in 0.4 ml of buffer. Chromatography of the incubation mixture showed the presence of a third uv-absorbing spot corresponding in R_f to ADP and 2-chloro-ADP.

Acknowledgments.—The authors are grateful to Dr. J. Fildes (Australian National University) and Dr. E. Challen (University of New South Wales) who carried out the microanalyses. Mr. D. Nobbs synthesized 2-chloroadenosine.

Nucleosides of 2-Fluoroadenine[1]

JOHN A. MONTGOMERY AND KATHLEEN HEWSON

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

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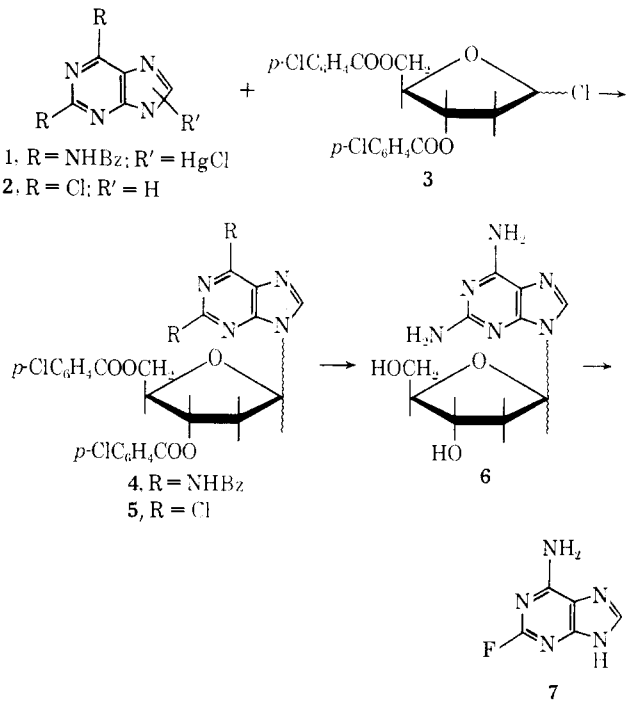
The preparation of the anomeric 9-(2-deoxy-D-*erythro*-pentofuranosyl)-2-fluoroadenines and 9-D-arabinofuranosyl-2-fluoroadenines from 2,6-dichloropurine is described. The cytotoxicity of these compounds, and also of 3′-deoxy-2-fluoroadenosine and 9-β-D-xylofuranosyl-2-fluoroadenine, to a number of HEp-2 cell lines in culture has been determined. The data permit certain conclusions concerning the probable metabolism and mechanism of action of these nucleosides.

2-Fluoroadenosine[2] is readily anabolized[3-5] but not catabolized[6-8] in cells in culture or *in vivo*. It is highly cytotoxic,[2] highly toxic to rodents,[10] and has broad-spectrum antibacterial activity.[11,12] It has a synergistic effect on the antimicrobial action of actinobolin[13] and is an inhibitor of blood-platelet aggregation.[14] The broad and high-level biologic activity of 2-fluoroadenosine has made the study of other nucleosides of 2-fluoroadenine desirable.

The preparation of 2-amino-2′-deoxyadenosine (β-**6**) in 1.7% over-all yield and its α anomer in 1.5% over-all yield from 2-amino-6-chloropurine by the conventional chloromercuri procedure has been reported.[15] In an effort to improve the yields of both anomers of **6** and to obtain analytical samples of these compounds, their preparation from the chloromercuri derivative of 2-benzamido-N-benzoyladenine[16] and 3,5-di-O-(*p*-chlorobenzoyl)-2-deoxy-D-*erythro*-pentofuranosyl chloride[17] was investigated and found to give **4** as an approximately 1:1 mixture of α and β anomers in a total yield of 36% (Scheme I). Treatment of **4** with NaOMe in the usual manner resulted in decomposition of the nucleoside, whereas treatment with methanolic NH_3 at 5° removed only the *p*-chlorobenzoyl groups. The anomeric mixture of 9-(2-deoxy-D-ribofuranosyl)-2-aminoadenine (**6**) was finally obtained in 31% yield by heating **4** with methanolic NH_3 at 100° for 6 hr in a bomb. The α and β anomers of **6**[15] were separated by fractional crystallization; about 8 parts α to 1 part β were isolated. Treatment of α-**6** with $NaNO_2$ in 48% fluoroboric acid resulted in replacement of the 2-amino group by fluorine but also in cleavage of the glycosyl linkage giving only 2-fluoroadenine (**7**),[18] a result not too unexpected in view of the known acid lability of purine 2′-deoxyribonucleosides.[19]

SCHEME I



Because of the acid lability of **6** and because of the difficulties in obtaining pure β-**6**[15] from the reaction of **1** and **3**, another route[20] to 2-fluoro-2′-deoxyadenosine

(1) This work was supported by funds from the Southern Research Institute, the C. F. Kettering Foundation, and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

(2) J. A. Montgomery and K. Hewson, *J. Amer. Chem. Soc.*, **79**, 4559 (1957).

(3) B. Lindberg, H. Klenow, and K. Hansen, *J. Biol. Chem.*, **242**, 350 (1967).

(4) H. P. Schnebli, D. L. Hill, and L. L. Bennett, Jr., *ibid.*, **242**, 1997 (1967).

(5) H. T. Shigeura, C. E. Boxer, S. D. Sampson, and M. L. Meloni, *Arch. Biochem. Biophys.*, **111**, 713 (1965).

(6) O. P. Chilson and J. R. Fischer, *ibid.*, **102**, 77 (1963).

(7) A. Block and C. A. Nichol, *Antimicrobial Agents Chemotherapy, 1964*, 530 (1965).

(8) J. C. Cory and R. J. Suhadolnik, *Biochemistry*, **4**, 1729 (1965).

(9) S. Frederiksen, *Arch. Biochem. Biophys.*, **113**, 383 (1966).

(10) H. E. Skipper, J. A. Montgomery, J. R. Thomson, and F. M. Schabel, Jr., *Cancer Res.*, **19**, 425 (1959).

(11) R. F. Pittillo, C. Monerief, R. W. Brockman, and P. Chambers, *Antimicrobial Agents Chemotherapy, 1964*, 474 (1965).

(12) R. F. Pittillo and M. Lucas, *Nature*, **205**, 824 (1965).

(13) R. F. Pittillo, F. M. Schabel, Jr., and B. G. Quinnelly, *Antibiot. Chemotherapy*, **11**, 501 (1961).

(14) G. V. R. Born, R. J. Haslam, M. Goldman, and R. D. Lowe, *Nature*, **205**, 678 (1965).

(15) R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963). The hydrogen analysis reported for the dihydrate of the α anomer was 0.75% low and that reported for the β anomer was 1.06% low, raising some question about the purity of these samples.

(16) J. Davoll and B. A. Lowy, *J. Amer. Chem. Soc.*, **73**, 1650 (1951).

