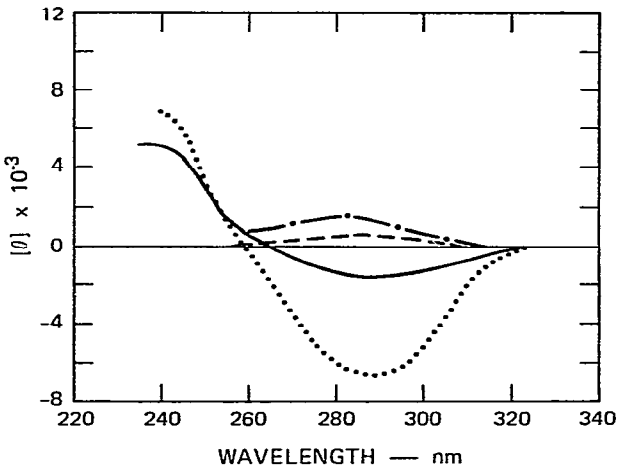


Fig 1. Circular dichroism spectra in ethanol. **1** (———); β-**24** (·····); **2** (– – –); α-**24** (—·—·—·).

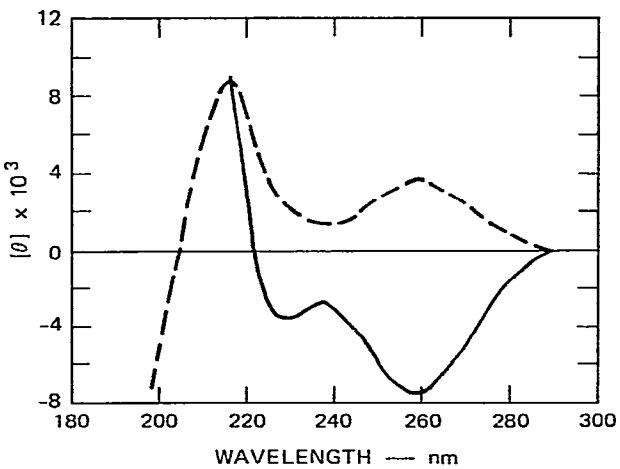


Fig. 2. Circular dichroism spectra in ethanol (from Ingwall, ref. 21): 9-(β-D-arabinofuranosyl)-adenine (———); 9-(α-D-arabinofuranosyl)adenine (– – –).

dichroic behavior as that recently observed by Ingwall[21] for anomeric D-arabinofuranosyl *N*-nucleosides of adenine (Fig. 2). Negative Cotton effects for β-anomers and positive ones for α-anomers were found for the D-pentofuranosyl nucleosides of adenine in general (near 260 nm, the wavelength of the major absorption peak). Furthermore, *N*-nucleosides having the C-1′–C-2′ substituents *cis* (such as the β-arabinoside, Fig. 2) had the largest molar ellipticites (θ values), and those having the C-1′–C-2′ substituents *trans* (such as the α-arabinoside, Fig. 2) had the smallest molar ellipticities. This is also observed with the arabinosyl *C*-nucleosides in Fig. 1. Thus the previously observed relationships between nucleoside structure and c.d. spectra[21] also hold for *C*-nucleosides. Even a quantitative similarity was observed. The ratio θ_{max} of β:θ_{max} of α was about 2:1 for the *N*-arabinosyl derivatives (Fig. 2); it was 2.7:1 for the *C*-arabinosides **1** and **2** (Fig. 1). The usefulness of this method should not be underestimated. In the present case it provides unequivocal support for the anomeric structures assigned to **1** and **2**.

BIOLOGICAL RESULTS

Preliminary evaluation of antitumor properties was done by Drug Research and Development, National Cancer Institute, Bethesda, Md., according to its protocols[22], and the results are shown in Table I. The analog **3** showed activity against lymphoid leukemia L1210 implanted in mice, when injected intraperitoneally in 9 daily doses of 50 and 100 mg/kg; toxic results were observed at 5 daily doses of 100 and 200 mg/kg. In the single test to date, **1** was both inactive and nontoxic at 200 mg/kg in the 5-dose regimen.