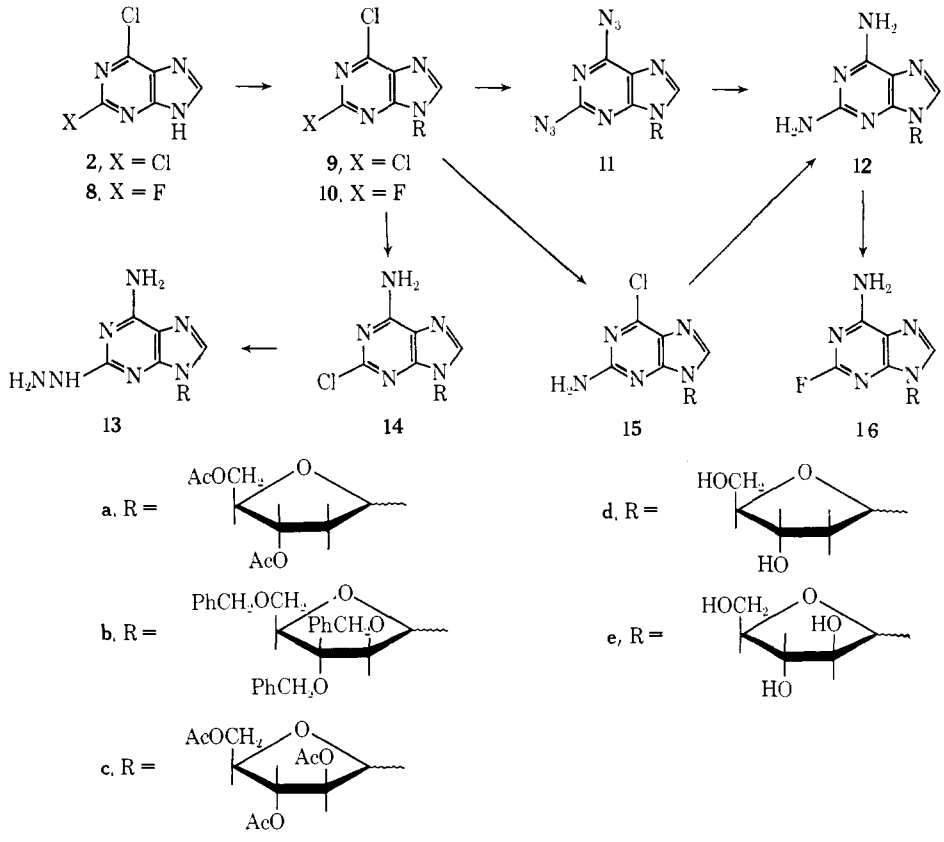
(17) J. J. Fox, N. C. Yung, I. Wempen, and M. Hoffer, *ibid.*, **83**, 4066 (1961).

(18) J. A. Montgomery and K. Hewson, *ibid.*, **82**, 463 (1960).

(19) J. A. Montgomery and H. J. Thomas, *Advan. Carbohydrate Chem.*, **17**, 301 (1962).

(20) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **33**, 432 (1968).

SCHEME II



(β-**16d**) was sought (Scheme II). Fusion of 1,3,5-tri-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranose[21] with 2,6-dichloropurine (**2**)[22] resulted in an 85% conversion to a 1.4 α to 1 β anomeric mixture of 9-(3,5-di-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranosyl)-2,6-dichloropurines (**9a**), which could be resolved by a combination of fractional crystallization and column chromatography.[23] The two anomers were identified by their pmr spectra.[24] Fusion of 1,3,5-tri-*O*-acetyl-2-dexoy-D-*erythro*-pentofuranose with 2,6-diazidopurine also gave a mixture of anomers slightly richer in the α anomer.

In an effort to obtain the pure β anomer of a protected 9-(2-deoxy-D-*erythro*-pentofuranosyl)-2,6-dichloropurine in a less tedious manner, **3** was allowed to react with 2,6-dichloropurine (**2**) in CH_2Cl_2 using a molecular sieve as the proton acceptor, conditions that favor the SN2 reaction, which should proceed with Walden inversion to give the β anomer (β-**5**), if **3** is the α-chloride.[25,27] Contrary to the results of Keller, *et al.*,[28] in the reaction of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride[29] with 2,6-dichloropurine (**2**) under SN2 conditions (*vide infra*), the reaction of **3** with **2** gave an α,β anomer mixture of **5** in the ratio of 6:5. The reaction of **3** with **2** in refluxing benzene in the presence of molecular sieve gave essentially the same results as obtained in CH_2Cl_2 at room temperature, although Bardos, *et al.*,[27] found that in refluxing benzene **3** reacted with 5-acetylmercapto-2,4-bis-*O*-(trimethylsilyl)uracil to give the β anomer only.[27] Furthermore, fusion of **3** with **2** proceeded poorly and again gave an anomer ratio of about 1, even though fusion of **3** with 5-allyl-2,4-bis-*O*-(trimethylsilyl)uracil gave a β/α ratio of about 2.5, from which the β anomer was readily isolated.[30]

Since the anomer ratio of **5** was no more favorable than that of **9a**, the reaction of α- and β-**9a** separately with NaN_3 was carried out to give α- and β-**11a**, which were reduced catalytically to 3′,5′-di-*O*-acetyl-2-amino-2′-deoxyadenosine (β-**12a**) (yield from β-**9a**, 65%) and its α anomer (α-**12a**) (yield from α-**9a**, 91%). The increased acid stability imparted to α- and β-**12a** by the *O*-acetyl groups permitted the preparation of 3′,5′-di-*O*-acetyl-2-fluoro-2′-deoxyadenosine (β-**16a**) and its α anomer (α-**16a**) by diazotization of β- and α-**12a** in 48% fluoroboric acid at −10°, although considerable cleavage of the glycosidic bond still occurred. Treatment of β- and α-**16a** with methanolic NH_3 at 0° gave 2-fluoro-2′-deoxyadenosine (β-**16d**) and its α anomer (α-**16d**).

Prior to the development of the route to 2-aminoadenine nucleosides described above (Scheme II), another procedure was investigated for the synthesis of the D-arabinofuranosides. 6-Chloro-2-fluoropurine (**8**)[18] upon fusion with 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose[31] gave 9-(2,3,5-tri-*O*-acetyl-D-arabinofuranosyl)-6-chloro-2-fluoropurine (**10c**) (Scheme II). Treatment of

(21) Purchased from Cyclo Chemical Corp., Los Angeles, Calif.

(22) J. A. Montgomery, *J. Amer. Chem. Soc.*, **78**, 1928 (1956).

(23) After this work had been completed, M. J. Robins and R. K. Robins [*J. Amer. Chem. Soc.*, **87**, 4934 (1965)] reported the isolation of the α anomer only from this reaction.

(24) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).

(25) Evidence for the α configuration of the related halogenoses. 3,5-di-*O*-(*p*-nitrobenzoyl)-2-deoxy-D-*erythro*-pentofuranosyl chloride and 3,5-di-*O*-(*p*-toluoyl)-2-deoxy-D-*erythro*-pentofuranosyl chloride, has been presented.[26] The pmr spectrum of **3**, a sharp-melting crystalline solid, does not permit assignment of its anomeric configuration.

(26) A. K. Bhattacharya, R. K. Ness, and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 426 (1963).

(27) T. J. Bardos, M. P. Kotick, and C. Szantay, *Tetrahedron Lett.*, 1759 (1966).

(28) F. Keller, I. J. Botviniek, and J. E. Bunker, *J. Org. Chem.*, **32**, 1644 (1967).

(29) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **87**, 4636 (1965).

(30) J. A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, **2**, 313 (1965).

(31) W. E. Fitzgibbon and S. J. Clayton, unpublished data

10c with NH_3–EtOH at 100° resulted in decomposition of the nucleoside, but at 5° for 3 days a good yield of 2-amino-9-D-arabinofuranosyl-6-chloropurine (**15e**) was obtained. Upon treatment with NH_3–EtOH at 100°, **15e** was converted into 2-amino-9-D-arabinofuranosyladenine (**12e**). The assumed α configuration of these nucleosides was firmly established by comparison of **12e** with an authentic sample of 2-amino-9-α-D-arabinofuranosyladenine (α-**12e**) prepared as described below.

Despite the successful preparation of α-**12e** from 6-chloro-2-fluoropurine, a sequence similar to that developed for the preparation of **12a** from **2** appeared preferable for α-**12c** and was carried out (Scheme II, **c** series, over-all yield 47%). In the modified Schiemann reaction using excess $NaNO_2$, α-**12c** gave primarily 9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)isoguanine (31%) and 9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2,6-difluoropurine (23%), along with a small amount of 9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2-fluoroadenine (α-**16c**) (<5%). The difluoropurine nucleoside and α-**16c** were converted to 9-(α-D-arabinofuranosyl)-2-fluoroadenine in the usual manner.[20]

For the preparation of the β anomer, the reaction of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride[29] with 2,6-dichloropurine (**2**) was investigated. In the presence of mercuric cyanide the reaction gave **9b** as an anomeric mixture (2.5α:1β) from which both anomers were isolated in the pure state; in the presence of molecular sieve, as described by Keller, *et al.*,[28] the reaction gave a 52% yield of β-**9b**, along with a small amount of the α anomer. These anomers could be readily identified by their pmr spectra since the signal from the anomeric proton of the *cis* or β anomer appeared downfield from the signal from the anomeric proton of the *trans* or α anomer.[32] Treatment of β-**9b** with NH_3–MeOH at ambient temperature for 3 days gave 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-chloroadenine (β-**14b**),[28] which was catalytically debenzylated to β-**14e**,[28] which in turn was acetylated with Ac_2O in pyridine to give β-**14c**. A number of unsuccessful attempts were made to cause β-**14c** to react with NaN_3, NH_4N_3, and LiN_3. The only reaction observed was deacetylation of β-**14c** to β-**14e**. The failure of β-**14b** to react with NaN_3 shows that facile replacement of both the 6- and the 2-chlorines of **9** and similar compounds[26] by the azide ion is due more to the fact that the 6-azido group does not deactivate the 2-chloro group than to the greater nucleophilicity of the azide ion compared to ammonia. On the other hand, displacement of the 2-chloro group of β-**14c** by hydrazine was readily accomplished, but attempts to acetylate β-**13e** gave a mixture of at least four products. An attempt to cleave the acetylated hydrazino group of 2-acetylhydrazino-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)adenine, presumed to be one of the products of the acetylation reaction, was not successful. This sequence was studied after all attempts to selectively O-acetylate 2-amino-9-β-D-ribofuranosyladenine failed—either no acetylation occurred or acetylation of the 2-amino group could not be prevented.

Because of these difficulties, we turned again to the reaction of NaN_3 with the 2,6-dichloropurine nucleoside, in this case β-**9b**. β-**11b** was thus prepared in good yield, and the azide groups of β-**11b** were reduced catalytically without removal of the *O*-benzyl groups to give 2-amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)adenine (β-**12b**) (over-all yield from **2**, 35%). The insolubility of β-**12b** in 48% HBF_4 necessitated the use of a heterogeneous, two-phase ($CHCl_3$ 48% HBF_4 mixture for the modified Schiemann reaction which gave a 36% yield of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (β-**16b**). Treatment of β-**16b** with Na and liquid NH_3 gave the desired 9-β-D-arabinofuranosyl-2-fluoroadenine (β-**16e**). α-**9b** was converted into α-**11b** and then to α-**12b**. Debenzylation of α-**12b** gave 2-amino-9-α-D-arabinofuranosyladenine (α-**12e**), identical with the sample prepared from **15e** *vide supra*).

Biologic Data.—The cytotoxicity of 2-fluoroadenine and its nucleosides was evaluated against cultures of HEp-2 cells and a number of sublines resistant to various agents[33,34] (see Table I). Thus 2-fluoroadenine (**7**) is highly cytotoxic to the sensitive strain of HEp-2 and also to the strain resistant to 6-mercaptopurine (HEp-2 MP), which has no IMP–GMP pyrophosphorylase, and to the strain resistant to 6-methylthiopurine ribonucleoside (HEP-2 MeMPR), which has no adenosine kinase. The mutant resistant to 2-fluoroadenine (HEp-2 FA) has no AMP pyrophosphorylase and the double mutant resistant to 2-fluoroadenine and to 2-fluoroadenosine (HEp-2 FA FAR) has no AMP pyrophosphorylase and no adenosine kinase. The fact that 2-fluoroadenosine is active against the cell line lacking adenosine kinase (HEp-2 MeMPR) indicates that it may be cleaved to 2-fluoroadenine, which can be converted to the nucleotide by AMP pyrophosphorylase. This viewpoint is supported by the 20-fold resistance of the HEp-2 FA mutant, which has adenosine kinase but no AMP pyrophosphorylase. 3′-Deoxy-2-fluoroadenosine[35] is active against the line lacking adenosine kinase (HEp-2 MeMPR), but is not active against the line lacking both the kinase and AMP pyrophosphorylase. These results indicate that the activity of 3′-deoxy-2-fluoroadenosine is a result of its cleavage to 2-fluoroadenine. In contrast, the mutant lacking both the kinase and AMP pyrophosphorylase (HEp-2 FA FAR) is only slightly resistant to 2′-deoxy-2-fluoroadenosine (β-**16d**). This result indicates that β-**16d** may act at the nucleoside level or, more likely, is phosphorylated by another kinase. The slight degree of resistance of the HEp-2 FA FAR cell line but not of the HEp-2 MeMPR cell line may indicate some cleavage of β-**16d** to 2-fluoroadenine. The ED_{50} value for 2-fluoro-9-β-D-xylofuranosyladenine, 38 μmoles/l., is about 800–900 times that of 2-fluoroadenosine, but about the same as that for 9-β-xylofuranosyladenine. In contrast, the ED_{50} value for 9-β-D-arabinofuranosyl-2-fluoroadenine, 5.1 μmoles/l., is about 250 times that of 2-fluoroadenosine but only one-thirteenth that of 9-β-D-arabinofuranosyladenine.

It is of general interest that a second change in the structure of a known antimetabolite (*i.e.*, the introduction of fluorine into the 2 position of *ara*-adenine) makes it a more effective antimetabolite. This in-

(32) J. A. Montgomery and K. Hewson, *J. Med. Chem.*, 11, 48 (1968).

(33) L. L. Bennett, Jr., H. P. Schnebli, M. H. Vail, P. W. Allen, and J. A. Montgomery, *Mol. Pharmacol.*, 2, 369 (1966).

(34) L. L. Bennett, Jr., M. H. Vail, S. Chumley, and J. A. Montgomery, *Biochem. Pharmacol.*, 15, 1719 (1966).

(35) A sample of this nucleoside was kindly provided by Dr. F. W. Holler

TABLE I

CYTOTOXICITY OF 2-FLUOROADENINE AND ITS NUCLEOSIDES TO HEp-2 CELLS IN CULTURE

Compd	HEp-2/0 ED_{50}, μmole/l.[a]	Degree of resistance: ED50 ratio of resistant/sensitive HEp-2/- MP	FA	MeMPR	FA/FAR
2-Fluoroadenine	0.03	1	>2000	1	>2000
2-Fluoroadenosine	0.02	1	20	1–2	>2000
2′-Deoxy-2-fluoroadenosine (β-**16d**)	0.2			1	2.4
9-(2′-Deoxy-α-D-ribofuranosyl)-2-fluoroadenine (α-**16d**)	6.5				
3′-Deoxy-2-fluoroadenosine[b]	2		12	1	>10
3′-Deoxyadenosine	80		1	1	
2-Fluoro-9-β-D-xylofuranosyladenine	38				
9-β-D-Xylofuranosyladenine	30				
9-β-D-Arabinofuranosyl-2-fluoroadenine (β-**16e**)	8.0				
9-β-D-Arabinofuranosyladenine	104				
9-α-D-Arabinofuranosyl-2-fluoroadenine (α-**16e**)	38				
9-α-D-Arabinofuranosyladenine	37				

[a] The concentration required to inhibit the growth of cells, as measured by clone counts, to 50% of untreated controls. [b] See ref 35.

creased cytotoxicity may be due to the fact that the fluorine at C-2 prevents deamination of the nucleoside, since it is known that 2-fluoroadenosine is not deaminated[8] but *ara*-adenine is.[36] The α anomers of 2-fluoro-2′-deoxyadenosine and 9-β-D-arabinofuranosyl-2-fluoroadenine are much less cytotoxic, the ED_{50} values being 6.5 and 38 μmoles/l., respectively.

Experimental Section[37]

2-Benzamido-N-benzoyl-9-[3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-α,β-D-*erythro*-pentofuranosyl]adenine (4).—Chloromercuri-2-benzamido-N-benzoyladenine[16] (36.6 g of 48% chloromercuri–Celite mixture) was azeotropically dried in C_6H_6 (600 ml) and allowed to react with 3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-*erythro*-pentofuranosyl chloride[17] (12.8 g, 30 mmoles) under reflux. After the usual work-up requiring filtration, evaporation, and extraction (30% aqueous KI) of a $CHCl_3$ solution of the reaction residue, the crude product was obtained as a glass, the pmr spectrum of which indicated it was approximately a 1:1 mixture of α- and β-**4**. An EtOH (100 ml) solution of the crude product was diluted with Et_2O until a filterable solid precipitated. Collection of the solid gave the purified product which was washed and dried; yield 2.7 g (12%), mp 140–145°. Tlc (99:1 $CHCl_3$–MeOH) showed trace impurities.