TABLE I

TEST RESULTS VS. L1210 MURINE LEUKEMIA

Structure	*Accession No.*[a]	*Dose Schedule*	*Dose (mg/kg)*	*T/C (%)*[b]
3	NSC-136727	QD 5	200[c]	—
			100[d]	101
		QD 9	100	188
			50	131
			25	96
1	NSC-164010	QD 5	200	111

[a]Assigned by the National Cancer Institute. [b]Ratios of survival times of treated mice over control mice; a ratio ≥125 is a positive result denoting activity. [c]Toxic dose, 1 out of 6 treated mice survived. [d]Four out of 6 treated mice survived.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns hot-stage and are uncorrected. I.r. spectra were determined in Nujol mulls or as liquid

films. U.v. spectra were determined with a Cary Model 11 recording spectrophotometer. Solutions for u.v. spectra were prepared in ethanol as stock solutions and were then diluted 1:5 with ethanol, with 0.1M HCl, and with 0.1M NaOH. P.m.r. spectra were determined at 60 or 100 MHz with Varian A-60A and HA-100 instruments, with the samples in chloroform-*d* solution unless otherwise noted. Tetramethylsilane (1%) was the internal reference (δ 0.00), except in D_2O, where it was used as external reference. Signals are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Mass spectra were determined on an LKB model 9000 spectrometer at 70 eV. Circular dichroism spectra were determined on a Durrum–Jasco ORD/UV-5 spectropolarimeter equipped with a Sproul Scientific SS-20 c.d. modification and a programmed 15-Å slit-width control (Durrum Instrument Co., Palo Alto, California) as described by Ingwall[21]. Path lengths of 1.0 cm and a concentration range of 0.1–0.4mM were used. Molar ellipticity, [θ], is given in deg $l.mol^{-1}.cm^{-1}$. T.l.c. was done with silica gel HF (E. Merck) on 5 × 20-cm glass plates. The spots were detected under u.v. light, and with sulfuric acid spray and charring. Preparative t.l.c. was done with 2-mm layers on 20 × 20-cm plates. In processing reaction mixtures, organic solutions were dried with magnesium sulfate, which was removed by filtration. Solutions were concentrated or evaporated *in vacuo* with a spin evaporator.

*2,3,5-Tri-*O*-benzyl-*D*-arabinofuranosyl bromide* (**4**). — The procedure of Fletcher and Barker[5] was modified slightly by saturating 120 ml of dichloromethane at 0° with anhydrous hydrogen bromide and adding a solution of 8.00 g (14.0 mmoles) of 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-α,β-D-arabinofuranose (Pfanstiehl Laboratories, Inc.) in 20 ml of dichloromethane. After being stirred for not more than 30 min at room temperature, the mixture was filtered. The filtrate was evaporated *in vacuo* (bath not over 30°) and the residual bromide (quantitative yield) was used immediately; p.m.r. δ 6.58 d (H-1 of β-anomer, $J_{1,2}$ 3.5 Hz), 6.48 s (H-1 of α-anomer), 4.1–3.9 m (assigned to H-2), 3.65 d (5-H_2, J 4.5 Hz). The ratio α:β was about 17:3.

*2,5-Anhydro-3,4,6-tri-*O*-benzyl-*D*-glucononitrile* (**5**). — *A. From the bromo sugar* **4**. Compound **4** (from 14.0 mmoles of 1-*p*-nitrobenzoate) was dissolved in 200 ml of dry benzene, and the solution was treated with 10.8 g (43.2 mmoles) of mercuric cyanide (previously dried *in vacuo* for 24 h at 100°). The suspension was stirred for 3 h at 25°, refluxed for 2 h, and filtered. The filtrate was concentrated, and the residue was dissolved in 100 ml of dichloromethane. Mercury salts were removed by filtration and by washing the filtrate with two 50-ml portions of 30% potassium iodide solution. The dichloromethane layer was washed with water, dried, and concentrated to 5.8 g (96%) of a syrup; t.l.c. (benzene–ether, 19:1) showed one major component, R_F 0.70, with the D-*manno* epimer **6** (R_F 0.75) and the non-nitrogenous by-product (R_F 0.63) as minor contaminants. Elemental analysis (N found/N calc. = 2.78/3.26) indicated the presence of ~85% nitrile and ~15% non-nitrogenous sugar. The p.m.r. spectrum (in C_6D_6 at 60 MHz) showed a doublet at δ 3.48 (6-H_2, J 6.0 Hz) and a quartet at δ 3.73 (furanose ring H, unassigned, J 2.7 and 5.0 Hz) that were characteristic; clarity of these signals was the necessary criterion for purity of **5**. A weak doublet at δ 3.38 (6-H_2, J 5.5 Hz) was evidence for a little of the D-*manno*

epimer **6**. The epimeric ratio was best determined by ^{13}C resonance, assuming the stronger singlet was from **5**; c.m.r. (in 1,4-dioxane at 25.15 MHz on the HA-100 spectrometer, p.p.m. from Me_4Si): δ 118.2 s (CN of D-*manno* **6**), 117.2 s (CN of D-*gluco* **5**); the integrated ratio of *gluco*:*manno* was 4:1.

*B. From 2,5-anhydro-*D*-gluconamide* (**14**). The amide **14** was *O*-benzylated with α-bromotoluene and sodium hydride in dimethyl sulfoxide to yield 67% of syrup, by the procedure[23] used with 2,5-anhydro-1-deoxy-1-ureido-D-allitol. T.l.c. in benzene showed an elongated spot. R_F 0.1–0.2, with a distinct contaminant, R_F 0.85. This contaminant was separated by column chromatography on silica gel with benzene as eluent, and appeared to be dibenzyl ether. Elution with 1:4 benzenemethanol yielded the product (48%); i.r. 3.0 broad (NH_2), 5.9 μm (C=O). This crude *2,5-anhydro-3,4,6-tri-*O*-benzyl-*D*-gluconamide* (**16**) was dehydrated with *p*-toluenesulfonyl chloride in pyridine[24], but even after a second treatment there still remained a weak amide C=O band in the i.r. spectrum. The crude nitrile (0.36 g) was separated from nearly overlapping contaminants by preparative t.l.c. on 3 plates developed with benzene to yield 65 mg, R_F 0.70, identical to **5** from *A*, foregoing, by direct comparison. The p.m.r. spectrum (in C_6D_6 at 100 MHz) was more clearly resolved than that (at 60 MHz) from procedure *A*, but provided identical data.

*2,5-Anhydro-3,4,6-tri-*O*-benzyl-*D*-mannononitrile* (**6**). — A solution of bromide **4** (from 7.00 mmoles of 1-*p*-nitrobenzoate) in 135 ml of dry nitromethane was treated with 2.71 g (10.8 mmoles) of mercuric cyanide and about 2 g of molecular sieve (Linde type 3A) and stirred for 20 h at 25°. Processing as for **5** (*A*) afforded 2.93 g (97%) of syrup. The three components having R_F 0.63, 0.70, and 0.75 (t.l.c. in 19:1 benzene–ether) were of about equal intensity, and there were minor contaminants having R_F 0.1. The p.m.r. spectrum (C_6D_6) showed three sharp doublets centered at δ 3.62, 3.48, and 3.38 that were diagnostic for the nitrogen-free by-product, the *gluco* nitrile **5**, and the *manno* nitrile **6**, respectively. Each doublet was assigned to 6-H_2 of the corresponding sugar. Preparative t.l.c. of 0.50 g on 4 plates in 19:1 benzene–ether afforded 118 mg of analytically pure **6**, R_F 0.75; p.m.r. (C_6D_6): δ 3.70 q (furanose ring H, unassigned, J 2.3 and 4.7 Hz), 3.38 d (6-H_2, J 5.5 Hz). The adjacent, slower-moving band afforded 196 mg that was still a mixture of the three components.

Anal. Calc. for $C_{27}H_{27}NO_4$: C, 75.5; H, 6.33; N, 3.26. Found: C, 75.6; H, 6.37; N, 3.17.

*2,5-Anhydro-*D*-gluconamide* (**14**). — Compound **14** ("chitaric acid amide") was prepared as described by Cox *et al.*[11], except that sodium nitrite was used in place of silver nitrite for diazotization of 2-amino-2-deoxy-D-gluconic acid (Pfanstiehl Laboratories, Inc.*), and esterification of 2,5-anhydro-D-gluconic acid (chitaric acid) was effected with methanolic hydrogen chloride. The amide **14** was not induced to crystallize (lit.[11] m.p. 170°), but both the methyl ester **12** and **14** were nicely charac-

*We are indebted to Mr. A. G. Holstein of Pfanstiehl for his interest in developing the preparation of 2-amino-2-deoxy-D-gluconic acid (glucosaminic acid) and for supplying 500-g lots of this versatile intermediate for these studies.

terized as tri-acetates by clearly resolved p.m.r. spectra: *methyl tri*-O-*acetyl-2,5-anhydro*-D-*gluconate* (**13**), δ 5.54 q (H-3), 5.13 t (H-4), 4.80 d (H-2), 4.6–4.1 m (H-5, 6-H_2), 3.78 s (CO_2CH_3), 2.12 s, 2.10 s, 2.08 s (three OAc groups), $J_{2,3}$ 4.8, $J_{3,4}$ 2.0 Hz; *tri*-O-*acetyl-2,5-anhydro*-D-*gluconamide* (**15**), δ 6.9–6.3 broad d (NH_2), 5.54 q (H-3), 4.99 q (H-4), 4.65 q (H-2), 4.5–4.0 m (H-5, 6-H_2), 2.13 s, 2.10 s (three OAc groups), $J_{2,3}$ 4.5, $J_{3,4}$ 1.2 Hz. If first completely exchanged to remove all OH or HOD, the amide **14** in D_2O exhibited a sharp doublet at δ 4.58 (H-2) and a sharp quartet at δ 4.32 (H-3), $J_{2,3}$ 4.2, $J_{3,4}$ 1.5 Hz.