An additional 6 g (26%) of less pure nucleoside was obtained as a glass by $CHCl_3$ extraction of the reaction mixture insoluble solids.

2-Amino-9-(2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)-adenine (6).—A solution of 2,6-dibenzamido-9-[3,5-di-*O*-(*p*-chlorobenzoyl)-D-*erythro*-pentofuranosyl]purine (**4**, 4.7 g, 6.2 mmoles) in MeOH–NH_3 (150 ml, saturated at 5°) was heated at 100° for 6 hr. The reaction solution was evaporated to dryness *in vacuo* and the residue, after trituration with $CHCl_3$, was crystallized by trituration with EtOH (20–30 ml). The mixture of crystalline anomers was collected by filtration, dried (500 mg, 31%), and recrystallized from 1:1 EtOH–MeOH (40 ml). Crystallization was allowed to take place slowly at room temperature and the crops were analyzed by tlc (5:1 $CHCl_3$–MeOH). The crops containing the slower traveling α anomer were combined (180 mg) and recrystallized from MeOH–H_2O. A second recrystallization from MeOH gave a sample of pure α-**6**: mp 230°; $[\alpha]^{27}D$ +73.9 ± 0.7° (*c* 0.75, H_2O); λ_{max} [mμ ($\epsilon \times 10^{-3}$)], pH 1—252 (10.6), 291 (9.8); pH 7, 13—256 (9.2), 279 (10.2) [lit.[15] $[\alpha]^{21}D$ +58.8 ± 1.0° (H_2O); λ_{max} [mμ ($\epsilon \times 10^{-3}$)], pH 7, 14—216 (25.0), 256 (8.9), 280 (9.9); mp 167°]. *Anal.* ($C_{10}H_{14}N_6O_3$) C, H, N.

Only a single tlc-homogeneous crop of the β anomer (24 mg) was obtained from the EtOH–MeOH crystallization solution. Evaporation of the combined filtrates from the EtOH–MeOH fractionation to dryness gave a residue which was dissolved in H_2O (3 ml). Fractionation of the aqueous solution resulted in the isolation of additional homogeneous product in the same anomeric ratio as the EtOH–MeOH crystallization.

2-Fluoroadenine (7).—2-Amino-9-(2-deoxy-α,β-D-*erythro*-pentofuranosyl)adenine (**6**, 270 mg, 1 mmole) was dissolved in 48% HBF_4 (30 ml at 0°), the solution was cooled to −15°, and $NaNO_2$ (100 mg, 1.7 mmoles) was added in small portions to the stirred reaction mixture. After the addition was complete (15 min), the mixture was stirred for 15 min at −10°, cooled to −30°, and neutralized (pH 6–7) with 50% NaOH. After evaporation to dryness *in vacuo*, the residue was triturated with EtOH (four 10-ml portions). Evaporation of the EtOH filtrate to dryness gave the crude product which was identified by tlc (5:1 $CHCl_3$–MeOH) as a mixture of 2-fluoroadenine and 2-deoxyribose.

9-(3,5-Di-*O*-acetyl-2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)-2,6-dichloropurine (α- and β-9a).—A mixture of 2,6-dichloropurine (10 g, 53 mmoles) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-*erythro*-pentofuranose (14 g, 54 mmoles) was fused, with stirring, *in vacuo* (10 mm) at 130–140° for 15 min. The reaction melt was dissolved in C_6H_6, the unreacted 2,6-dichloropurine that precipitated (2.2 g) was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in Et_2O, the solution was seeded with α-**9a**, and the crystals that formed were collected after 1 hr at room temperature, washed, and dried *in vacuo*; yield 4.83 g (23% α-**9a**): mp 125°; $[\alpha]^{25}D$ +4.5 ± 1.5° (*c* 0.97, $CHCl_3$) [lit.[24] mp 123.5–124.5°, $[\alpha]^{25}D$ +0.4° (*c* 1.13, MeOH)]; δ [ppm ($CDCl_3$)] 2.00 and 2.14 (CH_3 of acetyls), 2.82 m (two 2′-H), 4.25 m (two 5′-H), 4.63 m (4′-H), 5.33 m (3′-H), 6.55 (q, $J_{1'2'}$ = 6.5 Hz, $J_{1'2''}$ = 2.6 Hz, 1′-H), 8.37 (8-H). The filtrate and washings of α-**9a** were combined, the solution was evaporated to dryness, and the residue was dissolved in EtOH (100 ml). After treatment with Norit, the solution was seeded with crystalline α,β-**9a** and refrigerated overnight. The crystals that formed were collected and recrystallized from EtOH (100 ml); yield 6.4 g (31% 1α:1β-**9a**), mp 114–116°, $[\alpha]^{25}D$ 0 ± 1.0° (*c* 0.9, $CHCl_3$). The pmr spectrum of a solution of these crystals indicated that they are a 1:1 α:β mixture.

The EtOH filtrates from the isolation of crystalline 1α:1β-**9a** were combined and evaporated to dryness *in vacuo*. Trituration of the residue with C_6H_6 (25 ml) precipitated additional unreacted 2,6-dichloropurine which was removed by filtration. The filtrate was concentrated *in vacuo* and absorbed on a silica gel column (2.8 × 85 cm, packed and equilibrated (18 hr) with C_6H_6). The column was eluted with 9:1 $CHCl_3$–EtOAc and the fractions were analyzed by tlc (Et_2O). Fractions containing essentially homogeneous β-**9a** were combined and evaporated to dryness *in vacuo*. Recrystallization[39] of the residue from EtOH gave pure

(36) J. J. Brink and G. A. LePage, *Can. J. Biochem.*, **43**, 1 (1965).

(37) SilicAR-TLC-7 (Mallinckrodt) was used for column and thin layer (1-mm thick) chromatographic purifications. Silica gel H (Brinkmann) was used for thin layer (0.25-mm thick) analyses (tlc). Chromatographic homogeneity was established for all compounds using the solvent systems indicated. Spots were detected with either ultraviolet light (256 mμ) after spraying the plates with Ultraphor (WT, highly concentrated) (BASF Colors & Chemicals, Inc., Charlotte, N. C.) or heat charring after spraying with ammonium sulfate.[38] The ultraviolet spectra were determined in 0.1 *N* HCl, 0.1 *N* NaOH, and pH 7 buffer with a Cary Model 14 spectrophotometer; the infrared spectra were determined in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer. The pmr spectra were determined in the solvents specified with a Varian A-60A spectrometer using tetramethylsilane as an internal reference. Melting points, unless otherwise noted, were determined on a Kofler-Heizbank and are corrected.

(38) T. Ziminski and E. Borowski, *J. Chromatogr.*, **23**, 480 (1966).

(39) Seed crystals were obtained from a previous run by thin layer chromatography (Et_2O).

β-anomer: yield 2.3 g (11%); mp 108°; $[\alpha]^{25}D$ −6.0 ± 0.5° (*c* 0.97, $CHCl_3$); δ [ppm ($CDCl_3$)] 2.10 and 2.16 (CH_3 of acetyls), 2.79 m (two 2′-H), 4.39 m (two 5′-H and 4′-H), 5.41 m (3′-H), 6.47 (t, $J_{1'2',2''}$ = 6.95 ± 0.30 Hz, peak width 13.9 Hz, 1′-H), 8.41 (8-H). *Anal.* ($C_{14}H_{13}Cl_2N_4O_5 \cdot 0.5H_2O$) C, H, N.