1-Amino-2,5-anhydro-3,4,6-tri-O-*benzyl-1-deoxy*-D-*glucitol* (**7**). — A solution of 3.80 g (8.80 mmoles) of nitrile in 25 ml of anhydrous tetrahydrofuran was stirred and treated with 40 ml (40 mmoles) of M borane in tetrahydrofuran (Alfa). After 20 h, the excess borane was decomposed by adding methanol (7–10 ml) until there was no further effervescence. Concentration of the mixture afforded a syrup. To decompose the amine–borate complex into volatile methyl borate, the syrup was dissolved in 25 ml of methanol and 5 ml of benzene. The solution was acidified (pH 1–2) with 3M hydrochloric acid, refluxed for 3 h, and concentrated. If the residue still contained amine borate (i.r. 4.2 μm), the treatment was repeated. Finally, the syrup in 40 ml of benzene was washed with 5% sodium hydroxide solution, washed with water, dried, and recovered by concentration to yield 3.2 g (84%); R_F 0.5 in 1:4 methanol–benzene, detected under u.v. light and by ninhydrin spray, with an impurity having R_F 0.9 detected only by u.v. light. In the i.r., absorption for NH was evidenced only by a very weak peak at 2.94 with shoulders at 3.0 and 3.1 μm, and by a double peak at 6.21 and 6.28 μm that differed from the aryl bands of benzyl ethers only in increased intensity.

1-Acetamido-2,5-anhydro-3,4,6-tri-O-*benzyl-1-deoxy*-D-*glucitol* (**8**). — A solution of 25.0 g (57.6 mmoles) of amine **7** in 200 ml of dry pyridine was treated with 60 ml of acetic anhydride, stored overnight, and concentrated. A solution of the residual syrup in 250 ml of dichloromethane was washed with two 100-ml portions of 10% sulfuric acid, with saturated sodium hydrogen carbonate, with water, and was dried and concentrated to yield 26.2 g (95%), R_F 0.2 in 2:3 ether–benzene with impurities having R_F 0.85 (minor) and R_F 0.9 (major). A 2:3 ether–benzene solution (~40 ml) of the product was chromatographed on a column of 500 g of silica gel (90–200 mesh). Elution with 500 ml of ether–benzene separated the impurity (6.0 g). The amide was then eluted with 500 ml of 1:1 benzene–methanol, to yield 19.8 g (72%) of homogeneous syrup R_F 0.65 in 1:4 methanol–benzene *vs.* R_F 0.5 for **7**; i.r. 3.02 (NH), 3.28, 3.31, 3.43–3.50 (CH), 6.0 (amide C=O), 6.43 μm (amide II). A weak i.r. band persisted at 5.72 μm in all samples even after purification, when t.l.c. and n.m.r. evidenced no impurity, and may not have been due to any *O*-acetate contaminant. Similar weak bands at 5.76 μm occurred in all samples of the nitriles **5** and **6**, and may be related to the even weaker overtone bands at 5.1, 5.3, and 5.5 μm in spectra of all these of *O*-benzyl compounds. The p.m.r. spectrum gave δ 1.82 s (CH_3CO–N). There was no observed spectral or chromatographic resolution of the D-*manno* epimer (assumed 20% present).

Anal. Calc. for $C_{29}H_{33}NO_5$: C, 73.2; H, 7.00; N, 2.95. Found: C, 72.8; H, 6.89; N, 3.02.

*2,5-Anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-1-nitrosoacetamido-*D*-glucitol* (**10**). — To a solution of 5.70 g (12.0 mmoles) of the acetamide **8** in 100 ml of 1:1 carbon tetrachloride–glacial acetic acid was added 7.5 g of anhydrous sodium acetate[13] (buffer to take up the nitric acid to be liberated). The suspension was stirred and chilled to 0°, and liquid dinitrogen tetraoxide (nitrogen dioxide, Matheson; 3–4 ml required) was added until a green color persisted. The mixture was stirred for 2 h at 0° and was poured into ice and water, with addition of more sodium acetate buffer as needed to keep the pH at 3–4. Dichloromethane (75 ml) was added, and the organic layer was separated, washed with saturated aqueous sodium hydrogen carbonate until the acetic acid had been removed, washed with water, dried, and concentrated. The residual syrup (5.5 g, 91%) was homogeneous, R_F 0.8 in 1:3 ether–benzene, *vs.* R_F 0.1 for **8**; i.r. 5.75 (C=O), 6.6 μm broad (N=O) (a weak band always present at 6.05 μm was not considered indicative of unreacted **8**); p.m.r. δ 2.70 s (CH_3CO–N–NO; there was no resolution of a second singlet that might have been expected from the D-*manno* epimer). The nitrosoamide was used immediately, or stored at 0°. A sample decomposed partly after storage for 3 weeks at 25°, judging from the appearance of i.r. bands at 2.87 and 6.1 μm.