9-(2,3,5-Tri-*O*-benzyl-α- and -β-D-arabinofuranosyl)-2,6-dichloropurine (α- and β-9b). **A.**—A freshly prepared solution of 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride[29] (8.8 mmoles) in sieve-dried $MeNO_2$ (20 ml) was added with stirring to an azeotropically dried solution of 2,6-dichloropurine (**2**, 1.6 g, 8.3 mmoles) in $MeNO_2$ (100 ml) containing $Hg(CN)_2$ (2.5 g, 10 mmoles) and $CaSO_4$ (2.8 g, 20.3 mmoles of Drierite). The resulting reaction mixture was refluxed for 3 hr under anhydrous conditions and filtered through dry Celite, the filtrate was evaporated to dryness *in vacuo*, and the residue was triturated with C_6H_6. The insoluble solid that formed was removed by filtration and the filtrate was washed with H_2O, dried ($MgSO_4$), and concentrated *in vacuo*. The C_6H_6 concentrate (10 ml) was absorbed on a silica gel column (1.9 × 35 cm, packed and equilibrated with C_6H_6). The column was eluted with C_6H_6 (200 ml) and then with $CHCl_3$, and the fractions were analyzed by tlc ($CHCl_3$). The fractions containing the faster moving anomer were combined and evaporated to dryness *in vacuo* giving **β-9b** as a pigmented oil: yield 511 mg (11%); δ [ppm ($CDCl_3$)] 3.65 d (two 5′-H), 4.17 m and 4.57 d (4′-H, CH_2 of $PhCH_2$, and 2′-H), 6.39 (d, $J_{1'2'}$ = 4.3 Hz, 1′-H), 7.1 m and 7.33 d (phenyl H and 3′-H), 8.41 (8-H).

Evaporation of the fractions containing the slower moving anomer to dryness *in vacuo* gave **α-9b** as a colorless oil: yield 1.2 g (25%); δ [ppm ($CDCl_3$)] 3.65 d (two 5′-H), 4.15 m (4′-H), 4.50 m (CH_2 of $PhCH_2$ and 2′-H), 6.22 (d, $J_{1'2'}$ = 1.7 Hz, 1′-H), 7.05 m (3′-H), 7.32 d (phenyl H), 8.23 (8-H).

The conversion of **β-9b** to 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-chloroadenine[28] confirmed its configuration: yield 290 mg (60%), mp 137°, $[\alpha]^{25}D$ +43.7 ± 0.4° (*c* 0.51, $CHCl_3$) [lit.[28] mp 135–136°, $[\alpha]^{23.2}D$ +49.6° (*c* 0.5, $CHCl_3$)].

B.—The reaction carried out as described by Keller, *et al.*,[28] gave a 52% yield of **β-9b** and a small amount of **α-9b**, which were separated by column chromatography.

9-(2,3,5-Tri-*O*-acetyl-α-D-arabinofuranosyl)-6-chloro-2-fluoropurine (α-10c).—A mixture of 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose (3.5 g, 11 mmoles) and 6-chloro-2-fluoropurine[18] (**8**, 1.7 g, 10 mmoles) was heated *in vacuo* (10 mm) with stirring at 150° until a homogeneous melt was obtained and vigorous gas evolution had ceased. After the reaction flask had cooled but before the melt solidified, the vacuum was broken and *p*-toluenesulfonic acid (35 mg) was added. The reaction mixture was again heated *in vacuo* with stirring at 140° for 30 min. An EtOAc solution of the resulting clear glass was washed with saturated $NaHCO_3$ solution and then H_2O, and dried ($MgSO_4$) before it was evaporated to dryness *in vacuo*. The residue was triturated with Et_2O and the insoluble amorphous pigments were removed by filtration. Evaporation of the Et_2O filtrate to dryness gave **α-10c** as a glass: yield 3.7 g (85%); tlc (1:1 $CHCl_3$–EtOAc); δ [ppm ($CDCl_3$)] 2.13 t (CH_3 of acetyl), 4.35 m (two 5′-H), 4.73 m (4′-H), 5.35 m (3′-H), 5.81 t (2′-H), 6.22 (d, $J_{1'2'}$ = 3 Hz, 1′-H), 8.27 (8-H).

2,6-Diazido-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (β-11b).—A solution of NaN_3 (1.16 g, 17.8 mmoles) in H_2O (6.6 ml) was added with stirring to a solution of 2,6-dichloro-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (**β-9b**, 4.8 g, 8.1 mmoles) in EtOH (100 ml). After refluxing for 1 hr, the reaction mixture was filtered to remove the precipitate that formed, and the filtrate was evaporated to dryness *in vacuo*. Trituration of the residue with C_6H_6 (50 ml) gave a mixture from which the remaining inorganic salts were removed by filtration. Evaporation of the filtrate to dryness *in vacuo* gave **β-11b** as a glass: yield 4.8 g (98%); $\bar{\nu}_{max}$ (cm^{-1}) 3085, 3060, 3025, 2920, 2860 (CH) 2160, 2125 (N≡N), 1595, 1565 (C=C, C=N), 1110–1065, 1020 (COC), 730, 680 (phenyl). Tlc ($CHCl_3$) showed only trace impurities.

3′,5′-Di-*O*-acetyl-2-amino-2′-deoxyadenosine (β-12a). **A.**—A solution of NaN_3 (760 mg, 11.7 mmoles, in 3.5 ml of H_2O) and 9-(3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-2,6-dichloropurine (**β-9a**, 2.25 g, 5.8 mmoles) in EtOH (53 ml) was refluxed for 1 hr before it was evaporated to dryness *in vacuo*. A C_6H_6 solution of the residue was filtered to remove inorganic salts and, after evaporation of the filtrate to dryness *in vacuo*, the residue (**β-11b**, 2.3 g, 5.8 mmoles) was redissolved in EtOH (200 ml). The EtOH solution was hydrogenated in the presence of 5% Pd–C (400 mg) under conditions detailed elsewhere (**β-12b**). After removal of the catalyst, the EtOH filtrate was concentrated *in vacuo* and refrigerated. The crystals were collected in several crops and recrystallized from EtOH (75 ml); yield 1.3 g (65%); mp 168°; $[\alpha]^{30}D$ −16.0 ± 0.3° (*c* 1.01, $CHCl_3$); tlc (9:1 $CHCl_3$–MeOH); λ_{max} [mμ (ε × 10^{-3})], pH 1—252 (11.7), 292 (10.0); pH 7, 13—256 (9.4), 279 (10.1); $\bar{\nu}_{max}$ (cm^{-1}) 3445, 3350, 3170, 2939 (NH, CH), 1730, 1710 (C=O), 1660, 1630, 1595, 1585 (NH_2, C=C, C=N), 1090, 1070, 1060, 1020 (COC); δ [ppm (DMSO-d_6)] 2.05, 2.11 (CH_3 of acetyls), 292 m (two 2′-H), 4.27 (4′-H and two 5′-H), 5.35 d (3′-H), 5.80 and 6.75 (NH_2), 6.22 (q, $J_{1'2'}$ = 6.3 Hz, $J_{1'2''}$ = 8.3 Hz, 1′-H), 7.91 (8-H). *Anal.* ($C_{14}H_{18}N_6O_5$) C, H, N.

B.—The reaction mixture resulting from refluxing (1 hr) crystalline 9-(3,5-di-*O*-acetyl-2-deoxy-α,β-D-*erythro*-pentofuranosyl)-2,6-dichloropurine (6.3 g, 16 mmoles) in EtOH (150 ml) containing NaN_3 (2.1 g, 32.6 mmoles in 10 ml of H_2O) was refrigerated overnight. The inorganic salts that precipitated were removed by filtration, and the filtrate was diluted with EtOH (400 ml). Hydrogenation of the resulting EtOH solution in the presence of 5% Pd–C (1.2 g) was carried out as described for **β-12b**. After removal of the catalyst and evaporation of the filtrate to dryness, the residue was dissolved in $CHCl_3$ (60 ml). Filtration of the $CHCl_3$ solution removed inorganic salts, and after concentration *in vacuo* the filtrate (40 ml) was absorbed on a silica gel column (2.9 × 115 cm, packed and equilibrated with $CHCl_3$). The column was eluted with 19:1 $CHCl_3$–MeOH and the fractions were analyzed by tlc (9:1 $CHCl_3$–MeOH). Fractions containing homogeneous **β-12a** were combined with additional homogeneous **β-12a** obtained by rechromatographing impure fractions from the first column. Recrystallization (EtOH) of the combined fractions gave pure **β-12a**, yield 1.6 g (27%).