*2,5-Anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-1-diazo-*D*-glucitol* (**11**). — A solution of nitrosoamide **10** (5.5 g, from 12 mmoles of acetamide **8**) in 75 ml of 1:1 1,2-dimethoxyethane–ether was chilled to 0°, 50 ml of 30% potassium hydroxide was added, and the layers were intimately mixed by vigorous stirring for 1 h in the cold. An aliquot (few drops) of the ether layer was allowed to evaporate on a sodium chloride plate; the i.r. spectrum of the resultant film showed a strong, sharp band at 4.75 (CHN_2), but a weak–medium band always remained at 5.75 μm. The ether layer was separated, and the aqueous base was washed with 30 ml of cold ether. The combined ether solutions were washed with cold water, and the cold ether layer (70–80 ml; no change in the i.r. spectrum of a second aliquot) was used immediately in the next step.

*Dimethyl 3-(2,3,5-tri-*O*-benzyl-α,β-*D*-arabinofuranosyl)pyrazole-4,5-dicarboxylate* (**20**). — A solution of 2.0 ml (15 mmoles) of dimethyl acetylenedicarboxylate in 50 ml of ether was stirred at 0–5° and treated gradually during 15 min with the ethereal diazo sugar **11** (prepared from 12 mmoles of acetamide **10**). The solution was stirred for 1 h at room temperature, washed with two 70-ml portions of water, dried, and concentrated. The residual syrup (5.7 g) was estimated to be 64% pure by comparing the integrated H-1′ doublet in the p.m.r. with the C_6H_5 signal; R_F 0.40 on silica gel in 2:3 ether–benzene with impurities at R_F 0.45 and 0.90. Unreacted dimethyl acetylenedicarboxylate could also be detected, in varying amounts from run to run, as an extraneous singlet (CO_2CH_3) in the p.m.r. at δ 3.82. The chromatographically fast-moving impurity was removed on a column of 180 g of silica gel (200–400 mesh) in 2:3 ether–benzene, in the first 150 ml of eluate. Further elution with ether–benzene afforded 3.6 g (52% yield based on **8**, assuming 100% purity), R_F 0.40–0.45; i.r. 3.05 (NH), 5.72, 5.8 sh (C=O) μm; $\lambda_{max}^{pH\,13}$ 257 nm (ε 9450). Purity estimated by

comparing H-1′ and C_6H_5 signals in the p.m.r. was of limited accuracy and never exceeded 85%; δ 7.35 s, broadened to 6.9 (C_6H_5), 5.63 d (H-1′, J 4.2 Hz), 3.95 s (5-CO_2CH_3), 3.75 s (4-CO_2CH_3).

Anal. Calc. for $C_{33}H_{34}N_2O_8$: C, 67.6; H, 5.84; N, 4.78. Found: C, 67.6; H, 5.69; N, 4.42.

*Methyl 3-(2,3,5-tri-*O*-benzyl-α,β-*D*-arabinofuranosyl)-5-carbamoylpyrazole-4-carboxylate* (**21**). — A solution of 18.0 g of unchromatographed diester **20** in 250 ml of anhydrous methanolic ammonia (methanol presaturated at 0° with ammonia) was kept for 2 days in a stoppered flask at room temperature. Concentration afforded 17.7 g of a syrup, R_F 0.55 on silica gel in 1:4 methanol–benzene with an impurity at R_F 0.80 (but free of **20**, R_F 0.90). The syrup in 100 ml of 1:1 ether–benzene was chromatographed on a column of 500 g of silica gel (90–200 mesh) in ether. Elution with 1.6 l of ether separated the impurity (4.5 g), and the product was then eluted with 1.6 l of 1:1 ether–methanol to yield 12.6 g of homogeneous syrup. The β:α ratio could be only roughly estimated as 7:3 from the p.m.r. singlets for 4-CO_2Me (δ 3.77 for α, 3.62 for β), owing to overlap with sugar-proton signals, but it was consistent with the ratio (4:1) expected from the mixture of **5** and **6**. Purity estimated by comparing the integrated H-1′ doublet (δ 5.52) with the C_6H_5 signal was about 75% (that is assuming that at least 9.45 g of **21** was present in the syrup, the overall yield based on acetamide **8** was 42%).

Separation of anomers. — A column of 260 g of silica gel (200–325 mesh, Mallinkrodt SilicAR CC-7) in 2·3 acetone–benzene was prepared, and a solution of 8.0 g of amide ester **21** in 50 ml of acetone–benzene was added. The flow rate was adjusted to approximately 100 ml/h with nitrogen under low pressure while the column was eluted with more 2:3 acetone–benzene, and 50-ml fractions were collected.