2-Amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-adenine (β-12b).—Pd–C (5%) (500 mg) was added to a solution of 2,6-diazido-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (**β-11b**, 4.8 g, 8.0 mmoles) in EtOH (500 ml), and the resulting mixture was hydrogenated at atmospheric pressure for 6 hr. The H_2 atmosphere was changed after 30 min and after 1 hr. The catalyst was removed by filtration and was washed with $CHCl_3$ to dissolve precipitated product. The combined filtrate and washings was evaporated to dryness *in vacuo* and the resulting residue was dissolved in boiling EtOH (80 ml). The crystals that formed on cooling the EtOH solution were collected by filtration, washed with EtOH, and dried *in vacuo*: yield 3.3 g (75%), mp 161–162°, tlc (19:1 $CHCl_3$–MeOH). The analytically pure product was obtained from a previous preparation: yield 69%; mp 162°; $[\alpha]^{25}D$ +44.4 ± 0.7° (*c* 1.06, $CHCl_3$); λ_{max} [mμ (ε × 10^{-3})], pH 1—254 (10.9), 292 (9.0); EtOH—256 (10.8), 283 (9.0); pH 7, 13—256 (9.0), 279 (9.1); $\bar{\nu}_{max}$ (cm^{-1}) 3460, 3350, 3310, 3140 (NH, CH), 2940, 2915, 2890, 2865 (CH), 1660 (NH), 1590 (C=C, C=N), 1085, 1045, 1015 (COC), 730, 690 (phenyl). *Anal.* ($C_{38}H_{39}N_6O_4$) C, H, N.

2-Amino-9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-adenine (α-12c).—A mixture of 1,2,3,5-tetra-*O*-acetylarabinofuranose[30] (6 g, 19 mmoles) and 2,6-dichloropurine (**2**, 3.3 g, 18 mmoles) was fused *in vacuo* (10 mm) at 140° in the presence of *p*-toluenesulfonic acid (100 mg) under the conditions described for **α-10c**. After reaction was complete, the fusion melt was dissolved in C_6H_6 and the solution was extracted with $NaHCO_3$ solution and washed with H_2O before it was evaporated to dryness *in vacuo*. The residue was triturated with ligroin before it was dried *in vacuo*: yield of **α-9c**, 6 g (75%); δ [ppm ($CHCl_3$)] 2.12 t (CH_3 of acetyls), 4.40 m (two 5′-H), 5.37 m (3′-H), 5.82 t (2′-H), 6.27 (d, $J_{1'2'}$ = 3 Hz, 1′-H), 8.27 (8-H). The presence of a small amount of the β anomer was detected by bands at 6.6 (d, $J_{1'2'}$ = 4.5 Hz, 1′-H) and at 8.33 (8-H). The (Et_2O) indicated the product was suitable for use as an intermediate.

A solution of NaN_3 (1.7 g in 6 ml of H_2O) was added with stirring to **α-9c** (6 g, 13.4 mmoles) in EtOH (150 ml) and the mixture was refluxed for 1.25 hr before it was evaporated to dryness *in vacuo*. A C_6H_6 solution of the residue was filtered to remove inorganic salts and evaporation of the filtrate to dryness *in vacuo* gave **α-11c** as a glass: yield 6 g (100%); $\bar{\nu}_{max}$ (cm^{-1}) 3120, 2950, 2930 (CH), 2150, 2130 (N≡N), 1740 (C=O), 1600, 1570 (C=C, C=N), 1350, 1250–1220 (COC ester), 1050 (COC sugar). Tlc (Et_2O) indicated the material was suitable for use as an intermediate.

An EtOH solution (300 ml) of **α-11c** (6 g, 13 mmoles) was hydrogenated in the presence of 5% Pd–C (500 mg) as described for **β-12b**. After reaction was complete, the catalyst was removed by filtration, the filtrate was evaporated to dryness, and the residue (5.2 g, 98% crude yield) was redissolved in $CHCl_3$ (16 ml). The $CHCl_3$ solution was adsorbed on a silica gel column (2.8 ×

85 cm, packed and equilibrated (18 hr) with C_6H_6). The column was eluted with $CHCl_3$ (750 ml) before the eluent was changed to 19:1 $CHCl_3$–MeOH. The fractions were analyzed by tlc (19:1 $CHCl_3$–MeOH) and those fractions containing homogeneous α-**12c** were combined and evaporated to dryness *in vacuo;* yield 3.06 g (45%); δ [ppm ($CDCl_3$)] 2.08 and 2.11 (CH_3 of acetyls), 4.33 m (two 5′-H), 4.62 t (4′-H), 5.03 and 5.85 broad (NH_2), 5.32 m (3′-H), 6.06 m (2′-H and 1′-H), 7.68 (8-H).

2-Amino-9-α-D-arabinofuranosyladenine (α-**12e**). **A.**—A solution of NaN_3 (260 mg, 4 mmoles) in H_2O (1.5 ml) was added to 9-(2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl)-2,6-dichloropurine (α-**9b**, 1.2 g, 2 mmoles) in EtOH (30 ml) and the resulting reaction solution was refluxed for 1 hr. After removal of inorganic salts by filtration, the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in absolute EtOH (150 ml) and 5% Pd–C (200 mg) was added. The resulting reaction solution was hydrogenated at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated to dryness and the residue was dried *in vacuo*. The resulting crude α-**12b** was suspended with stirring in liquid NH_3. Na was added in small portions until a blue color persisted for 5 min. The reaction was quenched with the addition of NH_4Cl, and the NH_3 was removed in a stream of dry N_2. After trituration with C_6H_6, the residue was dissolved in H_2O (20 ml), and the solution was acidified with AcOH and filtered. The filtrate was concentrated *in vacuo*, and the crystals that formed were collected by filtration and dried *in vacuo* giving a 49% yield of product which showed a major spot [tlc (3:1 $CHCl_3$–MeOH)] identical with that prepared by method B (see below). Recrystallization of this product from EtOH–H_2O (4:1) followed by two recrystallizations from EtOH gave pure α-**12e**: yield 90 mg (16%); mp 213°; $[\alpha]^{50}D$ +42.3 ± 0.4° (*c* 1.1, H_2O); λ_{max} [mμ (ε × 10^{-3})], pH 1—253 (11.2), 292 (9.9); pH 7—256 (9.2), 280 (9.9); pH 13—256 (8.9), 279 (9.8); $\bar{\nu}_{max}$ (cm^{-1}) 3360, 3320, 3300, 3200–3100 (OH, NH), 2940–2900 (CH), 1660, 1615, 1600 (NH, C=C, C=N), 1090, 1050, 1010 (COC). *Anal.* ($C_{10}H_{14}N_6O_4$) C, H, N.

B.—A solution of 2-amino-6-chloro-9-α-D-arabinofuranosylpurine (α-**15e**, 500 mg, 1.6 mmoles) in EtOH–NH_3 (40 ml saturated at 5°) was heated in a Parr bomb at 100° for 18 hr. The residue from evaporation of the solution *in vacuo* was crystallized from H_2O (25 ml) with Norit treatment. The crystals were collected by filtration and dried *in vacuo;* yield 370 mg (80%); λ_{max} (mμ), pH 1—253, 292; pH 7, 13—256, 278.

9-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-2-chloroadenine (β-**14c**).—A solution of 9-β-D-arabinofuranosyl-2-chloroadenine[28] (β-**14e**, 1.1 g, 35 mmoles) in pyridine (14 ml) and Ac_2O (10 ml) was stirred at room temperature for 2 hr before it was evaporated to dryness *in vacuo*. A solution of the residue in EtOH was evaporated to dryness before the residue was crystallized from EtOH. The crystals were collected in several crops and recrystallized from EtOH giving β-**14c**: yield 1.1 g (71%), mp 160°; tlc (19:1 $CHCl_3$–MeOH) showed only trace impurities and indicated the material was suitable for use as an intermediate; λ_{max} (mμ), pH 1, 7—263, pH 13—264.5; $\bar{\nu}_{max}$ (cm^{-1}) 3360, 3310, 3260, 3175 (NH, CH), 3000, 2940 (CH), 1750, 1735 (C=O), 1650 (NH_2), 1590, 1570 (C=C, C=N), 1250–1210, 1110, 1060, 1040 (COC); δ [ppm ($CDCl_3$)] 1.94 and 2.15 d (CH_3 of acetyls), 4.37 m (two 5′-H and 4′-H), 5.04 m (3′-H and 2′-H), 6.37 broad (NH_2), 6.53 (d, $J_{1'2'}$ = 4.4 Hz, 1′-H), 7.98 (8-H).

2-Amino-6-chloro-9-α-D-arabinofuranosylpurine (α-**15e**).—A solution of 9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-6-chloro-2-fluoropurine (8, 3.5 g, 8 mmoles) in EtOH–NH_3 (200 ml, saturated at 5°) was refrigerated for 3 days before it was evaporated to dryness. The residue was dissolved in H_2O and extracted with $CHCl_3$. The crystals that precipitated from the H_2O solution on concentration were collected in several crops giving the crude product, which was recrystallized from H_2O; yield 860 mg (34%); mp 190° dec (Mel-Temp); tlc (3:1 $CHCl_3$–MeOH) showed only trace contaminants and indicated the product suitable for use as an intermediate; λ_{max} (mμ), pH 1—215, 246, 310; pH 7, 13—216, 247, 307; $\bar{\nu}_{max}$ (cm^{-1}) 3465, 3370, 3300, 3180, 3080 (OH, NH), 2940, 2920, 2840 (CH), 1630 (NH), 1615, 1555, 1510 (C=C, C=N), 1050, 1020 (COC).

9-(3,5-Di-*O*-acetyl-2-deoxy-α-D-ribofuranosyl)-2-fluoroadenine (α-**16a**).—A solution of 9-(3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranosyl)-2,6-dichloropurine (α-**9a**, 4.8 g, 12.5 mmoles) and NaN_3 (1.6 g, 25 mmoles, in 7 ml of H_2O) in EtOH (100 ml) was refluxed for 1.25 hr. After removal of inorganic salts by treatment with C_6H_6, an EtOH solution (250 ml) of the resulting α-**11a** (5 g, 12.5 mmoles) was hydrogenated in the presence of 5% Pd–C (500 mg) as described for β-**12a**. After removal of the catalyst and concentration of the EtOH filtrate, the α-**12a** precipitated and was collected by filtration, washed, and dried; yield 4 g (91%), mp 155°, tlc (9:1 $CHCl_3$–MeOH).

A $CHCl_3$ solution (50 ml) of 9-(3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranosyl-2-aminoadenine (α-**12a**, 3.5 g, 11 mmoles) was cooled to −20° and diluted with stirring with 48% HBF_4 (80 ml). The resulting mixture was treated with $NaNO_2$ (2 g, 30 mmoles, in 2 ml of H_2O) as described for β-**16b**. The neutral reaction emulsion was centrifuged and the reaction products were identified by tlc (EtOAc) and isolated as described (see β-**16a**) giving the following results: isoguanine (42%), 2-fluoroadenine (10%), 9-(3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranosyl)-2,6-difluoropurine (3%), and α-**16a** (10%, mp 183°).

Pure α-**16a** was isolated by tlc (EtOAc) followed by C_6H_6 recrystallization; yield 7%; mp 182°; $[\alpha]^{22}D$ +34.4 ± 0.8° (*c* 0.99, $CHCl_3$); λ_{max} [mμ (ε × 10^{-3})], pH 1—262 (13.4), 267 (sh); pH 7, 13—261 (14.8), 267 (sh); $\bar{\nu}_{max}$ (cm^{-1}) 3320, 3170 (NH, CH), 1750 (C=O), 1680, 1645, 1610, 1580 (NH, C=C, C=N), 1230, 1100, 1060, 1040 (COC); δ [ppm ($CDCl_3$)] 2.00 and 2.12 (CH_3 of acetyls), 2.80 (two 2′-H), 4.25 (two 5′-H), 4.59 (4′-H), 5.27 (3′-H), 6.22 broad (NH_2), 6.40 (q, $J_{1'2'}$ = 6.5 Hz, $J_{1'2''}$ = 3.2 Hz, 1′-H), 7.33 (benzene), 8.00 (8-H). *Anal.* ($C_{14}H_{16}FN_5O_5 \cdot {}^1/_{12}C_6H_6$) C, H, N.

The 9-(3,5-di-*O*-acetyl-2-deoxy-α-D-*erythro*-pentofuranosyl)-2,6-difluoropurine was purified by thin layer chromatography (1:1 $CHCl_3$–EtOAc) and its identity was confirmed by mass spectroscopy.

3′,5′-Di-*O*-acetyl-2-fluoro-2′-deoxyadenosine (β-**16a**).—3′,5′-Di-*O*-acetyl-2-amino-2′-deoxyadenosine (β-**12a**, 3.6 g, 10.4 mmoles) was dissolved with stirring in 48% HBF_4 (50 ml at −10°) and the solution was cooled to −20°. A solution of $NaNO_2$ (1.2 g, 17 mmoles, in 2 ml of H_2O) was added dropwise, and the reaction mixture was stirred at −20 to −10° for 20 min. After dilution with $CHCl_3$ (50 ml), the mixture was cooled to −30° and neutralized (pH 5–6) with 50% NaOH. The resulting emulsion was centrifuged to separate the insoluble gel from the liquid layers, which were each washed with more solvent (50 ml). The aqueous layers were discarded and the combined $CHCl_3$ solution was set aside. The gel was triturated with EtOH–Et_2O until a filterable solid was obtained, and, after the addition of more Et_2O, the solid was collected, triturated with H_2O, dried *in vacuo*, and identified by spectral analysis as isoguanine: yield 48%. The EtOH–Et_2O and H_2O filtrates were combined and evaporated to dryness *in vacuo*, and the residue was triturated with $CHCl_3$. The insoluble solid was collected and identified by spectral analyses as 2-fluoroadenine, yield 17%. The filtrate was combined with the $CHCl_3$ solution that had been set aside, and the resulting solution was washed with H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue was triturated with C_6H_6 and the insoluble solid was collected and recrystallized from $CHCl_3$ (40 ml); yield 536 mg (14.5%); mp 208°; tlc (EtOAc); δ [ppm ($CDCl_3$)] 2.07 and 2.11 (CH_3 of acetyls), 2.73 m (two 2′-H), 4.35 m (two 5′-H and 4′-H), 5.39 (3′-H), 6.03 broad (NH_2), 6.30 (t, $J_{1'2',2''}$ = 7.0 ± 0.2 Hz, peak width 13.5 Hz, 1′-H), 7.91 (8-H).

Tlc (EtOAc) indicated the benzene filtrate from the isolation of β-**16a** contained a small amount of 9-(3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-2,6-difluoropurine in addition to the sugar residues.

9-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (β-**16b**).—HBF_4 (48%) (150 ml) was added to a solution (5°) of 2-amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)adenine (β-**12b**, 4.6 g, 8.4 mmoles) in $CHCl_3$ (60 ml). The resulting mixture was cooled to −10° before a solution of $NaNO_2$ (1.7 g, 25 mmoles) in 4 ml of H_2O was added dropwise with stirring. After the addition was complete, the mixture was stirred an additional 40 min at −10 to −5° before it was diluted with $CHCl_3$ (75 ml), cooled to −20°, and neutralized (pH 5–6) with 50% NaOH. The $CHCl_3$ layer was separated, washed with H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue, primarily a mixture of β-**16b** and 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2,6-difluoropurine, identified by tlc (1:1 $CHCl_3$–EtOAc), was dissolved in absolute EtOH, and the solution was saturated at 5° with dry NH_3. After refrigeration overnight, the reaction solution was evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ (25 ml), and the solution was filtered through dry Celite to remove inorganic salts. The filtrate was adsorbed on a silica gel column (2.6 × 35 cm, packed and equilibrated with $CHCl_3$). The column was eluted with

1:1 $CHCl_3$–EtOAc. The fractions containing **β-16b** were combined and evaporated to dryness *in vacuo*, and the residue was recrystallized from C_6H_6 (40 ml): yield 1.7 g (36%); mp 157–159°; λ_{max} (mμ), pH 1, **7**, 13, and EtOH—261, 272 (sh); $\bar{\nu}_{max}$ (cm^{-1}) 3350, 3320, 3150 (NH), 2940, 2920, 2910, 2860 (CH), 1665, 1610, 1575 (NH, C=C, C=N), 1110, 1100, 1085, 1060 (COC), 730 (phenyl).