Fractions 3 and 4 contained α-**21**, 0.87 g, R_F 0.35 by t.l.c. in the same solvent, with a minor impurity at R_F 0.50; p.m.r. ($CDCl_3$): δ 7.28 s and 7.23 s (3 × C_6H_5), 6.5 broad (exchangeable, NH), 5.52 d (H-1′, J 4.2 Hz), 3.78 s (4-CO_2Me); $\lambda_{max}^{pH\,13}$ 258 nm (ε 8530).

Fractions 5 and 6 contained mixtures (2.54 g) of the anomers which could be resolved by further chromatography.

Fractions 7 through 10 contained β-**21**, and were combined to give 4.00 g, homogeneous on t.l.c. at R_F 0.25; p.m.r. ($CDCl_3$): δ 7.33 strong s, 7.3–7.2 m, 7.1–6.8 broad (3 × C_6H_5), 6.5 broad (exchangeable, NH), 5.52 d H-1′, J 4.2 Hz) 3.63 s (4-CO_2Me); $\lambda_{max}^{pH\,13}$ 257 nm (ε 9650).

The anomers were identical in the i.r.; 3.00, 3.12 (NH), 5.9 broad (C=O), 6.22 μm (amide II).

*3-Carbamoyl-5-(2,3,5-tri-*O*-benzyl-*D*-arabinofuranosyl)pyrazole-4-carboxylic acid hydrazide* (**22**). — Amide ester **21** (the pure anomers or the anomeric mixture) was treated with ice-cold ≥95% hydrazine (25 ml/g). The nucleoside gradually dissolved, and the orange-brown solution was kept for 2 h at room temperature, clarified by filtration, and concentrated (bath <50°). The residual syrup was dissolved in benzene

(25 ml/g) and washed 3 times with water to completely remove hydrazine. The benzene solution was dried and concentrated, and the hydrazide amide was recovered as a glass in 80–90% yield. The anomers were indistinguishable by the usual analytical methods, and it was assumed that anomeric composition was not affected by this process; R_F 0.60 in ethyl acetate (*vs.* R_F 0.45 for **21**); i.r. 3.1 (NH), 5.97, 6.1, 6.30 μm; p.m.r. ($CDCl_3$): δ 5.96 d (H-1′, J estimated 4 Hz).

A sample of β-**22** (from β-**21**) was analytically pure; $\lambda_{max}^{pH\,13}$ 262 nm (ε 9450).

Anal. Calc. for $C_{31}H_{33}N_5O_6$: C, 65.1; H, 5.82; N, 12.2. Found: C, 65.2; H, 5.96; N, 12.2.

*3-(2,3,5-Tri-*O*-benzyl-β-*D*-arabinofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione* (β-**24**). — According to the procedure for the D-*ribo* analog[14], 4.71 g of β-hydrazide amide (β-**22**, obtained from β-**21** in 91% yield) was treated, adding the 17 ml of 2M sodium nitrite in 4 portions during 30 min. The intermediate azide β-**23** was a yellow syrup; i.r. 4.68 (N_3), 6.00 μm (C=O). It was immediately diluted with toluene to 530 ml, and the solution was refluxed for 2 h and concentrated. The residue was redissolved in benzene, and recovered as a glass, 4.4 g (98%); R_F 0.6 on silica gel in 1:1 benzene–ethyl acetate with impurities at R_F 0.0–0.1 and a trace impurity at R_F 0.9. The i.r. spectrum showed a sharp impurity band of medium intensity at 6.43 μm.

A 5.2-g sample of crude β-**24** was purified by chromatography on a column of 210 g of silica gel (90–200 mesh) in 1:1 benzene–ethyl acetate, eluted with the same solvent. The first 250 ml of eluent afforded 1.44 g of gum, containing the impurity having the sharp i.r. band at 6.43 μm (strong), R_F 0.9, and some β-**24**. The next 275 ml contained 1.34 g (24%) of β-**24**, R_F 0.6 showing a trace spot at R_F 0; i.r. 3.13 (NH), 3.4–3.5 (CH), 5.84, 5.92 μm (C=O); p.m.r. ($CDCl_3$): δ 7.5–6.9 m (C_6H_5), 5.47 d (H-1′, $J_{1',2'}$ 4.8 Hz); mass spectrum (introduced by a direct probe at 150–220°): *m/e* 645 (M+benzyl), 555 (M+H), 554 (M), 463 (M-benzyl) 357, 181 (B+30).

Anal. Calc. for $C_{31}H_{30}N_4O_6$: C, 67.1; H, 5.45; N, 10.10. Found: C, 67.0; H, 5.63; N, 9.68.

Perhaps owing to solvation, the purity of this fraction was only 91%, as estimated from the u.v. extinctions compared with those of another otherwise identical sample isolated by t.l.c. (27% yield from β-**22**); λ_{max}^{EtOH} 285 nm (ε 4960); $\lambda_{max}^{pH\,13}$ 258 nm (ε 7930), 286 (4650).

Further elution of the column with 375 ml of solvent afforded an additional 0.61 g of β-**24**, identical by i.r. spectrum but appreciably contaminated with the impurity at R_F 0.0–0.1. Finally, 0.73 g of this nonmobile impurity was eluted with 350 ml of ethyl acetate (mol. wt. by osmometer, 777); it was misleadingly indistinguishable from β-**24** by its i.r. spectrum.

*3-(2,3,5-Tri-*O*-benzyl-α-*D*-arabinofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione* (α-**24**). — A 1.90-g sample of α-hydrazide amide (α-**22**, obtained from α-**21** in 93% yield) was treated as for β-**22**. Again, the intermediate azide α-**23** showed bands at 4.68 (N_3) and 6.00 μm (C=O). Column chromatography of the product,

after elution with 1:1 benzene–ethyl acetate of the mobile impurity (strong, sharp i.r. band at 6.43 μm), afforded 0.80 g (43%) of homogeneous α-**24**; p.m.r. δ 7.27 s and 7.22 s (C_6H_5), 5.30 d (H-1′, $J_{1',2'}$ 2.8 Hz); λ_{max}^{EtOH} 284 nm (ε 3360), $\lambda_{max}^{pH\,13}$ 257 nm (ε 6150). It was indistinguishable from β-**24** in the i.r. spectrum or by t.l.c. Elemental analysis, even after drying at 100° *in vacuo*, indicated purity of only ~83% by wt. owing to solvation.

Anal. Calc. for $C_{31}H_{30}N_4O_6 \cdot 0.8C_6H_6 \cdot 0.6CH_3CO_2Et$: C, 68.5; H, 5.96; N, 8.36. Found: C, 68.4; H, 6.04; N, 8.15.

In another run, a sample was purified by t.l.c. (19% yield from α-**22**); λ_{max}^{EtOH} 284 nm (ε 3660), $\lambda_{max}^{pH\,13}$ 257 (ε 7180), 300 (sh) nm.

*3-(2,3,5-Tri-*O*-benzyl-α,β-*D*-arabinofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)dione* (α,β-**24**). — A 4.9-g sample was obtained in 40% overall yield from the amide ester (α,β-**21**; β:α ratio estimated 7:3) *via* the hydrazide α,β-**22** and after chromatographic purification; λ_{max}^{EtOH} 286 nm (ε 4360). T.l.c. suggested weak contamination with the impurity having R_F 0.0–0.1.

*3-α,β-*D*-Arabinofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7(4*H*,6*H*)dione* (**1**, **2**). — A suspension of 0.5 g of palladium chloride (59% minimum palladium, Matheson, Coleman, and Bell) in 59 ml of anhydrous methanol was pre-reduced with hydrogen at 3 atm in a shaker for 45 min, 2.3 g (4.1 mmol) of α,β-**24** suspended in 10 ml of methanol was added, and the mixture was further hydrogenated for 5 h. Removal of the catalyst and evaporation of the filtrate afforded 1.4 g of a foamed glass. It was treated with 25 ml of water, and the solution was decanted from insoluble gum and stirred with 4 g of Dowex 2x-8 resin (CO_3^{2-}) for 2 h. Removal of the resin and concentration (bath 70–80°) yielded 0.74 g (63%) of a tan solid, of 59% purity by u.v. Recrystallization from ethanol removed impurities having R_F 0.0 and afforded 0.27 g, m.p. 148–154°, R_F 0.50 on silica gel in 1:4 methanol–chloroform; $\lambda_{max}^{pH\,1}$ 286 nm (ε 4230); $\lambda_{max}^{pH\,13}$ 253 nm (ε 5440). The β:α ratio was estimated to be 2.2:1 from p.m.r. (D_2O): δ 5.28 d (H-1′ of β, $J_{1',2'}$ 4.5 Hz), 5.02 d (H-1′ of α, $J_{1',2'}$ 6.5 Hz). There was no further resolution of anomers in later crops (0.12 g, m.p. 136–147°) or on attempted fractional recrystallization.