In a previous preparation a sample of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2,6-difluoropurine was isolated by thin layer chromatography. The identity of the sample was confirmed by spectral analyses: λ_{max} (mμ), EtOH—257; $\bar{\nu}_{max}$ (cm^{-1}) 3105, 3085, 3060, 3030, 2920, 2865 (CH), 1620, 1580 (C=C, C=N), 1110–1060 (COC), 730, 690 (phenyl).

9-(2-Deoxy-α-D-ribofuranosyl)-2-fluoroadenine (α-16d). A solution of 9-(3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranosyl)-2-fluoroadenine (**α-16a**, 700 mg, 2 mmoles) in EtOH–NH_3 (300 ml saturated at 5°) was refrigerated for 3 days before it was evaporated to dryness *in vacuo*. After trituration with $CHCl_3$, the reaction residue was crystallized from EtOH–Et_2O and the crude product was collected and recrystallized with Norit treatment from EtOH (70 ml). A second recrystallization from EtOH gave the pure material: yield 320 mg (42%); mp 232°; $[\alpha]^{25}D$ −24.8 ± 1.6° (*c* 0.51, EtOH); tlc (9:1 $CHCl_3$–MeOH); λ_{max} [mμ (ε × 10^{-3})], pH 1—262.5 (13.0), 267 (12.6, sh); pH **7**, 13—261 (14.9), 268 (11.8, sh); $\bar{\nu}_{max}$ (cm^{-1}) 3430, 3320, 3170 (OH, NH), 2930, 2870, 2770 (CH), 1690 (NH_2), 1640, 1620, 1580 (C=C, C=N), 1120, 1100, 1085, 1070 (COC). *Anal.* ($C_{10}H_{12}FN_5O_3$) C, H, N.

2′-Deoxy-2-fluoroadenosine (β-16d). An EtOH–NH_3 solution (100 ml saturated at 5°) of 3′,5′-di-*O*-acetyl-2′-deoxy-2-fluoroadenosine (**β-16a**, 500 mg, 1.4 mmoles) was refrigerated for 3 days before it was evaporated to dryness *in vacuo*. The residue, after $CHCl_3$ trituration, was dissolved in EtOH (30 ml) and treated with Norit, and the filtrate was evaporated to dryness *in vacuo*. Trituration of the residue with Me_2CO gave the pure material in two crops: yield 180 mg (48%); melting point indefinite, >210°; $[\alpha]^{25}D$ −19.2 ± 0.4° (*c* 0.9, EtOH); tlc (9:1 $CHCl_3$–MeOH); λ_{max} [mμ (ε × 10^{-3})], pH 1—262 (13.2), 267 (11.9, sh); pH 7, 13—261 (14.5), 267 (11.6, sh); $\bar{\nu}_{max}$ (cm^{-1}) 3400, 3340–3305, 3140 (OH, NH), 2915 (CH), 1690, 1650 (NH), 1605, 1575 (C=C, C=N), 1090, 1085, 1065, 1045 (COC). *Anal.* ($C_{10}H_{12}FN_5O_3$) C, H, N.

9-α-D-Arabinofuranosyl-2-fluoroadenine (α-16e). A mixture of 9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2-aminoadenine (**α-12c**, 3 g, 7.3 mmoles), $CHCl_3$ (25 ml), and 48% HBF_4 (65 ml) was treated with $NaNO_2$ solution (1.5 g, 22.2 mmoles in 2 ml of H_2O) under the modified Schiemann reaction conditions described in the preparation of **β-16b**. The dry $CHCl_3$ residue was triturated with C_6H_6 (20 ml) and the insoluble solid was removed by filtration. Evaporation of the filtrate to dryness *in vacuo* gave primarily 9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2,6-difluoropurine: yield 700 mg (23%); δ [ppm (CDCl₃)] 2.11, 2.13, 2.17 (CH_3 of acetyls), 4.38 m (two 5′-H), 4.70 m (4′-H), 5.36 m (3′-H), 5.83 t (2′-H), 6.25 (d, $J_{1'2'}$ = 2.8 Hz, 1′-H), 8.24 d (8-H). The 8-proton appears to be coupled with one or both F atoms. Tlc, (9:1 C_6H_6–MeOH) indicated that **α-16c** was the major contaminant.

An EtOH–NH_3 solution (100 ml saturated at 5°) of 9-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2,6-difluoropurine (700 mg, 1.7 mmoles) was refrigerated for 3 days before it was evaporated to dryness *in vacuo*. The residue was triturated first with $CHCl_3$ and dried *in vacuo* before it was triturated in boiling EtOH (30 ml) and the EtOH mixture was refrigerated. The EtOH-insoluble solid was collected and recrystallized from boiling H_2O (30 ml) giving the crude product. Recrystallization of the crude product from H_2O (40 ml) gave pure **α-16e** in two crops: yield 152 mg (31%); mp 275° (Mel-Temp); $[\alpha]^{25}D$ −73 ± 4° (*c* 0.05, EtOH); tlc (4:1 $CHCl_3$–MeOH); λ_{max} [mμ (ε × 10^{-3})], pH 1—262 (13.5); pH 7, 13—262 (14.9); $\bar{\nu}_{max}$ (cm^{-1}) 3420–3180 (OH, NH), 1695 (NH_2), 1620, 1575 (C=C, C=N), 1050, 1040 (COC). *Anal.* ($C_{10}H_{12}FN_5O_4$) C, H, N.

9-β-D-Arabinofuranosyl-2-fluoroadenine (β-16e). 9-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (**β-16b**, 1.7 g, 3 mmoles) was suspended in liquid NH_3 (75 ml) with stirring and Na (410 mg, 18 g-atoms) was added in small portions. When a purple color persisted for a few minutes after the addition of Na, the mixture was neutralized with NH_4Cl (920 mg, 18 mmoles). The NH_3 was evaporated in a stream of dry N_2, and the residue was triturated with Et_2O (75 ml). The insoluble solid that formed was collected by filtration and washed with H_2O (three 3-ml portions) before it was recrystallized from H_2O (65 ml) giving the crude product (479 mg), which was shown by tlc (4:1 $CHCl_3$–MeOH) to be primarily a mixture of **β-16e** and 9-β-D-arabinosyladenine. A solution of the crude product in H_2O (1 ml/mg) was percolated through an ion-exchange column [10 g of Amberlite CG50, 100–200 mesh)/mmole of nucleoside] at a rate not exceeding 0.5 ml/min, and the column was eluted with H_2O until elution of **β-16e** was complete. The fractions containing homogeneous **β-16e** were combined and evaporated to dryness. The residue was recrystallized from H_2O: yield 290 mg (34%); mp 265–267° (Mel-Temp).

An analytically pure sample was obtained from a previous synthesis. The crude product was first purified by tlc, and pure **β-16e** was obtained after three recrystallizations (EtOH–H_2O) of the tlc isolated material: mp 260° (Mel-temp); $[\alpha]^{25}D$ +17.0 ± 2.5° (*c* 0.1, EtOH); λ_{max} [mμ (ε × 10^{-3})], pH 1—262 (13.2); pH 7—261 (14.8); pH 13—262 (15.0); $\bar{\nu}_{max}$ (cm^{-1}) 3450, 3305, 3180, 3025 (OH, NH), 2915, 2900 (CH), 1630 (NH), 1610, 1590 (C=C, C=N), 1115, 1085, 1055, 1020, 1000 (COC); δ [ppm (DMSO-d_6)] 3.70 m (two 5′-H and 4′-H), 4.13 m (3′-H and 2′-H), 4.98 (5′-OH), 5.5 m (2′-OH and 3′-OH), 6.13 (d, $J_{1'2'}$ = 4.2 Hz, 1′-H), 7.2 broad (NH_2), 8.16 (8-H). *Anal.* ($C_{10}H_{12}FN_5O_4$) C, H, N.

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