*3-(2,3,5-Tri-*O*-acetyl-β-*D*-arabinofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione* (β-**24**, R = Ac). — *A. From* **1**+**2** *mixtures.* A solution of 0.48 g (1.6 mmol) of the recrystallized mixture of **1**+**2** in 15 ml of pyridine was treated with 1.5 ml of acetic anhydride, kept for 2 days at 25°, and concentrated. The residual syrup was partitioned between water and dichloromethane, and was evaporated twice from methanol solutions containing 2% of pyridine, but the product (R_F 0.4 on silica gel in 2:3 acetone–benzene still showed a second spot (R_F 0.6) indicative of an *N*-acetyl derivative (weak p.m.r. singlet at δ 2.78 in $CDCl_3$). Boiling in 49:1 methanol–pyridine and evaporation afforded 0.56 g (81%) of the α,β-mixture as a homogeneous brown glass, R_F 0.4. The β-anomer (0.20 g) crystallized from hot acetonitrile (5 ml) and was recrystallized from 5 ml of 95% ethanol to give 0.11 g (16%), m.p. 231–232°, R_F 0.6 on silica gel in 4:1 ethyl acetate–cyclohexane; $\lambda_{max}^{pH\,1}$ 285 nm (ε 5700), $\lambda_{max}^{pH\,13}$ 255 (ε 6430), 297 nm (4670); i.r. 3.15 (NH), 5.70, 5.80, 5.91 μm (C=O); p.m.r.

(acetone-d_6–methanol-d_4): δ 5.47 s (two H), 5.19 d (one H), 2.18 s, 2.17 s, 1.92 s (three OAc); in dimethyl sulfoxide-d_6 there was resolution of the signal at δ 5.47 into a rough triplet, $J \sim 5$ Hz.

Anal. Calc. for $C_{16}H_{18}N_4O_9$: C, 46.8; H, 4.42; N, 13.7. Found: C, 46.9; H, 4.59; N, 13.7.

B. From β-**24**. A 1.8-g sample of β-**24** was debenzylated as was α,β-**24** to give, without neutralization, 0.96 g of residual product. Acetylation of an 0.85-g portion yielded 1.1 g which was recrystallized to give 0.70 g (58% from β-**24**), m.p. 227–228.5°, having an i.r. spectrum identical to that of the analytical sample.

3-β*-*D*-Arabinofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7(4*H*,6*H*)dione* (**1**). — A solution of 0.70 g (1.7 mmol) of triacetate β-**24** (R = Ac) in 50 ml of anhydrous methanol was treated with 2.7 ml of diisopropylamine, refluxed for 3.5 h, and concentrated. To remove amine that persisted even after several evaporations from methanol, the residue was triturated with chloroform, and then evaporated from water solution several times until the product lost its solubility in water. Finally, a suspension in 6 ml of water was heated to boiling, chilled, and filtered to yield 0.30 g (62%), dried at 100° *in vacuo*, m.p. 263–264°, R_F 0.25 on silica gel in 1:4 methanol–benzene; λ_{max}^{EtOH} 285 nm (ε 5480), λ_{max}^{pH1} 287 nm (ε 5520), λ_{max}^{pH13} 254 (ε 6420), 297 nm (4720); i.r. 5.87 μm broad (C=O); p.m.r. (dimethyl sulfoxide-d_6 with 2 drops of acetic acid-d_4): δ 5.20 d (H-1′, $J_{1',2'}$ 4.5 Hz), 4.0 m (H-2′ and H-3′), 3.7 m (H-4′ and H_2-5′); the addition[25] of acetic acid-d_4 was generally useful in sharpening the entire spectra of these *C*-nucleosides. The mass spectrum of the per(trimethylsilyl) derivative, injected directly from a gas chromatogram, gave *m/e* 716 (M), 701, 626, 611, 584, 536, 523, 483, 469, 397 (B+30), 217, 147, 73.

Anal. Calc. for $C_{10}H_{12}N_4O_6$: C, 42.2; H, 4.26; N, 19.7. Found: C, 42.1; H, 4.52; N, 19.4.

3-α*-*D*-Arabinofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7(4*H*,6*H*)dione* (**2**). — A 0.75-g sample of α-**24** (1.3 mmol) that had been purified by column chromatography was debenzylated as for α,β-**24**. The crude residual product, without neutralization, was crystallized from 8 ml of 95% ethanol to give 97 mg (25%) in two crops. The first crop (74 mg) melted at 156–163°; λ_{max}^{EtOH} 286 nm (ε 4584); λ_{max}^{pH1} 286 nm (ε 4695); λ_{max}^{pH13} 253 (ε 6146), 306 nm (3925); p.m.r. (D_2O): δ 5.02 d (H-1′, $J_{1',2'}$ 6.5 Hz). Even though this and other samples were dried at 100° *in vacuo*, elemental analyses always indicated some solvation and hydration.

Anal. Calc. for $C_{10}H_{12}N_4O_6 \cdot 0.5C_2H_5OH \cdot 0.75H_2O$: C, 41.2; H, 5.18; N, 17.4. Found: C, 41.0; H, 4.83; N, 17.1.

Acetylation afforded a non-crystalline, homogeneous triacetate that was not resolved from its β-anomer (β-**24**, R = Ac) by t.l.c. The mass spectrum of **2** was recorded from another sample, as its per(trimethylsilyl) derivative, injected directly from a gas chromatogram; it differed from that of the β-anomer only in the weakness of peaks at *m/e* 584, 469, and 397.

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